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22 TOXICOLOGICAL CHEMISTRY

22.1. INTRODUCTION TO TOXICOLOGY AND TOXICOLOGICAL CHEMISTRY

Ultimately, most pollutants and hazardous substances are of concern because of their toxic effects. The general aspects of these effects are addressed in this chapter under the heading of toxicological chemistry; the toxicological chemistry of specific classes of chemical substances is addressed in Chapter 23. In order to understand toxicological chemistry, it is essential to have some understanding of biochemistry, the science that deals with chemical processes and materials in living systems. Biochemistry was summarized in Chapter 21.

Toxicology

A **poison**, or **toxicant**, is a substance that is harmful to living organisms because of its detrimental effects on tissues, organs, or biological processes. **Toxicology** is the science of poisons. These definitions are subject to a number of qualifications. Whether a substance is poisonous depends upon the type of organism exposed, the amount of the substance, and the route of exposure. In the case of human exposure, the degree of harm done by a poison can depend strongly upon whether the exposure is to the skin, by inhalation, or through ingestion.

Toxicants to which subjects are exposed in the environment or occupationally may be in several different physical forms. This may be illustrated for toxicants that are inhaled. **Gases** are substances such as carbon monoxide in air that are normally in the gaseous state under ambient conditions of temperature and pressure. **Vapors** are gas-phase materials that have evaporated or sublimed from liquids or solids. **Dusts** are respirable solid particles produced by grinding bulk solids, whereas **fumes** are solid particles from the condensation of vapors, often metals or metal oxides. **Mists** are liquid droplets.

Often a toxic substance is in solution or mixed with other substances. A substance with which the toxicant is associated (the solvent in which it is dissolved or

the solid medium in which it is dispersed) is called the **matrix**. The matrix may have a strong effect upon the toxicity of the toxicant.

There are numerous variables related to the ways in which organisms are exposed to toxic substances. One of the most crucial of these, **dose,** is discussed in Section 22.2. Another important factor is the **toxicant concentration**, which may range from the pure substance (100%) down to a very dilute solution of a highly potent poison. Both the **duration** of exposure per exposure incident and the **frequency** of exposure are important. The **rate** of exposure and the total time period over which the organism is exposed are both important situational variables. The exposure **site** and **route** also affect toxicity.

It is possible to classify exposures on the basis of acute vs. chronic and local *vs.* systemic exposure, giving four general categories. **Acute local** exposure occurs at a specific location over a time period of a few seconds to a few hours and may affect the exposure site, particularly the skin, eyes, or mucous membranes. The same parts of the body can be affected by **chronic local** exposure, for which the time span may be as long as several years. **Acute systemic** exposure is a brief exposure or exposure to a single dose and occurs with toxicants that can enter the body, such as by inhalation or ingestion, and affect organs, such as the liver, that are remote from the entry site. **Chronic systemic** exposure differs in that the exposure occurs over a prolonged time period.

In discussing exposure sites for toxicants it is useful to consider the major routes and sites of exposure, distribution, and elimination of toxicants in the body as shown in Figure 22.1. The major routes of accidental or intentional exposure to toxicants by humans and other animals are the skin (percutaneous route), the lungs (inhalation, respiration, pulmonary route), and the mouth (oral route); minor routes of exposure are rectal, vaginal, and parenteral (intravenous or intramuscular, a common means for the administration of drugs or toxic substances in test subjects). The way that a toxic substance is introduced into the complex system of an organism is strongly dependent upon the physical and chemical properties of the substance. The pulmonary system is most likely to take in toxic gases or very fine, respirable solid or liquid particles. In other than a respirable form, a solid usually enters the body orally. Absorption through the skin is most likely for liquids, solutes in solution, and semisolids, such as sludges.

The defensive barriers that a toxicant may encounter vary with the route of exposure. For example, toxic elemental mercury is absorbed through the alveoli in the lungs much more readily than through the skin or gastrointestinal tract. Most test exposures to animals are through ingestion or gavage (introduction into the stomach through a tube). Pulmonary exposure is often favored with subjects that may exhibit refractory behavior when noxious chemicals are administered by means requiring a degree of cooperation from the subject. Intravenous injection may be chosen for deliberate exposure when it is necessary to know the concentration and effect of a xenobiotic substance in the blood. However, pathways used experimentally that are almost certain not to be significant in accidental exposures can give misleading results when they avoid the body's natural defense mechanisms.

An interesting historical example of the importance of the route of exposure to toxicants is provided by cancer caused by contact of coal tar with skin. The major barrier to dermal absorption of toxicants is the **stratum corneum**, or horny layer.

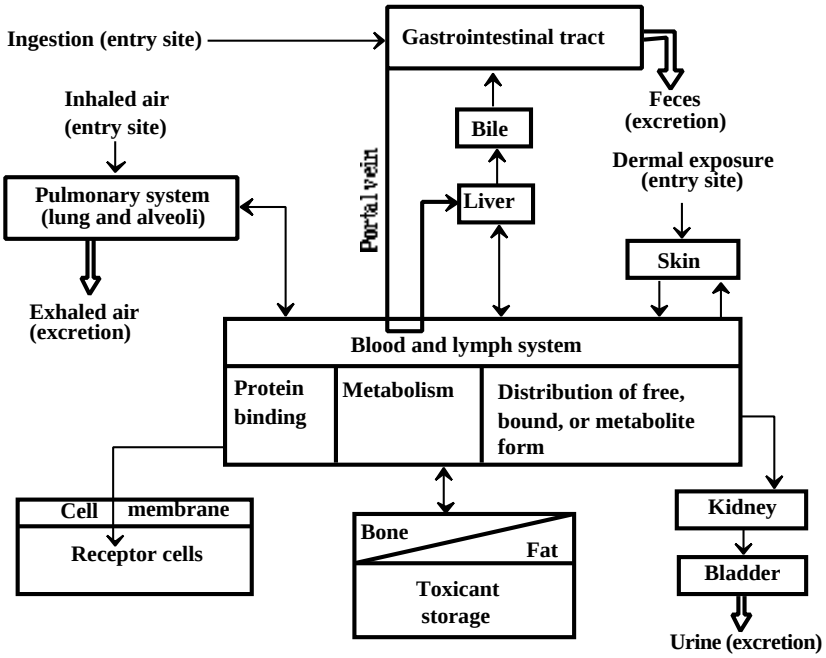


Figure 22.1. Major sites of exposure, metabolism, and storage, routes of distribution and elimination of toxic substances in the body.

The permeability of skin is inversely proportional to the thickness of this layer, which varies by location on the body in the order soles and palms > abdomen, back, legs, arms > genital (perineal) area. Evidence of the susceptibility of the genital area to absorption of toxic substances is to be found in accounts of the high incidence of cancer of the scrotum among chimney sweeps in London described by Sir Percival Pott, Surgeon General of Britain during the reign of King George III. The cancer-causing agent was coal tar condensed in chimneys. This material was more readily absorbed through the skin in the genital areas than elsewhere, leading to a high incidence of scrotal cancer. (The chimney sweeps' conditions were aggravated by their lack of appreciation of basic hygienic practices, such as bathing and regular changes of underclothing.)

