PART II

CLASSES OF TOXICANTS

CHAPTER 4

Exposure Classes, Toxicants in Air, Water, Soil, Domestic and Occupational Settings

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4.1 AIR POLLUTANTS

4.1.1 History

Air pollution probably occurred as soon as humans started to use wood fires for heat and cooking. For centuries fire was used in such a way that living areas were filled with smoke. After the invention of the chimney, combustion products and cooking odors were removed from living quarters and vented outside. Later, when soft coal was discovered and used for fuel, coal smoke became a problem in the cities. By the thirteenth century, records show that coal smoke had become a nuisance in London, and in 1273 Edward I made the first antipollution law, one that prohibited the burning of coal while Parliament was in session: "Be it known to all within the sound of my voice, whosoever shall be found guilty of burning coal shall suffer the loss of his head." Despite this and various other royal edicts, however, smoke pollution continued in London.

Increasing domestic and industrial combustion of coal caused air pollution to get steadily worse, particularly in large cities. During the twentieth century the most significant change was the rapid increase in the number of automobiles, from almost none at the turn of the century to millions within only a few decades. During this time few attempts were made to control air pollution in any of the industrialized countries until after World War II. Action was then prompted, in part, by two acute pollution episodes in which human deaths were caused directly by high levels of pollutants. One incident occurred in 1948 in Donora, a small steel mill town in western Pennsylvania. In late October, heavy smog settled in the area, and a weather inversion prevented the movement of pollutants out of the valley. Twenty-one deaths were attributed directly to the effects of the smog. The "Donora episode" helped focus attention on air pollution in the United States.

In London, in December 1952, a now infamous killer smog occurred. A dense fog at ground level coupled with smoke from coal fireplaces caused severe smog lasting more than a week. The smog was so heavy that daylight visibility was only a few

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meters, and bus conductors had to walk in front of the buses to guide the drivers through the streets. Two days after the smog began, the death rate began to climb, and between December 5 and December 9, there were an estimated 4000 deaths above the normal daily count. The chief causes of death were bronchitis, pneumonia, and associated respiratory complaints. This disaster resulted in the passage in Britain of the Clean Air Act in 1956.

In the United States the smog problem began to occur in large cities across the country, becoming especially severe in Los Angeles. In 1955 federal air pollution legislation was enacted, providing federal support for air pollution research, training, and technical assistance. Responsibility for the administration of the federal program now lies with the US Environmental Protection Agency (EPA). Technological interest since the mid-1950s has centered on automobile air pollution, pollution by oxides of sulfur and nitrogen, and the control of these emissions. Attention is also being directed toward the problems that may be caused by a possible greenhouse effect resulting from increased concentrations of carbon dioxide (CO_2) in the atmosphere, possible depletion of the stratospheric ozone layer, long-range transport of pollution, and acid deposition.

4.1.2 Types of Air Pollutants

What is clean air? Unpolluted air is a concept of what the air would be if humans and their works were not on earth, and if the air were not polluted by natural point sources such as volcanoes and forest fires. The true composition of "unpolluted" air is unknown because humans have been polluting the air for thousands of years. In addition there are many natural pollutants such as terpenes from plants, smoke from forest fires, and fumes and smoke from volcanoes. Table 4.1 lists the components that, in the absence of such pollution, are thought to constitute clean air.

Gaseous Pollutants. These substances are gases at normal temperature and pressure as well as vapors evaporated from substances that are liquid or solid. Among pollutants of greatest concern are carbon monoxide (CO), hydrocarbons, hydrogen sulfide (H_2S)

Table 4.1 Gaseous Components of Normal Dry Air

Compound	Percent by Volume	Concentration (ppm)
Nitrogen	78.09	780,900
Oxygen	20.94	209,400
Argon	0.93	9300
Carbon dioxide	0.0325	325
Neon	0.0018	18
Helium	0.0005	5.2
Methane	0.0001	1.1
Krypton	0.0001	1.0
Nitrous oxide		0.5
Hydrogen		0.5
Xenon		0.008
Nitrogen dioxide		0.02
Ozone		0.01–0.04

nitrogen oxides (N_xO_y), ozone (O_3) and other oxidants, sulfur oxides (S_xO_y), and CO_2. Pollutant concentrations are usually expressed as micrograms per cubic meter ($\mu g/m^3$) or for gaseous pollutants as parts per million (ppm) by volume in which 1 ppm = 1 part pollutant per million parts (10^6) of air.

Particulate Pollutants. Fine solids or liquid droplets can be suspended in air. Some of the different types of particulates are defined as follows:

- *Dust.* Relatively large particles about 100 μm in diameter that come directly from substances being used (e.g., coal dust, ash, sawdust, cement dust, grain dust).
- *Fumes.* Suspended solids less than 1 μm in diameter usually released from metallurgical or chemical processes, (e.g., zinc and lead oxides).
- *Mist.* Liquid droplets suspended in air with a diameter less than 2.0 μm, (e.g., sulfuric acid mist).
- *Smoke.* Solid particles (0.05–1.0 μm) resulting from incomplete combustion of fossil fuels.
- *Aerosol.* Liquid or solid particles (<1.0 μm) suspended in air or in another gas.

4.1.3 Sources of Air Pollutants

Natural Pollutants. Many pollutants are formed and emitted through natural processes. An erupting volcano emits particulate matter as well as gases such as sulfur dioxide, hydrogen sulfide, and methane; such clouds may remain airborne for long periods of time. Forest and prairie fires produce large quantities of pollutants in the form of smoke, unburned hydrocarbons, CO, nitrogen oxides, and ash. Dust storms are a common source of particulate matter in many parts of the world, and oceans produce aerosols in the form of salt particles. Plants and trees are a major source of hydrocarbons on the planet, and the blue haze that is so familiar over forested mountain areas is mainly from atmospheric reactions with volatile organics produced by the trees. Plants also produce pollen and spores, which cause respiratory problems and allergic reactions.

Anthropogenic Pollutants. These substances come primarily from three sources: (1) combustion sources that burn fossil fuel for heating and power, or exhaust emissions from transportation vehicles that use gasoline or diesel fuels; (2) industrial processes; and (3) mining and drilling.

The principal pollutants from combustion are fly ash, smoke, sulfur, and nitrogen oxides, as well as CO and CO_2. Combustion of coal and oil, both of which contain significant amounts of sulfur, yields large quantities of sulfur oxides. One effect of the production of sulfur oxides is the formation of acidic deposition, including acid rain. Nitrogen oxides are formed by thermal oxidation of atmospheric nitrogen at high temperatures; thus almost any combustion process will produce nitrogen oxides. Carbon monoxide is a product of incomplete combustion; the more efficient the combustion, the higher is the ratio of CO_2 to CO.

Transportation sources, particularly automobiles, are a major source of air pollution and include smoke, lead particles from tetraethyl lead additives, CO, nitrogen oxides, and hydrocarbons. Since the mid-1960s there has been significant progress in reducing exhaust emissions, particularly with the use of low-lead or no-lead gasoline as well as

the use of oxygenated fuels—for example, fuels containing ethanol or MTBE (methyl *t*-butyl ether).

Industries may emit various pollutants relating to their manufacturing processes—acids (sulfuric, acetic, nitric, and phosphoric), solvents and resins, gases (chlorine and ammonia), and metals (copper, lead, and zinc).

Indoor Pollutants. In general, the term "indoor air pollution" refers to home and nonfactory public buildings such as office buildings and hospitals. Pollution can come from heating and cooking, pesticides, tobacco smoking, radon, gases, and microbes from people and animals.

Although indoor air pollution has increased in developed nations because of tighter building construction and the use of building materials that may give off gaseous chemicals, indoor air pollution is a particular problem in developing countries. Wood, crop residues, animal dung, and other forms of biomass are used extensively for cooking and heating—often in poorly ventilated rooms. For women and children, in particular, this leads to high exposures of air pollutants such as CO and polycyclic aromatic hydrocarbons.

4.1.4 Examples of Air Pollutants

Most of the information on the effects of air pollution on humans comes from acute pollution episodes such as the ones in Donora and London. Illnesses may result from chemical irritation of the respiratory tract, with certain sensitive subpopulations being more affected: (1) very young children, whose respiratory and circulatory systems are poorly developed, (2) the elderly, whose cardiorespiratory systems function poorly, and (3) people with cardiorespiratory diseases such as asthma, emphysema, and heart disease. Heavy smokers are also affected more adversely by air pollutants. In most cases the health problems are attributed to the combined action of particulates and sulfur dioxides (SO_2); no one pollutant appears to be responsible. Table 4.2 summarizes some of the major air pollutants and their sources and effects.

Carbon Monoxide. Carbon monoxide combines readily with hemoglobin (Hb) to form carboxyhemoglobin (COHb), thus preventing the transfer of oxygen to tissues. The affinity of hemoglobin for CO is approximately 210 times its affinity for oxygen. A blood concentration of 5% COHb, equivalent to equilibration at approximately 45 ppm CO, is associated with cardiovascular effects. Concentrations of 100 ppm can cause headaches, dizziness, nausea, and breathing difficulties. An acute concentration of 1000 ppm is invariably fatal. Carbon monoxide levels during acute traffic congestion have been known to be as high as 400 ppm; in addition, people who smoke elevate their total body burden of CO as compared with nonsmokers. The effects of low concentrations of CO over a long period are not known, but it is possible that heart and respiratory disorders are exacerbated.

Sulfur Oxides. Sulfur dioxide is a common component of polluted air that results primarily from the industrial combustion of coal, with soft coal containing the highest levels of sulfur. The sulfur oxides tend to adhere to air particles and enter the inner respiratory tract, where they are not effectively removed. In the respiratory tract, SO_2 combines readily with water to form sulfurous acid, resulting in irritation of mucous

Table 4.2 Principal Air Pollutants, Sources, and Effects

Pollutant	Sources	Significance
Sulfur oxides, particulates	Coal and oil power plants Oil refineries, smelters Kerosene heaters	Main component of acid deposition Damage to vegetation, materials Irritating to lungs, chronic bronchitis
Nitrogen oxides	Automobile emissions Fossil fuel power plants	Pulmonary edema, impairs lung defenses Important component of photochemical smog and acid deposition
Carbon monoxide	Motor vehicle emissions Burning fossil fuels Incomplete combustion	Combines with hemoglobin to form carboxyhemoglobin, poisonous Asphyxia and death
Carbon dioxide	Product of complete combustion	May cause "greenhouse effect"
Ozone (O_3)	Automobile emissions Photochemical smog	Damage to vegetation Lung irritant
Hydrocarbons, C_xH_y	Smoke, gasoline fumes Cigarette smoke, industry Natural sources	Contributes to photochemical smog Polycyclic aromatic hydrocarbons, lung cancer
Radon	Natural	Lung cancer
Asbestos	Asbestos mines Building materials Insulation	Asbestosis Lung cancer, mesothelioma
Allergens	Pollen, house dust Animal dander	Asthma, rhinitis
Arsenic	Copper smelters	Lung cancer

membranes and bronchial constriction. This irritation in turn increases the sensitivity of the airway to other airborne toxicants.

Nitrogen Oxides. Nitrogen dioxide (NO_2), a gas found in photochemical smog, is also a pulmonary irritant and is known to lead to pulmonary edema and hemorrhage. The main issue of concern is its contribution to the formation of photochemical smog and ozone, although nitrogen oxides also contribute to acid deposition.

Ozone. A highly irritating and oxidizing gas is formed by photochemical action of ultraviolet (UV) light on nitrogen dioxide in smog. The resulting ozone can produce pulmonary congestion, edema, and hemorrhage.

$$NO_2 + \text{UV light} \longrightarrow NO + O^{\bullet}$$

$$O^{\bullet} + O_2 \longrightarrow O_3$$

At this point it is worth distinguishing between "good" and "bad" ozone. *Tropospheric ozone* occurs from 0 to 10 miles above the earth's surface, and is harmful. *Stratospheric ozone*, located about 30 miles above the earth's surface, is responsible for filtering out incoming UV radiation and thus is beneficial. It is the decrease in the stratospheric ozone layer that has been of much concern recently. It is estimated that a 1% decrease in stratospheric ozone will increase the amount of UV radiation reaching the earth's

surface by 2% and cause a 10% increase in skin cancer. Major contributors to damage to stratospheric ozone are thought to be the chlorofluorocarbons (CFCs). Chlorine is removed from the CFC compounds in the upper atmosphere by reaction with UV light and is then able to destroy the stratospheric ozone through self-perpetuating free radical reactions.

$$Cl + O_3 \longrightarrow ClO + O_2$$
$$ClO + O \longrightarrow Cl + O_2$$

Before being inactivated by nitrogen dioxide or methane, each chlorine atom can destroy up to 10,000 molecules of ozone. Use of CFC compounds is now being phased out by international agreements.

Hydrocarbons (HCs) or Volatile Organic Compounds (VOCs). These are derived primarily from two sources: approximately 50% are derived from trees as a result of the respiration process (biogenic); the other 45% to 50% comes from the combustion of fuel and from vapor from gasoline. Many gasoline pumps now have VOC recovery devices to reduce pollution.

Lead. One of the most familiar of the particulates in air pollutants is lead, with young children and fetuses being the most susceptible. Lead can impair renal function, interfere with the development of red blood cells, and impair the nervous system, leading to mental retardation and even blindness. The two most common routes of exposure to lead are inhalation and ingestion. It is estimated that approximately 20% of the total body burden of lead comes from inhalation.

Solid Particles. Dust and fibers from coal, clay, glass, asbestos, and minerals can lead to scarring or fibrosis of the lung lining. Pneumoconiosis, a condition common among coal miners that breathe coal dust, silicosis caused by breathing silica-containing dusts, and asbestosis from asbestos fibers are all well-known industrial pollution diseases.

4.1.5 Environmental Effects

Vegetation. Pollutants may visibly injure vegetation by bleaching, other color changes, and necrosis, or by more subtle changes such as alterations in growth or reproduction. Table 4.3 lists some of the more common visual effects of air pollutants on vegetation. Air pollution can also result in measurable effects on forest ecosystems, such as reduction in forest growth, change in forest species, and increased susceptibility to forest pests. High-dose exposure to pollutants, which is associated with point source emissions such as smelters, frequently results in complete destruction of trees and shrubs in the surrounding area.

Domestic Animals. Although domestic animals can be affected directly by air pollutants, the main concern is chronic poisoning as a result of ingestion of forage that has been contaminated by airborne pollutants. Pollutants important in this connection are

Table 4.3 Examples of Air Pollution Injury to Vegetation

Pollutant	Symptoms
Sulfur dioxide	Bleached spots, interveinal bleaching
Ozone	Flecking, stippling, bleached spotting
Peroxyacetylnitrate (PAN)	Glazing, silvering, or bronzing on lower leaf surfaces
Nitrogen dioxide	White or brown collapsed lesion near leaf margins
Hydrogen fluoride	Tip and margin burns, dwarfing

arsenic, lead, and molybdenum. Fluoride emissions from industries producing phosphate fertilizers and derivatives have damaged cattle throughout the world. The raw material, phosphate rock, can contain up to 4% fluoride, some of which is released into the air and water. Farm animals, particularly cattle, sheep, and swine, are susceptible to fluoride toxicity (fluorosis), which is characterized by mottled and soft teeth, and osteroflouritic bone lesions, which lead to lameness and, eventually, death.

Materials and Structures. Building materials have become soiled and blackened by smoke, and damage by chemical attack from acid gases in the air has led to the deterioration of many marble statues in western Europe. Metals are also affected by air pollution; for example, SO_2 causes many metals to corrode at a faster rate. Ozone is known to oxidize rubber products, and one of the effects of Los Angeles smog is cracking of rubber tires. Fabrics, leather, and paper are also affected by SO_2 and sulfuric acid, causing them to crack, become brittle, and tear more easily.

Atmospheric Effects. The presence of fine particles (0.1–1.0 mm in diameter) or NO_2 in the atmosphere can result in atmospheric haze or reduced visibility due to light scattering by the particles. The major effect of atmospheric haze has been degradation in visual air quality and is of particular concern in areas of scenic beauty, including most of the major national parks such as Great Smoky Mountain, Grand Canyon, Yosemite, and Zion Parks.

There is also concern over the increase in CO_2 in the atmosphere because CO_2 absorbs heat energy strongly and retards the cooling of the earth. This is often referred to as the greenhouse effect; theoretically an increase in CO_2 levels would result in a global increase in air temperatures. In addition to CO_2, other gases contributing to the greenhouse effect include methane, CFCs, nitrous oxide, and ozone.

Acidic Deposition. Acidic deposition is the combined total of wet and dry deposition, with wet acidic deposition being commonly referred to as acid rain. Normal uncontaminated rain has a pH of about 5.6, but acid rain usually has a pH of less than 4.0. In the eastern United States, the acids in acid rain are approximately 65% sulfuric, 30% nitric, and 5% other, whereas in the western states, 80% of the acidity is due to nitric acid.

Many lakes in northeastern North America and Scandinavia have become so acidic that fish are no longer able to live in them. The low pH not only directly affects fish but also contributes to the release of potentially toxic metals, such as aluminum, from the soil. The maximum effect occurs when there is little buffering of the acid by soils or rock components. Maximum fish kills occur in early spring due to the "acid shock"

from the melting of winter snows. Much of the acidity in rain may be neutralized by dissolving minerals in the soil such as aluminum, calcium, magnesium, sodium, and potassium, which are leached from the soil into surface waters. The ability of the soil to neutralize or buffer the acid rain is very dependent on the alkalinity of the soil. Much of the area in eastern Canada and the northeastern United States is covered by thin soils with low acid neutralizing capacity. In such areas the lakes are more susceptible to the effects of acid deposition leading to a low pH and high levels of aluminum, a combination toxic to many species of fish.

A second area of concern is that of reduced tree growth in forests. The leaching of nutrients from the soil by acid deposition may cause a reduction in future growth rates or changes in the type of trees to those able to survive in the altered environment. In addition to the change in soil composition, there are the direct effects on the trees from sulfur and nitrogen oxides as well as ozone.

4.2 WATER AND SOIL POLLUTANTS

With three-quarters of the earth's surface covered by water and much of the remainder covered by soil, it is not surprising that water and soil serve as the ultimate sinks for most anthropogenic chemicals. Until recently the primary concern with water pollution was that of health effects due to pathogens, and in fact this is still the case in most developing countries. In the United States and other developed countries, however, treatment methods have largely eliminated bacterial disease organisms from the water supply, and attention has been turned to chemical contaminants.

4.2.1 Sources of Water and Soil Pollutants

Surface water can be contaminated by *point* or *nonpoint* sources. An effluent pipe from an industrial plant or a sewage-treatment plant is an example of a point source; a field from which pesticides and fertilizers are carried by rainwater into a river is an example of a nonpoint source. Industrial wastes probably constitute the greatest single pollution problem in soil and water. These contaminants include organic wastes such as solvents, inorganic wastes, such as chromium and many unknown chemicals. Contamination of soil and water results when by-product chemicals are not properly disposed of or conserved. In addition industrial accidents may lead to severe local contamination. For a more in-depth discussion of sources and movements of water pollutants, see Chapter 27.

Domestic and municipal wastes, both from sewage and from disposal of chemicals, are another major source of chemical pollutants. At the turn of the twentieth century, municipal wastes received no treatment and were discharged directly into rivers or oceans. Even today, many older treatment plants do not provide sufficient treatment, especially plants in which both storm water and sewage are combined. In addition to organic matter, pesticides, fertilizers, detergents, and metals are significant pollutants discharged from urban areas.

Contamination of soil and water also results from the use of pesticides and fertilizers. Persistent pesticides applied directly to the soil have the potential to move from the soil into the water and thus enter the food chain from both soil and water. In a similar way

fertilizers leach out of the soil or runoff during rain events and flow into the natural water systems.

Pollution from petroleum compounds has been a major concern since the mid-1960s. In 1967 the first major accident involving an oil tanker occurred. The *Torrey Canyon* ran onto rocks in the English Channel, spilling oil that washed onto the shores of England and France. It is estimated that at least 10,000 serious oil spills occur in the United States each year. In addition, flushing of oil tankers plays a major role in marine pollution. Other sources, such as improper disposal of used oil by private car owners and small garages, further contribute to oil pollution.

4.2.2 Examples of Pollutants

Metals that are of environmental concern fall into three classes: (1) metals that are suspected carcinogens, (2) metals that move readily in soil, and (3) metals that move through the food chain.

Lead. The heavy metals of greatest concern for health with regard to drinking water exposure are lead and arsenic. The sources of lead in drinking water that are most important are from lead pipes and lead solder. Also of concern is the seepage of lead from soil contaminated with the fallout from leaded gasoline and seepage of lead from hazardous-waste sites. Lead poisoning has been common in children, particularly in older housing units and inner city dwellings, in which children may consume chips of lead contaminated paint. Lead and associated toxic effects are discussed more fully in Chapter 5.

Arsenic. Drinking water is at risk for contamination by arsenic from the leaching of inorganic arsenic compounds formerly used in pesticide sprays, from the combustion of arsenic-containing fossil fuels, and from the leaching of mine tailings and smelter runoff. Chronic high-level exposures can cause abnormal skin pigmentation, hyperkeratosis, nasal congestion, and abdominal pain. At lower levels of chronic exposure, cancer is the major concern. Epidemologic studies have linked chronic arsenic exposure to various cancers, including skin, lungs, and lymph glands.

Cadmium. One of the most significant effects of metal pollution is that aquatic organisms can accumulate metals in their tissues, leading to increased concentrations in the food chain. Concern about long-term exposure to cadmium intensified after recognition of the disease Itai-Itai (painful-painful) in certain areas of Japan. The disease is a combination of severe kidney damage and painful bone and joint disease and occurs in areas where rice is contaminated with high levels of cadmium. This contamination resulted from irrigation of the soil with water containing cadmium released from industrial sources. Cadmium toxicity in Japan has also resulted from consumption of cadmium-contaminated fish taken from rivers near smelting plants.

Mercury. In Japan in the 1950s and 1060s, wastes from a chemical and plastics plant containing mercury were discharged into Minamata Bay. The mercury was converted to the readily absorbed methylmercury by bacteria in the aquatic sediments. Consumption of fish and shellfish by the local population resulted in numerous cases of mercury poisoning, or Minamata disease. By 1970, at least

107 deaths had been attributed to mercury poisoning, and 800 cases of Minamata disease were confirmed. Even though the mothers appeared healthy, many infants born to these mothers who had eaten contaminated fish developed cerebral palsy-like symptoms and mental deficiency.

Pesticides are also a major source of concern as water and soil pollutants. Because of their stability and persistence, the most hazardous pesticides are the organochlorine compounds such as DDT, aldrin, dieldrin, and chlordane. Persistent pesticides can accumulate in food chains; for example, shrimp and fish can concentrate some pesticides as much as 1000- to 10,000-fold. This bioaccumulation has been well documented with the pesticide DDT, which is now banned in many parts of the world. In contrast to the persistent insecticides, the organophosphorus (OP) pesticides, such as malathion, and the carbamates, such as carbaryl, are short-lived and generally persist for only a few weeks to a few months. Thus these compounds do not usually present as serious a problem as the earlier insecticides. Herbicides, because of the large quantity used, are also of concern as potential toxic pollutants. Pesticides are discussed in more detail in Chapter 5.

Nitrates and phosphates are two important nutrients that have been increasing markedly in natural waters since the mid-1960s. Sources of nitrate contamination include fertilizers, discharge from sewage treatment plants, and leachate from septic systems and manure. Nitrates from fertilizers leach readily from soils, and it has been estimated that up to 40% of applied nitrates enter water sources as runoff and leachate. Fertilizer phosphates, however, tend to be absorbed or bound to soil particles, so that only 20% to 25% of applied nitrates are leached into water. Phosphate detergents are another source of phosphate, one that has received much media attention in recent years.

The increase in these nutrients, particularly phosphates, is of environmental concern because excess nutrients can lead to “algal blooms” or eutrophication, as it is known, in lakes, ponds, estuaries, and very slow moving rivers. The algal bloom reduces light penetration and restricts atmospheric reoxygenation of the water. When the dense algal growth dies, the subsequent biodegradation results in anaerobic conditions and the death of many aquatic organisms. High phosphate concentrations and algal blooms are generally not a problem in moving streams, because such streams are continually flushed out and algae do not accumulate.

There are two potential adverse health effects from nitrates in drinking water: (1) nitrosamine formation and (2) methemoglobinemia. Ingested nitrates can be converted to nitrites by intestinal bacteria. After entering the circulatory system, nitrite ions combine with hemoglobin to form methemoglobin, thus decreasing the oxygen-carrying capacity of the blood and resulting in anemia or blue-baby disease. It is particularly severe in young babies who consume water and milk-formula prepared with nitrate-rich water. Older children and adults are able to detoxify the methemoglobin as a result of the enzyme methemoglobin reductase, which reverses the formation of methemoglobin. In infants, however, the enzyme is not fully functional. Certain nitrosamines are known carcinogens.

