1

General considerations

People are exposed to a great variety of natural and synthetic substances. Under certain conditions such exposures produce adverse health effects, ranging in severity from subtle biological changes to death. Society's ever-increasing desire to identify and prevent these effects has prompted the dramatic evolution of toxicology as a study of poisons to the present-day complex science.

DEFINITION AND PURPOSE OF TOXICOLOGY

Toxicology is the study of the nature and mechanisms underlying toxic effects exerted directly or indirectly by substances such as biological, chemical, physical, genetic or psychological agents on living organisms and other biological systems. Toxicology also deals with quantitative or qualitative assessment of the adverse effects in relation to the concentration or dosage, duration, and frequency of exposure of the organisms.

The assessment of health hazards of industrial chemicals, environmental pollutants, and other substances represents an important element in the protection of the health of the workers and members of communities. In-depth studies of the nature and mechanism of the effects of toxicants are invaluable in the invention of specific antidotes and other ameliorative measures. Along with other sciences, toxicology contributes to the development of safer chemicals used as drugs, food additives, and pesticides, as well as many useful industrial chemicals for computers, cellular phones, televisions, and electronic equipment. Even the adverse effects per se are exploited in the pursuit of more effective insecticides, anthelmintics, antimicrobials, antivirals, and warfare agents. The purpose of toxicology is to protect human health or ecosystems from the exposure to hazardous substances.

SCOPE AND SUBDISCIPLINES

Toxicology is a science that has a broad scope. It deals with toxicity studies of substances used (*i*) in medicine for diagnostic, preventive, and therapeutic purposes; (*ii*) in the food industry as direct and indirect additives; (*iii*) in agriculture

as pesticides, growth regulators, artificial pollinators, and animal feed additives; and (*iv*) in the chemical industry as solvents, components, and intermediates of plastics, components of electronic devices and many other types of chemicals. It is also concerned with the health effects of metals (as in mines and smelters), radiation, petroleum products, paper and pulp, flame retardants, toxic plants, and animal toxins. Overall, toxicology covers general safety issues in our life.

Because of its broad scope as well as the need to accomplish different goals, toxicology has a number of subdisciplines. For example, a person may be exposed, accidentally or otherwise, to excessively large amounts of a toxicant and become severely intoxicated. If the identity of the toxicant is not known, *Analytical Toxicology* will be called upon to identify the toxicant through analysis of body fluids, stomach contents, suspected containers, etc. Those engaged in *clinical toxicology* administer antidotes, if available, to counter some specific toxicity, and take other measures to ameliorate the symptoms and signs and hasten the elimination of the toxicant from the body. There may also be legal implications, and that will be the task of *forensic toxicology*.

Intoxication may occur as a result of occupational exposure to toxicants. This may result in acute or chronic adverse effects. In either case, the problem is in the domain of *occupational toxicology*. The general public is exposed to a variety of toxicants, via air, water, and soil, contact with skin as well as from food as additives, pesticides, and contaminants, often at low levels that may be harmless acutely but may have long-term adverse effects. In pregnancy, the fetus is exposed via the maternal circulation while a lactating infant is exposed via breast milk. The sources of these substances, their transport, degradation, and bioconcentration in the environment, and their effects on humans are dealt with in *environmental toxicology*. *Regulatory toxicology* attempts to protect the public by setting laws, regulations, and standards to limit or suspend the use of very toxic chemicals as well as defines use conditions for others. Some of the relevant laws in the United States are listed in Appendix 1.

To set meaningful regulations and standards, extensive profiles of the toxic effects are essential. Such profiles can be established only with a great variety of relevant and comprehensive toxicological data derived from in-vitro, in-vivo, and human studies, which form the foundation of regulatory toxicology.

The basic part of such studies is referred to as *conventional toxicology*. In addition, knowledge of the mechanism of action, provided by *mechanistic toxicology*, enhances the toxicological evaluation and provides a basis for other branches of toxicology. The knowledge gained is then utilized to assess the risk of adverse effects to the environment and humans and is termed as risk assessment. A health risk assessment constitutes a written document that is based upon all pertinent scientific information regarding toxicology, human experiences, environmental fate, and exposure scenario. These data are subject to critique and interpretation. The aim of risk assessment is to estimate the potential of an adverse effect that occurs in humans and wildlife ecological system posed by exposure to a specific amount of toxic substances. Risk assessments include several elements such as

(*i*) description of the potential adverse health effects based on an evaluation of results of epidemiological, clinical, preclinical, and environmental research; (*ii*) extrapolation from these results to predict the type and estimate the extent of adverse health effects in humans under given conditions of exposure; (*iii*) judgments as to the number and characteristics of individuals exposed at various intensities and durations; (*iv*) and summary judgments on the existence and overall magnitude of the public health problem (Paustenbach, 2002). Risk characterization represents the final and the most critical step in the risk assessment process whereby data on the dose–response relationship of a chemical are integrated with estimates of the degree of exposure in a population to characterize the likelihood and severity of a health risk outcome (Williams and Paustenbach, 2002).

EARLY DEVELOPMENTS

The ancient man was well aware of the toxic effects of a number of substances, such as venom of snakes, the poisonous plants like hemlock and aconite, and the toxic heavy metals such as arsenic, lead, and antimony. Some of these were actually used intentionally for their toxic effects to commit homicide and suicide. For centuries, homicides with toxic substances were common in Europe. To protect against poisoning, there were continual efforts directed toward the discovery and development of preventive and antidotal measures. However, a more critical evaluation of these measures was only begun by Maimonides (1135–1204) with his famous medical work *Poisons and Their Antidotes*, published in 1198.

More significant contributions to the evolution of toxicology were made in the sixteenth century and later. Paracelsus stated: "No substance is a poison by itself. It is the dose (the amount of the exposure) that makes a substance a poison" and "the right dose differentiates a poison from a remedy." These statements laid the foundation for the concept of the "dose–response relation" and the "therapeutic index" developed later. In addition, he described in his book *Bergsucht* (1533–1534) the clinical manifestations of chronic arsenic and mercury poisoning as well as miner's disease. He might be considered the forefather of occupational toxicology. Orfila wrote an important treatise (1814–1815) describing a systematic correlation between the chemical and biological information on certain poisons.

He also devised methods for detecting poisons and pointed to the necessity for chemical analysis for legal proof of lethal intoxication. The introduction of this approach ushered in a specialty area of modern toxicology, namely, forensic toxicology.

RECENT DEVELOPMENTS

In the face of growing population, modern society demands improvements in health and living conditions, including nutrition, clothing, dwelling, and transportation. To meet this goal, a great variety of chemicals, many of them in large

quantities, must be manufactured and used. It has been estimated that tens of thousands of different chemicals are in commercial production in industrialized countries. In one way or another, these chemicals come in contact with various segments of the population: individuals are engaged in their manufacture, handling, use (e.g., painters, applicators of pesticides), consumption (e.g., drugs, food additives, natural food products or neutraceuticals), or misuse (e.g., suicide, accidental poisoning, and environmental disasters). Furthermore, people may be exposed to more persistent chemicals via various environmental media and be affected more insidiously. To illustrate the devastating effects of toxicants, some examples of massive acute and long-term poisonings are listed in Appendix 2. In some of these episodes, a considerable amount of sophisticated toxicological investigation was conducted before the etiology was ascertained. These and other tragic outbreaks of massive chemical poisonings have resulted in intensified testing programs, which have revealed the great diversity of the nature and site of toxic effects. This revelation, in turn, has called for more studies using a greater number of animals, a greater number of indicators of toxicity, biomonitoring of chemicals, etc. There is, therefore, a need to render the task of toxicologically assessing the vast number of chemicals by using increasingly more complex testing procedures to be made more manageable. As an attempt to fulfill this need, criteria have been proposed and adopted for the selection of chemicals to be tested according to their priority. In addition, the "tier systems" allow decisions to be made at different stages of toxicological testing, thus avoiding unnecessary studies. If a chemical is found to be exceedingly toxic using a simplified tier 1 screen such as loss of renal creatinine excretion and histopathology damage, then the compound is removed from the market and does not require more sophisticated tier 2 or 3 testing. This procedure is particularly remarkable because the current testing system for carcinogenicity, mutagenicity, and immunotoxicity, and reproductive capacity is quite expensive and involves a multitude of tests (chaps. 7, 8, 11, and 18, respectively).

Because the number of individuals exposed to these chemicals being large, society cannot defer appropriate control until serious injuries have appeared. The modern toxicologist, therefore, must attempt to identify, where possible, indicators of exposure, and early, reversible signs of adverse health effects. These will permit the formulation of decisions at the appropriate step to safeguard the health of individuals, either as occupational workers or in exposed communities. The achievements in these areas have assisted responsible personnel in instituting appropriate medical surveillance of occupational workers and other exposed populations. Notable examples are the use of cholinesterase inhibition as an indicator of exposure to organophosphorous pesticides or various biochemical parameters to monitor exposure to lead. Such "biological markers" or "biomarkers" are intended to measure exposure to toxicants or their effects as well as to detect susceptible population groups (NRC, 1987); they are used for clinical diagnosis, monitoring of occupational workers, and facilitating safety/risk assessment (WHO, 1993). Recently, systems toxicology (i.e., toxicogenomics, toxicoproteomics, and toxicometabolomics) has been considered a promising approach to develop new biomarkers comprehensively for predicting stage-dependent toxicity of substances and risk assessment (McHale et al., 2010; Yang et al., 2010).