Organisms can serve as indicators of various kinds of pollutants. In this application, organisms are known as **biomonitors**. For example, higher plants, fungi, lichens, and mosses can be important biomonitors for heavy metal pollutants in the environment.

Synergism, Potentiation, and Antagonism

The biological effects of two or more toxic substances can be different in kind and degree from those of one of the substances alone. One of the ways in which this can occur is when one substance affects the way in which another undergoes any of the steps in the kinetic phase as discussed in Section 22.7 and illustrated in Figure 22.9. Chemical interaction between substances may affect their toxicities. Both substances may act upon the same physiologic function, or two substances may

compete for binding to the same receptor (molecule or other entity acted upon by a toxicant). When both substances have the same physiologic function, their effects may be simply **additive** or they may be **synergistic** (the total effect is greater than the sum of the effects of each separately). **Potentiation** occurs when an inactive substance enhances the action of an active one, and **antagonism** when an active substance decreases the effect of another active one.

22.2. DOSE-RESPONSE RELATIONSHIPS

Toxicants have widely varying effects upon organisms. Quantitatively, these variations include minimum levels at which the onset of an effect is observed, the sensitivity of the organism to small increments of toxicant, and levels at which the ultimate effect (particularly death) occurs in most exposed organisms. Some essential substances, such as nutrient minerals, have optimum ranges above and below which detrimental effects are observed (see Section 22.5 and Figure 22.4).

Factors such as those just outlined are taken into account by the **dose-response** relationship, which is one of the key concepts of toxicology. **Dose** is the amount, usually per unit body mass, of a toxicant to which an organism is exposed. **Response** is the effect upon an organism resulting from exposure to a toxicant. In order to define a dose-response relationship, it is necessary to specify a particular response, such as death of the organism, as well as the conditions under which the response is obtained, such as the length of time from administration of the dose. Consider a specific response for a population of the same kinds of organisms. At relatively low doses, none of the organisms exhibits the response (for example, all live), whereas at higher doses all of the organisms exhibit the response (for example, all die). In between, there is a range of doses over which some of the organisms respond in the specified manner and others do not, thereby defining a dose-response curve. Dose-response relationships differ among different kinds and strains of organisms, types of tissues, and populations of cells.

Figure 22.2 shows a generalized dose-response curve. Such a plot may be obtained, for example, by administering different doses of a poison in a uniform manner to a homogeneous population of test animals and plotting the cumulative percentage of deaths as a function of the log of the dose. The dose corresponding to the mid-point (inflection point) of the resulting S-shaped curve is the statistical estimate of the dose that would kill 50 percent of the subjects and is designated as LD_{50}. The estimated doses at which 5 percent (LD_5) and 95 percent (LD_{95}) of the test subjects die are obtained from the graph by reading the dose levels for 5 percent and 95 percent fatalities, respectively. A relatively small difference between LD_5 and LD_{95} is reflected by a steeper **S**-shaped curve, and vice versa. Statistically, 68 percent of all values on a dose-response curve fall within ± 1 standard deviation of the mean at LD_{50} and encompass the range from LD_{16} to LD_{84}.

22.3. RELATIVE TOXICITIES

Table 22.1 illustrates standard **toxicity ratings** that are used to describe estimated toxicities of various substances to humans. In terms of fatal doses to an adult human of average size, a “taste” of a supertoxic substances (just a few drops or less)

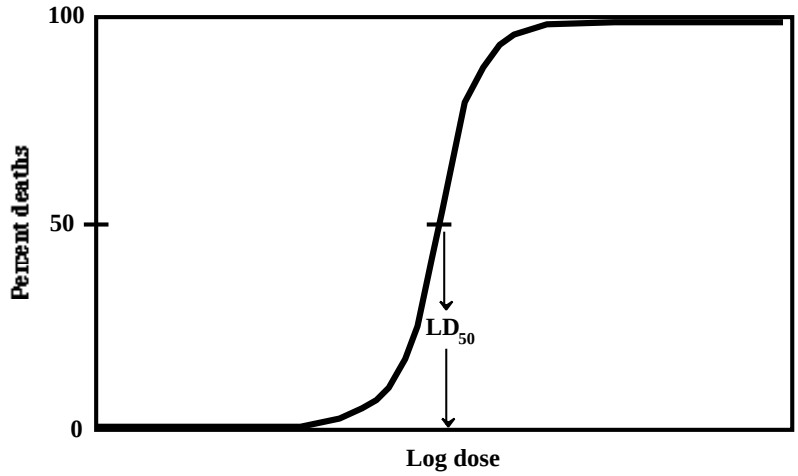


Figure 22.2. Illustration of a dose-response curve in which the response is the death of the organism. The cumulative percentage of deaths of organisms is plotted on the Y axis.

is fatal. A teaspoonful of a very toxic substance could have the same effect. However, as much as a quart of a slightly toxic substance might be required to kill an adult human.

When there is a substantial difference between LD_{50} values of two different substances, the one with the lower value is said to be the more **potent**. Such a comparison must assume that the dose-response curves for the two substances being compared have similar slopes.

Nonlethal Effects

So far, toxicities have been described primarily in terms of the ultimate effect—deaths of organisms or lethality. This is obviously an irreversible consequence of exposure. In many, and perhaps most, cases, **sublethal** and **reversible** effects are of greater importance. This is obviously true of drugs, where death from exposure to a registered therapeutic agent is rare, but other effects, both detrimental and beneficial, are usually observed. By their very nature, drugs alter biological processes; therefore, the potential for harm is almost always present. The major consideration in establishing drug dose is to find a dose that has an adequate therapeutic effect without undesirable side effects. A dose-response curve can be established for a drug that progresses from noneffective levels through effective, harmful, and even lethal levels. A low slope for this curve indicates a wide range of effective dose and a wide **margin of safety** (see Figure 22.3). This term applies to other substances, such as pesticides, for which it is desirable to have a large difference between the dose that kills a target species and that which harms a desirable species.

22.4. REVERSIBILITY AND SENSITIVITY

Sublethal doses of most toxic substances are eventually eliminated from an organism's system. If there is no lasting effect from the exposure, it is said to be **reversible**. However, if the effect is permanent, it is termed **irreversible**. Irrevers-

ible effects of exposure remain after the toxic substance is eliminated from the organism. Figure 22.3 illustrates these two kinds of effects. For various chemicals and different subjects, toxic effects may range from the totally reversible to the totally irreversible.