Oils and petroleum are ever-present pollutants in the modern environment, whether from the used oil of private motorists or spillage from oil tankers. At sea, oil slicks are responsible for the deaths of many birds. Very few birds that are badly contaminated recover, even after de-oiling and hand feeding. Oil is deposited on rocks and sand as

well, thus preventing the beaches from being used for recreation until after costly clean up. Shore animals, such as crabs, shrimp, mussels, and barnacles, are also affected by the toxic hydrocarbons they ingest. The subtle and perhaps potentially more harmful long-term effects on aquatic life are not yet fully understood.

Volatile organic compounds (VOCs) are other common groundwater contaminants. They include halogenated solvents and petroleum products, collectively referred to as VOCs. Both groups of compounds are used in large quantities by a variety of industries, such as degreasing, dry cleaning, paint, and the military. Historically petroleum products were stored in underground tanks that would erode, or were spilled onto soil surfaces. The EPA's National Priority List includes 11 VOCs: trichloroethylene, toluene, benzene, chloroform, tetrachloroethylene, 1,1,1-trichloroethane, ethylbenzene, trans-1,2-dichloroethane, xylene, dichloromethane, and vinyl chloride.

The physical and chemical properties of VOCs permit them to move rapidly into groundwater, and almost all of the previously mentioned chemicals have been detected in groundwater near contaminant sites. High levels of exposure can cause headache, impaired cognition, and kidney toxicities. At levels of exposure most frequently encountered, cancer and reproductive effects are of most concern, particularly childhood leukemia.

Low molecular weight chlorinated hydrocarbons are a by-product of the chlorination of municipal water. Chlorine reacts with organic substances commonly found in water to generate trihalomethanes (THMs), such as chloroform. The main organics that have been detected are chloroform, bromodichloromethane, dibromochloromethane, bromoform, carbon tetrachloride, and 1,2-dichloroethane. These compounds are associated with an increased risk of cancer. Studies in New Orleans in the mid-1970s showed that tap water in New Orleans contained more chlorinated hydrocarbons than did untreated Mississippi River water or well water. In addition chlorinated hydrocarbons, including carbon tetrachloride, were detected in blood plasma from volunteers who drank treated tap water. Epidemiologic studies indicated that the cancer death rate was higher among white males who drank tap water that among those who drank well water.

Radioactive contamination as some background radiation from natural sources, such as radon, occurs in some regions of the world, but there is particular concern over the contamination of surface water and groundwater by radioactive compounds generated by the production of nuclear weapons and by the processing of nuclear fuel. Many of these areas have remained unrecognized because of government secrecy.

Acids present in rain or drainage from mines, are major pollutants in many freshwater rivers and lakes. Because of their ability to lower the pH of the water to toxic levels and release toxic metals into solution, acids are considered particularly hazardous (see Chapter 5).

PCB organic compounds found as soil and water contaminants continue to grow each year. They include polychlorinated biphenyls (PCBs), phenols, cyanides, plasticizers, solvents, and numerous industrial chemicals. PCBs were historically used as coolants in electrical transformers and are also known by-products of the plastic, lubricant, rubber, and paper industries. They are stable, lipophilic, and break down only slowly in tissues. Because of these properties they accumulate to high concentrations in fish and waterfowl; in 1969 PCBs were responsible for the death of thousands of birds in the Irish Sea.

Dioxin has contaminated large areas of water and soil in the form of extremely toxic TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) through industrial accidents and through

widespread use of the herbicide 2,4,5-T. Small amounts of TCDD were contained as a contaminant in herbicide manufacturing. The US Army used this herbicide, known as Agent Orange, extensively as a defoliant in Vietnam. TCDD is one of the most toxic synthetic substances known for laboratory animals: LD50 for male rats, 0.022 mg/kg; LD50 for female rats, 0.045 mg/kg; LD50 for female guinea pigs (the most sensitive species tested), 0.0006 mg/kg. In addition it is fetotoxic to pregnant rats at a dose of only 1/400 of the LD50, and has been shown to cause birth defects at levels of 1 to 3 ng/kg. TCDD is a proven carcinogen in both mice and rats, with the liver being the primary target. Although TCDD does not appear to be particularly acutely toxic to humans, chronic low-level exposure is suspected of contributing to reproductive abnormalities and carcinogenicity.

4.3 OCCUPATIONAL TOXICANTS

Assessment of hazards in the workplace is a concern of occupational/industrial toxicology and has a history that dates back to ancient civilizations. The Greek historian Strabo, who lived in the first century AD, gave a graphic description of the arsenic mines in Pantus: “The air in mines is both deadly and hard to endure on account of the grievous odor of the ore, so that the workmen are doomed to a quick death.” With the coming of the industrial revolution in the nineteenth century, industrial diseases increased, and new ones, such as chronic mercurialism caused by exposure to mercuric nitrate used in “felting” animal furs, were identified. Hat makers, who were especially at risk, frequently developed characteristic tremors known as “hatters’ shakes,” and the expression “mad as a hatter” was coined. In recent years concern has developed over the carcinogenic potential of many workplace chemicals.

4.3.1 Regulation of Exposure Levels

The goal of occupational toxicology is to ensure work practices that do not entail any unnecessary health risks. To do this, it is necessary to define suitable permissible levels of exposure to industrial chemicals, using the results of animal studies and epidemiological studies. These levels can be expressed by the following terms for allowable concentrations.

Threshold limit values (TLVs) refer to airborne concentrations of substances and represent conditions under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse effect. Because of wide variation in individual susceptibility, a small percentage of workers may experience discomfort from some substances at or below the threshold limit; a smaller percentage may be affected more seriously by aggravation of a preexisting condition or by development of an occupational illness. Threshold limits are based on the best available information from industrial experience, from experimental human and animal studies, and when possible, from a combination of the three. The basis on which the values are established may differ from substance to substance; protection against impairment of health may be a guiding factor for some, whereas reasonable freedom from irritation, narcosis, nuisance, or other forms of stress may form the basis for others. Three categories of TLVs follow:

Threshold limit value–time-weighted average (TLV-TWA) is the TWA concentration for a normal 8-hour workday or 40-hour workweek to which nearly all workers may be repeatedly exposed, day after day, without adverse effect. Time-weighted averages allow certain permissible excursions above the limit provided that they are compensated by equivalent excursions below the limit during the workday. In some instances the average concentration is calculated for a workweek rather than for a workday.

Threshold limit value–short-term exposure limit (TLV-STEL) is the maximal concentration to which workers can be exposed for a period up to 15 minutes continuously without suffering from (1) irritation, (2) chronic or irreversible tissue change, or (3) narcosis of sufficient degree that would increase accident proneness, impair self-rescue, or materially work efficiency, provided that no more than four excursions per day are permitted, with at least 60 minutes between exposure periods, and provided that the daily TLV-TWA is not exceeded.

Threshold limit value–ceiling (TLV-C) is the concentration that should not be exceeded even instantaneously. For some substances—for instance, irritant gases—only one category, the TLV-ceiling, may be relevant. For other substances, two or three categories may be relevant.

Biologic limit values (BLVs) represent limits of amounts of substances (or their affects) to which the worker may be exposed without hazard to health or well-being as determined by measuring the worker's tissues, fluids, or exhaled breath. The biologic measurements on which the BLVs are based can furnish two kinds of information useful in the control of worker exposure: (1) measure of the worker's overall exposure and (2) measure of the worker's individual and characteristic response. Measurements of response furnish a superior estimate of the physiological status of the worker, and may consist of (1) changes in amount of some critical biochemical constituent, (2) changes in activity or a critical enzyme, and (3) changes in some physiological function. Measurement of exposure may be made by (1) determining in blood, urine, hair, nails, or body tissues and fluids the amount of substance to which the worker was exposed; (2) determining the amount of the metabolite(s) of the substance in tissues and fluids; and (3) determining the amount of the substance in the exhaled breath. The biologic limits may be used as an adjunct to the TLVs for air, or in place of them.

Immediately dangerous to life or health (IDLH) conditions pose a threat of severe exposure to contaminants, such as radioactive materials, that are likely to have adverse cumulative or delayed effects on health. Two factors are considered when establishing IDLH concentrations. The worker must be able to escape (1) without loss of life or without suffering permanent health damage within 30 minutes and (2) without severe eye or respiratory irritation or other reactions that could inhibit escape. If the concentration is above the IDLH, only highly reliable breathing apparatus is allowed.

4.3.2 Routes of Exposure

The principal routes of industrial exposure are dermal and inhalation. Occasionally toxic agents may be ingested, if food or drinking water is contaminated. Exposure to the skin often leads to localized effects known as "occupation dermatosis" caused by either irritating chemicals or allergenic chemicals. Such effects include scaling,

eczema, acne, pigmentation changes, ulcers, and neoplasia. Some chemicals may also pass through the skin; these include aromatic amines such as aniline and solvents such as carbon tetrachloride and benzene.

Toxic or potentially toxic agents may be inhaled into the respiratory tract where they may cause localized effects such as irritation (e.g., ammonia, chlorine gas), inflammation, necrosis, and cancer. Chemicals may also be absorbed by the lungs into the circulatory system, thereby leading to systemic toxicity (e.g., CO, lead).

4.3.3 Examples of Industrial Toxicants

Carcinogen exposure is largely due to lifestyle, such as cigarette smoking, but occupation is an important source of exposure to carcinogens. Table 4.4 lists some occupational chemical hazards and the cancers associated with them.

Cadmium is a cumulative toxicant with a biologic half-life of up to 30 years in humans. More than 70% of the cadmium in the blood is bound to red blood cells; accumulation occurs mainly in the kidney and the liver, where cadmium is bound to metallothionein. In humans the critical target organ after long-term exposure to cadmium is the kidney, with the first detectable symptom of kidney toxicity being an increased excretion of specific proteins.

Chromium toxicity results from compounds of hexavalent chromium that can be readily absorbed by the lung and gastrointestinal (GI) tract and to a lesser extent by the skin. Occupational exposure to chromium (Cr^{6+}) causes dermatitis, ulcers on the hands and arms, perforation of the nasal septum (probably caused by chromic acid), inflammation of the larynx and liver, and bronchitis. Chromate is a carcinogen causing bronchogenic carcinoma; the risk to chromate plant workers for lung cancer is 20 times greater than that for the general population. Compounds of trivalent chromium

Table 4.4 Some Occupational Hazards and Associated Cancers

Agent	Tumor Sites	Occupation
Asbestos	Lung, pleura, peritoneum	Miners, manufacturers, users
Arsenic	Skin, lung, liver	Miners and smelters, oil refinery, pesticide workers
Benzene	Hemopoietic tissue	Process workers, textile workers
Cadmium	Lung, kidney, prostate	Battery workers, smelters
Chloroethers	Lung	Chemical plant workers, process workers
Chromium	Lung, nasal cavity, sinuses	Process and production workers, pigment workers
Mustard gas	Bronchi, lung, larynx	Production workers
Naphthylamines	Bladder	Dyestuff makers and workers, Chemical workers, printers
Nickel	Lung, nasal sinuses	Smelters and process workers
Polycyclic aromatic hydrocarbons	Respiratory system, bladder	Furnace, foundry, shale, and gas workers; chimney sweeps
Radon, radium, uranium	Skin, lung, bone tissue, bone marrow	Medical and industrial chemists, miners
UV radiation	Skin	Outdoor exposure
X rays	Bone marrow, skin	Medical and industrial workers

are poorly absorbed. Chromium is not a cumulative chemical, and once absorbed, it is rapidly excreted into the urine.

Lead is a ubiquitous toxicant in the environment, and consequently the normal body concentration of lead is dependent on environmental exposure conditions. Approximately 50% of lead deposited in the lung is absorbed, whereas usually less than 10% of ingested lead passes into the circulation. Lead is not a major occupational problem today, but environmental pollution is still widespread. Lead interferes in the biosynthesis of porphyrins and heme, and several screening tests for lead poisoning make use of this interaction by monitoring either inhibition of the enzyme δ-aminolevulinic acid dehydratase (ALAD) or appearance in the urine of aminolevulinic acid (ALA) and coproporphorin (UCP). The metabolism of inorganic lead is closely related to that of calcium, and excess lead can be deposited in the bone where it remains for years. Inorganic lead poisoning can produce fatigue, sleep disturbances, anemia, colic, and neuritis. Severe exposure, mainly of children who have ingested lead, may cause encephalopathy, mental retardation, and occasionally, impaired vision.

Organic lead has an affinity for brain tissue; mild poisoning may cause insomnia, restlessness, and GI symptoms, whereas severe poisoning results in delirium, hallucinations, convulsions, coma, and even death.

Mercury is widely used in scientific and electrical apparatus, with the largest industrial use of mercury being in the chlorine-alkali industry for electrolytic production of chlorine and sodium hydroxide. Worldwide, this industry has been a major source of mercury contaminations. Most mercury poisoning, however, has been due to methylmercury, particularly as a result of eating contaminated fish. Inorganic and organic mercury differ in their routes of entry and absorption. Inhalation is the principal route of uptake of metallic mercury in industry, with approximately 80% of the mercury inhaled as vapor being absorbed; metallic mercury is less readily absorbed by the GI route. The principal sites of deposition are the kidney and brain after exposure to inorganic mercury salts. Organic mercury compounds are readily absorbed by all routes. Industrial mercurialism produces features such as inflammation of the mouth, muscular tremors (hatters' shakes), psychic irritation, and a nephritic syndrome characterized by proteinuria. Overall, however, occupational mercurialism is not a significant problem today.

Benzene was used extensively in the rubber industry as a solvent for rubber latex in the latter half of the nineteenth century. The volatility of benzene, which made it so attractive to the industry, also caused high atmospheric levels of the solvent. Benzene-based rubber cements were used in the canning industry and in the shoe manufacturing industry. Although cases of benzene poisoning had been reported as early as 1897 and additional reports and warnings were issued in the 1920s, the excellent solvent properties of benzene resulted in its continued extensive use. In the 1930s cases of benzene toxicity occurred in the printing industry in which benzene was used as an ink solvent. Today benzene use exceeds 11 billion gallons per year.

Benzene affects the hematopoietic tissue in the bone marrow and also appears to be an immunosuppressant. There is a gradual decrease in white blood cells, red blood cells, and platelets, and any combination of these signs may be seen. Continued exposure to benzene results in severe bone marrow damage and aplastic anemia. Benzene exposure has also been associated with leukemia.

Asbestos and other fibers of naturally occurring silicates will separate into flexible fibers. Asbestos is the general name for this group of fibers. Chrysotile is the most

important commercially and represents about 90% of the total used. Use of asbestos has been extensive, especially in roofing and insulation, asbestos cements, brake linings, electrical appliances, and coating materials. Asbestosis, a respiratory disease, is characterized by fibrosis, calcification, and lung cancer. In humans, not only is there a long latency period between exposure and development of tumors but other factors also influence the development of lung cancer. Cigarette smoking, for example, enhances tumor formation. Recent studies have shown that stomach and bowel cancers occur in excess in workers (e.g., insulation workers) exposed to asbestos. Other fibers have been shown to cause a similar disease spectrum, for instance, zeolite fibers.

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CHAPTER 5

Classes of Toxicants: Use Classes

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5.1 INTRODUCTION

As discussed in Chapter 1, use classes include not only chemicals currently in use but also the toxicological aspects of the development of new chemicals for commercial use, chemicals produced as by-products of industrial processes, and chemicals resulting from the use and/or disposal of chemicals. Because any use class may include chemicals from several different chemical classes, this classification is not sufficient for mechanistic considerations. It is, however, essential for an understanding of the scope of toxicology and, in particular, is essential for many applied branches of toxicology such as exposure assessment, industrial hygiene, public health toxicology and regulatory toxicology.

5.2 METALS

5.2.1 History

Although most metals occur in nature in rocks, ores, soil, water, and air, levels are usually low and widely dispersed. In terms of human exposure and toxicological significance, it is anthropogenic activities that are most important because they increase the levels of metals at the site of human activities.

Metals have been used throughout much of human history to make utensils, machinery, and so on, and mining and smelting supplied metals for these uses. These activities increased environmental levels of metals. More recently metals have found a number of uses in industry, agriculture, and medicine. These activities have increased exposure not only to metal-related occupational workers but also to consumers of the various products.

Despite the wide range of metal toxicity and toxic properties, there are a number of toxicological features that are common to many metals. Some of the more important aspects are discussed briefly in the following sections. For a metal to exert its toxicity, it must cross the membrane and enter the cell. If the metal is in a lipid soluble form such as methylmercury, it readily penetrates the membrane; when bound to proteins

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such as cadmium-metallothionein, the metal is taken into the cell by endocytosis; other metals (e.g., lead) may be absorbed by passive diffusion. The toxic effects of metals usually involve interaction between the free metal and the cellular target. These targets tend to be specific biochemical processes and/or cellular and subcellular membranes.

5.2.2 Common Toxic Mechanisms and Sites of Action

Enzyme Inhibition/Activation. A major site of toxic action for metals is interaction with enzymes, resulting in either enzyme inhibition or activation. Two mechanisms are of particular importance: inhibition may occur as a result of interaction between the metal and sulfhydryl (SH) groups on the enzyme, or the metal may displace an essential metal cofactor of the enzyme. For example, lead may displace zinc in the zinc-dependent enzyme δ-aminolevulinic acid dehydratase (ALAD), thereby inhibiting the synthesis of heme, an important component of hemoglobin and heme-containing enzymes, such as cytochromes.

Subcellular Organelles. Toxic metals may disrupt the structure and function of a number of organelles. For example, enzymes associated with the endoplasmic reticulum may be inhibited, metals may be accumulated in the lysosomes, respiratory enzymes in the mitochondria may be inhibited, and metal inclusion bodies may be formed in the nucleus.

Carcinogenicity. A number of metals have been shown to be carcinogenic in humans or animals. Arsenic, certain chromium compounds, and nickel are known human carcinogens; beryllium, cadmium, and cisplatin are probable human carcinogens. The carcinogenic action, in some cases, is thought to result from the interaction of the metallic ions with DNA (see Chapter 11 for a detailed discussion of carcinogenesis).

Kidney. Because the kidney is the main excretory organ of the body, it is a common target organ for metal toxicity. Cadmium and mercury, in particular, are potent nephrotoxicants and are discussed more fully in the following sections and in Chapter 15.

Nervous System. The nervous system is also a common target of toxic metals; particularly, organic metal compounds (see Chapter 16). For example, methylmercury, because it is lipid soluble, readily crosses the blood-brain barrier and enters the nervous system. By contrast, inorganic mercury compounds, which are more water soluble, are less likely to enter the nervous system and are primarily nephrotoxicants. Likewise organic lead compounds are mainly neurotoxicants, whereas the first site of inorganic lead is enzyme inhibition (e.g., enzymes involved in heme synthesis).

Endocrine and Reproductive Effects. Because the male and female reproductive organs are under complex neuroendocrine and hormonal control, any toxicant that alters any of these processes can affect the reproductive system (see Chapters 17 and 20). In addition metals can act directly on the sex organs. Cadmium is known to produce testicular injury after acute exposure, and lead accumulation in the testes is associated with testicular degeneration, inhibition of spermatogenesis, and Leydig-cell atrophy.

Respiratory System. Occupational exposure to metals in the form of metal dust makes the respiratory system a likely target. Acute exposure may cause irritations and

inflammation of the respiratory tract, whereas chronic exposure may result in fibrosis (aluminum) or carcinogenesis (arsenic, chromium, nickel). Respiratory toxicants are discussed more fully in Chapter 18.

Metal-Binding Proteins. The toxicity of many metals such as cadmium, lead, and mercury depends on their transport and intracellular bioavailability. This availability is regulated to a degree by high-affinity binding to certain cytosolic proteins. Such ligands usually possess numerous SH binding sites that can outcompete other intracellular proteins and thus mediate intracellular metal bioavailability and toxicity. These intracellular "sinks" are capable of partially sequestering toxic metals away from sensitive organelles or proteins until their binding capacity is exceeded by the dose of the metal. *Metallothionein* (MT) is a low molecular weight metal-binding protein (approximately 7000 Da) that is particularly important in regulating the intracellular bioavailability of cadmium, copper, mercury, silver, and zinc. For example, in vivo exposure to cadmium results in the transport of cadmium in the blood by various high molecular weight proteins and uptake by the liver, followed by hepatic induction of MT. Subsequently cadmium can be found in the circulatory system bound to MT as the cadmium-metallothionein complex (CdMT).

5.2.3 Lead

Because of the long-term and widespread use of lead, it is one of the most ubiquitous of the toxic metals. Exposure may be through air, water, or food sources. In the United States the major industrial uses, such as in fuel additives and lead pigments in paints, have been phased out, but other uses, such as in batteries, have not been reduced. Other sources of lead include lead from pipes and glazed ceramic food containers.

Inorganic lead may be absorbed through the GI tract, the respiratory system, and the skin. Ingested inorganic lead is absorbed more efficiently from the GI tract of children than that of adults, readily crosses the placenta, and in children penetrates the blood-brain barrier. Initially, lead is distributed in the blood, liver, and kidney; after prolonged exposure, as much as 95% of the body burden of lead is found in bone tissue.

The main targets of lead toxicity are the hematopoietic system and the nervous system. Several of the enzymes involved in the synthesis of heme are sensitive to inhibition by lead, the two most susceptible enzymes being ALAD and heme synthetase (HS). Although clinical anemia occurs only after moderate exposure to lead, biochemical effects can be observed at lower levels. For this reason inhibition of ALAD or appearance in the urine of ALA can be used as an indication of lead exposure.

The nervous system is another important target tissue for lead toxicity, especially in infants and young children in whom the nervous system is still developing (Chapter 16). Even at low levels of exposure, children may show hyperactivity, decreased attention span, mental deficiencies, and impaired vision. At higher levels, encephalopathy may occur in both children and adults. Lead damages the arterioles and capillaries, resulting in cerebral edema and neuronal degeneration. Clinically this damage manifests itself as ataxia, stupor, coma, and convulsions.

Another system affected by lead is the reproductive system (Chapter 20). Lead exposure can cause male and female reproductive toxicity, miscarriages, and degenerate offspring.

5.2.4 Mercury

Mercury exists in the environment in three main chemical forms: elemental mercury (Hg^0), inorganic mercurous (Hg^+) and mercuric ($Hg2^+$) salts, and organic methylmercury (CH_3Hg) and dimethylmercury (CH_3HgCH_3) compounds. Elemental mercury, in the form of mercury vapor, is almost completely absorbed by the respiratory system, whereas ingested elemental mercury is not readily absorbed and is relatively harmless. Once absorbed, elemental mercury can cross the blood-brain barrier into the nervous system. Most exposure to elemental mercury tends to be from occupational sources.

Of more concern from environmental contamination is exposure to organic mercury compounds. Inorganic mercury may be converted to organic mercury through the action of sulfate-reducing bacteria, to produce methylmercury, a highly toxic form readily absorbed across membranes. Several large episodes of mercury poisoning have resulted from consuming seed grain treated with mercury fungicides or from eating fish contaminated with methylmercury. In Japan in the 1950s and 1960s wastes from a chemical and plastics plant containing mercury were drained into Minamata Bay. The mercury was converted to the readily absorbed methylmercury by bacteria in the aquatic sediments. Consumption of fish and shellfish by the local population resulted in numerous cases of mercury poisoning or Minamata disease. By 1970 at least 107 deaths had been attributed to mercury poisoning, and 800 cases of Minamata disease were confirmed. Even though the mothers appeared healthy, many infants born to mothers who had eaten contaminated fish developed cerebral palsy-like symptoms and mental deficiency. Organic mercury primarily affects the nervous system, with the fetal brain being more sensitive to the toxic effects of mercury than adults.

Inorganic mercury salts, however, are primarily nephrotoxicants, with the site of action being the proximal tubular cells. Mercury binds to SH groups of membrane proteins, affecting the integrity of the membrane and resulting in aliguria, anuria, and uremia.

5.2.5 Cadmium

Cadmium occurs in nature primarily in association with lead and zinc ores and is released near mines and smelters processing these ores. Industrially cadmium is used as a pigment in paints and plastics, in electroplating, and in making alloys and alkali storage batteries (e.g., nickel-cadmium batteries). Environmental exposure to cadmium is mainly from contamination of groundwater from smelting and industrial uses as well as the use of sewage sludge as a food-crop fertilizer. Grains, cereal products, and leafy vegetables usually constitute the main source of cadmium in food. Reference has already been made to the disease Itai-Itai resulting from consumption of cadmium-contaminated rice in Japan (see Chapter 4, Section 4.2.2).