Advances made in biochemical and toxicokinetic studies as well as those in genetic toxicology, immunotoxicology, reproductive toxicology, morphological studies on a subcellular level, and biochemical studies on a molecular and genetic level have all contributed to a better understanding of the nature, site, and mechanisms of action of toxicants. For example, technological breakthroughs enabled in-vitro studies to demonstrate whether hepatocytes or nonparenchymal cells affected by a chemical carcinogen are related to differences in their ability to repair the DNA damage induced by the chemical. Studies using isolated nephrons have provided insight into the site and mode of action of nephrotoxicants (chap. 14). Various other types of in-vitro studies have demonstrated the possibility of their use in screening toxicants for specific effects such as mutagenicity and dermal irritancy. Numerous studies have shown that the responses to toxicants are better correlated with the effective dose, that is, the concentration of the toxicant at the target site of action, rather than the administered dose. Furthermore, it is important that we know whether the effects observed result mainly or entirely from an active metabolite, or from the concentration of the metabolite formed rather than that of the parent chemical.

An important function of toxicology is to determine safe levels of exposure to natural and synthetic chemicals (see the section "Scopes and Subdisciplines" that discusses risk assessment), thereby preventing the adverse effects of exposures to toxicants. One of the earliest official actions in this field was taken by the U.S. Food and Drug Administration. It stipulated that a 100-fold margin was required for a food additive to be permitted for use. In other words, a chemical additive should not occur in the total human diet in a quantity greater than one-hundredth of the amount that is the maximum safe dosage in long-term animal experiments (Lehman and Fitzhugh, 1954). For several reasons this approach was not practicable on an international level (chap. 20). While evaluating a number of food additives in 1961, WHO coined the term "acceptable daily intake (ADI)" (WHO, 1962). Using the ADI procedure, WHO has since convened annual meetings of experts on food additives, contaminants, residues of veterinary drugs, and pesticide residues. Assessment of these chemicals resulted, where appropriate, in the assignment of ADIs (chap. 20). The term "ADI" and the WHO evaluations have been adopted by regulatory agencies in many nations. The inception, evolution, and application of ADI have been outlined by Lu (1988). For toxicants in the occupational settings, quantitative assessments are provided in terms of "threshold limit values" (Federal Register, 1971).

These determinations involve comprehensive studies of the toxic properties, demonstration of dosages that produce no observable adverse effects, establishment of dose–effect and dose–response relationships, and toxicokinetic and biotransformation studies. The greatly increased scope and the multiplicity of subdisciplines as outlined above provide a vivid view of recent progress in toxicology.

SOME CHALLENGES AND SUCCESSES

The so-called aniline tumors were reported by Rehn (1895), a German surgeon, in the urinary bladders of three men who had worked in an aniline factory. The role of aniline and aniline dyes as etiological agents was confirmed only some 40 years later, after much experimental investigation in animals (e.g., Hueper et al., 1938), and extensive epidemiological studies by Case et al. (1954) had been carried out. This discovery led to improved occupational standards and more stringent controls of food colors derived from coal tar.

In the late 1950s, thalidomide was widely used as a sedative. It has a very low acute toxicity and readily met the toxicity-testing protocol prevailing at that time. However, a rare form of congenital malformation, phocomelia (the virtual absence of extremities), was observed among some offspring of mothers who had taken this drug during the first trimester (Lenz and Knapp, 1962). This tragedy led to the explosive development of teratology (developmental toxicology), an important specialty area of toxicology. The importance of modifying factors has been dramatized by the tragic effect of cobalt among heavy beer drinkers (chap. 5).

The once prevalent lead poisoning in certain areas of industrialized countries has now largely disappeared. This great accomplishment in the field of public health has resulted from the implementation of control measures devised on the basis of the knowledge gained from the numerous toxicological studies of lead. However, this has now raised new concerns. Lead has been replaced by the gasoline additive methylcyclopentadienyl manganese tricarbonyl (MMT). Combustion of MMT-containing gasoline generates tailpipe emissions of manganese and studies have shown that manganese produces central nervous system disturbances (Gwiadza et al., 2007). Thus, it should be borne in mind that removal of one chemical and replacement with another does not necessarily reduce the risk for development of adverse effects.

Many cases of serious illness that culminated in permanent paralysis and death were reported in Minamata and Niigata in Japan in the 1950s and 1960s, respectively (Study Group of Minamata Disease, 1968; Tsubaki and Irukayama, 1977). The cause of the illness was eventually traced to methyl mercury in the fish caught locally. The fish were contaminated with this chemical, which had been discharged as such, that is, untreated into the water by a factory, or the contaminant was derived from elemental mercury discharged by the factory and methylated through microorganisms in the mud. Measures to rehabilitate the surviving patients and legal control of the factories have been instituted.

On the other hand, the cause of another mysterious illness in Japan, known as *itai-itai* disease, remains unsolved, although cadmium apparently played a role. The patients had resided for many years in areas that were in the vicinity of mines and where the cadmium levels in rice and water were excessive.

A more solid foundation in the assessment of risks of chemical carcinogens resulted from recent advances in epidemiological studies, long-term animal studies, short-term mutagenesis/carcinogenesis tests, and mechanistic studies, as well as the realization that carcinogens differ in their potency, latency, and mode of action depending on species, strains, and sex (chaps. 7 and 25).

TOXICITY VS. OTHER CONSIDERATIONS

In general, exposure to toxic substances is to be avoided. However, the severity of the effects varies greatly; some chemicals induce mild, transient, reversible effects, whereas those of others may be irreversible, serious, and even fatal. Exposure to the former type of substances might thus be acceptable, but, as a rule, not the latter. Examples of the exceptions: methyl mercury, which is extremely toxic, is present in many species of fish. Because of the nutritional value of fish, permissible levels of methyl mercury are established to minimize the risk, yet not deny this valuable source of nutrients. Aflatoxin B_1 is one of the most potent carcinogens but is present in a variety of foods. Yet the contaminated food is not banned as long as the toxin does not exceed the permissible levels.

The complex nature of assessing the toxicity of a substance in light of its benefits is also exemplified by the toxicology seen in lactation and over-the-counter (OTC) products. The former involves weighing the benefits of breast feeding versus the toxicity of certain potential contaminants. OTC products, when improperly used, present toxicological problems. However, the value of these products in general cannot be ignored. There is a growing debate of natural food products (nutraceuticals), where the benefits are accepted by some, yet these products have not been assessed toxicologically and there are reports of adverse effects. The therapeutic value of these products is subject to debate and needs extensive study as the number of consumers is in the millions, but the toxicity remains unknown.

The perception of risk and the benefits to society are crucial. It is clearly documented that with the introduction of chemicals to control infectious diseases and diminished occupational exposure through the use of protective gear have increased the life expectancy (or benefit) for humans. However, to completely eliminate any risk at all requires excessively high costs. Hence, it may not be realistic to derive any benefits if society demands that all risk be removed no matter what the cost is. In essence, by completely removing all chemicals and potential risks, the life expectancy will decrease and mortality will rise. The concept of acceptable risk and benefit ratio needs to be borne in mind. The dilemmas involved in these topics are further described and discussed in other chapters.

FUTURE PROSPECTS

The need of new substances will undoubtedly continue. Some of them will treat or prevent a variety of diseases, which are currently untreatable or unpreventable. Others will render food more plentiful, tastier, and hopefully healthier. Still others will improve living conditions in various ways. At the same time, people are more conscious of subtle adverse effects on health and expect the new substances to be

"absolutely safe." Furthermore, the disposal of these substances and their by-products is expected to produce no environmental hazards, adversely affecting humans and the ecosystem.

To satisfy these seemingly irreconcilable societal demands, a toxicologist must carry out a series of studies on each substance: is it readily absorbed, distributed to specific organs, stored, and/or readily excreted? Is it detoxicated or bioactivated? What kind of adverse effects does it induce and what are the host and environmental factors, termed confounding factors, which can alter these effects? How does it produce the effect on a cellular and molecular level? What type of "general toxicity" does it produce? What organs are its targets? What is its predominant mechanism of action? How or can it be eliminated from the organism? Answers to these and other questions will provide a scientific basis for assessing its safety and risk for the intended use.

It is evident that the multitude of studies involved will place an increasing demand on the limited facilities for toxicological testing and on the short supply of qualified personnel. It is of utmost importance, therefore, that toxicity data generated anywhere be accepted internationally. However, to ensure general acceptance, the data must meet certain standards. The "Good Laboratory Practice" promulgated by the U.S. Food and Drug Administration (FDA, 1980) and the Organization of Economic Cooperation and Development (OECD, 1982) should be adopted by all countries involved in toxicological testing.

To streamline the long and costly testing of each chemical separately, there have been schemes that test a representative chemical extensively and verify the results on other members of the group with minimal testing. This practice has been adopted successfully when the substances included in a group are essentially similar. A proposal to ban all chlorine compounds, however, appears to have gone too far in ignoring the great diversity of the toxic nature and potency of such a large group of substances (Karol, 1995). Similar calls for the ban of substances containing bromine, especially flame retardants present in furniture, computers, and televisions, which are crucial for protection against hazards from fire damage, have been instituted by a segment of the population based on the adverse effects of these chemicals. It will be a major challenge to determine how diverse a variety of chemicals can be rationally grouped for toxicological testing and assessment purposes.

Other trends designed to simplify and hasten the testing include a reduction in the use of laboratory animals and supplement or supplant them with in-vitro studies. This is done partly in response to a societal call on humane grounds.