Table 22.1. Toxicity Scale with Example Substances[1]

Substance	Approximate LD_{50}	Toxicity rating
	-10^5	1. Practically nontoxic $> 1.5 \times 10^4$ mg/kg
DEHP[2] →	–	
Ethanol →	-10^4	2. Slightly toxic, 5×10^3 to 1.5×10^4 mg/kg
Sodium chloride →	–	
Malathion →	-10^3	3. Moderately toxic, 500 to 5000 mg/kg
Chlordane →	–	
Heptachlor →	-10^2	4. Very toxic, 50 to 500 mg/kg
	–	
Parathion →	–10	
	–	5. Extremely toxic, 5 to 50 mg/kg
TEPP[3] →	– 1	
	–	
Tetrodotoxin[4] →	-10^{-1}	
	–	
	-10^{-2}	6. Supertoxic, <5 mg/kg
	–	
TCDD[5] →	-10^{-3}	
	–	
	-10^{-4}	
	–	
Botulinus toxin →	-10^{-5}	

[1] Doses are in units of mg of toxicant per kg of body mass. Toxicity ratings on the right are given as numbers ranging from 1 (practically nontoxic) through 6 (supertoxic) along with estimated lethal oral doses for humans in mg/kg. Estimated LD_{50} values for substances on the left have been measured in test animals, usually rats, and apply to oral doses.

[2] Bis(2-ethylhexyl)phthalate

[3] Tetraethylpyrophosphate

[4] Toxin from pufferfish

[5] TCDD represents 2,3,7,8,-tetrachlorodibenzodioxin, commonly called “dioxin.”

Hypersensitivity and Hyposensitivity

Examination of the dose-response curve shown in Figure 22.2 reveals that some subjects are very sensitive to a particular poison (for example, those killed at a dose corresponding to LD_5), whereas others are very resistant to the same substance (for example, those surviving a dose corresponding to LD_{95}). These two kinds of responses illustrate **hypersensitivity** and **hyposensitivity**, respectively; subjects in the mid-range of the dose-response curve are termed **normals**. These variations in response tend to complicate toxicology in that there is no specific dose guaranteed to yield a particular response, even in a homogeneous population.

In some cases hypersensitivity is induced. After one or more doses of a chemical, a subject may develop an extreme reaction to it. This occurs with penicillin, for example, in cases where people develop such a severe allergic response to the antibiotic that exposure is fatal if countermeasures are not taken.

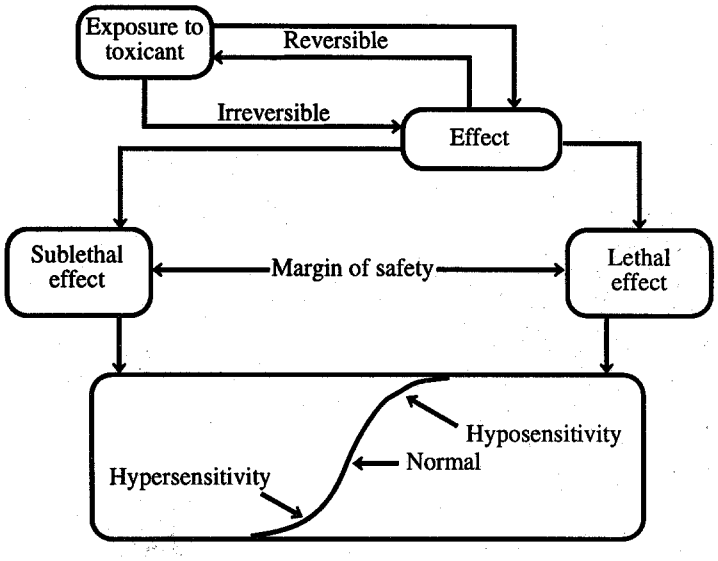


Figure 22.3. Effects of and responses to toxic substances.

22.5. XENOBIOTIC AND ENDOGENOUS SUBSTANCES

Xenobiotic substances are those that are foreign to a living system, whereas those that occur naturally in a biologic system are termed **endogenous**. The levels of endogenous substances must usually fall within a particular concentration range in order for metabolic processes to occur normally. Levels below a normal range may result in a deficiency response or even death, and the same effects may occur above the normal range. This kind of response is illustrated in Figure 22.4.

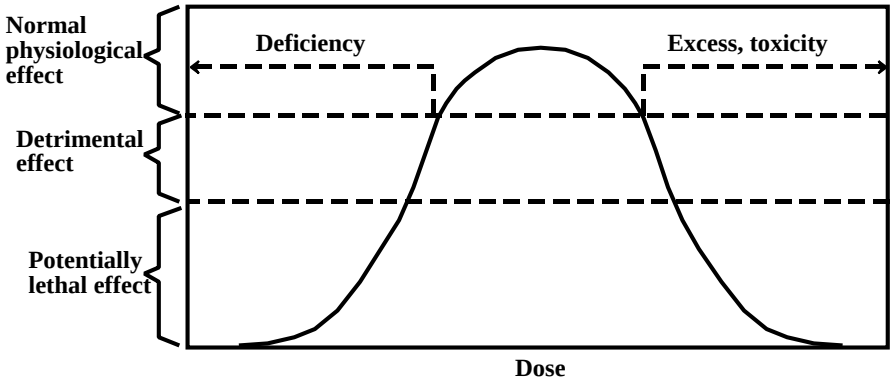


Figure 22.4. Biological effect of an endogenous substance in an organism showing optimum level, deficiency, and excess.

Examples of endogenous substances in organisms include various hormones, glucose (blood sugar), and some essential metal ions, including Ca^{2+}, K^{+}, and Na^{+}. The optimum level of calcium in human blood serum occurs over a rather narrow range of 9 – 9.5 milligrams per deciliter (mg/dL). Below these values a deficiency response known as hypocalcemia occurs, manifested by muscle cramping. At serum levels above about 10.5 mg/dL hypercalcemia occurs, the major effect of which is kidney malfunction.

22.6. TOXICOLOGICAL CHEMISTRY

Toxicological Chemistry

Toxicological chemistry is the science that deals with the chemical nature and reactions of toxic substances, including their origins, uses, and chemical aspects of exposure, fates, and disposal.[1] Toxicological chemistry addresses the relationships between the chemical properties and molecular structures of molecules and their toxicological effects. Figure 22.5 outlines the terms discussed above and the relationships among them.

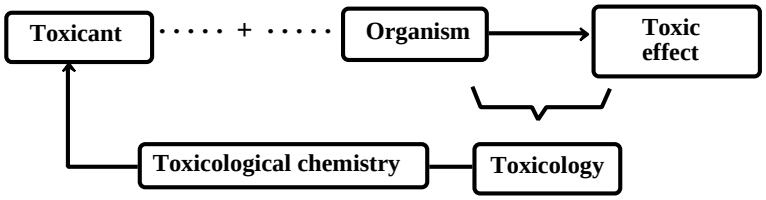


Figure 22.5. Toxicology is the science of poisons. Toxicological chemistry relates toxicology to the chemical nature of toxicants.