Acute effects of exposure to cadmium result primarily from local irritation. After ingestion, the main effects are nausea, vomiting, and abdominal pain. Inhalation exposure may result in pulmonary edema and chemical pneumonitis.

Chronic effects are of particular concern because cadmium is very slowly excreted from the body, with a half-life of about 30 years. Thus low levels of exposure can result in considerable accumulation of cadmium. The main organ of damage following long-term exposure is the kidney, with the proximal tubules being the primary site of

action. Cadmium is present in the circulatory system bound primarily to the metal-binding protein, metallothionein, produced in the liver. Following glomerular filtration in the kidney, CdMT is re-absorbed efficiently by the proximal tubule cells, where it accumulates within the lysosomes. Subsequent degradation of the CdMT complex releases Cd^{+2}, which inhibits lysosomal function, resulting in cell injury.

5.2.6 Chromium

Because chromium occurs in ores, environmental levels are increased by mining, smelting, and industrial uses. Chromium is used in making stainless steel, various alloys, and pigments. The levels of this metal are generally very low in air, water, and food, and the major source of human exposure is occupational. Chromium occurs in a number of oxidation states from Cr^{+2} to Cr^{+6}, but only the trivalent (Cr^{+3}) and hexavalent (Cr^{+6}) forms are of biological significance. Although the trivalent compound is the most common form found in nature, the hexavalent form is of greater industrial importance. In addition hexavalent chromium, which is not water soluble, is more readily absorbed across cell membranes than is trivalent chromium. In vivo the hexavalent form is reduced to the trivalent form, which can complex with intracellular macromolecules, resulting in toxicity. Chromium is a known human carcinogen and induces lung cancers among exposed workers. The mechanism of chromium (Cr^{+6}) carcinogenicity in the lung is believed to be its reduction to Cr^{+3} and generation of reactive intermediates, leading to bronchogenic carcinoma.

5.2.7 Arsenic

In general, the levels of arsenic in air and water are low, and the major source of human exposure is food. In certain parts of Taiwan and South America, however, the water contains high levels of this metalloid, and the inhabitants often suffer from dermal hyperkeratosis and hyperpigmentation. Higher levels of exposure result in a more serious condition; gangrene of the lower extremities or "blackfoot disease." Cancer of the skin also occurs in these areas.

Approximately 80% of arsenic compounds are used in pesticides. Other uses include glassware, paints, and pigments. Arsine gas is used in the semiconductor industry. Arsenic compounds occur in three forms: (1) pentavalent, As^{+5}, organic or arsenate compounds (e.g., alkyl arsenates); (2) trivalent, As^{+3}, inorganic or arsenate compounds (e.g., sodium arsenate, arsenic trioxide); and (3) arsine gas, AsH_3, a colorless gas formed by the action of acids on arsenic. The most toxic form is arsine gas with a TLV-TWA of 0.05 ppm. Microorganisms in the environment convert arsenic to dimethylarsenate, which can accumulate in fish and shellfish, providing a source for human exposure. Arsenic compounds can also be present as contaminants in well water. Arsenite (As^{+3}) compounds are lipid soluble and can be absorbed following ingestion, inhalation, or skin contact. Within 24 hours of absorption, arsenic distributes over the body, where it binds to SH groups of tissue proteins. Only a small amount crosses the blood-brain barrier. Arsenic may also replace phosphorus in bone tissue and be stored for years.

After acute poisoning, severe GI gastrointestinal symptoms occur within 30 minutes to 2 hours. These include vomiting, watery and bloody diarrhea, severe abdominal pain,

Table 5.1 Examples of Chelating Drugs Used to Treat Metal Toxicity

British antilewisite (BAL[2,3–dimercaptopropanol]), dimercaprol
DMPS (2,3-dimercapto-1-propanesulfonic acid)
DMSA (meso-2,3-dimercaptosuccinic acid)
EDTA (ethylenediaminetetraacetic acid, calcium salt)
DTPA (diethylenetriaminepentaacetic acid, calcium salt)
DTC (dithiocarbamate)
Penicillamine (β-β-dimethylcysteine), hydrolytic product of penicillin

and burning esophageal pain. Vasodilatation, myocardial depression, cerebral edema, and distal peripheral neuropathy may also follow. Later stages of poisoning include jaundice and renal failure. Death usually results from circulatory failure within 24 hours to 4 days.

Chronic exposure results in nonspecific symptoms such as diarrhea, abdominal pain, hyperpigmentation, and hyperkeratosis. A symmetrical sensory neuropathy often follows. Late changes include gangrene of the extremities, anemia, and cancer of the skin, lung, and nasal tissue.

5.2.8 Treatment of Metal Poisoning

Treatment of metal exposure to prevent or reverse toxicity is done with chelating agents or antagonists. Chelation is the formation of a metal ion complex, in which the metal ion is associated with an electron donor ligand. Metals may react with O-, S-, and N-containing ligands (e.g., –OH, –COOH, –S–S–, and $–NH_2$). Chelating agents need to be able to reach sites of storage, form nontoxic complexes, not readily bind essential metals (e.g., calcium, zinc), and be easily excreted.

One of the first clinically useful chelating drugs was British antilewisite (BAL [2,3-dimercaptopropanol]), which was developed during World War II as an antagonist to arsenical war gases. BAL is a dithiol compound with two sulfur atoms on adjacent carbon atoms that compete with critical binding sites involved in arsenic toxicity. Although BAL will bind a number of toxic metals, it is also a potentially toxic drug with multiple side effects. In response to BAL's toxicity, several analogues have now been developed. Table 5.1 lists some of the more common chelating drugs in therapeutic use.

5.3 AGRICULTURAL CHEMICALS (PESTICIDES)

5.3.1 Introduction

Chemicals have been used to kill or control pests for centuries. The Chinese used arsenic to control insects, the early Romans used common salt to control weeds and sulfur to control insects. In the 1800s pyrethrin (i.e., compounds present in the flowers of the chrysanthemum, *Pyrethrum cineraefolium*) was found to have insecticidal properties. The roots of certain Derris plant species, (*D. elliptica* and *Lonchocarpus* spp.) were used by the Chinese and by South American natives as a fish poison. The active ingredient, rotenone, was isolated in 1895 and used for insect control. Another material

developed for insect control in the 1800s was Paris Green, a mixture of copper and arsenic salts. Fungi were controlled with Bordeaux Mixture, a combination of lime and copper sulfate.

However, it was not until the 1900s that the compounds we identify today as having pesticidal properties came into being. Petroleum oils, distilled from crude mineral oils were introduced in the 1920s to control scale insects and red spider mites. The 1940s saw the introduction of the chlorinated hydrocarbon insecticides such as DDT and the phenoxy acid herbicides such as 2,4-*D*). Natural compounds such as Red Squill, derived from the bulbs of red squill, *Urginea (Scilla) maritima*, were effective in controlling rodents. Triazine herbicides, such as atrazine, introduced in the late 1950s, dominated the world herbicide market for years. Synthetic pyrethrins or pyrethroid insecticides (e.g., resmethrin) became and continue to be widely used insecticides due to their low toxicity, enhanced persistence compared to the pyrethrins and low application rates. New families of fungicides, herbicides, and insecticides continue to be introduced into world markets as older compounds lose their popularity due to pest resistance or adverse health effects.

Pesticides are unusual among environmental pollutants in that they are used deliberately for the purpose of killing some form of life. Ideally pesticides should be highly selective, destroying target organisms while leaving nontarget organisms unharmed. In reality, most pesticides are not so selective. In considering the use of pesticides, the benefits must be weighed against the risk to human health and environmental quality. Among the benefits of pesticides are control of vector-borne diseases, increased agricultural productivity, and control of urban pests. A major risk is environmental contamination, especially translocation within the environment where pesticides might enter both food chains and natural water systems. Factors to be considered in this regard are persistence in the environment and potential for bioaccumulation.

5.3.2 Definitions and Terms

The term "agricultural chemicals" has largely been replaced by the term "pesticides," defined as economic poisons, regulated by federal and state laws, that are used to control, kill, or repel pests. Depending on what a compound is designed to do, pesticides have been subclassified into a number of categories (Table 5.2). The primary classes of pesticides in use today are fumigants, fungicides, herbicides, and insecticides with total US production of 1.2 billion pounds (1997: US Environmental Protection Agency's latest figures) and production of some 665 million pounds of wood preservatives. Table 5.3 describes the relative use of different classes of pesticides in the United States.

Generally, it takes some five to seven years to bring a pesticide to market once its pesticidal properties have been verified. Many tests must be conducted to determine such things as the compound's synthesis, its chemical and physical properties, and its efficacy. In addition, in order for registration for use by the US EPA, numerous toxicity tests are undertaken including those for acute toxicity, those for chronic effects such as reproductive anomalies, carcinogenesis, and neurological effects and those for environmental effects.

The mandated pesticide label contains a number of specified items, including the concentration and/or percentage of both the active (A.I.) and inert ingredients; proper mixing of the formulation with water to obtain the application rate of A.I., what the A.I.

Table 5.2 Classification of Pesticides, with Examples

Class	Principal Chemical Type	Example, Common Name
Algicide	Organotin	Brestar
Fungicide	Dicarboximide	Captan
	Chlorinated aromatic	Pentchlorophenol
	Dithiocarbamate	Maneb
	Mercurial	Phenylmercuric acetate
Herbicide	Amides, acetamides	Propanil
	Bipyridyl	Paraquat
	Carbamates, thiocarbamates	Barban
	Phenoxy	2,4-D
	Dinitrophenol	DNOC
	Dinitroaniline	Trifluralin
	Substitute urea	Monuron
	Triazine	Atrazine
Nematocide	Halogenated alkane	Ethylene dibromide (EDB)
Molluscicide	Chlorinated hydrocarbon	Bayluscide
Insecticide	Chlorinated hydrocarbons	
	DDT analogous	DDT
	Chlorinated alicyclic	BHC
	Cyclodiene	Aldrin
	Chlorinated terpenes	Toxaphene
	Organophosphorus	Chlorpyrifos
	Carbamate	Carbaryl
	Thiocyanate	Lethane
	Dinitrophenols	DNOC
	Fluoroacetate	Nissol
	Botanicals	
	Nicotinoids	Nicotine
	Rotenoids	Rotenone
	Pyrethroids	Pyrethrin
	Synthetic pyrethroids	Fenvalerate
	Synthetic nicotinoids	Imidacloprid
	Fiproles	Fipronil
	Juvenile hormone analogs	Methroprene
	Growth regulators	Dimilin
	Inorganics	
	Arsenicals	Lead arsenate
	Fluorides	Sodium fluoride
	Microbials	Thuricide, avermectin
Insecticide synergists	Methylenedioxyphenyl	Piperonyl butoxide
	Dicarboximides	MGK-264
Acaricides	Organosulfur	Ovex
	Formamidine	Chlordimeform
	Dinitrophenols	Dinex
	DDT analogs	Chlorbenzilate
Rodenticides	Anticoagulants	Warfarin
	Botanicals	
	Alkaloids	Strychine sulfate
	Glycosides	Scillaren A and B
	Fluorides	Fluoroacetate
	Inorganics	Thallium sulfate
	Thioureas	ANTU

will control, and how and when to apply it. In addition the label describes environmental hazards, proper storage of the material, re-entry intervals (REIs) for application sites, and the personal protective equipment (PPE) that must be worn during application or harvesting.

Depending on the toxicity, formulation concentration, and use patterns, pesticides can be classified as "general" or "restricted" use. A general use pesticide will cause no unreasonable, adverse effects when used according to the label and can be purchased and applied by anyone. A restricted use pesticide, defined as generally causing undesirable effects on the environment, applicator, or workers can only be purchased and applied by an individual who is licensed by the state.

The US EPA has developed "category use" definitions based on toxicity. Category I pesticides are highly hazardous, are classified as restricted use and have an oral LD50 less than or equal to 1.0/kg of body weight; category II pesticides are moderately toxic and have an oral LD50 less than or equal to 500 mg/kg; category III pesticides are generally nontoxic and have an oral LD50 less than or equal to 15,000 mg/kg. In addition the US EPA has developed a "carcinogenicity categorization" to classify pesticides for carcinogenicity.

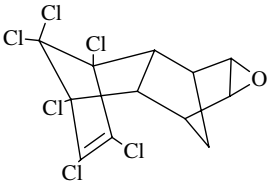
5.3.3 Organochlorine Insecticides

The chlorinated hydrocarbon insecticides were introduced in the 1940s and 1950s and include familiar insecticides such as DDT, methoxychlor, chlordane, heptachlor, aldrin, dieldrin, endrin, toxaphene, mirex, and lindane. The structures of two of the more familiar ones, DDT and dieldrin, are shown in Figure 5.1. The chlorinated hydrocarbons are neurotoxicants and cause acute effects by interfering with the transmission of nerve impulses. Although DDT was synthesized in 1874, its insecticidal properties were not noted until 1939, when Dr. Paul Mueller, a Swiss chemist, discovered its effectiveness as an insecticide and was awarded a Nobel Prize for his work. During World War II the United States used large quantities of DDT to control vector-borne diseases, such as typhus and malaria, to which US troops were exposed. After the war DDT use became widespread in agriculture, public health, and households. Its persistence, initially considered a desirable attribute, later became the basis for public concern. The publication of Rachel Carson's book *The Silent Spring* in 1962 stimulated this concern and eventually led to the ban of DDT and other chlorinated insecticides in the United States in 1972.

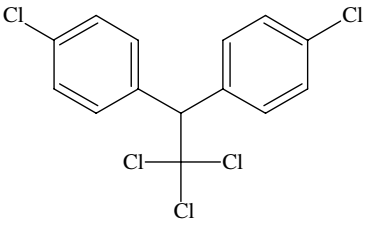
Table 5.3 Use Patterns of Pesticides in the United States

Class	Percentage of Total Pesticide Use
Herbicides	47
Insecticides	19
Fungicides	13
Others[a]	21

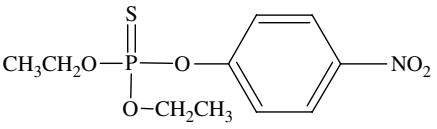
Note: Most recent data: for 1997, published by US EPA in 2001.
[a]Includes fumigants and wood preservates.



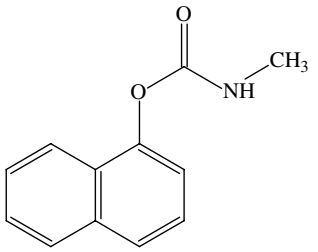
Dieldrin



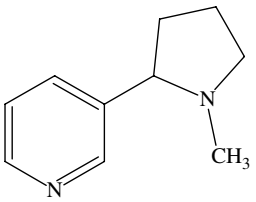
DDT



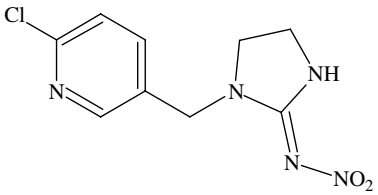
Parathion



Carbaryl



Nicotine



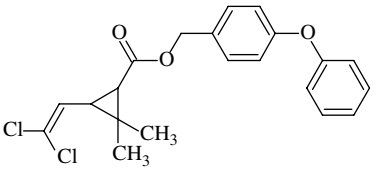
Imidacloprid

Figure 5.1 Some examples of chemical structures of common pesticides.

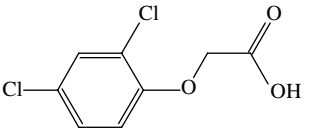
DDT, as well as other organochlorines, were used extensively from the 1940s through the 1960s in agriculture and mosquito control, particularly in the World Health Organization (WHO) malaria control programs. The cyclodiene insecticides, such as chlordane were used extensively as termiticides into the 1980s but were removed from the market due to measurable residue levels penetrating into interiors and allegedly causing health problems. Residue levels of chlorinated insecticides continue to be found in the environment and, although the concentrations are now so low as to approach the limit of delectability, there continues to be concern.

5.3.4 Organophosphorus Insecticides

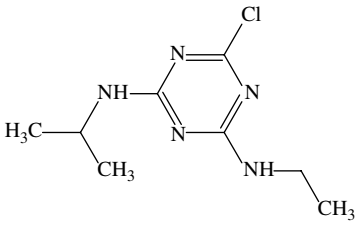
Organophosphorus pesticides (OPs) are phosphoric acid esters or thiophosphoric acid esters (Figure 5.1) and are among the most widely used pesticides for insect control. During the 1930s and 1940s Gerhard Schrader and coworkers began investigating OP compounds. They realized that the insecticidal properties of these compounds and by the end of the World War II had made many of the insecticidal OPs in use today,



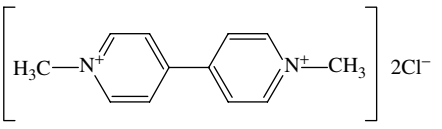
Permethrin



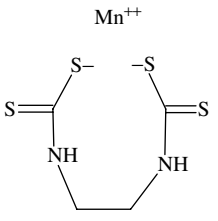
2,4-D



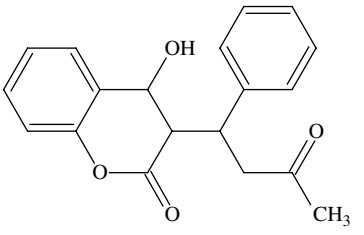
Atrazine



Paraquat



Maneb



Warfarin

Figure 5.1 (*continued*)

such as ethyl parathion [*O*,*O*-diethyl *O*-(4-nitrophenyl)phosphorothioate]. The first OP insecticide to find widespread use was tetraethylpyrophosphate (TEPP), approved in Germany in 1944 and marketed as a substitute for nicotine to control aphids. Because of its high mammalian toxicity and rapid hydrolysis in water, TEPP was replaced by other OP insecticides.

Chlorpyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] became one of the largest selling insecticides in the world and had both agricultural and urban uses. The insecticide could be purchased for indoor use by homeowners, but health-related concerns caused USEPA to cancel home indoor and lawn application uses in 2001. The only exception is its continued use as a termiticide.

Parathion was another widely used insecticide due to its stability in aqueous solutions and its broad range of insecticidal activity. However, its high mammalian toxicity through all routes of exposure led to the development of less hazardous compounds. Malathion [diethyl (dimethoxythiophosphorylthio)succinate], in particular, has low mammalian toxicity because mammals possess certain enzymes, the carboxylesterases, that readily hydrolyze the carboxyester link, detoxifying the compound. Insects, by

contrast, do not readily hydrolyze this ester, and the result is its selective insecticidal action.

OPs are toxic because of their inhibition of the enzyme acetylcholinesterase. This enzyme inhibition results in the accumulation of acetylcholine in nerve tissue and effector organs, with the principal site of action being the peripheral nervous system (PNS) (see Chapter 16). In addition to acute effects, some OP compounds have been associated with delayed neurotoxicity, known as organophosphorus-induced delayed neuropathy (OPIDN). The characteristic clinical sign is bilateral paralysis of the distal muscles, predominantly of the lower extremities, occurring some 7 to 10 days following ingestion (see Chapter 16). Not all OP compounds cause delayed neuropathy. Among the pesticides associated with OPIDN are leptophos, mipafox, EPN, DEF, and trichlorfon. Testing is now required for OP substances prior to their use as insecticides.

The OP and carbamate insecticides are relatively nonpersistent in the environment. They are applied to the crop or directly to the soil as systemic insecticides, and they generally persist from only a few hours to several months. Thus these compounds, in contrast to the organochlorine insecticides, do not represent a serious problem as contaminants of soil and water and rarely enter the human food chain. Being esters, the compounds are susceptible to hydrolysis, and their breakdown products are generally nontoxic. Direct contamination of food by concentrated compounds has been the cause of poisoning episodes in several countries.

5.3.5 Carbamate Insecticides

The carbamate insecticides are esters of *N*-methyl (or occasionally *N*,*N*-dimethyl) carbamic acid (H_2NCOOH). The toxicity of the compound varies according to the phenol or alcohol group. One of the most widely used carbamate insecticides is carbaryl (1-napthyl methylcarbamate), a broad spectrum insecticide (Figure 5.1). It is used widely in agriculture, including home gardens where it generally is applied as a dust. Carbaryl is not considered to be a persistent compound, because it is readily hydrolyzed. Based on its formulation, it carries a toxicity classification of II or III with an oral LD50 of 250 mg/kg (rat) and a dermal LC50 of >2000 mg/kg.

An example of an extremely toxic carbamate is aldicarb [2-methyl-2-(methylthio) propionaldehyde]. Both oral and dermal routes are the primary portals of entry, and it has an oral LD50 of 1.0 mg/kg (rat)and a dermal LD50 of 20 mg/kg (rabbit). For this reason it is recommended for application to soils on crops such as cotton, citrus, and sweet potatoes. This compound moves readily through soil profiles and has contaminated groundwater supplies.

Like the OP insecticides, the mode of action of the carbamates is acetylcholinesterase inhibition with the important difference that the inhibition is more rapidly reversed than with OP compounds.

5.3.6 Botanical Insecticides

Extracts from plants have been used for centuries to control insects. Nicotine [(*S*)-3-(1-methyl-2-pyrrolidyl)pyridine] (Figure 5.1) is an alkaloid occurring in a number of plants and was first used as an insecticide in 1763. Nicotine is quite toxic orally as well as dermally. The acute oral LD50 of nicotine sulfate for rats is 83 mg/kg and

the dermal LD50 is 285 mg/kg. Symptoms of acute nicotine poisoning occur rapidly, and death may occur with a few minutes. In serious poisoning cases death results from respiratory failure due to paralysis of respiratory muscles. In therapy attention is focused primarily on support of respiration.

Pyrethrin is an extract from several types of chrysanthemum, and is one of the oldest insecticides used by humans. There are six esters and acids associated with this botanical insecticide. Pyrethrin is applied at low doses and is considered to be nonpersistent.

Mammalian toxicity to pyrethrins is quite low, apparently due to its rapid breakdown by liver microsomal enzymes and esterases. The acute LD50 to rats is about 1500 mg/kg. The most frequent reaction to pyrethrins is contact dermatitis and allergic respiratory reactions, probably as a result of other constituents in the formulation. Synthetic mimics of pyrethrins, known as the pyrethroids, were developed to overcome the lack of persistence.

5.3.7 Pyrethroid Insecticides

As stated, pyrethrins are not persistent, which led pesticide chemists to develop compounds of similar structure having insecticidal activity but being more persistent. This class of insecticides, known as pyrethroids, have greater insecticidal activity and are more photostable than pyrethrins. There are two broad classes of pyrethroids depending on whether the structure contains a cyclopropane ring [e.g., cypermethrin {(±)-α-cyano-3-phenoxybenzyl (±)-*cis*,*trans*-3-(2,2-dichlorovinyl_2,2-dimethyl cyclopropanecarboxylate)}] or whether this ring is absent in the molecule [e.g., fenvalerate{(*RS*)-α-cyano-3-phenoxybenzyl(*RS*)-2-(4-chlorophenyl)-3-methylbutyrate}]. They are generally applied at low doses (e.g., 30 g/Ha) and have low mammalian toxicities [e.g., cypermethrin, oral (aqueous suspension) LD50 of 4,123 mg/kg (rat) and dermal LD50 of >2000 mg/kg (rabbit)]. Pyrethroids are used in both agricultural and urban settings (e.g., termiticide; Figure 5.1).

Pyrethrins affect nerve membranes by modifying the sodium and potassium channels, resulting in depolarization of the membranes. Formulations of these insecticides frequently contain the insecticide synergist piperonyl butoxide [5-{2-(2-butoxyethoxy) ethoxymethyl}-6-propyl-1,3-benzodioxole], which acts to increase the efficacy of the insecticide by inhibiting the cytochrome P450 enzymes responsible for the breakdown of the insecticide.

5.3.8 New Insecticide Classes

There are new classes of insecticides that are applied at low dosages and are extremely effective but are relatively nontoxic to humans. One such class is the fiproles, and one of these receiving major attention is fipronil [(5-amino-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-4-((1,*R*,*S*)-(trifluoromethyl)su-1-*H*-pyrasole-3-carbonitrile)]. Although it is used on corn, it is becoming a popular termiticide because of its low application rate (ca. 0.01%) and long-term effectiveness. Another class of insecticides, the chloronicotinoids, is represented by imidacloprid [1-(6-chloro-3-pyridin-3-ylmethyl)-*N*-nitroimidazolidin-2-ylidenamine] (Figure 5.1), which also is applied at low dose rates to soil and effectively controls a number of insect species, including termites.