Isolated organs, cultured tissues and cells, and lower forms of life will be increasingly used. Furthermore, such test systems will likely be faster and less expensive, and will augment the variety of studies, especially those related to the mechanism of toxicity. An understanding of the mechanism of action of a chemical is often valuable in providing a sounder basis for the assessment of its safety and risk. Other types of improvements of the testing procedure with respect to simplicity and reliability will continue to be made. Currently there is a major

movement termed "Toxicity Testing in the 21st Century" where computational models are being developed to predict the toxicity of chemicals based upon some in-vitro data (Rhomberg, 2010; Roggen, 2011). Although this will go a long way in reducing animal usage, the computational model cannot always predict effects in vivo in animals and humans. One must bear in mind that this is only a model and can have a useful function as a tier 1 screen as in-vivo testing will still be required.

As noted earlier, to provide a basis for proper assessment of the safety or risk of a chemical and for a variety of other purposes, toxicology is increasingly becoming a multifaceted science. To facilitate the acquisition of a broad knowledge of toxicology, this book covers four major areas.

Part I describes general topics related to absorption, distribution, and excretion of toxicants, their transformation in the body, the various types of toxic effects they exert, and the host and environmental factors that modify these effects.

Procedures used in determining the general and specific effects are described in Part II.

Part III describes the organ/system-specific toxicants and the procedures commonly used to detect their effects. Part IV discusses several major groups of toxicants such as food additives and contaminants, pesticides, metals, OTC preparations, various environmental pollutants, and toxicants in the workplace. The last chapter outlines the widely adopted approaches to the assessment of safety and risk of noncarcinogenic and carcinogenic chemicals. In addition, two indices are appended listing chemicals and subjects, respectively, to assist the reader in retrieving relevant parts of the text.

REFERENCES

Case RAM, Hosker ME, McDonald DB, et al. (1954). Tumours of the urinary bladder in workmen engaged in the manufacture and use of certain dyestuff intermediates in the British chemical industry. Br J Ind Med 11, 75–104.

FDA. (1980). Code of Federal Regulations, Title 21, Food and Drugs. Part 58. Washington, DC: US. Government Printing Office.

Federal Register. (1971). Threshold Limit Values Adopted by the American Conference of Governmental Industrial Hygienists, 1968, vol. 36, no. 105, May 29. Washington, DC: US. Government Printing Office.

Gwiadza R, Lucchini R, Smith D (2007). Adequacy and consistency of animal studies to evaluate the neurotoxicity of chronic low-level manganese exposure in humans. J Toxicol Environ Health A 70, 594–605.

Hueper WC, Wiley FH, Wolfe HD (1938). Experimental production of bladder tumors in dogs by administration of beta-naphthylamine. J Ind Hyg Toxicol 20, 46–84.

Karol MH (1995). Toxicologic principles do not support the banning of chlorine: a society of toxicology position paper. Fundam Appl Toxicol 24, 1–2.

Lehman AJ, Fitzhugh OG (1954). 100-fold margin of safety. Q Bull Assoc Food Drug Officials U.S. 18, 33–5.

Lenz W, Knapp K (1962). Thalidomide embryopathy. Arch Environ Health 5, 100–5.

Lu FC (1988). Acceptable daily intakes: inception, evolution and application. Regul Toxicol Pharmacol 8, 45–60.
McHale CM, Zhang L, Hubbard AE, et al. (2010). Toxicogenomic profiling of chemically exposed humans in risk assessment. Mutat Res. 705, 172–83.
NRC Committee on Biological Markers. (1987). Biological markers in environmental health research. Environ Health Perspect 74, 3–9.
OECD. (1982). Good Laboratory Practice in the Testing of Chemicals. Paris Cedex, France: Organization of Economic Cooperation and Development.
Paustenbach DJ (2002). Human and Ecological Risk Assessment. Theory and Practice. New York, NY: John Wiley and Sons, Inc.
Rehn L (1895). Blasengeschwulste bei fuchsin-arbeiten. Arch Klin Chir 50, 588.
Rhomberg LR (2010). Toxicity testing in the 21st century: how will it affect risk assessment? J Toxicol Environ Health B Crit Rev. 13,361–75.
Roggen EL (2011). In vitro toxicity testing in the twenty-first century. Front Pharmacol. 2, 1–3.
Study Group of Minamata Disease. (1968). Minamata Disease. Minamata, Japan: Minamata University.
Tsubaki T, Irukayama K (1977). Minamata Disease: Methyl Mercury Poisoning in Minamata and Niigata, Japan. New York, NY: Elsevier Scientific.
WHO. (1962). Sixth Report of the Joint FAD/WHO Expert Committee on Food Additives. Geneva, Switzerland: World Health Organization.
WHO. (1993). Biomarkers and risk assessment: concepts and principles. Environ Health Criteria 155.
Williams PRD, Paustenbach DJ (2002). Risk characterization: principles and practice. J Toxicol Environ Health B 5, 337–406.
Yang B, Wang Q, Lei R, et al. (2010). Systems toxicology used in nanotoxicology: mechanistic insights into the hepatotoxicity of nano-copper particles from toxicogenomics. J Nanosci Nanotechnol 10, 8527–37.

Appendix 1 U.S. Laws that have a Basis in Toxicology

Responsible Agency	Law
Food and Drug Administration	Federal Food, Drug, and Cosmetic Act
Environmental Protection Agency	Federal Insecticide, Fungicide, and Rodenticide Act
	Clean Air Act
	Safe Drinking Water Act
	Toxic Substances Control Act
Consumer Product Safety Commission	Consumer Product Safety Act
	Federal Hazardous Substances Act
Occupational Safety and Health Administration	Occupational Safety and Health Act

Appendix 2 Examples of Outbreaks of Mass Poisoning in Humans

Location and Year	Toxicant	Adverse Effect	Number Affected
Detroit, MI., 1930	Tri-*o*-cresyl phosphate in "ginger jake"	Delayed neurotoxicity	16,000
London, UK, 1952	Sulfur dioxide and suspended particulate matter Win air	Increased deaths from heart and lung diseases	3000
Toyama, Japan, 1950s	Cadmium in rice	Severe kidney and bone disease ("*itai-itai* disease")	200[a]
Minamata, Japan, 1950s	Methyl mercury in fish	Severe neurological disease ("Minamata disease")	200[a,b]
Southeast Turkey, 1956	Hexachlorobenzene in wheat	Porphyria, neurological diseases	4000
Morocco, 1959	Tri-*o*-cresyl phosphate in adulterated oil	Delayed neurotoxicity	10,000
Japan, 1956–1977	Clioquinol	Subacute myelo-opticoneuropathy	10,000
Western Europe, late 1950s and early 1960s	Thalidomide	Phocomelia	10,000
Fukuoka, Japan, 1968	Polychlorinated biphenyls	Skin disease, general weakness	1700
Iraq, 1972	Methyl mercury in wheat	Deaths from neurological disease	500
		Nonfatal cases	50,000
Madrid, Spain, 1981	Toxic oil in food	Deaths with various symptoms and signs	340
		Nonfatal cases	20,000
Bhopal, India, 1984	Methyl isocyanate released into air	Deaths from acute lung disease	6000
		Nonfatal cases	200,000
Chernobyl	Radioactivity release	Deaths	31
		Childhood leukemia	75[a]
			Normally less than 1

[a]Many more cases with mild or moderate symptoms and signs of intoxication.
[b]Hundreds of cases occurred in Niigata, Japan, in the 1960s from the same source, fish.

2

Absorption, distribution, and excretion of toxicants

INTRODUCTION

A toxicant, apart from causing local effects at the site of contact, can be detrimental only after it is absorbed by the organism. Absorption can occur systematically through the skin, the gastrointestinal (GI) tract, the lungs, and several minor routes. Furthermore, the nature and intensity of the effects of a chemical on an organism depend on its concentration in the target organs. The concentration depends not only on the administered dose but also on other factors, including absorption, distribution, metabolism, receptor binding, storage, and excretion. For a chemical to be absorbed, distributed, degraded, and eventually excreted, a toxicant must pass through a number of cell membranes. A cell membrane generally consists of a biomolecular layer of lipid molecules with proteins scattered throughout the membrane (Fig. 2.1).

There are four mechanisms by which a toxicant may pass through a cell membrane; the most important of them is passive diffusion through the membrane. The others are filtration through the membrane pores, carrier-mediated transport, and engulfing by the cell. The last two mechanisms are different in that the cell takes an active part in the transfer of a toxicant across its membranes.

Passive Diffusion

Most toxicants cross cell membranes by simple, passive diffusion. The rate of passage is related directly to the concentration gradient across the membrane and to the lipid solubility. For example, mannitol is hardly absorbed (<2%), acetylsalicylic acid is fairly well absorbed (21%), and thiopental is even more readily absorbed (67%). It is noteworthy that the chloroform:water partition of the non-ionized forms of these chemicals is, respectively, <0.002, 2, and 100. For references to this and other examples, see Hogben et al. (1958).