Toxicants in the Body

The processes by which organisms metabolize xenobiotic species are enzyme-catalyzed Phase I and Phase II reactions, which are described briefly here.[2,3]

Phase I Reactions

Lipophilic xenobiotic species in the body tend to undergo **Phase I reactions** that make them more water-soluble and reactive by the attachment of polar functional groups, such as –OH (Figure 22.6). Most Phase I processes are "microsomal mixed-function oxidase" reactions catalyzed by the cytochrome P-450 enzyme system associated with the **endoplasmic reticulum** of the cell and occurring most abundantly in the liver of vertebrates.[4]

Phase II Reactions

A **Phase II reaction** occurs when an endogenous species is attached by enzyme action to a polar functional group which often, though not always, is the result of a Phase I reaction on a xenobiotic species. Phase II reactions are called **conjugation**

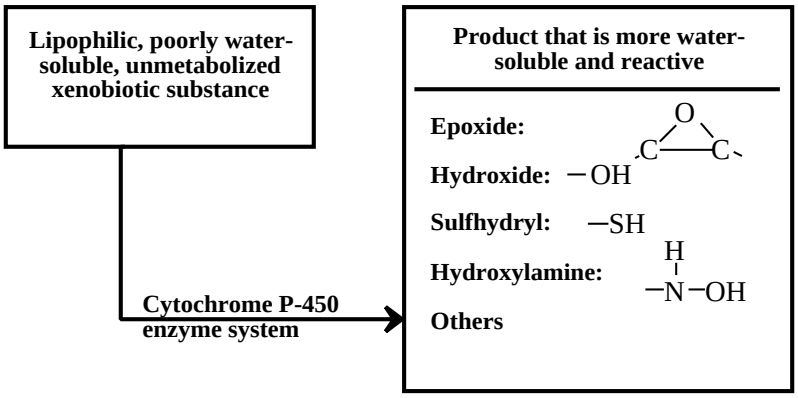


Figure 22.6. Illustration of Phase I reactions.

reactions in which enzymes attach **conjugating agents** to xenobiotics, their Phase I reaction products, and nonxenobiotic compounds (Figure 22.7). The **conjugation product** of such a reaction is usually less toxic than the original xenobiotic compound, less lipid-soluble, more water-soluble, and more readily eliminated from the body. The major conjugating agents and the enzymes that catalyze their Phase II reactions are glucuronide (UDP glucuronyltransferase enzyme), glutathione (glutathionetransferase enzyme), sulfate (sulfotransferase enzyme), and acetyl (acetylation by acetyltransferase enzymes). The most abundant conjugation products are glucuronides. A glucuronide conjugate is illustrated in Figure 22.8, where -X-R represents a xenobiotic species conjugated to glucuronide, and R is an organic moiety. For example, if the xenobiotic compound conjugated is phenol, HXR is HOC_6H_5, X is the O atom, and R represents the phenyl group, C_6H_5.

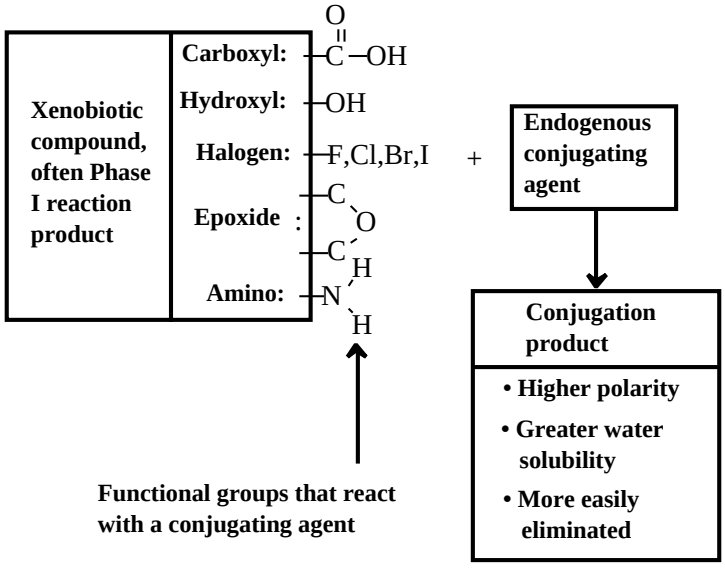


Figure 22.7. Illustration of Phase II reactions.

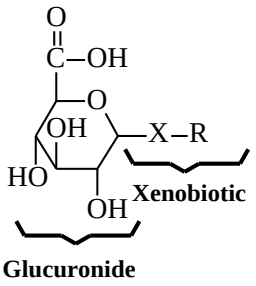


Figure 22.8. Glucuronide conjugate formed from a xenobiotic, HX-R.

22.7. KINETIC PHASE AND DYNAMIC PHASE

Kinetic Phase

The major routes and sites of absorption, metabolism, binding, and excretion of toxic substances in the body are illustrated in Figure 22.1. Toxicants in the body are metabolized, transported, and excreted; they have adverse biochemical effects; and they cause manifestations of poisoning. It is convenient to divide these processes into two major phases, a kinetic phase and a dynamic phase.

In the **kinetic phase**, a toxicant or the metabolic precursor of a toxic substance (**protoxicant**) may undergo absorption, metabolism, temporary storage, distribution, and excretion, as illustrated in Figure 22.9. A toxicant that is absorbed may be passed through the kinetic phase unchanged as an **active parent compound**, metabolized to a **detoxified metabolite** that is excreted, or converted to a toxic **active metabolite**. These processes occur through Phase I and Phase II reactions discussed above.

Dynamic Phase

In the **dynamic phase** (Figure 22.10) a toxicant or toxic metabolite interacts with cells, tissues, or organs in the body to cause some toxic response. The three major subdivisions of the dynamic phase are the following:

- **Primary reaction** with a receptor or target organ
- A **biochemical response**
- **Observable effects.**

Primary Reaction in the Dynamic Phase

A toxicant or an active metabolite reacts with a receptor. The process leading to a toxic response is initiated when such a reaction occurs. A typical example is when benzene epoxide, the initial product of the Phase I reaction of benzene (see Chapter 23, Figure 23.1), forms an adduct with a nucleic acid unit in DNA (receptor) resulting in alteration of the DNA. (Many species that cause a toxic response are reactive intermediates, such as benzene epoxide, which have a brief lifetime but a strong tendency to undergo reactions leading to a toxic response while they are around.[5])

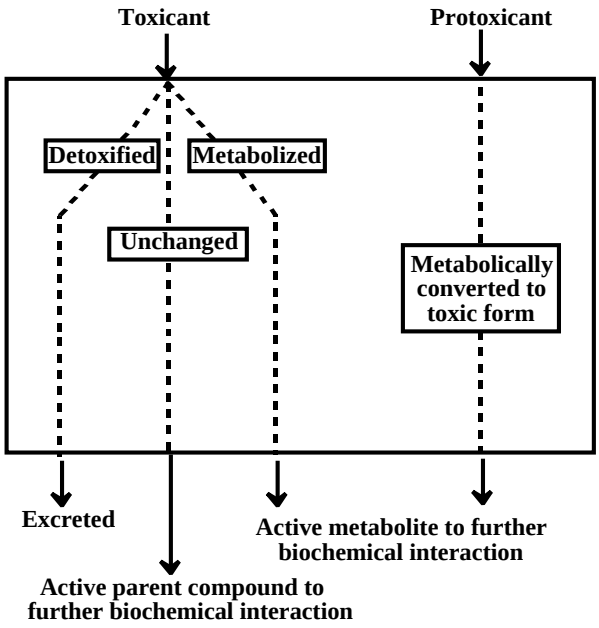


Figure 22.9. Processes involving toxicants or protoxicants in the kinetic phase.