5.3.9 Herbicides

Herbicides control weeds and are the most widely used class of pesticides. The latest US EPA data show that some 578 million pounds of herbicides were used in the United States in 1997 and accounts for some 47% of pesticides used. This class of pesticide can be applied to crops using many strategies to eliminate or reduce weed populations. These include preplant incorporation, pre- and postemergent applications. New families of herbicides continue to be developed, and are applied at low doses, are relatively nonphytotoxic to beneficial plants and are environmentally friendly. Some of the newer families such as the imidazolinones inhibit the action of acetohydroxyacid synthase that produces branched-chain amino acids in plants. Because this enzyme is produced only in plants, these herbicides have low toxicities to mammals, fish, insects, and birds.

The potential for environmental contamination continues to come from families of herbicides that have been used for years. The chlorophenoxy herbicides such as 2,4-*D* (2,4-dichlorophenoxy acetic acid) and 2,4,5-*T* (2,4,5-trichlorophenoxy-acetic acid) (Figure 5.1) are systemic acting compounds to control broadleaf plants and have been in use since the 1940s. The oral toxicities of these compounds are low.

A mixture of 2,4-*D* and 2,4,5-*T*, known as Agent Orange, was used by the US military as a defoliant during the Vietnam conflict, and much controversy has arisen over claims by military personnel of long-term health effects. The chemical of major toxicological concern was identified as a contaminant, TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), that was formed during the manufacturing process. TCDD is one of the most toxic synthetic substances known in laboratory animals. The LD50 for male rats is 0.022 mg/kg, and for female guinea pigs (the most sensitive species tested) the LD50 is 0.0006 mg/kg. In addition it is toxic to developing embryos in pregnant rats at a dose of only 1/400 of the LD50, and has been shown to cause birth defects at levels of 1 to 3 ng/kg of body weight. TCDD is a proven carcinogen in both mice and rats, with the liver being the primary target. This chemical has also been shown to alter the immune system and enhance susceptibility in exposed animals.

Another family of herbicides, the triazines, continues to cause concern to environmentalists and toxicologists because of the contamination of surface and groundwater supplies that become public drinking water. The herbicide, atrazine [6-chloro-*N*-ethyl-*N*-(1-methylethyl)-1,2,5-triazine-2,4-diamine (Figure 5.1) is used primarily on corn and has an MCL of 3.0 μg/L. This herbicide has been found in surface and groundwaters worldwide with widely varying concentrations (e.g., 1 to >130 μg/L). It, along with two other triazines, cyanazine [2-{{4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl}amino}-2-methylpropanenitrile] and simazine (6-chloro-*N*,*N*-diethyl-1,3,5-triazine-2,4-diamine) (MCL of 4.0 μg/L). The uses of cyanazine were canceled in 2001 and no further use was permitted after 2002. Although relatively nontoxic [e.g., atrazine, oral LD50 of 3,100 mg/kg (rat)], the major concern with these types of compounds is their carcinogenic effects, and US EPA considers these three triazines as possible human carcinogens (category C).

A member of the bipyridylium family of herbicides is the compound paraquat (1,1-dimethyl-4,4-bipyridinium ion as the chloride salt) (Figure 5.1). It is a very water-soluble contact herbicide that is active against a broad range of plants and is used as a defoliant on many crops. The compound binds tightly to soil particles following

application and becomes inactivated. However, this compound is classified as a class I toxicant with an oral LD50 of 150 mg/kg (rat). Most poisoning cases, which are often fatal, are due to accidental or deliberate ingestion of paraquat. Toxicity results from lung injury resulting from both the preferential uptake of paraquat by the lungs and the redox cycling mechanism.

5.3.10 Fungicides

Every year fungi cause crop losses in the United States amounting to millions of dollars. In addition recent studies have shown that toxins and other airborne organic compounds released from fungi inhabiting the interior of dwellings probably are responsible for a number of adverse health effects. Compounds produced to combat these losses and adverse health effects are called fungicides, and a number of these families have been around for years.

The fungicide, chlorothalonil (tetrachloroisophthalonitrile), is a broad-spectrum fungicide which is used widely in urban environments. It is relatively cheap and controls some 140 species of organisms. As a result of the popularity of this compound, it is found routinely in surface waters entering public drinking water supplies. In the formulation that can be purchased by the general public, it is relatively nontoxic.

One family of fungicides that is of concern are the dithiocarbamates, sulfur derivatives of dithiocarbamic acid and include the metallic dimethyldithiocarbamates. The latter group includes mancozeb (a coordination product of zinc ion and manganese ethylene bisdithiocarbamate), maneb (manganese ethylenebisdithiocarbamate)(Figure 5.1), and zineb (zinc ethylenebisdithiocarbamate). All are effective fungicides and are used on a variety of crops including grapes, sugar beets, and ornamental plants. Although relatively nontoxic, they do hydrolyze producing known carcinogens such as ethylthiourea (ETU).

5.3.11 Rodenticides

This class of compounds is used to control rodents that cause yearly losses of 20% to 30% in grain and other food storage facilities. These pests harbor diseases in the form of fleas that carry bacteria and other organisms. A number of the rodenticides have been used for years and include warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin] (Figure 5.1), an anticoagulant. This is a potent toxicant with an oral LD50 of 3.0 mg/kg (rat). As the rats navigate through narrow passages, they bruise themselves, developing small hemorrhages. Anticoagulants prevent the blood from clotting, and the animals bleed to death in about a week. Humans who are exposed to this class of compounds are given vitamin K, and if the poisoning is severe, blood transfusions as a treatment. Other rodenticides poison the animal and many times are applied along with an attractant such as peanut butter to overcome bait shyness. Fluoroacetamide is a fast acting poison with an oral LD50 (rat) of 15 mg/kg. This material is supplied as bait pellets or grains. ANTU ($\acute{\alpha}$ -naphthylthiourea), strychnine, and thallium salts are other fast acting poisons, and have been on the market for many years. Most of the rodenticides are classified as restricted use and are applied only by licensed pest

control operators. Human poisonings associated with rodenticides usually result from accidental or suicidal ingestion of the compounds.

5.3.12 Fumigants

Fumigants are extremely toxic gases used to protect stored products, especially grains, and to kill soil nematodes. These materials are applied to storage warehouses, freight cars, and houses infested with insects such as powder post beetles. They present a special hazard due to inhalation exposure and rapid diffusion into pulmonary blood; thus extreme care must be taken when handling and applying this class of pesticides. All fumigants are classified as restricted use compounds and require licensed applicators to handle them.

One of the most effective fumigants is methyl bromide. It essentially sterilizes soil when applied under a ground covering, because it kills insects, nematodes, and weed seed but also is used to fumigate warehouses. Overexposure to this compound causes respiratory distress, cardiac arrest, and central nervous effects. The inhalation LC50 is 0.06 mg/L (15 min) of air (rat) and 7900 ppm (1.5 h) (human). Methyl bromide has been classified as an ozone depleter under the Clean Air Act and is due to be phased out of use by 2005.

Chloropicrin (trichloronitromethane) is another soil/space fumigant that has been used for many years. It has an inhalation LC50 of 150 ppm (15 min). Thus it is highly toxic by inhalation, can injure the heart, and cause severe eye damage.

5.3.13 Conclusions

This section has covered only a few of the pesticides available today on the United States and world markets. An understanding of the basic chemical processes affected by pesticides has led to the discovery and production of new families of chemicals. Today's modern pesticide is generally safe to use if the directions on the label are followed. Advances in instrumentation and an understanding of how adverse health effects are produced have resulted in the production of many environmentally friendly but effective pesticides.

5.4 FOOD ADDITIVES AND CONTAMINANTS

Chemicals are added to food for a number of reasons: as preservatives with antibacterial, antifungal, or antioxidant properties; to change physical characteristics, particularly for processing; to change taste; to change color; and to change odor. In general, food additives have proved to be safe and without chronic toxicity. Many were introduced when toxicity testing was relatively unsophisticated, however, and some of these have been subsequently shown to be toxic. Table 5.4 gives examples of different types of organic food additives. Inorganics, the most important of which are nitrate and nitrite, are discussed later. Certainly hundreds, and possibly thousands, of food additives are in use worldwide, many with inadequate testing. The question of synergistic interactions between these compounds has not been explored adequately. Not all toxicants in food are synthetic; many examples of naturally occurring toxicants in the human diet are known, including carcinogens and mutagens.

Table 5.4 Examples of Organic Chemicals Used as Food Additives

Function	Class	Example
Preservatives	Antioxidants	Butylatedhydroxyanisole
		Ascorbic acid
	Fungistatic agents	Methyl *p*-benzoic acid
		Propionates
		Bactericides Sodium nitrite
Processing aids	Anticaking agents	Calcium silicate
		Sodium aluminosilicate
	Emulsifiers	Propylene glycol
		Monoglycerides
	Chelating agents	EDTA
		Sodium tartrate
	Stabilizers	Gum ghatti
		Sodium alginate
	Humectants	Propylene glycol
		Glycerol
Flavor and taste modification	Synthetic sweeteners	Saccharin
		Mannitol
		Aspartame
	Synthetic flavors	Piperonal
		Vanillin
Color modification	Synthetic dyes	Tartrazine (FD&C yellow5)
		Sunset Yellow
Nutritional supplements	Vitamins	Thiamin
		Vitamin D3
	Amino acids	Alanine
		Aspartic acid
	Inorganics	Manganese sulfate
		Zinc sulfate

5.5 TOXINS

5.5.1 History

A discussion of toxins first necessitates the understanding and distinction between the toxicological terms toxicant and toxin. A *toxicant* is any chemical, of natural or synthetic origin, capable of causing a deleterious effect on a living organism. A *toxin* is a toxicant that is produced by a living organism and is not used as a synonym for toxicant—all toxins are toxicants, but not all toxicants are toxins. Toxins, whether produced by animals, plants, insects, or microbes are generally metabolic products that have evolved as defense mechanisms for the purpose of repelling or killing predators or pathogens. The action of natural toxins has long been recognized and understood throughout human history. For example, ancient civilizations used natural toxins for both medicinal (therapeutic) and criminal purposes. Even today, we continue to discover and understand the toxicity of natural products, some for beneficial pharmaceutical or therapeutic purposes whose safety and efficacy are tested, and some for other less laudable purposes like biological or chemical warfare. Toxins may be

classified in various ways depending on interest and need, such as by target organ toxicity or mode of action, but are commonly classified according to source.

5.5.2 Microbial Toxins

The term "microbial toxin" is usually reserved by microbiologists for toxic substances produced by microorganisms that are of high molecular weight and have antigenic properties; toxic compounds produced by bacteria that do not fit these criteria are referred to simply as poisons. Many of the former are proteins or mucoproteins and may have a variety of enzymatic properties. They include some of the most toxic substances known, such as tetanus toxin, botulinus toxin, and diphtheria toxin. Bacterial toxins may be extremely toxic to mammals and may affect a variety of organ systems, including the nervous system and the cardiovascular system. A detailed account of their chemical nature and mode of action is beyond the scope of this volume.

The range of poisonous chemicals produced by bacteria is also large. Again, such compounds may also be used for beneficial purposes, for example, the insecticidal properties of *Bacillus thuringiensis*, due to a toxin, have been utilized in agriculture for some time.

5.5.3 Mycotoxins

The range of chemical structures and biologic activity among the broad class of fungal metabolites is large and cannot be summarized briefly. Mycotoxins do not constitute a separate chemical category, and they lack common molecular features.

Mycotoxins of most interest are those found in human food or in the feed of domestic animals. They include the ergot alkaloids produced by *Claviceps* sp., aflatoxins and related compounds produced by *Aspergillus* sp., and the tricothecenes produced by several genera of fungi imperfecti, primarily *Fusarium* sp.

The ergot alkaloids are known to affect the nervous system and to be vasoconstrictors. Historically they have been implicated in epidemics of both gangrenous and convulsive ergotism (St. Anthony's fire), although such epidemics no longer occur in humans due to increased knowledge of the cause and to more varied modern diets. Outbreaks of ergotism in livestock do still occur frequently, however. These compounds have also been used as abortifacients. The ergot alkaloids are derivatives of ergotine, the most active being, more specifically, amides of lysergic acid.

Aflatoxins are products of species of the genus *Aspergillus*, particularly *A flavus*, a common fungus found as a contaminant of grain, maize, peanuts, and so on. First implicated in poultry diseases such as Turkey-X disease, they were subsequently shown to cause cancer in experimental animals and, from epidemiological studies, in humans. Aflatoxin B1, the most toxic of the aflatoxins, must be activated enzymatically to exert its carcinogenic effect.

Tricothecenes are a large class of sesquiterpenoid fungal metabolites produced particularly by members of the genera *Fusarium* and *Tricoderma*. They are frequently acutely toxic, displaying bactericidal, fungicidal, and insecticidal activity, as well as causing various clinical symptoms in mammals, including diarrhea, anorexia, and ataxia. They have been implicated in natural intoxications in both humans and animals, such as Abakabi disease in Japan and Stachybotryotoxicosis in the former USSR, and

are the center of a continuing controversy concerning their possible use as chemical warfare agents.

Mycotoxins may also be used for beneficial purposes. The mycotoxin avermectin is currently generating considerable interest both as an insecticide and for the control of nematode parasites of domestic animals.

5.5.4 Algal Toxins

Algal toxins are broadly defined to represent the array chemicals derived from many species of cyanobacteria (blue-green bacteria), dinoflagellates, and diatoms. The toxins produced by these freshwater and marine organisms often accumulate in fish and shellfish inhabiting the surrounding waters, causing both human and animal poisonings, as well as overt fish kills. Unlike many of the microbial toxins, algal toxins are generally heat stable and, therefore, not altered by cooking methods, which increases the likelihood of human exposures and toxicity. Many of the more common algal toxins responsible for human poisonings worldwide are summarized herein.

Amnesic Shellfish Poisoning (ASP) was first identified in 1987 from Prince Edward Island, Canada after four people died from eating contaminated mussels. It is caused by domoic acid produced by several species of *Pseudonitzschia* diatoms. The main contamination problems include mussels, clams, and crabs of the Pacific Northwest of the United States and Canada.

Paralytic Shellfish Poisoning (PSP) was first determined to be a problem in 1942 after three people and many seabirds died from eating shellfish on the west coast of the United States, near the Columbia River. It is caused by the saxitoxin family (saxitoxin + 18 related compounds) produced by several species of *Alexandrium* dinoflagellates. The main contamination problems include mussels, clams, crabs, and fish of the Pacific Northwest and Northeast Atlantic.

Neurotoxic Shellfish Poisoning (NSP) is caused by a red-tide producer that was first identified in 1880 from Florida, with earlier historical references. It causes sickness in humans lasting several days. NSP is not fatal to humans; however, it is known to kill fish, invertebrates, seabirds, and marine mammals (e.g., manatees). It is caused by the brevetoxin family (brevetoxin + 10 related compounds produced by the dinoflagellate *Karenia brevis* a.k.a. *Gymnodinium breve*. The main contamination problems include oysters, clams, and other filter feeders of the Gulf of Mexico and southeast Atlantic, including North Carolina.

Diarrheic Shellfish Poisoning (DSP). Human poisonings were first identified in the 1960s. It causes sickness in humans lasting several days but is not fatal. It is caused by chemicals of the okadaic acid family (okadaic acid + 4 related compounds) produced by several species of *Dinophysis* dinoflagellates. The main contamination problems include mussels, clams, and other bivalves of the cold and warm temperate areas of the Atlantic and Pacific Oceans, mainly in Japan and Europe. Only two cases of DSP have been documented in North America.

Ciguatera Fish Poisoning (CFP) was first identified in 1511, CFP is a tropical-subtropical seafood poisoning that affects up to 50,000 people each year and is the most often reported foodborne disease of a chemical origin in the United States. Caused by consumption of reef fishes (e.g., grouper, snapper), sickness in

humans lasts several days to weeks, but the human fatality rate is low. It is caused by the ciguatoxin family (ciguatoxin + 3 or more related compounds) and produced by several species of dinoflagellates including *Gambierdiscus*, *Prorocentrum*, *and Ostreopsis*. The main contamination problems include herbivorous tropical reef fish worldwide.

Cyanobacterial (Blue-Green Bacteria) Toxins. Cyanobacterial poisonings were first recognized in the late 1800s. Human poisonings are rare; however, kills of livestock, other mammals, birds, fish, and aquatic invertebrates are common. It is caused by a variety of biotoxins and cytotoxins, including anatoxin, microcystin, and nodularin produced by several species of cyanobacteria, including *Anabaena*, *Aphanizomenon*, *Nodularia*, *Oscillatoria*, *and Microcystis*. The main contamination problems include all eutrophic freshwater rivers, lakes, and streams.

Ambush Predator (Pfiesteria piscicida and Toxic Pfiesteria Complex) Toxins. Members belonging to this group of organisms were first identified in 1991 from estuaries in North Carolina. They were believed to produce a toxin that has been implicated in several large fish kills and is suspect in causing adverse human health effects. However, the toxin or toxins are not yet identified and toxicity tests are not universally conclusive. Produced by several dinoflagellate species including, *Pfiesteria piscicida*, *Pfiesteria shumwayae*, and perhaps several other unidentified, un-named dinoflagellates belonging to the potentially toxic *Pfiesteria* complex. Main problems include major fish kills in North Carolina and Maryland and potential human health problems. The range may extend from the Gulf of Mexico to the Atlantic estuarine waters, including Florida, North Carolina, Maryland, and Delaware, and possibly outward to Europe.

5.5.5 Plant Toxins

The large array of toxic chemicals produced by plants (phytotoxins), usually referred to as secondary plant compounds, are often held to have evolved as defense mechanisms against herbivorous animals, particularly insects and mammals. These compounds may be repellent but not particularly toxic, or they may be acutely toxic to a wide range of organisms. They include sulfur compounds, lipids, phenols, alkaloids, glycosides, and many other types of chemicals. Many of the common drugs of abuse such as cocaine, caffeine, nicotine, morphine, and the cannabinoids are plant toxins. Many chemicals that have been shown to be toxic are constituents of plants that form part of the human diet. For example, the carcinogen safrole and related compounds are found in black pepper. Solanine and chaconine, which are cholinesterase inhibitors and possible teratogens, are found in potatoes, and quinines and phenols are widespread in food. Livestock poisoning by plants is still an important veterinary problem in some areas.

5.5.6 Animal Toxins

Some species from practically all phyla of animals produce toxins for either offensive or defensive purposes. Some are passively venomous, often following inadvertent ingestion, whereas others are actively venomous, injecting poisons through specially

adapted stings or mouthparts. It may be more appropriate to refer to the latter group only as venomous and to refer to the former simply as poisonous. The chemistry of animal toxins extends from enzymes and neurotoxic and cardiotoxic peptides and proteins to many small molecules such as biogenic amines, alkaloids, glycosides, terpenes, and others. In many cases the venoms are complex mixtures that include both proteins and small molecules and depend on the interaction of the components for the full expression of their toxic effect. For example, bee venom contains a biogenic amine, histamine, three peptides, and two enzymes (Table 5.5). The venoms and defensive secretions of insects may also contain many relatively simple toxicants or irritants such as formic acid, benzoquinone, and other quinines, or terpenes such as citronellal. Bites and stings from the Hymenoptera (ants, bees, wasps, and hornets) result in 5 to 60 fatal anaphylactic reactions each year in the United States. According to experts, about 0.3% to 3.0% of the US population experiences anaphylactic reactions from insect stings and bites.

Snake venoms have been studied extensively; their effects are due, in general, to toxins that are peptides with 60 to 70 amino acids. These toxins are cardiotoxic or neurotoxic, and their effects are usually accentuated by the phospholipases, peptidases, proteases, and other enzymes present in venoms. These enzymes may affect the blood-clotting mechanisms and damage blood vessels. Snake bites are responsible for less than 10 deaths per year in the United States but many thousand worldwide.

Many fish species, over 700 species worldwide, are either directly toxic or upon ingestion are poisonous to humans. A classic example is the toxin produced by the puffer fishes (*Sphaeroides* spp.) called tetrodotoxin (TTX). Tetrodotoxin is concentrated in the gonads, liver, intestine, and skin, and poisonings occurs most frequently in Japan and other Asian countries where the flesh, considered a delicacy, is eaten as "fugu." Death occurs within 5 to 30 minutes and the fatality rate is about 60%. TTX is an inhibitor of the voltage-sensitive Na channel (like saxitoxin); it may also be found in some salamanders and may be bacterial in origin.

Toxins and other natural products generally provide great benefit to society. For example, some of the most widely used drugs and therapeutics like streptomycin, the aminoglycoside antibiotic from soil bacteria, and acetylsalicylic acid (aspirin), the nonsteroidal anti-inflammatory from willow tree bark, are used by millions of people everyday to improve health and well-being. On the other hand, adverse encounters with toxins like fish and shellfish toxins, plant, and insect toxins do result in harm to humans.

Table 5.5 Some Components of Bee Venom

Compound	Effect
Biogenic amine	
Histamine	Pain, vasodilation, increased capillary permeability
Peptides	
Apamine	CNS effects
Melittin	Hemolytic, serotonin release, cardiotoxic
Mast cell degranulating peptide	Histamine release from mast cells
Enzymes	
Phospholipase A	Increased spreading and penetration of tissues
Hyaluronidase	

5.6 SOLVENTS

Although solvents are more a feature of the workplace they are also found in the home. In addition to cutaneous effects, such as defatting and local irritation, many have systemic toxic effects, including effects on the nervous system or, as with benzene, on the blood-forming elements. Commercial solvents are frequently complex mixtures and may include nitrogen- or sulfur-containing organics—gasoline and other oil-based products are examples of this. The common solvents fall into the following classes:

Aliphatic Hydrocarbons, such as hexane. These may be straight or branched-chain compounds and are often present in mixtures.

Halogenated Aliphatic Hydrocarbons. The best-known examples are methylene dichloride, chloroform, and carbon tetrachloride, although chlorinated ethylenes are also widely used.

Aliphatic Alcohols. Common examples are methanol and ethanol.

Glycols and Glycol Ethers. Ethylene and propylene glycols, for example, in antifreeze give rise to considerable exposure of the general public. Glycol ethers, such as methyl cellosolve, are also widely used.

Aromatic Hydrocarbons. Benzene is probably the one of greatest concern, but others, such as toluene, are also used.

5.7 THERAPEUTIC DRUGS

Although the study of the therapeutic properties of chemicals fall within the province of pharmacology, essentially all therapeutic drugs can be toxic, producing deleterious effects at some dose. The danger to the individual depends on several factors, including the nature of the toxic response, the dose necessary to produce the toxic response, and the relationship between the therapeutic dose and the toxic dose. Drug toxicity is affected by all of factors that affect the toxicity of other xenobiotics, including individual (genetic) variation, diet, age, and the presence of other exogenous chemicals.

Even when the risk of toxic side effects from a particular drug has been evaluated, it must be weighed against the expected benefits. The use of a very dangerous drug with only a narrow tolerance between the therapeutic and toxic doses may still be justified if it is the sole treatment for an otherwise fatal disease. However, a relatively safe drug may be inappropriate if safer compounds are available or if the condition being treated is trivial.

The three principal classes of cytotoxic agents used in the treatment of cancer all contain carcinogens, for example, Melphalen, a nitrogen mustard, adriamycin, an antitumor antibiotic, and methotrexate, an antimetabolite. Diethylstilbestrol (DES), a drug formerly widely used, has been associated with cancer of the cervix and vagina in the offspring of treated women.

Other toxic effects of drugs can be associated with almost every organ system. The stiffness of the joints accompanied by damage to the optic nerve (SMON—subacute myelo-optic neuropathy) that was common in Japan in the 1960s was apparently a toxic side effect of chloroquinol (Enterovioform), an antidiarrhea drug. Teratogenosis

can also be caused by drugs, with thalidomide being the most alarming example. Skin effects (dermatitis) are common side effects of drugs, an example being topically applied corticosteroids.

A number of toxic effects on the blood have been documented, including agranulocytosis caused by chlorpromazine, hemolytic anemia caused by methyldopa, and megaloblastic anemia caused by methotrexate. Toxic effects on the eye have been noted and range from retinotoxicity caused by thioridazine to glaucoma caused by systemic corticosteroids.

5.8 DRUGS OF ABUSE

All drugs are toxic at some dose. Drugs of abuse, however, either have no medicinal function or are taken at dose levels higher than would be required for therapy. Although some drugs of abuse may affect only higher nervous functions—mood, reaction time, and coordination—many produce physical dependence and have serious physical effects, with fatal overdoses being a frequent occurrence.

The drugs of abuse include central nervous system depressants such as ethanol, methaqualone (Quaalude), and secobarbital; central nervous system stimulants, such as cocaine, methamphetamine (speed), caffeine, and nicotine; opioids, such as heroin and mependine (demerol); and hallucinogens such as lysergic acid diethylamide (LSD), phencyclidine (PCP), and tetrahydrocannabinol, the most active principal of marijuana. A further complication of toxicological significance is that many drugs of abuse are synthesized in illegal and poorly equipped laboratories with little or no quality control. The resultant products are therefore often contaminated with compounds of unknown, but conceivably dangerous, toxicity. The structures of some of these chemicals are shown in Figure 5.2.