Many toxicants are ionizable. The ionized form is often unable to penetrate the cell membrane because of its low lipid solubility. On the other hand, the

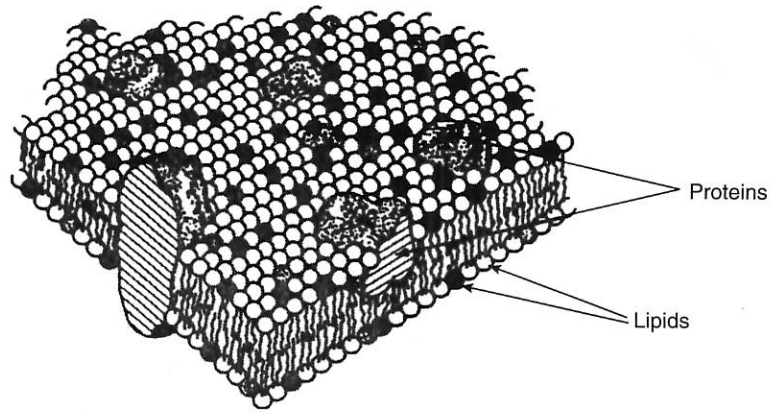

Figure 2.1 Schematic diagram of a biological membrane. Spheres represent head groups (phosphatidylcholine) and zigzag lines indicate tail ends of lipids. Black, white, and stippled spheres indicate different kinds of lipids. Large bodies represent proteins; some are located on the surface, others within the membrane. *Source*: From Singer and Nicholson (1972).

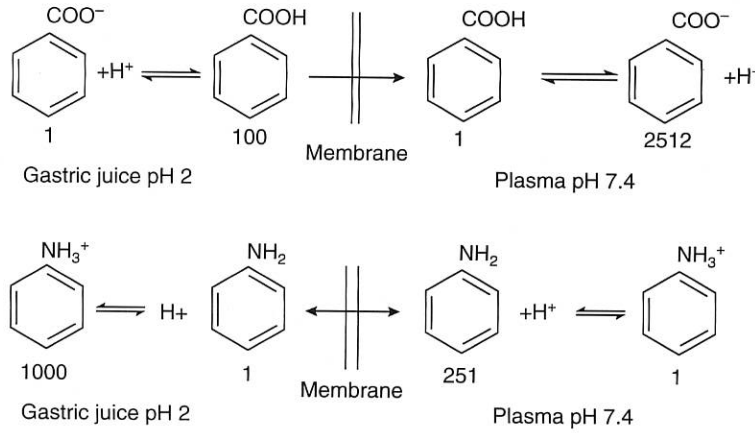

Figure 2.2 Disposition of benzoic acid and aniline in gastric juice and plasma. Figures immediately below the structural formulas represent proportions of ionized and non-ionized forms. *Source*: From Timbrell (1991).

non-ionized form is likely lipid-soluble enough to do so, and its rate of penetration depends on the lipid solubility. The extent of ionization of weak organic acids and bases depends on the pH of the medium. Thus, for the former, such as benzoic acid, diffusion is facilitated in acidic environment, where they exist mainly in the non-ionized form; for the latter, such as aniline, diffusion is facilitated in basic environment (Figs. 2.2 and 2.3).

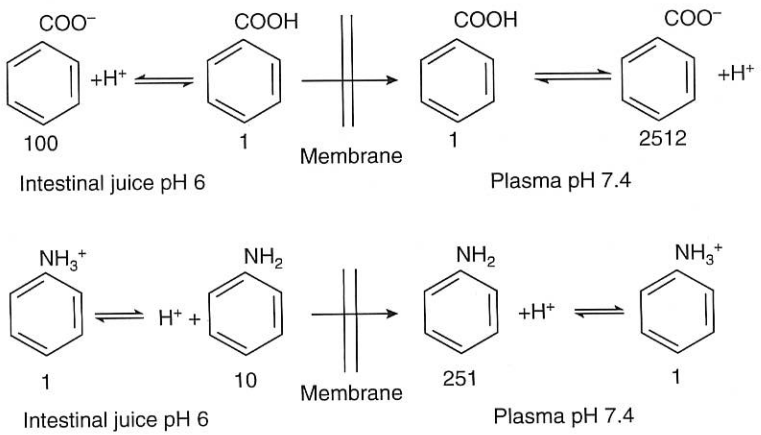

Figure 2.3 Disposition of benzoic acid and aniline in intestinal juice and plasma. *Source*: From Timbrell (1991).

Filtration

The membranes of the capillaries and the glomeruli have relatively large pores (about 70 nm) and allow molecules smaller than albumin (molecular weight 60,000 Da) to pass through. Bulk flow of water through these pores results from hydrostatic and/or osmotic pressure and can act as a carrier of toxicants. The pores in most cells, however, are relatively small (about 4 nm) and allow chemicals only up to a molecular weight of 100–200 Da to pass through. Chemicals of larger molecules, therefore, can filter into and out of the capillaries. They can, therefore, establish equilibrium between the concentrations in the plasma and in the extracellular fluid, but they cannot do so by filtration between the extracellular and intracellular fluids.

Carrier-Mediated Transport

This involves the formation of a complex between the chemical and a macromolecular carrier on one side of the membrane. The complex then diffuses to the other side of the membrane, where the chemical is released. Thereafter, the carrier returns to the original surface to repeat the transport process. The carrier has a limited capacity. When it is saturated, the rate of transport is no longer dependent on the concentration of the chemical and assumes zero order kinetics. Structure, conformation, size, and charge are important in determining the affinity of a chemical for a carrier site, and competitive inhibition can occur among chemicals with similar characteristics.

Active transport involves a carrier that moves molecules across a membrane against a concentration gradient, or, if the molecule is an ion, against an electrochemical gradient. It requires the expenditure of metabolic energy and can be inhibited by poisons that interfere with cell metabolism.

Facilitated diffusion is similar to active transport but does not move molecules against a concentration gradient. Furthermore, it is not energy-dependent, and metabolic poisons will not inhibit this process. Therefore, facilitated diffusion is classified as passive transport system.

Engulfing by the Cell (Endocytosis)

Particles may be engulfed by cells. When the particles are solid, the process is called phagocytosis and when they are liquid, it is called pinocytosis. This process is also called active transport and requires energy. Such special transport systems are important for the removal of particulate matter from the alveoli and of certain toxic substances from the blood by the reticuloendothelial system. Carrageenans (molecular weight about 10,000 ~ 40,000 Da) are also absorbed from the gut by this process.

ABSORPTION

The main routes by which toxicants are absorbed are the GI tract, lungs, and skin. However, in toxicologic studies, special routes such as intravenous, intraperitoneal, intradermal, intramuscular, and subcutaneous injections are also used. Other minor routes include intracardiac and intra-articular injection, and intraosseous infusion.

Gastrointestinal Tract

Many toxicants can enter the GI tract along with food and water or alone as drugs or other types of chemicals. With the exception of those that are caustic or very irritating to the mucosa, most toxicants do not exert any toxic effect unless they are absorbed. Absorption can take place along the entire GI tract. For example, certain drugs are administered as sublingual tablets and suppositories to be absorbed in the mouth and the rectum, respectively. However, the mouth and rectum are, in general, insignificant sites of absorption of environmental chemicals.

The stomach is a significant site of absorption, especially for weak acids, which will exist in the diffusible, non-ionized, and lipid-soluble form. On the other hand, weak bases will be highly ionized in the acidic gastric juice and therefore not readily absorbable. The difference in absorption is further amplified by the circulating plasma. Weak acids will exist mainly in ionized form in plasma and be carried away, whereas weak bases will exist in non-ionized form and can diffuse back to the stomach. The influence of these factors, using benzoic acid and aniline as examples, is shown in Figure 2.2.

In the intestine, weak acids will exist mainly in the ionized form; hence they are less readily absorbable. However, upon entering the blood, they become ionized and thus will not readily diffuse back. On the other hand, weak bases will exist

mainly in the non-ionized form; hence more readily absorbable, as shown in Figure 2.3. It should be noted that intestinal absorption is further enhanced by the long contact time and the large surface area provided by the villi and microvilli.

In the intestine, there are special carrier-mediated transport systems that are responsible for the absorption of nutrients, such as monosaccharides, amino acids, and elements such as iron, calcium, and sodium. However, a few toxicants, such as 5-fluorouracil (5-FU), thallium, and lead, are known to be absorbable from the intestine by active transport systems. In addition, particulate matters such as those of the azo dyes and polystyrene latex can enter the intestinal cell by pinocytosis.

Respiratory Tract

The main site of absorption in the respiratory tract is the alveoli in the lungs. This is especially true for gases such as carbon monoxide, nitrogen oxides, and sulfur dioxide, and for vapors of volatile liquids such as benzene and carbon tetrachloride. Their ready absorption is related to the large alveolar area, high blood flow, and proximity of the blood to the alveolar air.

The rate of absorption is dependent on the solubility of the gas in the blood—the more soluble it is, the faster the absorption. However, equilibrium between the air and the blood is reached more slowly for more soluble chemicals, such as chloroform, compared with less soluble chemicals, such as ethylene. This is because the more soluble a chemical, the more of it can be dissolved in the blood. As the alveolar air can carry only a limited amount of the chemical, more respirations and hence a longer time will be required to attain equilibrium. It will take even longer if the chemical is also deposited in the fat tissue.

In addition to gases and vapors, liquid aerosols and airborne particles may also be absorbed. In general, large particles (>10 μm) do not enter the respiratory tract; when they do, they are deposited in the nose and disposed of by wiping, blowing, and sneezing. Very small particles (<0.01 μm) are likely to be exhaled. Those in the range of 0.01–10 μm are deposited in various parts of the respiratory tract. The larger ones are likely to be deposited in the nasopharynx and absorbed either through the epithelium of this region or through the GI tract epithelium after they are swallowed along with the mucus. Smaller particles are deposited in the trachea, bronchi, and bronchioli, and are then either aspirated onto the mucociliary escalator or engulfed by phagocytes. The particles carried up by the escalator will be coughed up or swallowed. The phagocytes with engulfed particles will be absorbed into the lymphatics. Some free particles can also migrate into the lymphatics. Soluble particles may be absorbed through the epithelium into the blood.