This reaction is an **irreversible** reaction between a toxicant and a receptor. A **reversible** reaction that can result in a toxic response is illustrated by the binding between carbon monoxide and oxygen-transporting hemoglobin (Hb) in blood:

$$O_2Hb + CO \leftrightarrows COHb + O_2 \quad (22.7.1)$$

Biochemical Effects in the Dynamic Phase

The binding of a toxicant to a receptor may result in some kind of biochemical effect. The major ones are the following:

- Impairment of enzyme function by binding to the enzyme, coenzymes, metal activators of enzymes, or enzyme substrates
- Alteration of cell membrane or carriers in cell membranes
- Interference with carbohydrate metabolism
- Interference with lipid metabolism resulting in excess lipid accumulation (“fatty liver”)
- Interference with respiration, the overall process by which electrons are transferred to molecular oxygen in the biological oxidation of energy-yielding substrates
- Stopping or interfering with protein biosynthesis by their action on DNA
- Interference with regulatory processes mediated by hormones or enzymes.

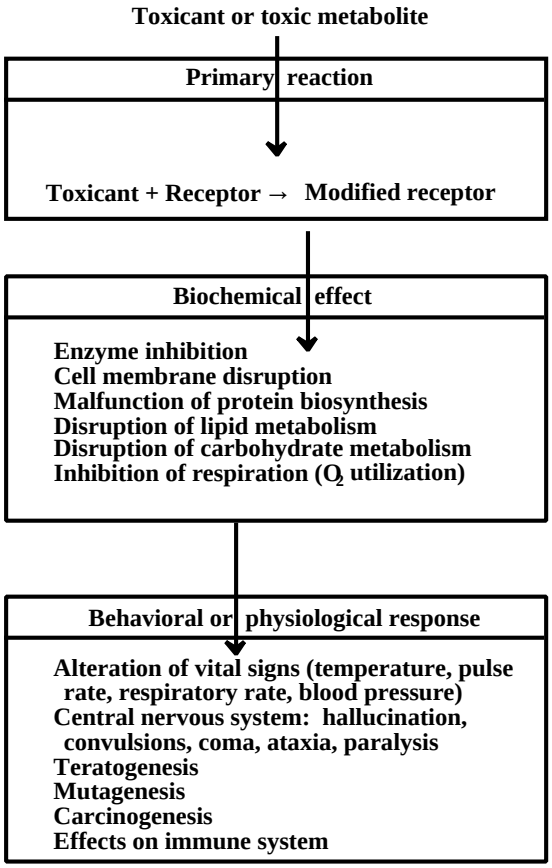


Figure 22.10. The dynamic phase of toxicant action.

Responses to Toxicants

Among the more immediate and readily observed manifestations of poisoning are alterations in the **vital signs** of **temperature**, **pulse rate**, **respiratory rate**, and **blood pressure**. Poisoning by some substances may cause an abnormal skin color (jaundiced, yellow skin from CCl_4 poisoning) or excessively moist or dry skin. Toxic levels of some materials or their metabolites cause the body to have unnatural **odors**, such as the bitter almond odor of HCN in tissues of victims of cyanide poisoning. Symptoms of poisoning manifested in the eye include **miosis** (excessive or prolonged contraction of the eye pupil), **mydriasis** (excessive pupil dilation), **conjunctivitis** (inflammation of the mucus membrane that covers the front part of the eyeball and the inner lining of the eyelids) and **nystagmus** (involuntary movement of the eyeballs). Some poisons cause a moist condition of the mouth, whereas others cause a dry mouth. Gastrointestinal tract effects including pain, vomiting, or paralytic ileus (stoppage of the normal peristalsis movement of the intestines) occur as a result of poisoning by a number of toxic substances.

Central nervous system poisoning may be manifested by **convulsions**, **paralysis**, **hallucinations**, and **ataxia** (lack of coordination of voluntary movements of the body), as well as abnormal behavior, including agitation, hyperactivity, disorientation, and delirium. Severe poisoning by some substances, including organophosphates and carbamates, causes **coma**, the term used to describe a lowered level of consciousness.

Prominent among the more chronic responses to toxicant exposure are mutations, cancer, and birth defects and effects on the immune system. Other observable effects, some of which may occur soon after exposure, include gastrointestinal illness, cardiovascular disease, hepatic (liver) disease, renal (kidney) malfunction, neurologic symptoms (central and peripheral nervous systems), and skin abnormalities (rash, dermatitis).

Often the effects of toxicant exposure are subclinical in nature. The most common of these are some kinds of damage to the immune system, chromosomal abnormalities, modification of functions of liver enzymes, and slowing of conduction of nerve impulses.

22.8. TERATOGENESIS, MUTAGENESIS, CARCINOGENESIS, AND EFFECTS ON THE IMMUNE AND REPRODUCTIVE SYSTEMS

Teratogenesis

Teratogens are chemical species that cause birth defects. These usually arise from damage to embryonic or fetal cells. However, mutations in germ cells (egg or sperm cells) may cause birth defects, such as Down's syndrome.

The biochemical mechanisms of teratogenesis are varied. These include enzyme inhibition by xenobiotics; deprivation of the fetus of essential substrates, such as vitamins; interference with energy supply; or alteration of the permeability of the placental membrane.

Mutagenesis

Mutagens alter DNA to produce inheritable traits. Although mutation is a natural process that occurs even in the absence of xenobiotic substances, most mutations are harmful. The mechanisms of mutagenicity are similar to those of carcinogenicity, and mutagens often cause birth defects as well. Therefore, mutagenic hazardous substances are of major toxicological concern.

Biochemistry of Mutagenesis

To understand the biochemistry of mutagenesis, it is important to recall from Chapter 21 that DNA contains the nitrogenous bases adenine, guanine, cytosine, and thymine. The order in which these bases occur in DNA determines the nature and structure of newly produced RNA, a substance generated as a step in the synthesis of new proteins and enzymes in cells. Exchange, addition, or deletion of any of the nitrogenous bases in DNA alters the nature of RNA produced and can change vital life processes, such as the synthesis of an important enzyme. This phenomenon,

which can be caused by xenobiotic compounds, is a mutation that can be passed on to progeny, usually with detrimental results.