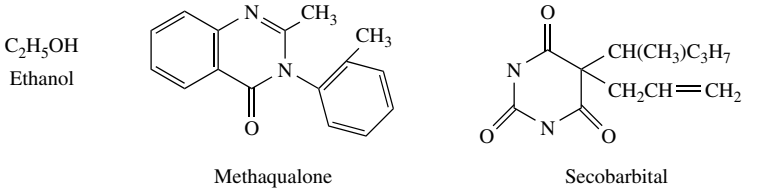
5.9 COMBUSTION PRODUCTS

While many air pollutants (see Chapter 4) are the products of natural or anthropomorphic combustion, some of the most important from the point of view of human health are polycyclic aromatic hydrocarbons. Although also found in natural products such as coal and crude oil, they are generally associated with incomplete combustion of organic materials and are found in smoke from wood, coal, oil, and tobacco, for example, as well as in broiled foods. Because some of them are carcinogens, they have been studied intensively from the point of view of metabolic activation, interactions with DNA, and other aspects of chemical carcinogenesis. Some are heterocyclic, containing nitrogen in at least one of the rings. Some representative structures of the most studied polycyclic aromatic hydrocarbons are shown in Figure 5.3.

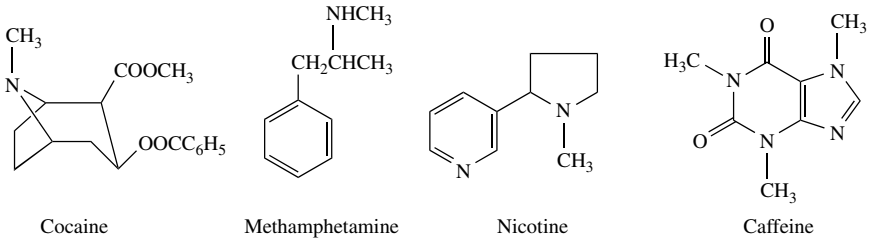
5.10 COSMETICS

The most common deleterious effects of modern cosmetics are occasional allergic reactions and contact dermatitis. The highly toxic and/or carcinogenic azo or aromatic

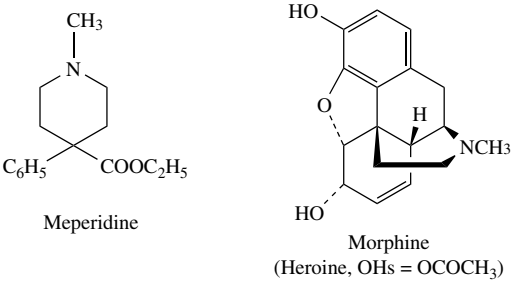
1. CNS Depressants



2. CNS Stimulants



3. Opioids



4. Hallucinogens

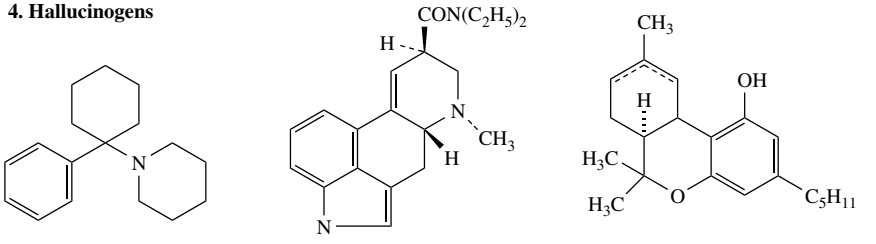
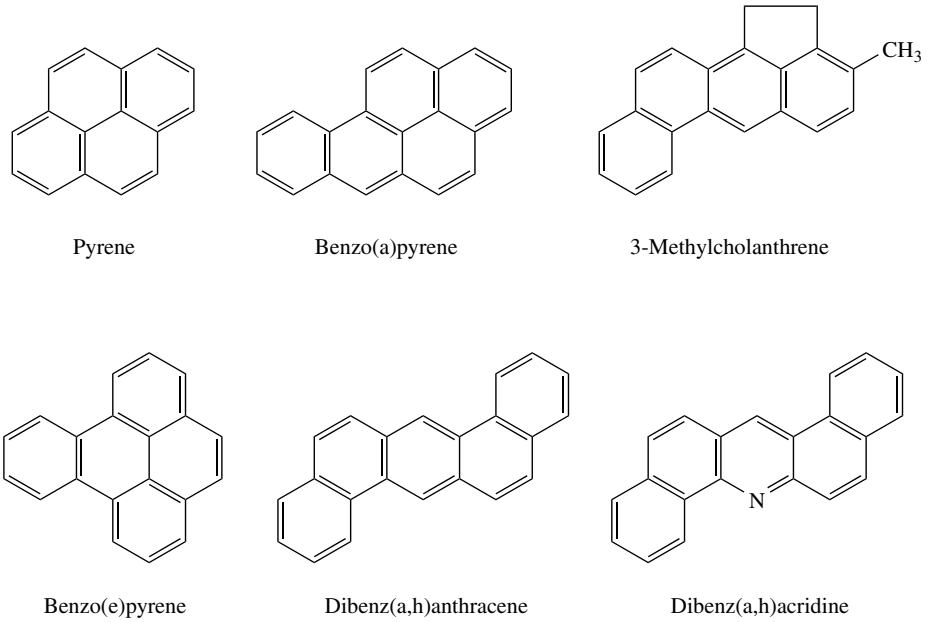


Figure 5.2 Some common drugs of abuse.

amine dyes are no longer in use, nor are the organometallics, used in even earlier times. Bromates, used in some cold-wave neutralizers, may be acutely toxic if ingested, as may the ethanol used as a solvent in hair dyes and perfumes. Thioglycolates and thioglycerol used in cold-wave lotion and depilatories and sodium hydroxide used in hair straighteners are also toxic on ingestion. Used as directed, cosmetics appear to



Pyrene | Benzo(a)pyrene | 3-Methylcholanthrene

Benzo(e)pyrene | Dibenz(a,h)anthracene | Dibenz(a,h)acridine

Figure 5.3 Some common polycyclic aromatic hydrocarbons.

present little risk of systemic poisoning, due in part to the deletion of ingredients now known to be toxic and in part to the small quantities absorbed.

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PART III

TOXICANT PROCESSING IN VIVO

CHAPTER 6

Absorption and Distribution of Toxicants

RONALD E. BAYNES and ERNEST HODGSON

6.1 INTRODUCTION

As illustrated in the previous chapter, the human body can be exposed to a variety of toxicants that may be present in various environmental media such as air, soil, water, or food. However, just simply being exposed to these hazardous chemicals does not necessarily translate into a toxicological response. The mammalian body has several inherent defense mechanisms and membrane barriers that tend to prevent the entry or absorption and distribution of these toxicants once an exposure event has occurred. However, if the toxicant is readily absorbed into the body, there are still other anatomical and physiological barriers that may prevent distribution to the target tissue to elicit a toxic response. As the toxicological response is often related to the exposed dose, interactions between the toxicant and the body's barriers and defense mechanisms will have an effect on toxicant movement in the body, and ultimately modulate the rate and extent of toxicant absorption and distribution to the target tissue.

The skin represents the largest organ in the human body, and one of its primary functions can be seen as a physical barrier to absorption of toxicants. The other major routes of toxicant entry into the body are through the respiratory and gastrointestinal tract, which can be seen to offer less resistance to toxicant absorption than the skin. In general, the respiratory tract offers the most rapid route of entry, and the dermal the least rapid. One reason for this major difference is primarily because membrane thickness, which is really the physical distance between the external environment (skin surface, air in the lung, or lumen of the gut) and the blood capillaries, varies across these portals of entry. The overall entry depends on both the amount present and the saturability of the transport processes involved.

Liver metabolism will have the most significant effect on toxicant bioavailability following gastrointestinal absorption, but microbial activity and various enzymes in the gastrointestinal tract and the skin can play a significant role in oral and dermal absorption, respectively. Physicochemical characteristics of the toxicant such as the chemical form can be a useful indicator of whether the toxicant will be absorbed and distributed in the body. In this regard toxicant molecular weight, ionization (pKa), and octanol/water partition coefficient (log P) are useful indexes of predicting chemical

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transport from an environmental media across biological membranes to the blood stream. The reader should also be aware that for those toxicants that are readily ionized, the pH gradient across membranes can determine the extent of toxicant transport and accumulation in tissues.

Once the toxicant has been absorbed, the toxicant molecules can move around the body in two ways: (1) by bulk flow transfer (i.e., in the blood stream) and (2) by diffusional transfer (i.e., molecule-by-molecule over short distances). Disposition is the term often used to describe the simultaneous effects of distribution and elimination processes subsequent to absorption. The cardiovascular system provides distribution of all toxicants, regardless of their chemical nature, to various organs and tissues with various levels of affinities for toxicants. It should be remembered that organ mass and blood perfusion can vary, which can account for differential distribution of toxicants. Toxicant disposition can also be influenced by plasma protein binding in the blood stream. The nature of this toxicant-protein interaction is dependent on the chemical nature of the toxicant, the presence of other toxicants or drugs in the blood stream, as well as plasma protein levels. However, what distinguishes one toxicant pharmacokinetically from another is its diffusional characteristics. That is, its ability to cross nonaqueous diffusional barriers (e.g., cell membranes) from an aqueous compartment. This usually involves movement across several compartments separated by lipid membranes. It is therefore important to understand the *mechanisms by which drugs cross membranes* and the *physicochemical properties* of molecules and membranes that influence the movement of drugs from the environment to the body via either oral, inhalation, or dermal routes. These factors also influence movement from one compartment to another within the body during distribution as well as metabolism, and excretion.

We can quantitate this movement or transport from one compartment to another using mathematical models to describe transport rates. This in fact is what we do in pharmacokinetic analysis and modeling. *Pharmaco- or toxicokinetics* is therefore the quantitation of the time course of toxicants in the body during the various processes of absorption, distribution, and elimination or clearance (metabolism and/or excretion) of the toxicant. Stated differently, this is a study of how the body "handles" the toxicant as it is reflected in the plasma concentration at various time points. The two most important pharmacokinetic parameters that describe the disposition of a chemical are volume of distribution and systemic (body) clearance. *Pharmaco- and toxicodynamics* is the study of the biochemical and physiological effects of drugs and toxicants and determines their mechanism of action. Physiologically based pharmaco- or toxicokinetic models are used to integrate this information and to predict disposition of toxicants for a given exposure scenario. These concepts will be introduced at the end of this chapter.

6.2 CELL MEMBRANES

During absorption, distribution, and elimination processes the toxicant will encounter various cell membranes before interacting with the target tissue. Each step of these process involves translocation of the chemical across various membrane barriers, from the skin or mucosa through the capillary membranes, and through the cellular and organelle membranes (Figure 6.1). These membrane barriers vary from the relatively thick areas of the skin to the relatively thin lung membranes. In all cases, however, the membranes of tissue, cell, and cell organelle are relatively similar.

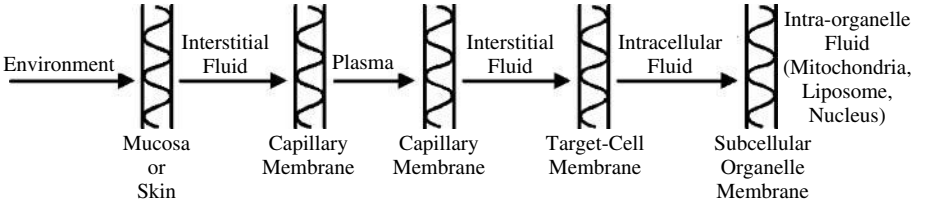


Figure 6.1 Schematic showing membranes that a chemical may need to cross during passage from the environment to the site of action. (Adapted from E. Hodgson and P. E. Levi, eds. *Introduction to Biochemical Toxicology*, 2nd ed., Appleton and Lange, 1994, p. 12.)

The cell membranes are predominantly a lipid matrix or can be considered a lipid barrier with an average width of a membrane being approximately 75 Å. The membrane is described as the fluid mosaic model (Figure 6.2) which consist of (1) a bilayer of phospholipids with hydrocarbons oriented inward (hydrophobic phase), (2) hydrophilic heads oriented outward (hydrophilic phase), and (3) associated intra- and extracellular proteins and transverse the membrane. The ratio of lipid to protein varies from 5:1 for the myelin membrane to 1:5 for the inner structure of the mitochondria. However, 100% of the myelin membrane surface is lipid bilayer, whereas the inner membrane of the mitochondria may have only 40% lipid bilayer surface. In this example the proportion of membrane surface that is lipid will clearly influence distribution of toxicants of varying lipophilicity.

The lipid constituents in the membrane permit considerable movement of macromolecules, and membrane constituents may move appreciably within membranes. Membrane fluidity, a function of lipid composition, can be altered by temperature and chemicals

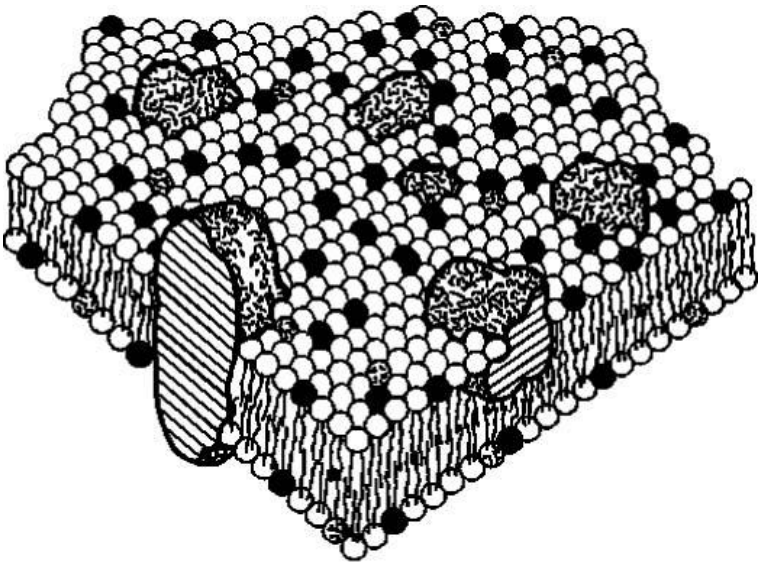


Figure 6.2 Schematic diagram of biological membrane. Head groups of lipids represented by spheres, tail ends by zigzag lines. Black, white, or stippled spheres indicate different kinds of lipids and illustrate asymmetry in certain cases. Large bodies are membrane-associated proteins. (Adapted from Singer and Nicolson, *Science* **175**:720, 1972.)

(e.g., anesthetics). Several types of lipids are found in membranes, with phospholipids and cholesterol predominating. Sphingolipids comprise the primary minor component. Phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine are the primary phosphatides, and their two fatty acid hydrocarbon chains (typically 16 to 18, but varying from 12 to 22) comprise the nonpolar region. Some of the fatty acids are unsaturated and contribute appreciably to the fluidity of the membrane.

Proteins, which have many physiological roles in normal cell function, are intimately associated with lipids and may be located throughout lipid bilayers. These proteins may be located on either the surface or traverse the entire structure. Hydrophobic forces are responsible for maintaining the structural integrity of proteins and lipids within membranes, but movement within the membranes may occur. External and internal membrane proteins can function as receptors. Many proteins that traverse the membrane are transport proteins, and are involved in translocation of ligands; that is, they are involved in active and facilitated transport.

Complexes of intrinsic membrane proteins and lipids can form hydrophilic or hydrophobic channels that allow transport of molecules with different physicochemical characteristics. The amphipathic nature of the membrane creates a barrier for ionized, highly polar drugs, although it does not completely exclude them. The presence of pores of approximately 4 Å are believed to allow for ready movement of small molecules such as water. Thus certain molecules that ordinarily would be excluded can rapidly traverse the highly lipid membrane barrier.

It is worth noting that differences among membranes, such as the presence of different lipids, the amount of surface lipid, differences in size and shape of proteins, or physical features of bonding, may cause differences in permeability among membranes. These biochemical and biophysical differences are thought to be responsible for permeability differences in skin from different anatomical regions of the body.

6.3 MECHANISMS OF TRANSPORT

In general, there are four main ways by which small molecules cross biological lipid membranes:

1. *Passive diffusion.* Diffusion occurs through the lipid membrane.
2. *Filtration.* Diffusion occurs through aqueous pores.
3. *Special transport.* Transport is aided by a carrier molecule, which act as a "ferry-boat."
4. *Endocytosis.* Transport takes the form of pinocytosis for liquids and phagocytosis for solids.

The first and third routes are important in relation to pharmacokinetic mechanisms. The aqueous pores are too small in diameter for diffusion of most drugs and toxicant, although important for movement of water and small polar molecules (e.g., urea). Pinocytosis is important for some macromolecules (e.g., insulin crossing the blood-brain barrier).

6.3.1 Passive Diffusion

Most drugs and toxicant pass through membranes by *simple diffusion* down a concentration gradient. The driving force being the concentration gradient across the membrane.

This diffusion process can continue until equilibrium, although in reality there is always movement but the net flux is zero. Eventually the concentration of unionized or unbound (free) toxicant is the same on either side of the membrane. In other words, there is no competition of molecules and there is generally a lack of saturation. Solubility in the lipid bilayer is important, and the greater the partition coefficient, the higher is the concentration in the membrane, and the greater is the rate of diffusion across the membrane. For ionized toxicants the steady state concentration is dependent on the differences in pH across the membrane. Most membranes are relatively permeable to water either by diffusion or by flow that results from hydrostatic or osmotic differences across the membrane, and bulk flow of water can also carry with it small and water soluble molecules by this mechanism. These substances generally have a molecular weight of less than 200. Although inorganic ions are small and will readily diffuse across some membranes, their hydrated ionic radius is relatively large. In such cases active transport is required (see below). Specific ion fluxes are also controlled by specific channels that are important in nerves, muscles, and signal transduction.

We can now quantitate the *rate* at which a toxicant can be transported by passive diffusion, and this can be described by Fick's law of diffusion as follows:

$$\text{Rate of diffusion} = \frac{D \times S_a \times P_c}{d}(C_H - C_L),$$

where D is the diffusion coefficient, S_a is the surface area of the membrane, P_c is the partition coefficient, d is the membrane thickness, and C_H and C_L are the concentrations at both sides of the membrane (high and low, respectively). The first part of this equation (DP_c/d) represents the permeability coefficient of the drug. The permeability expresses the ease of penetration of a chemical and has units of velocity, distance/time (cm/h).

The diffusion coefficient or diffusivity of the toxicant, D, is primarily dependent on solubility of the toxicant in the membrane and its molecular weight and molecular conformation. Depending on the membrane, there is a functional molecular size and/or weight cutoff that prevents very large molecules from being passively absorbed across any membrane. One would expect small molecular weight molecules to diffuse more rapidly than larger molecular weight toxicants. Therefore the magnitude of a toxicant's diffusion coefficient really reflects the ease with which it is able to diffuse through the membrane. The reader should also be aware that as a toxicant crosses from the donor or aqueous medium and through the membrane medium, there are really two diffusion environments and thus two diffusion coefficients to consider. Another important factor that can influence the diffusion coefficient is membrane viscosity. This physicochemical characteristic should remain constant in biological systems but can be modified in skin membranes exposed to various pharmaceutical or pesticide formulations. Formulation additives or excipients may enter the membrane barrier and reversibly or irreversibly change viscosity and thus diffusion coefficient of the drug or pesticide in the barrier membranes of the skin.

The partition coefficient, which will be described in more detail later in this chapter, is the relative solubility of the compound in lipid and water, and the compound's solubility really reflects the ability of the toxicant to move from a relatively aqueous environment across a lipid membrane. It is this factor that is often manipulated in pesticide and drug formulations to create a vehicle. Membrane permeability is therefore strongly correlated to the lipid solubility of the toxicant in the membrane as well as

the aqueous environment surrounding the membrane. Please be aware that there are instances where partition coefficient or lipid solubility of the toxicant may be very large, and there may be a tendency for the drug to sequester in the membrane. Membrane surface area and membrane thickness can also vary across different organs in the body, but one does not expect these two factors in Fick's equation to vary considerably. The final component of Fick's equation is the concentration gradient $(C_H - C_L)$ across the membrane, which is the **driving force for diffusion**, and as will be demonstrated below in our discussion on first-order kinetics, is the most important factor dictating the rate of transport across most biological membranes.

First-Order Kinetics. When the rate of a process is dependent on a **rate constant** and a concentration gradient, a linear or first-order kinetic process will be operative. The reader should be aware that there are numerous deviations from the first-order process when chemical transport in vivo is analyzed, and this can be deemed an *approximation* since, in many barriers, penetration is slow and a long period of time is required to achieve steady state.

The rate of movement of a toxicant across a membrane may be expressed as the *change in amount of toxicant, A, (dA) or toxicant concentration, C, (dC) per unit of time (dt), which equals* $d\mathbf{A}/d\mathbf{t}$. Calculus can be used to express instantaneous rates over very small time intervals (dt). Thus rate processes may then be generally expressed as

$$\frac{d\mathbf{A}}{d\mathbf{t}} = \mathbf{K}\mathbf{A}^n$$

where $d\mathbf{A}/d\mathbf{t}$ is the rate of chemical (X) movement (e.g. absorption, distribution, elimination), $\mathbf{K}$ is the rate constant of the process, and n is the kinetic order of the transport process (e.g., absorption). The n either equals 1 (first order) or 0 (zero order). Thus the first-order rate equation is written as

$$\frac{d\mathbf{A}}{d\mathbf{t}} = \mathbf{K}\mathbf{A}^1 = \mathbf{K}\mathbf{A},$$

and the zero-order rate equation as

$$\frac{d\mathbf{A}}{d\mathbf{t}} = \mathbf{K}\mathbf{A}^0 = \mathbf{K}.$$

We know from Fick's law that the rate of diffusion (now expressed as $d\mathbf{A}/d\mathbf{t}$) is

$$\frac{d\mathbf{A}}{d\mathbf{t}} = \frac{\mathbf{D}\cdot\mathbf{S_a}\cdot\mathbf{P_c}(\mathbf{A_1} - \mathbf{A_2})}{\mathbf{d}}.$$

Once a toxicant crosses a membrane, it is rapidly removed from the "receiving side" (compartment B in Figure 6.3) either by uptake into the blood stream or elimination from the organism. Thus it is A_1 that is the primary driving force, and if we replace this with A in all equations, then

$$\frac{d\mathbf{A}}{d\mathbf{t}} = \left(\frac{\mathbf{D}\cdot\mathbf{S_a}\cdot\mathbf{P_c}}{\mathbf{d}}\right)\mathbf{A}.$$

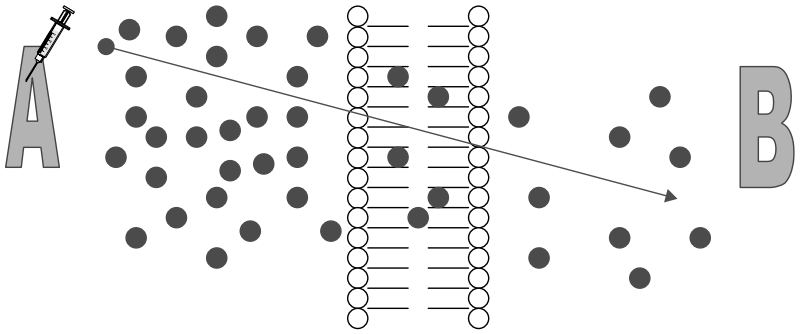


Figure 6.3 Illustration of concentration gradient generated by administration of a drug that can travel down this gradient from area A and across a biological membrane to area B.

If we let $\mathbf{K} = (\mathbf{D \cdot S_a \cdot P_c / d})$, then, since A is present in the equation, n must equal 1, so we have a first-order rate process. Fick's law of diffusion, which is important for quantitating rates of absorption, distribution, and elimination, is thus the basis for using first-order kinetics in most pharmacokinetic models.

Therefore in a first-order process, the rate of drug movement is directly proportional to the amount of drug (A) in the body, which is usually a function of the dose. **K** is the first-order fractional rate constant with units of liters/time (time^{-1}) and represents the *fraction* of drug that is transported *per unit of time*. Thus in a *first-order* process, the rate of drug movement is proportional to dose but the fraction moved per unit of time is constant and *independent of dose*.