A detailed examination of the deposition of particles of various sizes in different parts of the respiratory tract is provided by a Task Group on Lung Dynamics (1966). However, as a rough estimate, 25% of inhaled particles are exhaled, 50% are deposited in the upper respiratory tract, and 25% are deposited in the lower respiratory tract (Morrow et al., 1966).

Skin

In general, the skin is relatively impermeable, and therefore it constitutes a good barrier, separating the organism from its environment. However, some chemicals can be absorbed through the skin in sufficient quantities to produce systemic effects.

A chemical may be absorbed via the hair follicles or through the cells of the sweat glands or those of the sebaceous glands. These are, however, minor routes for absorption because they constitute only a small surface area of the skin. Therefore, the percutaneous absorption of a chemical is essentially through the skin proper, which consists of the epidermis and dermis (Fig. 15.1 of chap. 15).

The first phase of percutaneous absorption is diffusion of the toxicant through the epidermis, the most important barrier, and especially its stratum corneum. The stratum corneum consists of several layers of thin, cohesive, dead cells that contain chemically resistant material (protein filament). Small amounts of polar substances appear to diffuse through the outer surface of the protein filaments of the hydrated stratum corneum; non-polar substances dissolve in and diffuse through the lipid matrix between the protein filaments.

In the human stratum corneum, there are significant differences in structure and chemistry from one region of the body to another, which are reflected in the permeability to chemicals. For example, toxicants cross the scrotum readily, cross the abdominal skin less readily, and cross the sole and palm with great difficulty (Zbinden, 1976).

The second phase of percutaneous absorption is diffusion of the toxicant through the dermis, which contains a porous, non-selective, aqueous diffusion medium. Therefore, it is much less effective as a barrier than the stratum corneum; as a consequence, abrasion or removal of the latter causes a marked increase in the percutaneous absorption. Acids, alkalis, and mustard gases will also increase the absorption by injuring this barrier. Some solvents, notably dimethyl sulfoxide, also increase dermal permeability.

DISTRIBUTION

After a chemical enters the blood, it is distributed rapidly throughout the body. The rate of distribution to each organ is related to the speed and the amount of blood that flows through the organ, the ease with which the chemical crosses the local capillary wall and the cell membrane, and the affinity of components of the organ for the chemical.

Barriers

The *blood–brain barrier* (BBB) is located at the brain capillary wall. The capillary endothelial cells are tightly joined, leaving few or no pores between these cells (Bradbury, 1984).Thus, the toxicant has to pass through the capillary endothelium itself. A lack of vesicles in these cells further reduces their transport ability.

Finally, the protein concentration of the interstitial fluid in the brain is low, in contrast to that in other organs; protein binding therefore does not serve as a mechanism for the transfer of toxicants from the blood to the brain. For these reasons, the penetration of toxicants into the brain depends on their lipid solubility. An outstanding example is the toxicant methyl mercury, which enters the brain readily and the main toxicity of which is on the central nervous system. In contrast, inorganic mercury compounds are not lipid soluble, do not enter the brain readily, and exert their main adverse effects not on the brain but on the kidney. Glucose is transported across the brain capillary wall by glucose transporter the glucose transporter 2 (GLUT2).

The *placental barrier* differs anatomically among various animal species. There are six layers of cells between fetal and maternal blood in some species, whereas in others there is only one layer. Furthermore, the number of layers may change as the gestation progresses. Although the relationship of the number of layers of the placenta to its permeability needs quantitative determination, the placental barrier does impede the transfer of toxicants to the fetus, which is therefore protected to some extent. However, the concentration of a toxicant such as methyl mercury may be higher in certain fetal organs, such as the brain, because of the less-effective fetal blood–brain barrier. On the other hand, the fetal concentration of the food coloring amaranth is only 0.03–0.06% of that of the mother (Munro and Willes, 1978).

Other barriers are also present in organs such as the eyes and testes. In addition, the erythrocyte plays an interesting role in the distribution of certain toxicants. For example, its membrane acts as a barrier against the penetration of inorganic mercury compounds but not that of alkyl mercury. Furthermore, there is affinity of the erythrocyte cytoplasm for alkyl mercury compounds. Because of these factors, the concentration of inorganic mercury compounds in the erythrocytes is only about half that in the plasma, whereas that of methyl mercury in the erythrocyte is about 10 times that in the plasma (WHO, 1976).

Binding and Storage

As noted above, binding of a chemical in a tissue can result in a higher concentration in that tissue. There are two major types of binding. The covalent type of binding is irreversible and is, in general, associated with significant toxic effects. The non-covalent binding usually accounts for a major portion of the dose and is reversible. Therefore, this process plays an important role in the distribution of toxicants in various organs and tissues. There are several types of non-covalent binding as outlined by Guthrie (1980). In general, storage of toxicants in non-target organs may be considered as the first step to protect our body, but their chronic release from non-target organs into the body may produce chronic intoxication later.

Plasma proteins can bind normal physiological constituents in the body as well as many foreign compounds. Most of the latter are bound to the albumin and are therefore not immediately available for distribution to the extravascular space. However, since the binding is reversible, it permits the bound chemical to

dissociate from the protein, thereby replenishing the level of unbound chemical, which may then cross the capillary endothelium. The toxicologic significance of the binding can be illustrated by the possible induction of coma by the administration of sulfonamide drugs to patients who are taking antidiabetic drugs. The antidiabetic drugs are bound to the plasma proteins but can be replaced by the sulfonamide drugs, which have a greater affinity for the plasma protein. The antidiabetic drugs thus released may precipitate a hypoglycemic coma. Other types of plasma proteins for storage are lipoproteins (for vitamins, cholesterol, steroid, lipid soluble agents, and basic drugs), gamma globulins for antigens, transferrin for iron, and ceruloplasmin for copper.

The *liver* and *kidney* have a higher capacity for binding chemicals. This characteristic may be related to their metabolic and excretory functions. Certain proteins have been identified in these organs for their specific binding property, such as metallothionein (MT), which is important for the binding of cadmium in the liver and kidney, and possibly also for the transfer of the metal from the liver to the kidney. Binding of a substance can increase its concentration in an organ rapidly. For example, 30 minutes after a single administration of lead, its concentration in the liver is 50 times higher than that in the plasma. MTs can be also upregulated in various organs/tissues by inflammatory stimuli, suggesting the possible target of MTs in inflammatory diseases (Inoue et al., 2009). MTs (e.g., MT-I, MT-II, MT-III, and MT-IV, and other isoforms) have various biological effects on heavy metal homeostasis, cancer, circulation, diabetes, immune system, and antioxidative defense (Thirumoorthy et al., 2011).

The *adipose tissue* is an important storage depot for lipid-soluble substances such as dichlorodiphenyltrichloroethane (DDT), dieldrin, and polychlorinated biphenyls (PCBs). They appear to be stored in the adipose tissue by simple dissolution in the neutral fats. There exists the potential that the plasma concentration of the substances stored in the fat may increase sharply as a result of rapid mobilization of fat following starvation. Conjugation of fatty acid to toxicants, such as DDT, may also be a mechanism by which these chemicals are retained in the lipid-containing tissues and cells of the body (Leighty et al., 1980).

Bone is a major site for storage of such toxicants as fluoride, lead, and strontium. The storage takes place by an exchange adsorption reaction between the toxicants in the interstitial fluid and the hydroxyapatite crystals of bone mineral. By virtue of similarities in size and charge, F^- may readily replace OH^-, and calcium may be replaced by lead or strontium. These stored substances can be released by ionic exchange and by dissolution of bone crystals through osteoclastic activity.

EXCRETION

After absorption and distribution in the organism, toxicants are excreted, rapidly or slowly. A generally accepted indicator of the rate of elimination of a toxicant is its "half-life" ($t_{½}$), which is the time required to remove 50% of it from the bloodstream.

The toxicants are excreted as the parent chemicals, as their metabolites, and/or as conjugates of them. The principal routes of excretion are the urine and feces, but the liver and lungs are also important excretory organs for certain types of chemicals. In addition, there are a number of other minor routes for excretion, including, sweat, saliva, and milk.

Urinary Excretion

The kidney removes toxicants from the body by the same mechanisms as those used in the removal of end products of normal metabolism, namely, glomerular filtration, tubular diffusion, and tubular secretion.

The glomerular capillaries have large pores (70 nm); therefore, most toxicants will be filtered at the glomerulus, except those that are very large (greater than 60,000 Da) or those that are tightly bound to plasma protein. Once a toxicant enters the glomerular filtrate, it will either be passively reabsorbed across the tubular cells if it has a high lipid/water partition coefficient or remains in the tubular lumen and be excreted if it is a polar compound.

A toxicant can also be excreted through the tubules into the urine by passive diffusion. As urine is normally acidic, this process plays a role in the excretion of organic bases. On the other hand, organic acids are unlikely to be excreted by passive diffusion through the tubular cells. However, weak acids are often metabolized to stronger acids, thereby increasing the percentage of the ionic forms that are not reabsorbed through the tubular cells and are thus excreted.