There are several ways in which xenobiotic species may cause mutations. It is beyond the scope of this work to discuss these mechanisms in detail. For the most part, however, mutations due to xenobiotic substances are the result of chemical alterations of DNA, such as those discussed in the two examples below.

Nitrous acid, HNO_2, is an example of a chemical mutagen that is often used to cause mutations in bacteria. To understand the mutagenic activity of nitrous acid it should be noted that three of the nitrogenous bases—adenine, guanine, and cytosine—contain the amino group, $–NH_2$. The action of nitrous acid is to replace amino groups with a hydroxy group. When this occurs, the DNA may not function in the intended manner, causing a mutation to occur.

Alkylation, the attachment of a small alkyl group, such as $–CH_3$ or $–C_2H_5$, to an N atom on one of the nitrogenous bases in DNA is one of the most common mechanisms leading to mutation. The methylation of "7" nitrogen in guanine in DNA to form N–Methylguanine is shown in Figure 22.11. O-alkylation may also occur by attachment of a methyl or other alkyl group to the oxygen atom in guanine.

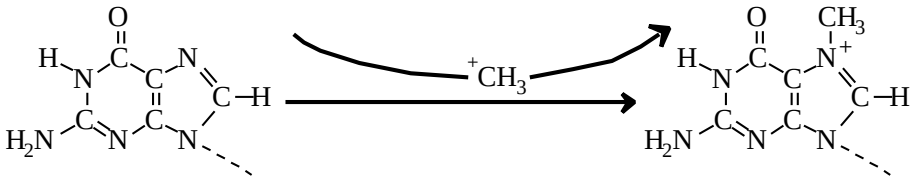


Figure 22.11. Alkylation of guanine in DNA.

A number of mutagenic substances act as alkylating agents. Prominent among these are the compounds shown in Figure 22.12.

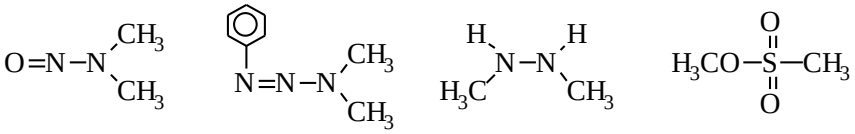
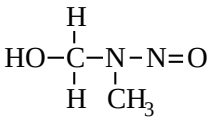
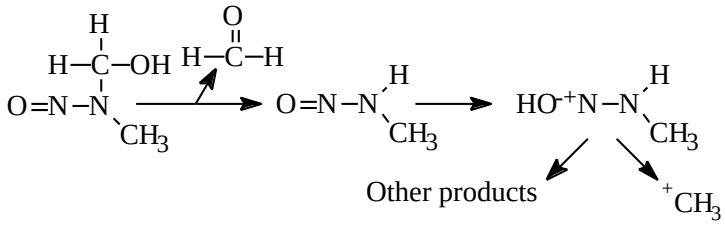


Figure 22.12. Examples of simple alkylating agents capable of causing mutations.

Alkylation occurs by way of generation of positively charged electrophilic species that bond to electron-rich nitrogen or oxygen atoms on the nitrogenous bases in DNA. The generation of such species usually occurs by way of biochemical and chemical processes. For example, dimethylnitrosamine (structure in Figure 22.12) is activated by oxidation through cellular NADPH to produce the following highly reactive intermediate:



This product undergoes several nonenzymatic transitions, losing formaldehyde and generating a methyl carbocation, $^{+}CH_3$, that can methylate nitrogenous bases on DNA



(22.7.1)

One of the more notable mutagens is tris(2,3-dibromopropyl)phosphate, commonly called “tris,” that was used as a flame retardant in children’s sleepwear. Tris was found to be mutagenic in experimental animals and metabolites of it were found in children wearing the treated sleepwear. This strongly suggested that tris is absorbed through the skin and its uses were discontinued.

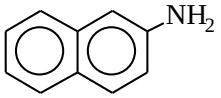
Carcinogenesis

Cancer is a condition characterized by the uncontrolled replication and growth of the body’s own (somatic) cells. **Carcinogenic agents** may be categorized as follows:

- Chemical agents, such as nitrosamines and polycyclic aromatic hydrocarbons
- Biological agents, such as hepadnaviruses or retroviruses
- Ionizing radiation, such as X-rays
- Genetic factors, such as selective breeding.

Clearly, in some cases, cancer is the result of the action of synthetic and naturally occurring chemicals. The role of xenobiotic chemicals in causing cancer is called **chemical carcinogenesis**. It is often regarded as the single most important facet of toxicology and is clearly the one that receives the most publicity.

Chemical carcinogenesis has a long history. As noted earlier in this chapter, in 1775 Sir Percival Pott, Surgeon General serving under King George III of England, observed that chimney sweeps in London had a very high incidence of cancer of the scrotum, which he related to their exposure to soot and tar from the burning of bituminous coal. Around 1900 a German surgeon, Ludwig Rehn, reported elevated incidences of bladder cancer in dye workers exposed to chemicals extracted from coal tar; 2-naphthylamine,



was shown to be largely responsible. Other historical examples of carcinogenesis include observations of cancer from tobacco juice (1915), oral exposure to radium from painting luminescent watch dials (1929), tobacco smoke (1939), and asbestos (1960).

Large expenditures of time and money on the subject in recent years have yielded a much better understanding of the biochemical bases of chemical carcinogenesis. The overall processes for the induction of cancer may be quite complex, involving numerous steps.[6] However, it is generally recognized that there are two major steps in carcinogenesis: an **initiation stage** followed by a **promotional stage**. These steps are further subdivided as shown in Figure 22.13.