When first-order kinetics hold, a simple relationship exists between the penetration rate constant, K, and $t_{0.5}$ (time necessary for one-half of the applied dose to penetrate):

$$K = \frac{0.693}{t_{0.5}},$$

where the units of K are a percentage of the change/time unit. We can also derive the concentration of the toxicant if we know the volume or volume of distribution (V_d) of the toxicant compartment as

$$V_d(\text{volume}) = \frac{A(\text{mass})}{C(\text{mass/volume})}.$$

(*V_d is discussed in more detail later in this chapter.*)

6.3.2 Carrier-Mediated Membrane Transport

This mechanism is important for compounds that lack sufficient lipid solubility to move rapidly across the membrane by simple diffusion. A membrane-associated protein is usually involved, specificity, competitive inhibition, and the saturation phenomenon and their kinetics are best described by *Michaelis-Menton enzyme kinetic models*. Membrane penetration by this mechanism is more rapid than simple diffusion and, in the case of active transport, may proceed beyond the point where concentrations are equal on both

sides of the membrane. Generally, there are two types of specialized carrier-mediated transport processes:

Passive facilitated diffusion involves movement down a concentration gradient without an input of energy. This mechanism, which may be highly selective for specific conformational structures, is necessary for transport of endogenous compounds whose rate of transport by simple diffusion would otherwise be too slow. The classical example of facilitated diffusion is transport of glucose into red blood cells.

Active transport requires energy, and transport is against a concentration. Maintenance against this gradient requires energy. It is often coupled to energy-producing enzymes (e.g., ATPase) or to the transport of other molecules (e.g., Na^+, Cl^-, H^+) that generate energy as they cross the membranes. Carrier-mediated drug transport can occur in only a few sites in the body, and the main sites are

- BBB, neuronal membranes, choroid plexus
- Renal tubular cells
- Hepatocytes, biliary tract

There are instances in which toxicants have chemical or structural similarities to endogenous chemicals that rely on these special transport mechanisms for normal physiological uptake and can thus utilize the same system for membrane transport. Useful examples of drugs known to be transported by this mechanism include levodopa, which is used in treating Parkinson's disease, and fluorouracil, a cytotoxic drug. Levodopa is taken up by the carrier that normally transports phenylalanine, and fluorouracil is transported by the system that carries the natural pyrimidines, thymine, and uracil. Iron is absorbed by a specific carrier in the mucosal cells of the jejunum, and calcium by a vitamin D-dependent carrier system. Lead may be more quickly moved by a transport system that is normally involved in the uptake of calcium.

For carrier-mediated transport, the rate of movement across a membrane will now be *constant*, since flux is dependent on the capacity of the membrane carriers and not the mass of the chemical to be transported. These processes are described by *zero-order* kinetic rate equations of the form:

$$\frac{d\mathbf{X}}{d\mathbf{t}} = \mathbf{K}\mathbf{X}^0 = \mathbf{K}_0.$$

$\mathbf{K}_0$ is now the *zero-order* rate constant and is expressed in terms of mass/time. In an active carrier-mediated transport process following zero-order kinetics, the rate of drug transport is always equal to $\mathbf{K}$ once the system is fully loaded or saturated. At subsaturation levels, the rate is *initially first order* as the carriers become loaded with the toxicant, but at concentrations normally encountered in pharmacokinetics, the rate becomes constant. Thus, as dose increases, the rate of transport does *not* increase in proportion to dose as it does with the fractional rate constant seen in first-order process. This is illustrated in the Table 6.1 where it is assumed that the first-order rate constant is 0.1 (10% per minute) and the zero-order rate is 10 mg/min.

In the case of first order, these amounts will subsequently diminish (10% of 900 is 90, etc.). In the case of zero order, the amount transported does not vary with time (constant rate of transport).

Table 6.1 Amount of Toxicant (mg) Transported in One Minute

Initial Toxicant Mass (mg)	First-Order Rate	Zero-Order Rate
1000	100	10
100	10	10
10	1	10

The plot in Figure 6.4 illustrates the differences in passive (linear) versus carrier-mediated (nonlinear) transport. At relatively low concentrations of drug, carrier-mediated processes may appear to be first order since the protein carriers are not saturated. However, at higher concentrations, zero-order behavior becomes evident. It is in plots such as this that the terms *linear* (first order) and *nonlinear* (zero order) come into existence.

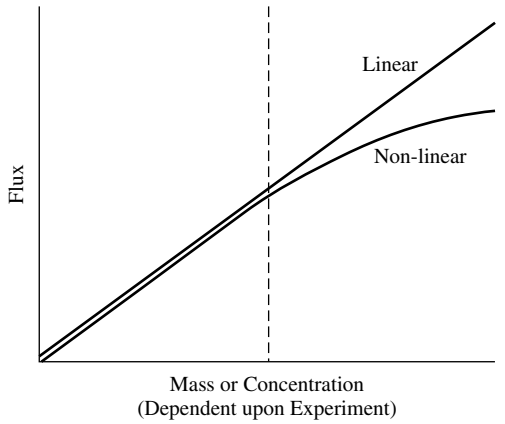


Figure 6.4 Plot depicting a linear relationship (first order) and nonlinear relationship (zero order) between chemical flux across a membrane and the initial mass or concentration of the chemical.

6.4 PHYSICOCHEMICAL PROPERTIES RELEVANT TO DIFFUSION

The following physicochemical properties are important for chemical diffusion. We have discussed several of these properties in previous sections of this chapter as they relate to the passive diffusion mechanism and its impacts on rate of toxicant transport across membranes.

Molecular size and shape
Solubility at site of absorption
Degree of ionization
Relative lipid solubility of ionized and unionized forms

Although molecular weight is important, it is less important than the drug's *lipid solubility* when it comes to assessing the rate of passive diffusion across membranes.

The permeability, $P (P = P_c \times D)$, of a nonpolar substance through a cell membrane is dependent on two physicochemical factors: (1) *solubility in the membrane* (P_c), which can be expressed as a partition coefficient of the drug between the aqueous phase and membrane phase, and (2) *diffusivity or diffusion coefficient (D)*, which is a measure of mobility of the drug molecules within the lipid. The latter may vary only slightly among toxicants, but the former is more important. Lipid solubility is therefore one of the most important determinants of the pharmacokinetic characteristics of a chemical, and it is important to determine whether a toxicants is readily ionized or not influenced by pH of the environment. If the toxicant is readily ionized, then one needs to understand its chemicals behavior in various environmental matrices in order to adequately assess its transport mechanism across membranes.

6.4.1 Ionization

For the purposes of this discussion on membrane transport, chemicals can be broadly categorized into those that are ionized and those that are not ionized. Many drugs (e.g., antibiotics) and several toxicants (e.g., strychnine) are either weak acids or weak bases and can exist in solution as a mixture of nonionized and ionized forms. Generally, these drugs and toxicants must be in the uncharged or nonionized form to be transported by passive diffusion across biological membranes. This is because biological membranes are of a lipid nature and are less permeable to the ionized form of the chemical. The pH of the environment (e.g., lumen of the gastrointestinal tract and renal tubules) can influence transfer of toxicant that are ionizable by increasing or decreasing the amount of nonionized form of the toxicant. Aminoglycosides (e.g., gentamicin) are the exception to this general rule in that the uncharged species is insufficiently lipid soluble to cross the membrane appreciably. This is due to a preponderance of hydrogen-bonding groups in the sugar moiety that render the uncharged molecule hydrophilic. Note that some amphoteric drugs (e.g., tetracyclines) may be absorbed from both acidic and alkaline environments. In essence, the amount of drug or toxicant in ionized or nonionized form depends on the pKa (pH at which 50% of the drug is ionized) of the drug and the pH of the solution in which the drug is dissolved. *The pKa, which is the negative logarithm of the dissociation constant of a weak acid or weak base, is a physicochemical characteristic of the drug or toxicant.* When the pH of the solution is equal to the pKa, then 50% of the toxicant is in the ionized form and 50% is in the nonionized form. The ionized and nonionized fractions can be calculated according to the *Henderson-Hasselbach* equations listed below:

$$\textit{For weak acids}: \quad \mathrm{pKa} - \mathrm{pH} = \log(\text{Nonionized form}/\text{Ionized form}),$$

$$\textit{For weak bases}: \quad \mathrm{pKa} - \mathrm{pH} = \log(\text{Ionized form}/\text{Nonionized form}).$$

For an organic acid ($\mathrm{RCOOH} \leftrightarrow \mathrm{RCOO^-} + \mathrm{H^+}$), acidic conditions (pH less than the pKa of the compound) will favor the formation of the nonionized RCOOH, whereas alkaline conditions (pH greater than pKa) will shift the equilibrium to the right. For an organic base ($\mathrm{RNH_2} + \mathrm{H^+} \Leftrightarrow \mathrm{RNH_3^+}$), the reverse is true, and decreasing the pH (increasing the concentration of H+) will favor formation of the ionized form, whereas increasing the pH (decreasing the concentration of $\mathrm{H^+}$) will favor formation of the nonionized form.

Table 6.2 Amount of Toxicant Absorbed at Various pH Values (%)

Compound	pKa	3.6–4.3	4.7–5.0	7.0–7.2	7.8–8.0
		Acids			
Nitrosalicyclic	2.3	40	27	<02	<02
Salicyclic	3.0	64	35	30	10
Benzoic	4.2	62	36	35	05
		Bases			
Aniline	4.6	40	48	58	61
Aminopyrene	5.0	21	35	48	52
Quinine	8.4	09	11	41	54

Memory aid: In general, weak organic acids readily diffuse across a biological membrane in an acidic environment, and organic bases can similarly diffuse in a basic environment. This is illustrated quite well in Table 6.2 for the chemical in rat intestine. There are the usual exceptions to the generalizations concerning ionization and membrane transport, and some compounds, such as pralidoxime (2-PAM), paraquat, and diquat, are absorbed to an appreciable extent even in the ionized forms. The mechanisms allowing these exceptions are not well understood.

Ion trapping can occur when at equilibrium the total (ionized + nonionized) concentration of the drug will be different in each compartment, with an acidic drug or toxicant being concentrated in the compartment with the relatively high pH, and vice versa. The pH partition mechanism explains some of the qualitative effects of pH changes in different body compartment on the pharmacokinetics of weakly basic or acidic drugs or toxicant as it relates to renal excretion and penetration of the blood-brain barrier. Alkalization of urine in the lumen of renal tubules can enhance elimination of weak acids. However, this phenomenon is not the main determinant of absorption of drugs or toxicants from the gastrointestinal tract. In the gastrointestinal tract the enormous absorptive surface area of the villi and microvilli in the ileum, compared to the smaller absorptive area of the stomach, is of overriding importance.

6.4.2 Partition Coefficients

A second physicochemical parameter influencing chemical penetration through membranes is the relative lipid solubility of the potential toxicant that can be ascertained from its known partition coefficient. The partition coefficient is a measure of the ability of a chemical to separate between two immiscible phases. The phases consist of an organic phase (e.g., octanol or heptane) and an aqueous phase (e.g., water). The lipid solvent used for measurement is usually octanol because it best mimics the carbon chain of phospholipids, but many other systems have been reported (chloroform/water, ether/water, olive oil/water). The lipid solubility and the water solubility characteristics of the chemical will allow it to proportionately partition between the organic and water phase. The partition coefficients can be calculated using the following equation:

$$P = \frac{V_w}{V_o}\left[\frac{(C_{wo} - C_w)}{C_w}\right],$$

where P is the partition coefficient and usually expressed in terms of its logarithmic value ($\log P$), V_w and V_o are the volumes of aqueous and oil or organic phase, respectively, and C_{wo} and C_w are drug or toxic concentrations in the aqueous phase before and after shaking, respectively.

The lower the partition coefficient, the more water soluble, and the least permeable the toxicant is across a membrane. Regarding dermal absorption, partition coefficients can be predictive of absorption. However, toxicants with extremely high partition coefficients tend to remain in the membrane or skin. This explains why a strong correlation between permeability and the partition coefficient can exist for a hypothetical series of analogous chemicals for a specific range of partition coefficients, but the correlation does not exists for $\log P$ values greater than 6 in many instances. A $\log P$ of around 1 is often taken as desirable for skin penetration. The reader should also recall that this parameter is operative as the chemical diffuses across membranes (Figure 6.1) of varying lipid content during absorption, distribution, and elimination processes.

6.5 ROUTES OF ABSORPTION

Primary routes of entry of toxicants to the human body are dermal, gastrointestinal, and respiratory. Methods for studying these different routes are numerous, but they are perhaps best developed for the study of dermal absorption because this route is subject to more direct methodology, whereas methods for studying respiratory or gastrointestinal absorption require more highly specialized instrumentation. Additional routes encountered in experimental studies include intraperitoneal, intramuscular, and subcutaneous routes. When direct entry into the circulatory system is desired, intravenous (IV) or intra-arterial injections can be used to bypass the absorption phase. Information from this more direct route of entry (e.g., IV) should, however, be used in addition to data from the extravascular route of interest to adequately assess the true extent of absorption of a toxicant.

6.5.1 Extent of Absorption

It is often useful to determine *how much of the drug* actually penetrates the membrane barrier (e.g., skin or gastrointestinal tract) and gets into the blood stream. This is usually determined experimentally for oral and dermal routes of administration. The *area under the curve (AUC)* of the concentration-time profiles for oral or dermal routes is compared with the AUC for IV routes of administration. The AUC is determined by breaking the curve up into a series of trapezoids and summing all of the areas with the aid of an appropriate computer program (Figure 6.5).

The intravenous correction is very important if absolute bioavailability is desired. The ratio of these AUC values is absolute bioavailability, F:

$$F = \frac{(\mathrm{AUC})_{\mathrm{route}}}{(\mathrm{AUC})_{\mathrm{IV}}}.$$

The relationship above holds if the same doses are used with both routes, but the bioavailability should be corrected if different doses are used:

$$F = \frac{\mathrm{AUC}_{\mathrm{route}} \times \mathrm{Dose}_{\mathrm{IV}}}{\mathrm{AUC}_{\mathrm{IV}} \times \mathrm{Dose}_{\mathrm{route}}}.$$

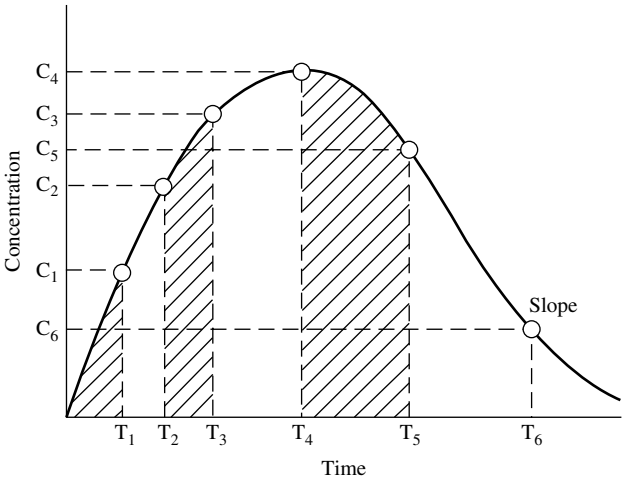


Figure 6.5 Plasma concentration time profile for oral exposure to a toxicant and depiction of AUCs determined by summation of trapezoids at several time periods.

Another technique is to monitor drug or toxicant excretion rather than blood concentrations, especially when blood or plasma concentrations are very low. Using the same equations, the AUC is now replaced by chemical concentrations in urine, feces, and expired air. Some chemicals are primarily excreted by the kidney and urine data alone may be necessary. The rate and extent of absorption are clearly important for therapeutic and toxicological considerations. For example, different formulations of the same pesticide can change the absorption rate in skin or gastrointestinal tract, and not bioavailability, but can result in blood concentrations near the toxic dose. Also different formulations can result in similar absorption rates but different bioavailability.

6.5.2 Gastrointestinal Absorption

The gastrointestinal tract (GIT) is a hollow tube (Figure 6.6a) lined by a layer of columnar cells, and usually protected by mucous, which offers minimal resistance to toxicant penetration. The distance from the outer membrane to the vasculature is about 40 μm, from which point further transport can easily occur. However, the cornified epithelium of the esophagus prevents absorption from this region of the GIT. Most of the absorption will therefore occur in the intestine (pH = 6), and to some extent in the stomach (pH = 1–3). Buccal and rectal absorption can occur in special circumstances. Note that secretions from the lachrymal duct, salivary gland, and nasal passages can enter the GIT via the buccal cavity. Therefore, following IV administration, a toxicant can enter the GIT if the drug is in these secretions.

The intestine can compensate the 2.5 log units difference between it and the stomach by the increased surface area in the small intestines. The presence of microvilli (Figure 6.6*b*) in the intestine is an increase of 600-fold in surface area compared to a hollow tube of comparable length. Note that there is no absorption, except for water, in the large intestine.

Most of the absorption in the GIT is by passive diffusion, except for nutrients; glucose, amino acids, and drugs that look like these substances are taken up by

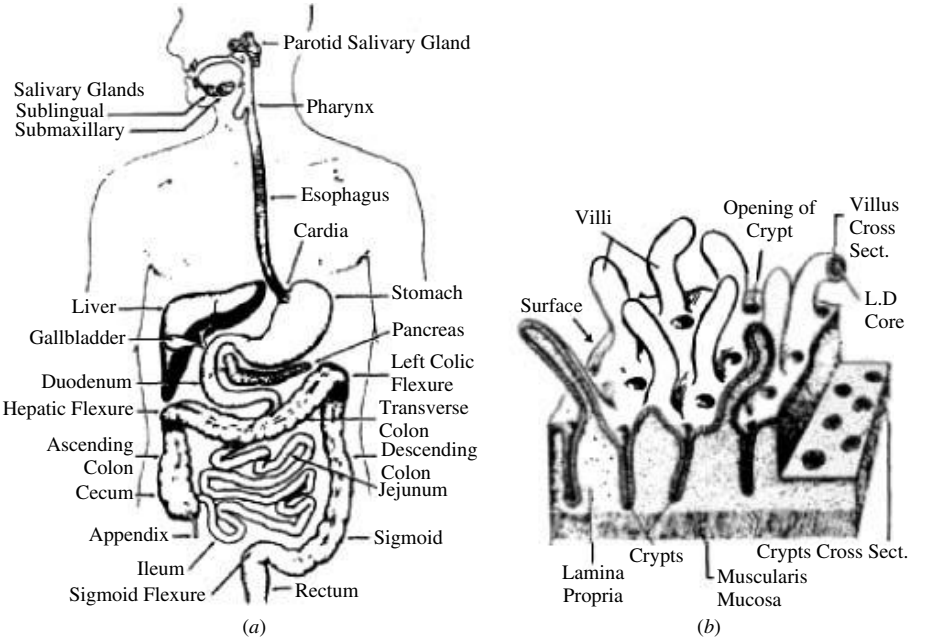


Figure 6.6 Schematic showing (*a*) alimentary canal and associated structures and (*b*) lining of the small intestine. (*Sources:*(*a*) Scholtelius and Scholtelius in *Textbook of Physiology*, Mosby, 1973; (*b*) Ham and Cormack, in *Histology*, 8th ed., Lippincott, 1979.)

active transport. For toxicants with structural similarities to compounds normally taken up by these active transport mechanisms, entry is enhanced. For example, cobalt is absorbed by the same active transport mechanism that normally transports iron, and 5-bromouracil is absorbed by the pyrimidine transport system.

Very lipid soluble toxicants and drugs, which are not miscible in the aqueous intestinal fluid, are presented as emulsions, and brought into solution through the action of detergent-like bile acids. The product of this mixing is large surface area micelles (hydrophobic interior) that deliver the lipids to the brush border of the intestine for diffusion across the membrane. As stated previously, the rate of passive transfer will be dependent on ionization and lipid solubility. Very strong bases (e.g., tubocurarine, succinylcholine) and strong acids are not readily absorbed in the GIT. These muscle relaxants therefore are given IV. The smaller the particle size of the toxicant, the greater is the absorption, and a chemical must be in aqueous solution for it to be absorbed in the GIT. A feature of the GIT that seems to contradict basic assumptions of absorption is the penetration of certain very large molecules. Compounds such as bacterial endotoxins, large particles of azo dyes, and carcinogens are apparently absorbed by endocytotic mechanisms.

GIT motility has a significant effect on GIT absorption of a toxicant. For example, excessively rapid movement of gut contents can reduce absorption by reducing residence time in the GIT, while the presence of food in the stomach can delay the progress of drugs from the stomach to the small intestine where most of the absorption will occur. Increased splanchnic blood flow after a meal can result in absorption of several drugs (e.g., propranolol), but in hypovolemic states, absorption can be reduced.

Biotransformation in the GIT prior to absorption can have a significant impact on bioavailability of a toxicant. The resident bacterial population can metabolize drugs in the GIT. Because of microbial fermentation in the rumen of ruminants and large intestine and cecum of horses and rabbits, its is often difficult to compare drug absorption profiles with carnivores (e.g., dogs) and omnivores (e.g., humans, pigs). Acid hydrolysis of some compounds can also occur, and enzymes in the intestinal mucosa can also have an effect on oral bioavailability. If the toxicant survive these microbial and chemical reactions in the stomach and small intestine, it is absorbed in the GIT and carried by the hepatic portal vein to the liver, which is the major site of metabolism. Chapters 7, 8, and 9 will discuss liver metabolism of toxicants in more detail. In brief, this activity in the liver can result in detoxification and/or bioactivation. Some drugs and toxicant that are conjugated (e.g., glucuronidation) in the liver are excreted via the biliary system back into the GIT. Once secreted in bile by active transport and excreted from the bile duct into the small intestine, this conjugated toxicant can be subjected to microbial beta-glucuronidase activity that can result in regeneration of the parent toxicant that is more lipophilic than the conjugate. The toxicant can now be reabsorbed by the GIT, prolonging the presence of the drug or toxicant in the systemic circulation. This is called *enterohepatic circulation*, which will be covered in greater detail in subsequent chapters.

6.5.3 Dermal Absorption

The skin is a complex multilayered tissue with a large surface area exposed to the environment. Skin anatomy, physiology, and biochemistry vary among species, within species, and even between anatomic sites within an individual animal or human. Logically these biological factors alone can influence dermal absorption. What is consistent is that the outer layer, the *stratum corneum* (SC), can provide as much as 80% of the resistance to absorption to most ions as well as aqueous solutions. However, the skin is permeable to many toxicants, and dermal exposure to agricultural pesticides and industrial solvent can result in severe systemic toxicity.

The anatomy of the skin is depicted in the schematic diagram of Figure 6.7. In mammalian skin there are really three distinct layers, which are the epidermis, dermis, and hypodermis or subcutaneous fat layer. Human skin is 3 mm thick, but it is the epidermis, which is only 0.1 to 0.8 mm, that provides the greatest resistance to toxicant penetration. The five layers of the epidermis, starting from the outside, are the stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale. The basal cells of the epidermis proliferate and differentiate as they migrate outward toward the surface of the skin. It requires about 2 to 28 days for cells to migrate from the basal layer to the stratum corneum, where they are eventually sloughed off. These dead, keratinized cells are, however, very water absorbant (hydrophilic), a property that keeps the skin soft and supple. Sebum, a natural oil covering the skin, functions in maintaining the water-holding ability of the epidermis. The stratum corneum is the primary barrier to penetration, and it consists primarily of these dead keratin-filled keratinocytes embedded in an extracellular lipid matrix. The lipids are primarily sterols, other neutral lipids, and ceramides. This association between lipids and dead keratinized cells, which is often referred to as the "brick and mortar" model as depicted in Figure 6.7*b*, is used to simplify the composition of the stratum corneum that is integral to chemical transport through skin.

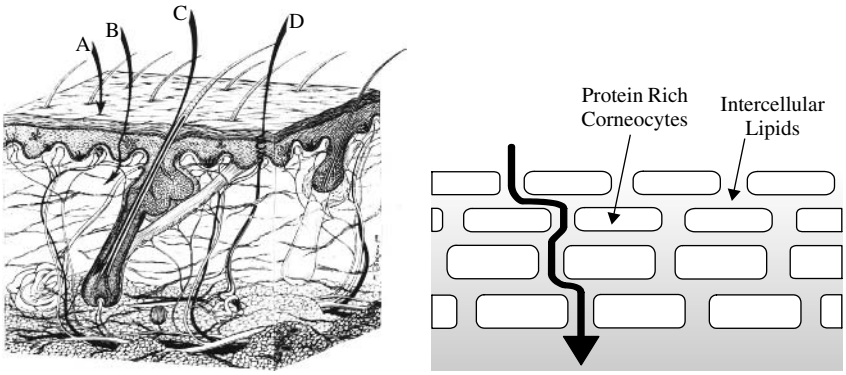


Figure 6.7 (*a*) Schematic diagram of the microstructure of mammalian skin and potential pathways for absorption by (A) intercellular, (B) transcellular, (C) transfollicular, or (D) sweat pore routes. (*b*) Brick-and-mortar" model of the stratum corneum depicting intercellular pathway (i.e., route A) between keratinocytes through the lipid domain of the stratum corneum.