Certain toxicants can be secreted by the cells of the proximal tubules into the urine. There are two distinct secretory mechanisms, one for organic acids (e.g., glucuronide and sulfate conjugates) and the other for organic bases. Protein-bound toxicants can also be secreted, provided the binding is reversible. Furthermore, chemicals of similar characteristics compete for the same transport system. For example, probenecid can increase the serum level of penicillin and prolong its activity by blocking its tubular excretion.

Biliary Excretion

The liver is also an important organ for the excretion of toxicants, especially for compounds with high polarity (anionic and cationic), conjugates of compounds bound to plasma proteins, and compounds with molecular weights greater than 300 Da. In general, once these compounds are in the bile, they are not reabsorbed into the blood and are excreted via the feces. However, there are exceptions, such as the glucuronide conjugates, which can be hydrolyzed by intestinal flora, enabling the reabsorption of the free toxicants.

The importance of the biliary route of excretion for some chemicals has been well demonstrated in experiments that showed a several-fold increase of the acute toxicity in animals with ligated bile ducts. Such chemicals include digoxin, indocyanine green, ouabain, and, most dramatically, diethylstilbestrol (DES).

The toxicity of DES is increased by a factor of 130 in rats with ligated bile ducts (Klaassen, 1973).

Lungs

Substances that exist in the gaseous phase at body temperature are excreted mainly by the lungs. Volatile liquids are also readily excreted via the expired air. Highly soluble liquids such as chloroform and halothane are excreted slowly because of their storage in the adipose tissue and the limited ventilation volume. Excretion of toxicants from the lung is accomplished by simple diffusion through cell membranes. The breakdown of compounds to constituents such as carbon dioxide results in excretion of this gas via the lungs.

Other Routes

The *gastrointestinal tract* is not a major route of excretion of toxicants. However, because the human stomach and intestine, each secretes about 3 L of fluid per day, some toxicants are excreted along with the fluid. The excretion is mainly by diffusion and thus the rate depends on the pK_a of the toxicant and the pH of the stomach and intestine.

The excretion of toxicants in mother's *milk* is not important as far as the host organism is concerned. However, the presence of toxic substances in milk may be toxicologically significant because they can be passed in the milk from the mother to the nursing child and from cows to humans. The excretion is also via simple diffusion. As milk is slightly acidic, basic compounds will reach a higher level in milk than in plasma, while the opposite is true with acidic compounds. Lipophilic compounds such as DDT and PCB also reach a higher level in milk because of its higher fat content.

Sweat and *saliva* are also minor routes of excretion of toxicants. The excretion also occurs by diffusion; thus it is confined to the non-ionized, lipid-soluble forms of the toxicants. Substances excreted in the saliva are usually swallowed and then become available for reabsorption in the GI tract.

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING

Physiologically based pharmacokinetic (PBPK) models represent the body as a set of functional compartments, for example blood, brain, liver, muscle, skin, and the remainder of the body. A schematic PBPK model for manganese is illustrated in Figure 2.4. All compartments need not necessarily be involved as shown in Figure 2.5, as a PBPK model for methyl ethyl ketone, where brain as opposed to manganese, is not a significant target. PBPK facilitates simulation of pharmacokinetic profiles of chemicals on the basis of available information on relevant physiological, physicochemical, and biochemical factors (Thompson et al., 2008). These models utilize biological information to predict the disposition of

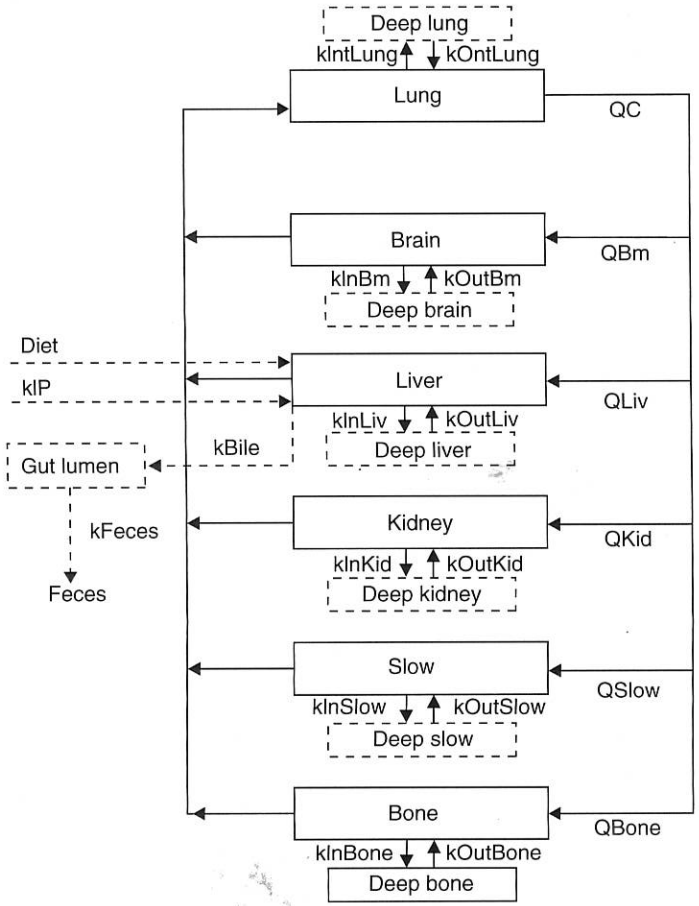

Figure 2.4 Schematic of physiologically based pharmacokinetic model for assessing tracer doses of radiolabeled and total manganese. *Source*: From Teeguarden et al. (2007).

chemicals for which limited human data are available. It is essential to understand that the chemical is present at low or pharmacological levels and the responses are linear. PBPK models are becoming increasingly important in chemical health risk assessment. In order to construct PBPK models to predict the disposition of chemicals, it is important to take into account genetic makeup, life stage, and health status, because these are confounding factors that may affect the disposition and subsequently the risk assessment. The PBPK model for an infant will be markedly different from an adult and thus the risk for a child in general is greater than that for an adult (Price et al., 2003). Governmental agencies have come to rely in recent years on PBPK modeling as an essential tool in the overall assessment of a chemical hazard for humans.

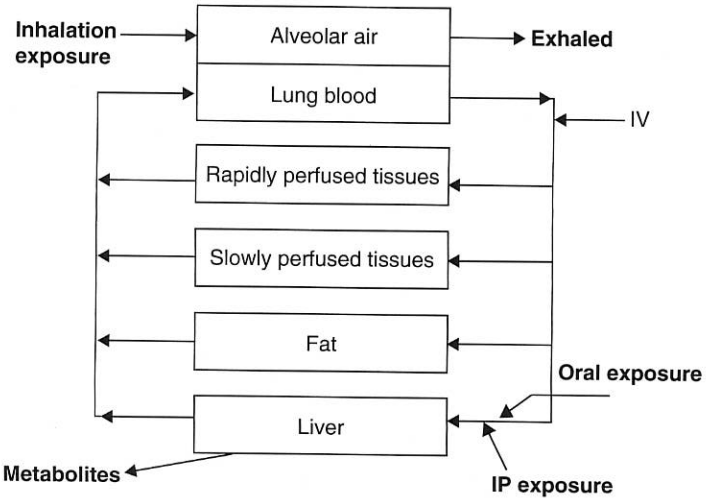

Figure 2.5 Schematic diagram of physiologically based pharmacokinetic model for methyl ethyl ketone. *Source*: From Thrall et al. (2002).

A further step is the use of physiologically based toxicokinetic (PBTK) models where the chemical is present in excessive or toxic levels and where the responses do not follow a linear pattern (Dixit et al., 2003). Toxicokinetic models are often defined based upon the nature of the rate processes, be it absorption across the GI tract, hepatic metabolism, or elimination by the kidney. A conceptual representation of a PBTK model for volatile organic chemicals is illustrated in Figure 2.6. At toxic concentrations, the solubility of chemicals is altered leading to different absorption patterns, excess bioavailability, and accumulation, and the predictability of chemical disposition becomes less reliable. A toxicokinetic model is only a tool to estimate chemical concentrations and generates parameters that are useful for further analysis and quantification of biological processes under study. Toxicokinetics serves as a bridge for extrapolating chemical concentrations across different species, from in-vitro to in-vivo studies, from chemical exposure to systemic dose in risk assessment, or as a link between physiology or genetics and chemical disposition in populations of animals and humans.