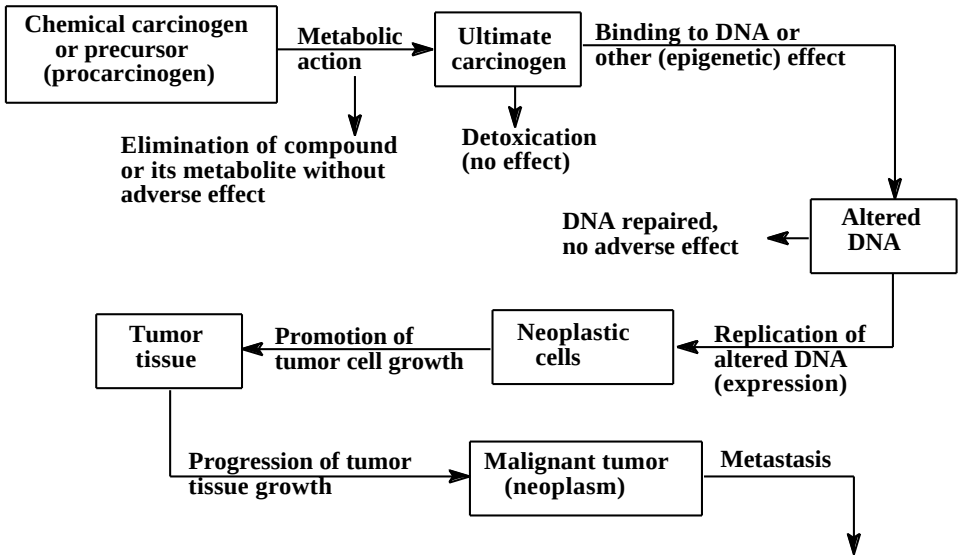
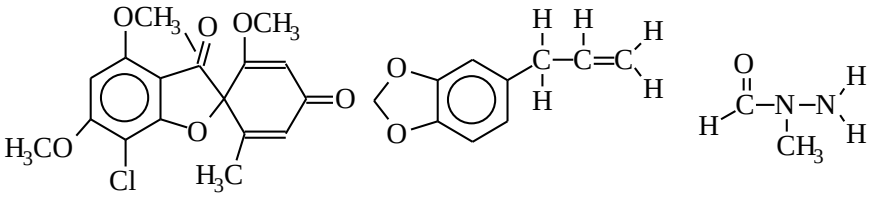


Figure 22.13. Outline of the process by which a carcinogen or procarcinogen may cause cancer.

Initiation of carcinogenesis may occur by reaction of a **DNA-reactive species** with DNA,[7] or by the action of an **epigenetic carcinogen** that does not react with DNA and is carcinogenic by some other mechanism.[8] Most DNA-reactive species are **genotoxic carcinogens** because they are also mutagens. These substances react irreversibly with DNA. They are either electrophilic or, more commonly, metabolically activated to form electrophilic species, as is the case with electrophilic $^{+}CH_3$ generated from dimethylnitrosamine, discussed under mutagenesis above. Cancer-causing substances that require metabolic activation are called **procarcinogens**. The metabolic species actually responsible for carcinogenesis is termed an **ultimate carcinogen**. Some species that are intermediate metabolites between precarcinogens and ultimate carcinogens are called **proximate carcinogens**. Carcinogens that do not require biochemical activation are categorized as **primary** or **direct-acting carcinogens**. Some procarcinogens and primary carcinogens are shown in Figure 22.14.

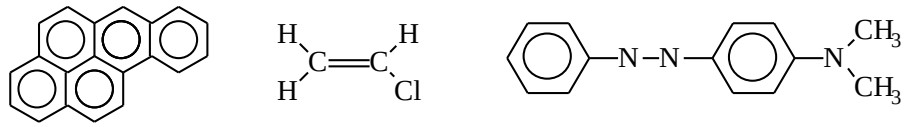
Most substances classified as epigenetic carcinogens are **promoters** that act after initiation. Manifestations of promotion include increased numbers of tumor cells and decreased length of time for tumors to develop (shortened latency period). Promoters do not initiate cancer, are not electrophilic, and do not bind with DNA. The classic example of a promoter is a substance known chemically as decanoyl phorbol acetate or phorbol myristate acetate, Which is extracted from croton oil.

Naturally occurring carcinogens that require bioactivation



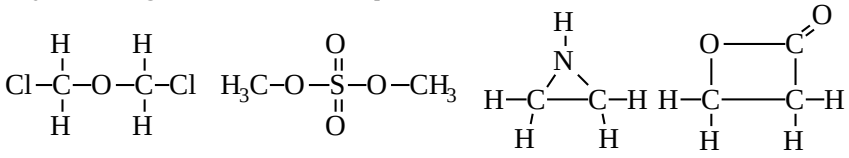
Griseofulvin (produced by *Penicillium griseofulvum*) **Saffrole (from sassafras)** **N-methyl-N-formylhydrazine (from edible false morel mushroom)**

Synthetic carcinogens that require bioactivation



Benzo(a)pyrene **Vinyl chloride** **4-Dimethylaminoazobenzene**

Primary carcinogens that do not require bioactivation

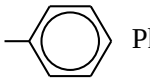


Bis(chloromethyl)-ether **Dimethyl sulfate** **Ethyleneimine** **β-Propioacetone**

Figure 22.14. Examples of the major classes of naturally occurring and synthetic carcinogens, some of which require bioactivation, and others which act directly.

Alkylating Agents in Carcinogenesis

Chemical carcinogens usually have the ability to form covalent bonds with macromolecular life molecules.[9] Such covalent bonds can form with proteins, peptides, RNA, and DNA. Although most binding is with other kinds of molecules, which are more abundant, the DNA adducts are the significant ones in initiating cancer. Prominent among the species that bond to DNA in carcinogenesis are the alkylating agents which attach alkyl groups—methyl (CH_3) or ethyl (C_2H_5)—to DNA. A similar type of compound, **arylating agents**, act to attach aryl moieties, such as the phenyl group



Phenyl group

to DNA. As shown by the examples in Figure 22.15, the alkyl and aryl groups become attached to N and O atoms in the nitrogenous bases that compose DNA. This alteration in DNA can trigger initiation of the sequence of events that results

in the growth and replication of neoplastic (cancerous) cells. The reactive species that donate alkyl groups in alkylation are usually formed by metabolic activation through the action of enzymes. This process was shown for conversion of dimethylnitrosamine to a methylating metabolic intermediate in the discussion of mutagenesis earlier in this section.

Testing for Carcinogens

Only a few chemicals have definitely been established as human carcinogens. A well documented example is vinyl chloride, CH_2=CHCl, which is known to have caused a rare form of liver cancer (angiosarcoma) in individuals who cleaned autoclaves in the polyvinylchloride fabrication industry. In some cases chemicals are known to be carcinogens from epidemiological studies of exposed humans. Animals are used to test for carcinogenicity, and the results can be extrapolated, although with much uncertainty, to humans.

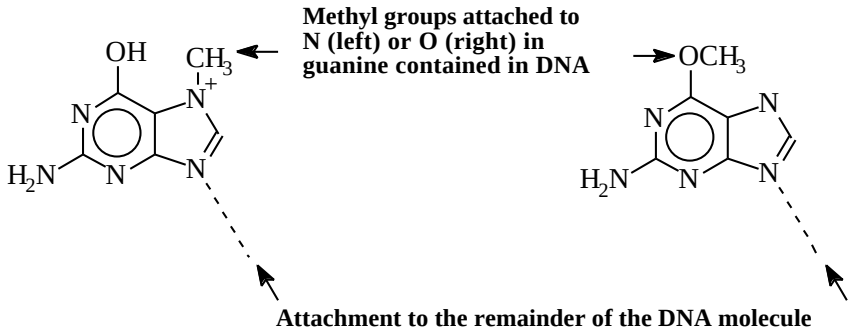


Figure 22.15. Alkylated (methylated) forms of the nitrogenous base guanine.