A number of appendages are associated with the skin, including hair follicles, sebaceous glands, eccrine and apocrine sweat glands, and nails. Recently it was found that removal of the stratum corneum does not allow complete absorption; thus it is apparent that some role, although of lesser importance, is played by other parts of the skin. The dermis and subcutaneous areas of the skin are less important in influencing penetration, and once a toxicant has penetrated the epidermis, the other layers are traversed rather easily. The dermis is highly vascular, a characteristic that provides maximal opportunity for further transport once molecules have gained entry through the epidermis or through skin appendages. Most of the systemic absorption occurs at the capillary loops located at the epidermis-dermis junction. The blood supply of the dermis is under neural and humoral influences whose temperature-regulating functions could thus affect penetration and distribution of toxicants. Vasoactive drugs or environmental temperature can also influence absorption by altering blood flow to these capillaries. The subcutaneous layer of the skin is highly lipid in nature and serves as a shock absorber, an insulator, and a reserve depot of energy. The pH of the skin varies between 4 and 7 and is markedly affected by hydration.

Cutaneous biotransformation is mostly associated with the stratum basale layer where there can be phase I and phase II metabolism. However, the skin is not very efficient, compared to the liver. The epidermal layer accounts for the major portion of biochemical transformations in skin, although the total skin activity is low (2–6% that of the liver). Where activity is based on epidermis alone, that layer is as active as the liver or, in the case of certain toxicants, several times more active. For some chemicals, metabolism can influence absorption, and transdermal delivery systems of drugs utilize this activity. For example prodrug such as lipid esters are applied topically, and cutaneous esterases liberate the free drug. These basal cells and extracellular esterases have been shown to be involved in detoxification of several pesticides and bioactivation of carcinogens such as benzo(a)pyrene. For rapidly penetrating substances, metabolism by the skin is not presently considered to be of major significance, but skin may have an important first-pass metabolic function, especially for compounds that are absorbed slowly.

The *intercellular pathway* is now accepted as the major pathway for absorption. Recall that the rate of penetration is often correlated with the partition coefficient. In fact this is a very tortuous pathway, and the h (skin thickness) in Fick's first law of diffusion is really 10× the measured distance. By placing a solvent (e.g., ether, acetone) on the surface or tape stripping the surface, the stratum corneum (SC) is removed, and absorption can be significantly increased by removing this outer barrier. This may not be the case for very lipophilic chemical. This is because the viable epidermis and dermis are regarded as aqueous layers compared to the SC. Note that the more lipophilic the drug, the more likely it will form a depot in the SC and be slowly absorbed over time and thus have a prolonged half-life.

The *transcellular pathway* has been discredited as a major pathway, although some polar substances can penetrate the outer surface of the protein filaments of hydrated stratum corneum. The *transfollicular pathway* is really an invagination of the epidermis into the dermis, and the chemical still has to penetrate the epidermis to be absorbed into the blood stream. This is also a regarded as *minor* route. *Sweat pores* are not lined with the stratum corneum layer, but the holes are small, and this route is still considered a minor route for chemical absorption. In general, the epidermal surface is 100 to 1000 times the surface area of skin appendages, and it is likely that only very small and/or polar molecules penetrate the skin via these appendages.

Variations in areas of the body cause appreciable differences in penetration of toxicants. The rate of penetration is in the following order:

$$\text{Scrotal} > \text{Forehead} > \text{Axilla} >= \text{Scalp} > \text{Back} = \text{Abdomen} > \text{Palm and plantar}.$$

The palmar and plantar regions are highly cornified and are 100 to 400 times thicker than other regions of the body. Note that there are differences in blood flow and to a lesser extent, hair density, that may influence absorption of more polar toxicants.

Formulation additives used in topical drug or pesticide formulations can alter the stratum corneum barrier. Surfactants are least likely to be absorbed, but they can alter the lipid pathway by fluidization and delipidization of lipids, and proteins within the keratinocytes can become denatured. This is mostly likely associated with formulations containing anionic surfactants than non-ionic surfactants. Similar effects can be observed with solvents. Solvents can partition into the intercellular lipids, thereby changing membrane lipophilicity and barrier properties in the following order: ether/acetone > DMSO > ethanol > water. Higher alcohols and oils do not damage the skin, but they can act as a depot for lipophilic drugs on the skin surface. The presence of water in several of these formulations can hydrate the skin. Skin occlusion with fabric or transdermal patches, creams, and ointments can increase epidermal hydration, which can increase permeability.

The reader should be aware of the animal model being used to estimate dermal absorption of toxicants in humans. For many toxicants, direct extrapolation from a rodent species to human is not feasible. This is because of differences in skin thickness, hair density, lipid composition, and blood flow. Human skin is the least permeable compared to skin from rats, mice, and rabbits. Pig skin is, however, more analogous to human skin anatomically and physiologically, and pig skin is usually predictive of dermal absorption of most drugs and pesticides in human skin. Human skin is the best model, followed by skin from pigs, primates, and hairless guinea pigs, and then rats, mice, and rabbits. In preliminary testing of a transdermal drug, if the drug does

not cross rabbit or mice skin, it is very unlikely that it will cross human skin. There are several in vitro experimental techniques such as static diffusion (Franz) cells or flow-through diffusion (Bronough) cells. There are several ex vivo methods including the isolated perfused porcine skin flap (IPPSF), which with its intact microvasculature makes this model unique. In vivo methods are the golden standard, but they are very expensive, and there are human ethical and animal rights issues to be considered.

There are other factors that can influence dermal absorption, and these can include environmental factors such as air flow, temperature, and humidity. Preexisting skin disease and inflammation should also be considered. The topical dose this is usually expressed in per unit surface area can vary, and relative absorption usually decreases with increase in dose.

6.5.4 Respiratory Penetration

As observed with the GIT and skin, the respiratory tract can be regarded as an external surface. However, the lungs, where gas/vapor absorption occurs, are preceded by protective structures (e.g., nose, mouth, pharynx, trachea, and bronchus), which can reduce the toxicity of airborne substances, especially particles. There is little or no absorption in these structures, and residual volume can occur in these sites. However, cells lining the respiratory tract may absorb agents that can cause a toxicological response. The absorption site, which is the alveoli-capillary membrane, is very thin (0.4–1.5 μm). The membranes to cross from the alveolar air space to the blood will include: *type I cells to basement membrane to capillary endothelial cells* (Figure 6.8). This short distance allows for rapid exchange of gases/vapors. The analogous absorption distance in skin is 100 to 200 μm, and in GIT it is about 30 μm. There is also a large surface area (50 times the area of skin) available for absorption as well as significant blood flow, which makes it possible to achieve rapid adjustments in plasma concentration.

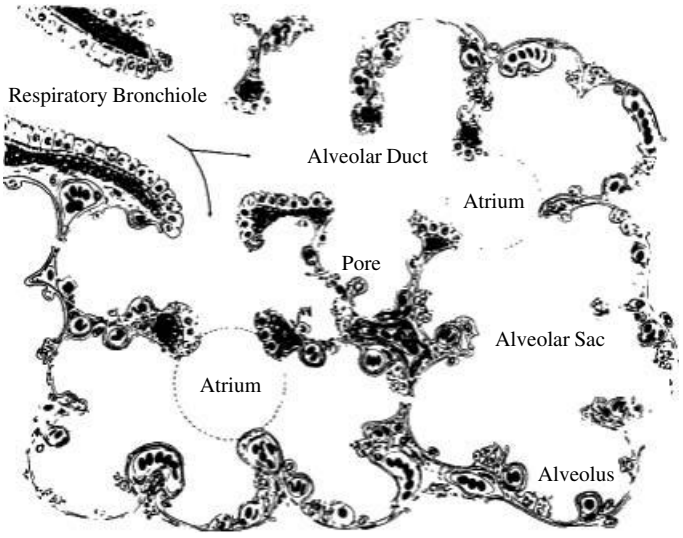


Figure 6.8 Schematic representation of the respiratory unit of the lung. (From Bloom and Fawcett, in *A Textbook of Histology*, Philadelphia: Saunders, 1975.)

Gases/vapors must get into solution in the thin fluid film in the alveoli for systemic absorption to occur. For this reason doses are often a measurement of partial pressures, which is important for gases/vapors.

The process of respiration involves the movement and exchange of air through several interrelated passages, including the nose, mouth, pharynx, trachea, bronchi, and successively smaller airways terminating in the alveoli, where gaseous exchange occurs. These alveoli consist mainly of type I pneumocytes, which represent 40% of all cells but cover > 90% of surface area, and type II pneumocytes, which represent 60% of all cells but cover 5% of surface area. Macrophages make up 90% of cells in alveolar space. The amount of air retained in the lung despite maximum expiratory effort is known as the residual volume. Thus toxicants in the respiratory air may not be cleared immediately because of slow release from the residual volume. The rate of entry of vapor-phase toxicants is controlled by the alveolar ventilation rate, with the toxicant being presented to the alveoli in an interrupted fashion approximately 20 times/min.

Airborne toxicants can be simplified to two general types of compounds, namely gases and aerosols. Compounds such as gases, solvents, and vapors are subject to gas laws and are carried easily to alveolar air. Much of our understanding of xenobiotic behavior is with anesthetics. Compounds such as aerosols, particulates, and fumes are not subject to gas laws because they are in particulate form.

The transfer of gas from alveoli to blood is the actual absorption process. Among the most important factors that determine rate and extent of absorption of a gas in lungs is the solubility of that gas. Therefore it is not the membrane partition coefficient that necessarily affects absorption as has been described for skin and GIT membranes, but rather the blood: gas partition coefficient or blood/gas solubility of the gas. A high blood: gas partition coefficient indicates that the blood can hold a large amount of gas. Keep in mind that it is the *partial pressure* at equilibrium that is important, so the more soluble the gas is in blood, the greater the amount of gas that is needed to dissolve in the blood to raise the partial pressure or tension in blood. For example, anesthetics such as diethyl ether and methoxyflurane, which are soluble (Table 6.3), require a longer period for this partial pressure to be realized. Again, the aim is to generate the same tension in blood as in inspired air. Because these gases are very soluble, detoxification is a prolonged process. In practice, anesthetic induction is slower, and so is recovery from anesthesia. For less soluble gases (e.g., NO, isoflurane, halothane), the partial pressure or tension in blood can be raised a lot easier to that of inspired gases, and detoxification takes less time than those gases that are more soluble.

There are several other important factors that can determine whether the gas will be absorbed in blood and then transported from the blood to the perfused tissue. The concentration of the gas in inspired air influences gas tension, and partial pressure can be increased by overventilation. In gas anesthesiology we know that the effects of

Table 6.3 Blood: Gas Partition Coefficient in Humans

Agent	Coefficient
Methoxyflurane	13.0–15.0
Halothane	2.3–2.5
Isoflurane	1.4
NO	0.5

respiratory rate on speed of induction are transient for gases that have low solubility in blood and tissues, but there is a significant effect for agents that are more soluble and take a longer time for gas tensions to equilibrate. In determining how much of the gas is absorbed, its important to consider what fraction of the lung is ventilated and what fraction is perfused. However, one should be aware that due to diseased lungs, there can be differences between these fractions. For example, decreased perfusion will decrease absorption, although there is agent in the alveoli, and vice versa. The rate at which a gas passes into tissues is also dependent on gas solubility in the tissues, rate of delivery of the gas to tissues, and partial pressures of gas in arterial blood and tissues. After uptake of the gas, the blood takes the gas to other tissues. The mixed venous blood returned to the lungs progressively begins to have more of the gas, and differences between arterial (or alveolar) and mixed venous gas tensions decreases continuously.

While gases are more likely to travel freely through the entire respiratory tract to the alveoli, passage of aerosols and particles will be affected by the upper respiratory tract, which can act as an effective filter to prevent particulate matter from reaching the alveoli. Mucous traps particles to prevent entry to alveoli, and the mucociliary apparatus in the trachea traps and pushes particles up the trachea to the esophagus where they are swallowed and possibly absorbed in the GI tract.

In addition to upper pathway clearance, lung phagocytosis is very active in both upper and lower pathways of the respiratory tract and may be coupled to the mucus cilia. Phagocytes may also direct engulfed toxicants into the lymph, where the toxicants may be stored for long periods. If not phagocytized, particles ≤ 1 μm may penetrate to the alveolar portion of the lung. Some particles do not desequamate but instead form a dust node in association with a developing network of reticular fibers. Overall, removal

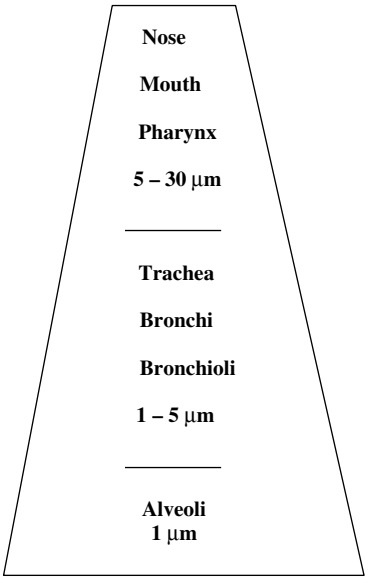


Figure 6.9 Schematic illustration of the regions where absorption may occur in the respiratory tract.

of alveolar particles is markedly slower than that achieved by the directed upper pulmonary mechanisms. This defense mechanism is not important for vapors/gases. The efficiency of the system is illustrated by the fact that on average, only 100 g of coal dust is found postmortem in the lungs of coal miners, although they inhale approximately 6000 g during their lifetime.

The deposition site of particles in the respiratory tract is primarily dependent on the *aerodynamic behavior* of the particles. The particle size, density, shape, hygroscopicity, breathing pattern, and lung airway structure are also important factors influencing the deposition site and efficiency. The *aerodynamic-equivalent diameter* (for particle $>$ 0.5 μm) and *diffusion-equivalent diameter* ($<$ 0.5 μm) are defined as the diameter of a *unit density sphere* having the same *settling velocity* (aerodynamic-equivalent) or the same *diffusion rate* (diffusion-equivalent) as the *irregularly shaped particle of interest*. Deposition occurs by five possible mechanisms: electrostatic precipitation, interception, impaction, sedimentation, impaction, and diffusion. The latter three are most important. Only particle sizes less than 10 to 20 μm that get pass the nasopharyngeal regions and reach the alveoli are of medical concern. As particle size decreases below 0.5 μm, the aerosol begins to behave like a gas (Figure 6.9). For these particles, diffusion becomes the primary mechanism of deposition in the respiratory tract before it finally reaches the alveoli.

6.6 TOXICANT DISTRIBUTION

6.6.1 Physicochemical Properties and Protein Binding

Absorption of toxicants into the blood needs to be high enough so that it will have a significant effect at the site of action in other areas of the body. The distribution process that takes the absorbed drug to other tissues is dependent on various physiological factors and physicochemical properties of the drug. This process is therefore a reversible movement of the toxicant between blood and tissues or between extracellular and intracellular compartments. There are, however, several complicating factors that can influence the distribution of a toxicant. For example, *perfusion* of tissues is an important physiological process, as some organs are better perfused (e.g., heart, brain) than others (e.g., fat). There can also be significant *protein binding* that affects delivery of drug to tissues. To further complicate the issue, elimination processes such as excretion and biotransformation (discussed at a later time) is occurring simultaneously to remove the toxicant from the blood as well as the target site.

There are several physiochemical properties of the toxicant that can influence its distribution. These include lipid solubility, pKa, and molecular weight, all of which were described earlier in this chapter (Section 6.4) and will not be described here. For many toxicants, distribution from the blood to tissues is by simple diffusion down a concentration gradient, and the absorption principles described earlier also apply here. The concentration gradient will be influenced by the partition coefficient or rather the ratio of toxicant concentrations in blood and tissue. Tissue mass and blood flow will also have a significant effect on distribution. For example, a large muscle mass can result in increased distribution to muscle, while limited blood flow to fat or bone tissue can limit distribution. The ratio of blood flow to tissue mass is also a useful indicator of how well the tissue is perfused. The well perfused tissues include liver,

kidney, and brain, and the low perfused tissues include fat and bone where there is slow elimination from these tissues. Initial distribution to well-perfused tissues (e.g., heart, brain) occurs within the first few minutes, while delivery of drug to other tissues (e.g., fat, skin) is slower.

If the affinity for the target tissue is high, then the chemical will accumulate or form a depot. The advantage here is that if this is a drug, there is no need to load up the central compartment to get to the active site. However, if the reservoir for the drug has a large capacity and fills rapidly, it so alters the distribution of the drug that larger quantities of the drug are required initially to provide a therapeutic effective concentration at the target organ. If this is a toxicant, this may be an advantageous feature as toxicant levels at the target site will be reduced. In general, lipid-insoluble toxicants stay mainly in the plasma and interstitial fluids, while lipid-soluble toxicants reach all compartments, and may accumulate in fat. There are numerous examples of cellular reservoirs for toxicants and drugs to distribute. Tetracycline antibiotics have a high affinity for calcium-rich tissues in the body. The bone can become a reservoir for the slow release of chemicals such as lead, and effects may be chronic or there may be acute toxicity if the toxicant is suddenly released or mobilized from these depots. The antimalaria drug quinacrine accumulates due to reversible intracellular binding, and the concentration in the liver can be several thousand times that of plasma. Another antimalaria drug, chloroquine, has a high affinity for melanin, and this drug can be taken up by tissues such as the retina, which is rich in melanin granules, and can cause retinitis with a drug overdose. Lipophilic pesticides and toxicants (e.g., PCBs) and lipid soluble gases can be expected to accumulate in high concentration in fat tissue.

There are unique anatomical barriers that can limit distribution of toxicants. A classical example of such a unique barrier is the blood-brain barrier (BBB), which can limit the distribution of toxicants into the CNS and cerebrospinal fluid. There are three main processes or structures that keep drug or toxicant concentrations low in this region: (1) The BBB, which consist of capillary endothelial tight junctions and glial cells, surrounds the precapillaries, reduces filtration, and requires that the toxicant cross several membranes in order to get to the CSF. (Note that endothelial cells in other organs can have intercellular pores and pinocytotic vesicles.) (2) Active transport systems in the choroid plexus allow for transport of organic acids and bases from the CSF into blood. (3) The continuous process of CSF production in the ventricles and venous drainage continuously dilutes toxicant or drug concentrations. Disease processes such as meningitis can disrupt this barrier and can allow for penetration of antibiotics (e.g., aminoglycosides) that would not otherwise readily cross this barrier in a healthy individual. Other tissue/blood barriers include prostate/blood, testicles/blood, and globe of eye/blood, but inflammation or infection can increase permeability of these barriers. Toxicants can cross the placenta primarily by simple diffusion, and this is most easily accomplished if the toxicants are lipid-soluble (i.e., nonionized weak acids or bases). The view that the placenta is a barrier to drugs and toxicants is inaccurate. The fetus is, at least to some extent, exposed to essentially all drugs even if those with low lipid solubility are taken by the mother.

As was indicated earlier, the circulatory system and components in the blood stream are primarily responsible for the transport of toxicants to target tissues or reservoirs. Erythrocytes and lymph can play important roles in the transport of toxicants, but compared to plasma proteins, their role in toxicant distribution is relatively minor for most toxicants. Plasma protein binding can affect distribution because only the unbound

toxicant is free or available to diffuse across the cell membranes. The toxicant-protein binding reaction is reversible and obeys the laws of mass action:

$$\underset{\text{(free)}}{\text{Toxicant}} + \text{Protein} \underset{k_2}{\overset{k_1}{\leftrightarrow}} \underset{\text{(bound)}}{\text{Toxicant-Protein}}$$

Usually the ratio of unbound plasma concentration (C_u) of the toxicant to total toxicant concentration in plasma (C) is the fraction of drug unbound, $\boldsymbol{f_u}$, that is,

$$f_u = \frac{C_u}{C}.$$

The constants k_1 and k_2 are the specific rate constants for association and dissociation, respectively. The association constant K_a will be the ratio k_1/k_2, and conversely, the dissociation constant, K_d will be k_2/k_1. The constants and parameters are often used to describe and, more important, to compare the relative affinity of xenobiotics for plasma proteins.

The are many circulating proteins, but those involved in binding xenobiotics include albumin, α_1-acid glycoprotein, lipoproteins, and globulins. Because many toxicants are lipophilic, they are likely to bind to plasma α- and β-lipoproteins. There are mainly three classes of lipoproteins, namely high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low density lipoprotein (VLDL). Iron and copper are known to interact strongly with the metal-binding globulins transferin and ceruloplasmin, respectively. Acidic drugs bind primarily to albumin, and basic drugs are bound primarily to α_1-acid glycoprotein and β-globulin. Albumin makes up 50% of total plasma proteins, and it reacts with a wide variety of drugs and toxicants. The α_1-acid glycoprotein does not have as many binding sites as albumin, but it has one high-affinity binding site. The amount of toxicant drug that is bound depends on free drug concentration, and its affinity for the binding sites, and protein concentration. Plasma protein binding is nonselective, and therefore toxicants and drugs with similar physicochemical characteristics can compete with each other and endogenous substances for binding sites. Binding to these proteins does not necessarily prevent the toxicant from reaching the site of action, but it slows the rate at which the toxicant reaches a concentration sufficient to produce a toxicological effect. Again, this is related to what fraction of the toxicant is free or unbound ($\boldsymbol{f_u}$).

Toxicants complex with proteins by various mechanisms. Covalent binding may have a pronounced effect on an organism due to the modification of an essential molecule, but such binding is usually a very minor portion of the total dose. Because covalently bound molecules dissociate very slowly, if at all, they are not considered further in this discussion. However, we should recognize that these interactions are often associated with carcinogenic metabolites. Noncovalent binding is of primary importance to distribution because the toxicant or ligand can dissociate more readily than it can in covalent binding. In rare cases the noncovalent bond may be so stable that the toxicant remains bound for weeks or months, and for all practical purposes, the bond is equivalent to a covalent one. Types of interactions that lead to noncovalent binding under the proper physiological conditions include ionic binding, hydrogen bonding, van der Waals forces, and hydrophobic interactions. There are, however, some transition metals that have high association constants and dissociation is slow.

We know more about ligand-protein interactions today because of the numerous protein binding studies performed with drugs. The major difference between drugs and most toxicants is the frequent ionizability and high water solubility of drugs as compared with the non-ionizability and high lipid solubility of many toxicants. Thus experience with drugs forms an important background, but one that may not always be relevant to other potentially toxic compounds.

Variation in chemical and physical features can affect binding to plasma constituents. Table 6.4 shows the results of binding studies with a group of insecticides with greatly differing water and lipid solubilities. The affinity for albumin and lipoproteins is inversely related to water solubility, although the relation may be imperfect. Chlorinated hydrocarbons bind strongly to albumin but even more strongly to lipoproteins. Strongly lipophilic organophosphates bind to both protein groups, whereas more water-soluble compounds bind primarily to albumin. The most water-soluble compounds appear to be transported primarily in the aqueous phase. Chlordecone (Kepone) has partitioning characteristics that cause it to bind in the liver, whereas DDE, the metabolite of DDT, partitions into fatty depots. Thus the toxicological implications for these two compounds may be quite different.

Although highly specific (high-affinity, low-capacity) binding is more common with drugs, examples of specific binding for toxicants seem less common. It seems probable that low-affinity, high-capacity binding describes most cases of toxicant binding. The number of binding sites can only be estimated, often with considerable error, because of the nonspecific nature of the interaction. The number of ligand or toxicant molecules bound per protein molecule, and the maximum number of binding sites, n, define the definitive capacity of the protein. Another consideration is the binding affinity K_{binding} (or $1/K_{\text{diss}}$). If the protein has only one binding site for the toxicant, a single value, K_{binding}, describes the strength of the interaction. Usually more than one binding site is present, each site having its intrinsic binding constant, $k_1, k_2, \ldots, k_n$. Rarely does one find a case where $k_1 = k_2 = \ldots = k_n$, where a single value would describe the affinity

Table 6.4 Relative Distribution of Insecticides into Albumin and Lipoproteins

		Percent Distribution of Bound Insecticide		
Insecticide	Percent Bound	Albumin	LOL	HDL
DDT	99.9	35	35	30
Deildrin	99.9	12	50	38
Lindane	98.0	37	38	25
Parathion	98.7	67	21	12
Diazinon	96.6	55	31	14
Carbaryl	97.4	99	<1	<1
Carbofuran	73.6	97	1	2
Aldicarb	30.0	94	2	4
Nicotine	25.0	94	2	4

Source: Adapted from B. P. Maliwal and F. E. Guthrie, *Chem Biol Interact* **35**:177–188, 1981.
Note: LOL, low-density lipoprotein; HOL, high-density lipoprotein.

constant at all sites. This is especially true when hydrophobic binding and van der Waals forces contribute to nonspecific, low-affinity binding. Obviously the chemical nature of the binding site is of critical importance in determining binding. The three-dimensional molecular structure of the binding site, the environment of the protein, the general location in the overall protein molecule, and allosteric effects are all factors that influence binding. Studies with toxicants, and even more extensive studies with drugs, have provided an adequate elucidation of these factors. Binding appears to be too complex a phenomenon to be accurately described by any one set of equations.