LEVELS OF TOXICANTS IN THE BODY

As noted above, the nature and intensity of the effects of a chemical depend on its concentration at the site of action, namely, the effective dose rather than the administered dose. The level in the target organ is, in general, a function of the blood level. However, binding of a toxicant in a tissue will increase its level, whereas tissue barriers tend to reduce the level. As the blood level is more readily

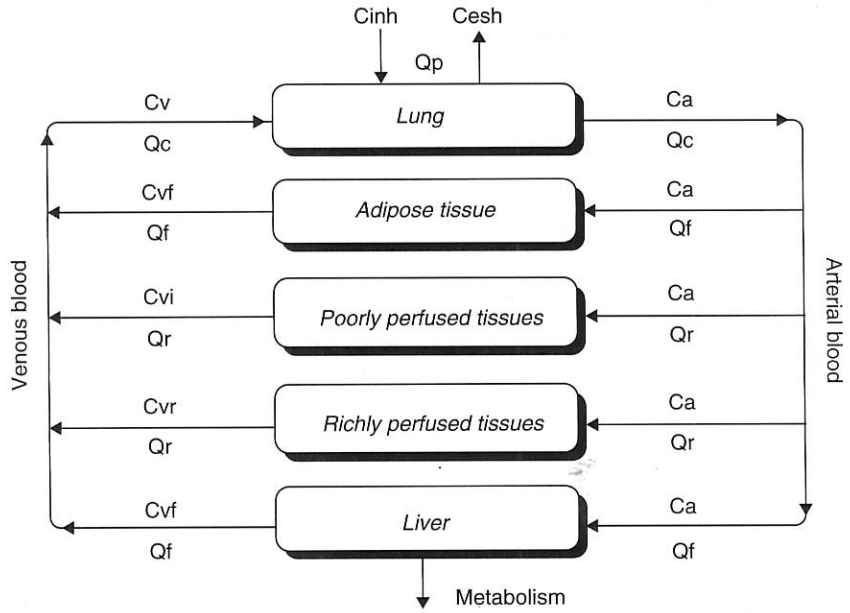

Figure 2.6 Conceptual representation of PBTK model for volatile organic chemicals. Qp and Qc refer to alveolar ventilation rate and cardiac output. Cinh, Cexh, Cv, and Ca refer to chemical concentration in inhaled air, exhaled air, venous blood, and arterial blood, respectively. Cvf and Qf refer to venous blood concentrations leaving tissue compartments and blood flow to tissues (i.e., f, adipose tissue; s, slowly perfused tissues; r, richly perfused tissues; and l, liver). *Source*: From Haddad et al. (2000).

determined, especially over a time period, it is the parameter often used in toxicokinetic studies.

While the toxicant is being absorbed, its blood level rises. In the meantime, the rates of its excretion, biotransformation (chap. 3), and the distribution to other organs and tissues also increase. The curve depicting the blood level against time and the area under that curve (AUC) are useful tools in toxicokinetics. In a series of experimental studies, Smyth and Hottendorf (1980) demonstrated that the AUC for a solution of a chemical is, in general, greater than that for its suspension, and it is greater for acidic than basic chemicals. They also illustrated the effects of the route of administration, dose level, and dosing vehicle on the AUC.

The influence of the rate of excretion on the blood level is vividly shown in Figure 2.7. Saccharin is rapidly excreted; hence its blood level drops rapidly, even after repeated administration. On the other hand, methyl mercury is excreted very slowly; its gradual accumulation culminates in a near plateau only after 270 days (Munro and Willes, 1978). It should be noted that the blood level of a chemical does not necessarily correlate with toxicity. In the case of blood lead levels, the concentration found was high but did not appear to correlate with any adverse

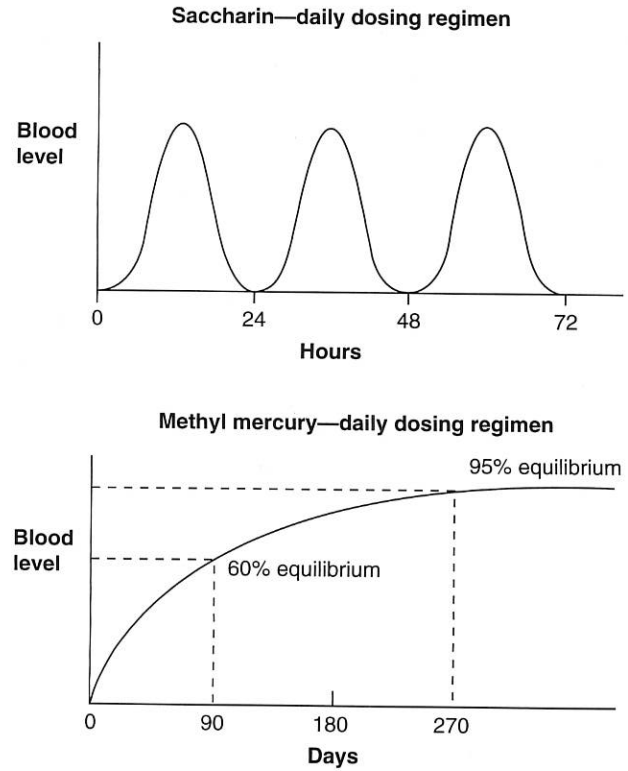

Figure 2.7 Comparative chemobiokinetics of saccharin and methyl mercury chloride. *Source*: From Munro and Willes (1978).

effects, especially in children. The blood chemical concentration is simply a marker of exposure and not necessarily toxicity.

Methods for determining the rate and extent of absorption, distribution, binding, storage, and excretion at various organs and tissues are readily found in the literature. A summary of some of them is given in a WHO publication and OECD guideline (WHO, 1986; OECD, 2009).

REFERENCES

Bradbury MWB (1984). The structure and function of the blood–brain barrier. Fed Proc 43, 186–90.

Dixit R, Riviere J, Krishnan K, et al. (2003). Toxicokinetics and physiologically based toxicokinetics in toxicology and risk assessment. J Toxicol Environ Health B 6, 1–40.

Guthrie FE (1980). Absorption and distribution. In: Hodgson E, Guthrie FE, eds. Introduction to Biochemical Toxicology. New York, NY: Elsevier.

Haddad S, Charest-Tardif G, Krishnan K (2000). Physiologically based modeling of the maximal effect of metabolic interactions on the kinetics of components of complex chemical mixtures. J Toxicol Environ Health A 61, 209–23

Hogben CAM, Tocco DJ, Brodie BB, et al. (1958). On the mechanism of intestinal absorption of drugs. J Pharmacol Exp Therap 125, 275–82.

Inoue K, Takano H, Shimada A, et al. (2009). Metallothionein as an anti-inflammatory mediator. Mediators Inflamm 2009, 7.

Klaassen CD (1973). Comparison of the toxicity of chemicals in newborn rats to bile duct-ligated and sham-operated rats and mice. Toxicol Appl Pharmacol 24, 37–44.

Leighty EG, Fentiman AF Jr, Thompson RM (1980). Conjugation of fatty acids to DDT in the rat: Possible mechanism for retention. Toxicology 15, 77–82.

Morrow PE, Hodge HC, Newman WF, et al. (1966). Deposition and retention models for internal dosimetry of the human respiratory tract. Health Phys 12, 173–207.

Munro IO, Willes AF (1978). Reproductive toxicity and the problems of in vitro exposure. In: Galli A, Paoletti R, Veterazzi G, eds. Chemical Toxicology of Food. Amsterdam, The Netherland: Elsevier/North Holland.

OECD. (2009). OECD guideline for the testing of chemicals draft proposal for a revised TG: 417 "Toxicokinetics". [online] Available from: http://www.oecd.org/dataoecd/26/32/44216274.pdf.

Price K, Haddad S, Krishnan K (2003). Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. J Toxicol Environ Health A 66, 417–33.

Singer SJ, Nicolson GC (1972). The fluid mosaic model of the structure of cell membranes. Science 175, 720–31.

Smyth AD, Hottendorf GH (1980). Application of pharmacokinetics and biopharmaceutics in the design of toxicological studies. Toxicol Appl Pharmacol 53, 179–95.

Task Group on Lung Dynamics. (1966). Deposition and retention models for internal dosimetry of the human respiratory tract. Health Phys 12, 173–207.

Thirumoorthy N, Shyam Sunder A, Manisenthil Kumar K, et al. (2011). A review of Metallothionein isoforms and their role in pathophysiology. World J Surg Oncol 20, 9–54.

Timbrell JA (1991). Principles of Biochemical Toxicology. London, UK: Taylor & Francis.

Thompson CM, Sonawane B, Barton HA, et al. (2008). Approaches for applications of physiologically based pharmacokinetic models in risk assessment. J Toxicol Environ Health B 11, 519–47.

WHO. (1976). Mercury: Environmental Health Criteria 1. Geneva, Switzerland: World Health Organization, p. 70.

WHO. (1986). Principles of Toxicokinetic Studies. Environmental Health Criteria 57. Geneva, Switzerland: World Health Organization.

Zbinden G (1976). Percutaneous drug permeation. In: Progress in Toxicology, vol. 2. New York, NY: Springer-Verlag.

Teeguarden JG, Gearhart J, Clewell HJ, et al. (2007). Pharmacokinetic modeling of manganese. III. Physiological approaches accounting for background and tracer kinetics. J Toxicol Environ Health A 70, 1515–26.

Thrall KD, Soelberg JJ, Weitz KK, et al. (2002). Development of a physiologically based pharmacokinetic model for methyl ethyl ketone in F344 rats. J Toxicol Environ Health A 65, 881–96.

3

Biotransformation of toxicants

GENERAL CONSIDERATIONS

As noted in the previous chapter, a toxicant can be absorbed into an organism through different routes. After absorption it is distributed to various parts of the body, including the excretory organs, and is thus available for excretion. Many chemicals are known to undergo biotransformation (metabolic transformation or metabolism), which occurs in the organs and tissues. The most important site of such reactions is the liver; the others being the lungs, stomach, intestine, skin, and kidneys. During the process of biotransformation, "xenobiotics" (foreign substances to an organism or toxic substances) can be either activated (toxic form) or inactivated (nontoxic form) to become reactive or nonreactive to biomolecules such as DNA, RNA, proteins, and lipids.