Bruce Ames Test

Mutagenicity used to infer carcinogenicity is the basis of the **Bruce Ames** test, in which observations are made of the reversion of mutant histidine-requiring *Salmonella* bacteria back to a form that can synthesize its own histidine.[10] The test makes use of enzymes in homogenized liver tissue to convert potential procarcinogens to ultimate carcinogens. Histidine-requiring *Salmonella* bacteria are inoculated onto a medium that does not contain histidine, and those that mutate back to a form that can synthesize histidine establish visible colonies that are assayed to indicate mutagenicity.

According to Bruce Ames, the pioneer developer of the test which bears his name, animal tests for carcinogens that make use of massive doses of chemicals have a misleading tendency to give results that cannot be accurately extrapolated to assess cancer risks from smaller doses of chemicals.[11] This is because the huge doses of chemicals used kill large numbers of cells, which the organism's body attempts to replace with new cells. Rapidly dividing cells greatly increase the likelihood of mutations that result in cancer simply as the result of rapid cell proliferation, not genotoxicity.

Immune System Response

The **immune system** acts as the body's natural defense system to protect it from xenobiotic chemicals; infectious agents, such as viruses or bacteria; and neoplastic cells, which give rise to cancerous tissue. Adverse effects on the body's immune system are being increasingly recognized as important consequences of exposure to hazardous substances.[12] Toxicants can cause **immunosuppression**, which is the impairment of the body's natural defense mechanisms. Xenobiotics can also cause the immune system to lose its ability to control cell proliferation, resulting in leukemia or lymphoma.

Another major toxic response of the immune system is **allergy** or **hypersensitivity**. This kind of condition results when the immune system overreacts to the presence of a foreign agent or its metabolites in a self-destructive manner. Among the xenobiotic materials that can cause such reactions are beryllium, chromium, nickel, formaldehyde, some kinds of pesticides, resins, and plasticizers.

Estrogenic Substances

A number of xenobiotic substances are thought to have adverse effects on animal and human reproductive systems by mimicking or interfering with the action of estrogens (see Section 21.5). Rodent experiments indicate that such substances, sometimes called exogenous estrogens or exoestrogens, may cause disorders of the reproductive tract and effects such as reduced sperm counts and semen production.[13] Another effect of some concern is the potential to cause hormone-dependent cancers. A wide variety of synthetic compounds, including phthalates, alkylphenols, organochlorine compounds, and polycyclic aromatic hydrocarbons, are suspected of being exoestrogens.

22.9. HEALTH HAZARDS

In recent years attention in toxicology has shifted away from readily recognized, usually severe, acute maladies that developed on a short time scale as a result of brief, intense exposure to toxicants, toward delayed, chronic, often less severe illnesses caused by long-term exposure to low levels of toxicants. Although the total impact of the latter kinds of health effects may be substantial, their assessment is very difficult because of factors such as uncertainties in exposure, low occurrence above background levels of disease, and long latency periods.

Assessment of Potential Exposure

A critical step in assessing exposure to toxic substances, such as those from hazardous waste sites is evaluation of potentially exposed populations. The most direct approach to this is to determine chemicals or their metabolic products in organisms. For inorganic species this is most readily done for heavy metals, radionuclides, and some minerals, such as asbestos. Symptoms associated with exposure to particular chemicals may also be evaluated. Examples of such effects include readily apparent effects, for example, skin rashes, or subclinical effects, such as chromosomal damage.

Epidemiological Evidence

Epidemiological studies applied to toxic environmental pollutants, such as those from hazardous wastes, attempt to correlate observations of particular illnesses with probable exposure to such wastes. There are two major approaches to such studies. One approach is to look for diseases known to be caused by particular agents in areas where exposure is likely from such agents in hazardous wastes. A second approach is to look for **clusters** consisting of an abnormally large number of cases of a particular disease in a limited geographic area, then attempt to locate sources of exposure to hazardous wastes that may be responsible. The most common types of maladies observed in clusters are spontaneous abortions, birth defects, and particular types of cancer.

Epidemiologic studies are complicated by long latency periods from exposure to onset of disease, lack of specificity in the correlation between exposure to a particular waste and the occurrence of a disease, and background levels of a disease in the absence of exposure to a hazardous waste capable of causing the disease.

Estimation of Health Effects Risks

An important part of estimating the risks of adverse health effects from exposure to toxicants involves extrapolation from experimentally observable data. Usually the end result needed is an estimate of a low occurrence of a disease in humans after a long latency period resulting from low-level exposure to a toxicant for a long period of time. The data available are almost always taken from animals exposed to high levels of the substance for a relatively short period of time. Extrapolation is then made using linear or curvilinear projections to estimate the risk to human populations. There are, of course, very substantial uncertainties in this kind of approach.

Risk Assessment

Toxicological considerations are very important in estimating potential dangers of pollutants and hazardous waste chemicals. One of the major ways in which toxicology interfaces with the area of hazardous wastes is in **health risk assessment**, providing guidance for risk management, cleanup, or regulation needed at a hazardous waste site based upon knowledge about the site and the chemical and toxicological properties of wastes in it. Risk assessment includes the factors of site characteristics; substances present, including indicator species; potential receptors; potential exposure pathways; and uncertainty analysis. It may be divided into the following major components:

- Identification of hazard
- Dose-response assessment
- Exposure assessment
- Risk characterization.

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QUESTIONS AND PROBLEMS

1. How are conjugating agents and Phase II reactions involved with some toxicants?
2. What is the toxicological importance of proteins, particularly as related to protein structure?
3. What is the toxicological importance of lipids? How are lipids related to hydrophobic pollutants and toxicants?
4. What are Phase I reactions? What enzyme system carries them out? Where is this enzyme system located in the cell?
5. Name and describe the science that deals with the chemical nature and reactions of toxic substances, including their origins, uses, and chemical aspects of exposure, fates, and disposal.
6. What is a dose-response curve?
7. What is meant by a toxicity rating of 6?
8. What are the three major subdivisions of the *dynamic phase* of toxicity, and what happens in each?
9. Characterize the toxic effect of carbon monoxide in the body. Is its effect reversible or irreversible? Does it act on an enzyme system?
10. Of the following, choose the one that is **not** a biochemical effect of a toxic substance: (a) impairment of enzyme function by binding to the enzyme, (b) alteration of cell membrane or carriers in cell membranes, (c) change in vital signs, (d) interference with lipid metabolism, (e) interference with respiration.
11. Distinguish among teratogenesis, mutagenesis, carcinogenesis, and immune system effects. Are there ways in which they are related?

12. As far as environmental toxicants are concerned, compare the relative importance of acute and chronic toxic effects and discuss the difficulties and uncertainties involved in studying each.
13. What are some of the factors that complicate epidemiologic studies of toxicants?