There are many methods for analyzing binding, but equilibrium dialysis is the most extensively used. Again, the focus of these studies is to determine the percentage of toxicant bound, the number of binding sites (n), and the affinity constant (K_a). The examples presented here are greatly simplified to avoid the undue confusion engendered by a very complex subject.

Toxicant-protein complexes that utilize relatively weak bonds (energies of the order of hydrogen bonds or less) readily associate and dissociate at physiological temperatures, and the law of mass action applies to the thermodynamic equilibrium:

$$K_{\text{binding}} = \frac{[TP]}{[T][P]} = \frac{1}{K_{\text{diss}}},$$

where K_{binding} is the equilibrium constant for association, $[TP]$ is the molar concentration of toxicant-protein complex, $[T]$ is the molar concentration of free toxicant, and $[P]$ is the molar concentration of free protein. This equation does not describe the binding site(s) or the binding affinity. To incorporate these parameters and estimate the extent of binding, double-reciprocal plots of $1/[TP]$ versus $1/[T]$ may be used to test the specificity of binding. The $1/[TP]$ term can also be interpreted as moles of albumin per moles of toxicant. The slope of the straight line equals $1/nK_a$ and the intercept of this line with the x-axis equals $-K_a$. Regression lines passing through the origin imply infinite binding, and the validity of calculating an affinity constant under these circumstances is questionable. Figure 6.10 illustrates one such case with four pesticides, and the insert illustrates the low-affinity, "unsaturable" nature of binding in this example.

The two classes of toxicant-protein interactions encountered may be defined as (1) specific, high affinity, low capacity, and (2) nonspecific, low affinity, high capacity. The term high affinity implies an affinity constant (K_{binding}) of the order of 10^8 M^{-1}, whereas low affinity implies concentrations of 10^4 M^{-1}. Nonspecific, low-affinity binding is probably most characteristic of nonpolar compounds, although most cases are not as extreme as that shown in Figure 6.10.

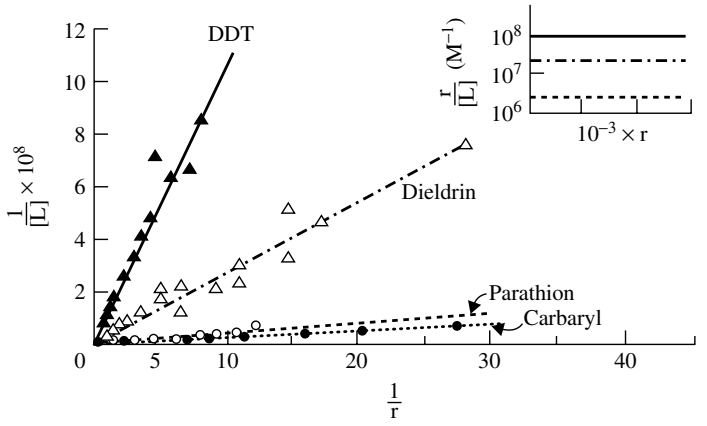
An alternative and well-accepted treatment for binding studies is the Scatchard equation especially in situations of high-affinity binding:

$$\nu = \frac{nk[T]}{1 + k[T]},$$

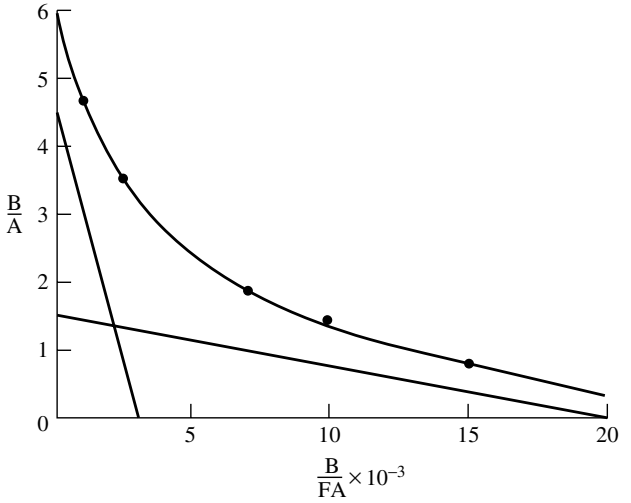
which is simplified for graphic estimates to

$$\frac{\nu}{[T]} = k(n - \nu),$$

where ν is the moles of ligand (toxicant) bound per mole of protein, $[T]$ is the concentration of free toxicant, k is the intrinsic affinity constant, and n is the number of sites exhibiting such affinity. When $\nu[T]$ is plotted against ν, a straight line is obtained



(*a*)



(*b*)

Figure 6.10 Binding of toxicants to blood proteins: (*a*) Double-reciprocal plot of binding of rat serum lipoprotein fraction with four insecticides. Insert illustrates magnitude of differences in slope with Scatchard plot. (*b*) Scatchard plot of binding of salicylate to human serum proteins. (Sources: (*a*) Skalsky and Guthrie, *Pest. Biochem. Physiol.* **7**: 289, 1977; (*b*) Moran and Walker, *Biochem. Pharmacol.* **17:** 153, 1968.)

if only one class of binding sites is evident. The slope is $-k$, and the intercept on the ν-axis becomes n (number of binding sites). If more than one class of sites occurs (probably the most common situation for toxicants), a curve is obtained from which the constants may be obtained. This is illustrated in Figure 6.10*b*, for which the data show not one but two species of binding sites: one with low capacity but high affinity, and another with about three times the capacity but less affinity. Commonly used computer programs usually solve such data by determining one line for the specific binding and one line for nonspecific binding, the latter being an average of many possible solutions.

When hydrophobic binding of lipid toxicants occurs, as is the case for many environmental contaminants, binding is probably not limited to a single type of plasma

protein. For example, the binding of the chlorinated hydrocarbon DDT is strongest for lipoproteins and albumin, but other proteins account for a significant part of overall transport. Similar results have been observed for several compounds with a range of physiochemical properties.

The presence of another toxicant and/or drug that can bind at the same site can also increase the amount of free or unbound drug. This is an example of drug interaction that can have serious toxicological or pharmacological consequences. In general, when bound concentrations are less than 90% of the total plasma concentrations, plasma protein binding has little clinical importance. Plasma protein binding *becomes important when it is more than 90%*. For example, if a toxicant is 99% bound to plasma proteins, then 1% is free, but if there is toxicant interaction (e.g., competitive binding) that results in 94% bound, 6% is now free. Note that because of this interaction, the amount of available toxicant to cause a toxicological response *has increased sixfold*. Such a scenario may result in severe acute toxicity. Extensive plasma protein binding can influence renal clearance if glomerular filtration is the major elimination process in the kidney, but not if it is by active secretion in the kidney. Binding can also affect drug clearance if the extraction ratio (*ER*) in the liver is low, but not if the *ER* is high for that toxicant. Plasma protein binding can vary between and within chemical classes, and it is also species specific. For example, humans tend to bind acidic drugs more extensively than do other species.

There are several other variables that can alter plasma protein concentrations. These include malnutrition, pregnancy, cancer, liver abscess, renal disease, and age can reduce serum albumin. Furthermore α_1-glycoprotein concentrations can increase with age, inflammation, infections, obesity, renal failure, and stress. Small changes in body temperature or changes in acid-base balance may alter chemical protein-binding characteristics. Although termination of drug or toxicant effect is usually by biotransformation and excretion, it may also be associated with redistribution from its site of action into other tissues. The classical example of this is when highly lipid-soluble drugs or toxicants that act on the brain or cardiovascular system are administered by IV or by inhalation.

6.6.2 Volume of Distribution (V_d)

Usually after a toxicant or drug is absorbed it can be distributed into various physiologic fluid compartments. The total body water represents 57% of total body mass (0.57 L/kg) (Table 6.5). The plasma, interstitial fluid, extracellular fluid, and intracellular fluid represent about 5, 17, 22, and 35% body weight, respectively. The extracellular fluid comprises the blood plasma, interstitial fluid, and lymph. Intracellular fluid includes

Table 6.5 Volume of Distribution into Physiological Fluid Compartments

Compartment	Volume of Distribution in L/kg Body Weight (Ls/70 kg Body weight)
Plasma	0.05(3.5 L)
Interstitial fluid	0.18(12.6 L)
Extracellular fluid	0.23(16.1 L)
Intracellular fluid	0.35(24.5 L)
Total body water	0.55(39 L)

the sum of fluid contents of all cells in the body. There is also transcellular fluid that represents 2% body weight, and this includes cerebrospinal, intraocular, peritoneal, pleural, and synovial fluids, and digestive secretions. Fat is about 20% body weight, while the GIT contents in monogastrics make up 1% body weight, and in ruminants it can constitute 15% body weight.

Its sometimes useful to quantitate how well a drug or toxicant is distributed into these various fluid compartments, and in this context the apparent volume of distribution can be a useful parameter. The apparent volume of distribution, V_d, is defined as the volume of fluid required to contain the total amount, A, of drug in the body at the same concentration as that present in plasma, C_p,

$$V_d = \frac{A}{C_p}.$$

In general, the V_d for a drug is to some extent descriptive of its distribution pattern in the body. For example, drugs or toxicants with relatively small V_d values may be confined to the plasma as diffusion across the capillary wall is limited. There are other toxicants that have a slightly larger V_d (e.g., 0.23 L/kg), and these toxicants may be distributed in the extracellular compartment. This includes many polar compounds (e.g., tubocurarine, gentamicin, $V_d = 0.2–0.4$ L/kg). These toxicants cannot readily enter cells because of their low lipid solubility. If the V_d for some of these toxicants is in excess of the theoretical value, this may be due to limited degree of penetration into cells or from the extravascular compartment. Finally there are many toxicants distributed throughout the body water ($V_d \geq 0.55$ L/kg) that may have V_d values much greater than that for total body water. This distribution is achieved by relatively lipid-soluble toxicants and drugs that readily cross cell membranes (e.g., ethanol, diazepam; $V_d = 1$ to 2 L/kg). Binding of the toxicant anywhere outside of the plasma compartment, as well as partitioning into body fat, can increase V_d beyond the absolute value for total body water. In general, toxicants with a large V_d can even reach the brain, fetus, and other transcellular compartments. In general, toxicants with large V_d are a consequence of extensive tissue binding. The reader should be aware that we are talking about tissue binding, and not plasma protein binding where distribution is limited to plasma for obvious reasons.

The fraction of toxicant located in plasma is dependent on whether a toxicant binds to both plasma and tissue components. Plasma binding can be measured directly, but not tissue binding. It can, however, be inferred from the following relationship:

$$\begin{array}{rcll} \text{Amount in body} & = & \text{Amount in plasma} & + \quad \text{Amount outside plasma} \\ V_d \times C & = & V_p \times C & + \quad V_{TW} \times C_{TW} \end{array}$$

where V_d is the apparent volume of distribution, V_p the volume of plasma, V_{TW} the apparent volume of tissue, and C_{TW} the tissue concentration. If the preceding equation is divided by C, it now becomes

$$V_d = V_p + V_{TW} \times \frac{C_{TW}}{C}$$

Recall that $f_u = C_u/C$ occurs with plasma, and also that the fraction unbound in tissues is $f_{uT} = C_{uT}/C_{TW}$.

Assuming at equilibrium that unbound concentration in tissue and plasma are equal, then we let the ratio of f_u/f_{uT} replace C_{TW}/C and determine the volume of distribution as follows:

$$V_d = V_p + V_{TW} \times \left(\frac{f_u}{f_{uT}}\right).$$

It is possible to predict what happens to V_d when f_u or f_{uT} changes as a result of physiological or disease processes in the body that change plasma and/or tissue protein concentrations. For example, V_d can increase with increased unbound toxicant in plasma or with a decrease in unbound toxicant tissue concentrations. The preceding equation explains why: because of both plasma and tissue binding, some V_d values rarely correspond to a real volume such as plasma volume, extracellular space, or total body water. Finally interspecies differences in V_d values can be due to differences in body composition of body fat and protein, organ size, and blood flow as alluded to earlier in this section. The reader should also be aware that in addition to V_d, there are volumes of distribution that can be obtained from pharmacokinetic analysis of a given data set. These include the volume of distribution at steady state ($V_{d,\mathrm{ss}}$), volume of the central compartment (V_c), and the volume of distribution that is operative over the elimination phase ($V_{d,\mathrm{area}}$). The reader is advised to consult other relevant texts for a more detailed description of these parameters and when it is appropriate to use these parameters.

6.7 TOXICOKINETICS

The explanation of the pharmacokinetics or toxicokinetics involved in absorption, distribution, and elimination processes is a highly specialized branch of toxicology, and is beyond the scope of this chapter. However, here we introduce a few basic concepts that are related to the several transport rate processes that we described earlier in this chapter. Toxicokinetics is an extension of pharmacokinetics in that these studies are conducted at higher doses than pharmacokinetic studies and the principles of pharmacokinetics are applied to xenobiotics. In addition these studies are essential to provide information on the fate of the xenobiotic following exposure by a define route. This information is essential if one is to adequately interpret the dose-response relationship in the risk assessment process. In recent years these toxicokinetic data from laboratory animals have started to be utilized in physiologically based pharmacokinetic (PBPK) models to help extrapolations to low-dose exposures in humans. The ultimate aim in all of these analyses is to provide an estimate of tissue concentrations at the target site associated with the toxicity.

Immediately on entering the body, a chemical begins changing location, concentration, or chemical identity. It may be transported independently by several components of the circulatory system, absorbed by various tissues, or stored; the chemical may effect an action, be detoxified, or be activated; the parent compound or its metabolite(s) may react with body constituents, be stored, or be eliminated—to name some of the more important actions. Each of these processes may be described by rate constants similar to those described earlier in our discussion of first-order rate processes that are associated with toxicant absorption, distribution, and elimination and occur

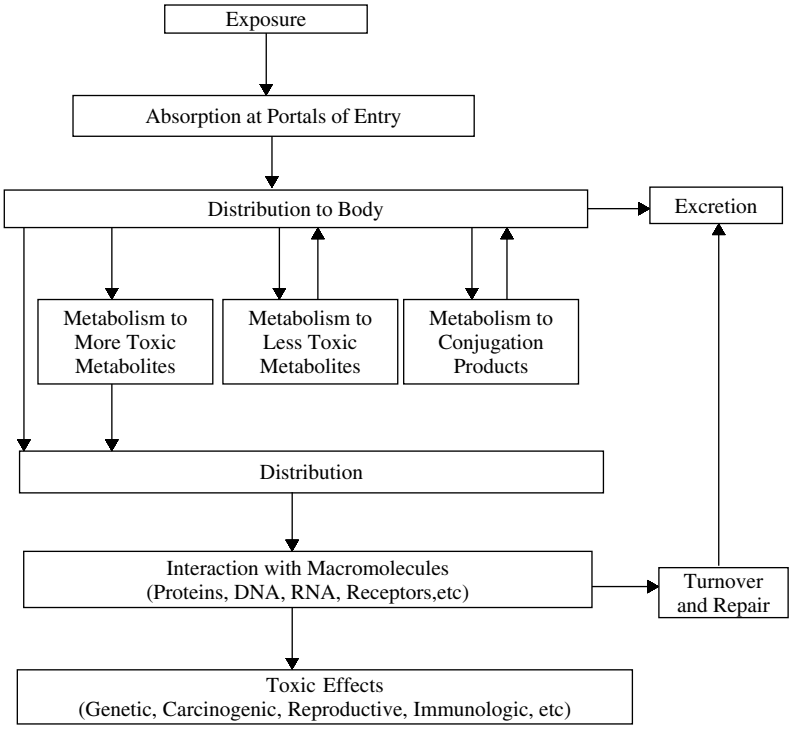


Figure 6.11 Sequence of events following exposure of an animal to exogenous chemicals.

simultaneously. Thus at no time is the situation stable but is constantly changing as indicated in Figure 6.11.

It should be noted, however, that as the toxicant is being absorbed and distributed throughout the body, it is being simultaneously eliminated by various metabolism and/or excretion mechanisms, as will be discussed in more detail in the following chapters. However, one should mention here that an important pharmacokinetic parameter known as *clearance* ($C\ell$) can be used to quantitatively assess elimination of a toxicant. Clearance is defined as the rate of toxicant excreted relative to its plasma concentration, C_p:

$$C\ell = \frac{\text{Rate of toxicant excretion}}{C_p}.$$

The rate of excretion is really the administered dose times the fractional elimination rate constant K_{el} described earlier. Therefore we can express the preceding equation in terms of K_{el} and administered dose as volume of distribution, V_d:

$$C\ell = K_{\text{el}} \cdot \frac{\text{Dose}}{C_p} = K_{\text{el}} \cdot (V_d \cdot C_p)/C_p = K_{\text{el}} \cdot V_d.$$

In physiological terms we can also define clearance as the volume of blood cleared of the toxicant by an organ or body per unit time. Therefore, as the equations above indicate, the body clearance of a toxicant is expressed in units of volume per unit time (e.g., L/h), and can be derived if we know the volume of distribution of the toxicant

and fractional rate constant. In many instances this can only be derived by appropriate pharmacokinetic analysis of a given data set following blood or urine sample collection and appropriate chemical analyses to determine toxicant concentrations in either of these biological matrices.

Each of the processes discussed thus far—absorption, distribution, and elimination—can be described as a rate process. In general, the process is assumed to be first order in that the rate of transfer at any time is proportional to the amount of drug in the body at that time. Recall that the rate of transport (dC/dt) is proportional to toxicant concentration (C) or stated mathematically:

$$\frac{dC}{dt} = KC,$$

where K is the rate constant (fraction per unit time). Many pharmacokinetic analyses of a chemical are based primarily on toxicant concentrations in blood or urine samples. It is often assumed in these analyses that the rate of change of toxicant concentration in blood reflects quantitatively the change in toxicant concentration throughout the body (first-order principles). Because of the elimination/clearance process, which also assumed to be a first-order rate process, the preceding rate equation now needs a negative sign. This is really a decaying process that is observed as a decline of toxicant concentration in blood or urine after intravenous (IV) administration. The IV route is preferred in these initial analyses because there is no absorption phase, but only chemical depletion phase. However, one cannot measure infinitesimal change of C or time, t; therefore there needs to be integration after rearrangement of the equation above:

$$\frac{-dC}{C} = kdt \quad \text{becomes} \quad \int \frac{-dC}{C} = k \int dt,$$

which can be expressed as

$$C = C^0 e^{-kt},$$

where e is the base of the natural logarithm. We can remove e by taking the ln of both sides:

$$\ln C^t = \ln C^0 - kt.$$

Note that K is the slope of the straight line for a semilog plot of toxicant concentration versus time (Figure 6.12). In the preceding equation it is the elimination rate constant that is related to the half-life of the toxicant described earlier in this chapter. The derived C^0 can be used to calculate the volume of distribution (V_d) of the toxicant as follows:

$$V_d = \frac{\text{Dose}}{C^0}.$$

However, toxicokinetic data for many toxicants do not always provide a straight line when plotted as described above. More complicated equations with more than one exponential term with rate constants may be necessary to mathematically describe the concentration-time profile. These numerous rate constants are indicative of chemical transport between various compartments in the body and not only to a single central compartment as suggested in the simple equation and semilog plot described in

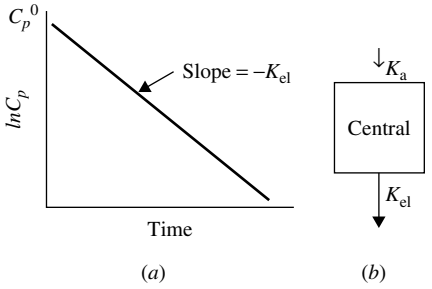


Figure 6.12 (*a*) Semilog plot of plasma concentration (C_p) versus time. $C_p{}^0$ is the intercept on the *y*-axis, and K_{el} is the elimination rate constant. (*b*) Single compartment model with rate constants for absorption, K_a and for elimination, K_{el}.

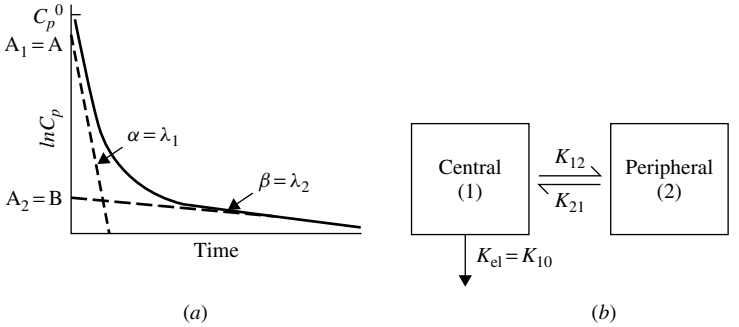


Figure 6.13 (*a*) Semilog plot of plasma concentration for (C_p) versus time representative of a two-compartment model. The curve can be broken down into an α or λ_1 distribution phase and β or λ_2 elimination phase. (*b*) Two-compartment model with transfer rate constants, K_{12} and K_{21}, and elimination rate constant, K_{el}.

Figure 6.12. In some instances the data may fit to a bi-exponential concentration-time profile (Figure 6.13). The equation to describe this model is

$$C = Ae^{-\alpha t} + Be^{-\beta t}.$$

In other instances, complex profiles may require a three- or multi-exponential concentration-time profile (Figure 6.14). The equation to describe the three-profile case is

$$C = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}.$$

In the physiological sense, one can divide the body into "compartments" that represent discrete parts of the whole-blood, liver, urine, and so on, or use a mathematical model describing the process as a composite that pools together parts of tissues involved in distribution and bioactivation. Usually pharmacokinetic compartments have no anatomical or physiological identity; they represent all locations within the body that have similar characteristics relative to the transport rates of the particular toxicant. Simple first-order kinetics is usually accepted to describe individual

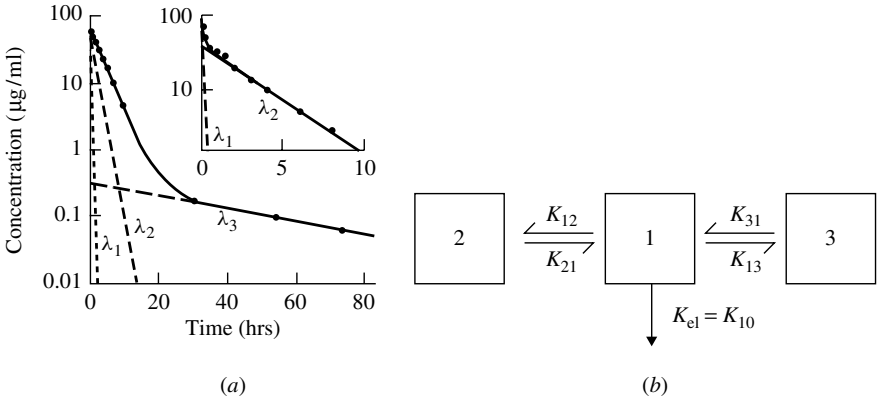


Figure 6.14 (*a*) Semilog plot of plasma concentration for (C_p) versus time representative of a three- or multi-compartment model. The curve can be broken down into three phases, λ_1, λ_2, and λ_3. (*b*) Three-compartment model with transfer rate constants, K_{12}, K_{21}, K_{13}, K_{31}, and elimination rate constant, K_{el}. As these models can get more complicated, the α, β, and γ nomenclature may get replaced with λ_n as indicated in the profile.

rate processes for the toxicant after entry. The resolution of the model necessitates mathematical estimates (as a function of time) concerning the absorption, distribution, biotransformation, and excretion of the toxicant.

Drugs and toxicants with multi-exponential behavior depicted in Figure 6.14 require calculation of the various micro constants. An alternative method involves using model-independent pharmacokinetics to arrive at relevant parameters. Very briefly, it involves determination of the area under the curve (AUC) of the concentration-time profiles. The emergence of microcomputers in recent years has greatly facilitated this approach.

In conclusion, pharmacokinetics is a study of the time course of absorption, distribution, and elimination of a chemical. We use pharmacokinetics as a tool to analyze plasma concentration time profiles after chemical exposure, and it is the derived rates and other parameters that reflect the underlying physiological processes that determine the fate of the chemical. There are numerous software packages available today to accomplish these analyses. The user should, however, be aware of the experimental conditions, the time frame over which the data were collected, and many of the assumptions embedded in the analyses. For example, many of the transport processes described in this chapter may not obey first-order kinetics, and thus may be nonlinear especially at toxicological doses. The reader is advised to consult other texts for more detailed descriptions of these nonlinear interactions and data analyses.

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