There are two types of biotransformation:

1. Phase I involving oxidation, reduction, and hydrolysis may be considered degradation reactions
2. Phase II involving the production of a compound (a conjugate) that is biosynthesized from the toxicant, or its metabolite, plus an endogenous metabolite may be considered conjugation reactions

Biotransformation is, therefore, a process that, in general, converts the parent compounds into metabolites and then forms conjugates, but it may involve only one of these reactions. For example, benzene undergoes oxidation, a phase I reaction, to form phenol, which conjugates with sulfate, a phase II reaction. However, when the administered chemical is phenol, it will conjugate with sulfate without a phase I reaction. The metabolites and conjugates are usually more water-soluble and more polar, hence more readily excretable. Biotransformation mediated by phase I and II metabolizing enzymes can, therefore, be considered a mechanism of detoxication (detoxication or bioinactivation) by the host organism.

However, it must be noted that in certain cases the metabolites are more toxic than the parent compounds. Such reactions are known as "bioactivation."

This is frequently noted with chemicals that induce cancer where the metabolite and not the parent compound is the culprit.

The rate of biotransformation and the type of biotransformation of a toxicant often differ from one species of animal to another and even from one strain to another, a fact that often accounts for the difference of toxicity in these animals (see chap. 5). The age and sex of the animal and its exposures to other chemicals may also alter the biotransformation. Genetic polymorphisms in xenobiotic-metabolizing enzymes and transporter have been considered a critical factor for individual susceptibility or variation in toxic responses (Ginsberg et al., 2009; Johansson and Ingelman-Sundberg, 2011). Knowledge of such factors is important in the design of toxicological studies and in the interpretation of health hazards of toxicants to humans.

PHASE I (DEGRADATION) REACTIONS

The three types of phase I reactions, namely, oxidation, reduction, and hydrolysis, are briefly described.

Oxidation

The biotransformation of a great variety of chemicals involves oxidative processes. The most important enzyme systems catalyzing the processes involve cytochrome P-450 and NADPH cytochrome P-450 reductase. In these reactions, one atom of molecular oxygen is reduced to water and the other is incorporated into the where substrate as follows: SH + O_2+ NADPH + H → SOH + HO + NAPD, S is the substrate.

The cytochrome-linked monooxygenases (oxidases) are located in the endoplasmic reticulum. When a cell is homogenized, the endoplasmic reticulum breaks down to small vesicles known as microsomes. Because of the location of these enzymes and the great variety of chemicals that they may catalyze, they are also known as microsomal, mixed-function oxidases (MFOs). The human P-450 MFO system consists of more than 30 isozymes. They catalyze a great variety of chemicals as shown in this chapter. In addition, oxidation of a number of toxicants is catalyzed by nonmicrosomal oxidoreductases that are located in the mitochondrial fraction in the 100,000 *g* supernatant of tissue homogenates.

Oxidation may take place in a variety of reactions, and often more than one metabolite is formed. The following are some examples.

Microsomal Oxidation

1. Aliphatic oxidation involves oxidation of the aliphatic side chains of aromatic chemicals: for example, *n*-propylbenzene → 3-phenylpropan-1-ol, 3-phenylpropan-2-ol, and 3-phenylpropan-3-ol, as well as aliphatic compounds such as *n*-hexane.

2. Aromatic hydroxylation generally proceeds through an epoxide intermediate: for example, naphthalene → naphthalene-1, 2-epoxide → 1-naphthol + 2-naphthol.
3. Epoxidation: for example, aldrin → dieldrin.
4. Oxidative deamination: for example, amphetamine → phenylacetone.
5. N-dealkylation: for example, *N,N*-dimethyl-*p*-nitrophenyl → carbamate *N*-methyl-*p*-nitrophenyl carbamate.
6. O-dealkylation: for example, *p*-nitroanisole → *p*-nitrophenol.
7. S-dealkylation: for example, 6-methylthiopurine → 6-mercaptothiopurine.
8. N-oxidation: for example, trimethylamine → trimethylamine oxide.
9. N-hydroxylation: for example, aniline → phenylhydroxylamine.
10. P-oxidation: for example, diphenylmethylphosphine → diphenylmethylphosphine oxide.
11. Sulfoxidation: for example, methiocarb → methiocarb sulfone.
12. Desulfuration involves the replacement of Sby O: for example, parathion → paraoxon.

Nonmicrosomal Oxidations Catalyzed by Enzymes in Mitochondria, Cytosol, and Nuclei

1. Amine oxidation: Monoamine oxidase is located in mitochondria and diamine oxidase is a cytosolic enzyme. Both are involved in the oxidation of the primary, secondary, and tertiary amines, such as 5-hydroxytryptamine and putrescine into their corresponding aldehydes.
2. Alcohol and aldehyde dehydrogenations are catalyzed, respectively, by alcohol dehydrogenase and aldehyde dehydrogenase: for example, ethanol → acetaldehyde → acetic acid.

Reduction

Toxicants may undergo reductions through the function of reductases. These reactions are less active in mammalian tissues but more so in intestinal bacteria. A notable example is the reduction of prontosil to sulfanilamide converting an inactive chemical to an effective antibacterial drug.

Microsomal Reduction

1. Nitro reduction: for example, nitrobenzene → nitrosobenzene → phenyl hydroxylamine -aniline.
2. Azo reduction: for example, azobenzene aniline.

Nonmicrosomal reductions occur via the reverse reaction of alcohol dehydrogenases (see the section, "Nonmicrosomal Oxidations Catalyzed by Enzymes in Mitochondria, Cytosol, and Nuclei").

Hydrolysis

Many toxicants contain ester-type bonds and are subject to hydrolysis. These are essentially esters, amides, and compounds of phosphate. Mammalian tissues, including the plasma, contain a large number of nonspecific esterases and amidases, which are involved in hydrolysis. The esterases, usually located in the soluble fraction of the cell, may be broadly categorized into four classes:

1. arylesterases, which hydrolyze aromatic esters;
2. carboxyl esterases, which hydrolyze aliphatic esters;
3. cholinesterases, which hydrolyze esters in which the alcohol moiety is choline; and
4. acetyl esterases, which hydrolyze esters in which the acid moiety is acetic acid.

In contrast to esterases, amidases cannot be classified according to substrate specificity. Furthermore, enzymatic hydrolysis of amides proceeds much more slowly than that of esters, probably a result of the lack of substrate specificity.

PHASE II (CONJUGATION) REACTIONS

Phase II reactions involve several types of endogenous metabolites that, as noted above, may form conjugates with the toxicants per se or their metabolites. These conjugates are generally more water soluble and more readily excretable. This is because the physiological transport mechanisms for the endogenous metabolites also recognize the conjugates and hence facilitate their excretion.

Glucuronide Formation

This is the most common and the most important type of conjugation. The enzyme catalyzing this reaction is uridine diphosphate -glucuronyl transferase and the coenzyme is uridine-5′-diphospho-α-d-glucuronic acid. This enzyme is also located in the endoplasmic reticulum. There are four classes of chemical compounds that are capable of forming conjugates with glucuronic acid: (*i*) aliphatic or aromatic alcohols, (*ii*) carboxylic acids, (*iii*) sulfhydryl compounds, and (*iv*) amines.

Sulfate Conjugation

This reaction is catalyzed by sulfotransferases. These enzymes are found primarily in the cytosolic fraction of liver, kidney, and intestine. The coenzyme is 3-phosphoadenosine-5′-phosphosulfate. The functional groups of the foreign compounds for sulfate transfer are phenols and aliphatic alcohols as well as aromatic amines.

Methylation

This reaction is catalyzed by methyl transferases. The coenzyme is S-adenosylmethionine. Methylation is not a major route of biotransformation of toxicants because of the broader availability of uridine-5′ -diphospho-α-d-glucuronic acid, which leads to the formation of glucuronides. Furthermore, it does not always increase the water solubility of the methylated products. The enzymes are located in both the mitochondrial and cytosolic fractions.

Acetylation

Acetylation involves transfer of acetyl groups to primary aromatic amines, hydrazines, hydrazides, sulfonamides, and certain primary aliphatic amines. The enzyme and coenzyme involved are, respectively, *N*-acetyl transferases and acetyl coenzyme A. In certain cases, such as isoniazid, acetylation results in a decrease in water solubility of an amine and an increase in toxicity.

Amino Acid Conjugation

This conjugation is catalyzed by amino acid conjugates and coenzyme A. Aromatic carboxylic acids, arylacetic acids, and aryl-substituted acrylic acids can form conjugates with not only α-amino acids, mainly glycine, but also with glutamine in humans and certain monkeys and ornithine in birds.

Glutathione Conjugation

This important reaction is affected by glutathione S-transferases and the cofactor glutathione (GSH, a tripeptide; γ-glu-cys-gly). Glutathione conjugates subsequently undergo enzymatic cleavage and acetylation, forming *N*-acetylcysteine (mercapturic acid) derivatives of the toxicants, which are readily excreted. Examples of chemicals such as epoxides and aromatic halogens that conjugate with glutathione are shown in Figure 3.1. In addition, glutathione can conjugate unsaturated aliphatic compounds and displace the nitro groups in chemicals.

In the process of biotransformation of toxicants, a number of highly reactive electrophilic metabolites are formed. In particular, the generation of reactive oxygen species (ROS) is believed to be responsible for the cancer produced by diesel exhaust particles. Some of these metabolites can react with cellular constituents and cause cell death, induce tumor formation, or affect immune function. The role of glutathione is to react with the electrophilic metabolites such as ROS and thus prevent their harmful effects on the cells. However, exposure to very large amounts of such reactive substances can deplete the glutathione, thereby resulting in marked toxic effects. An example of the depletion of glutathione by acetaminophen and the concomitant increase in covalent binding

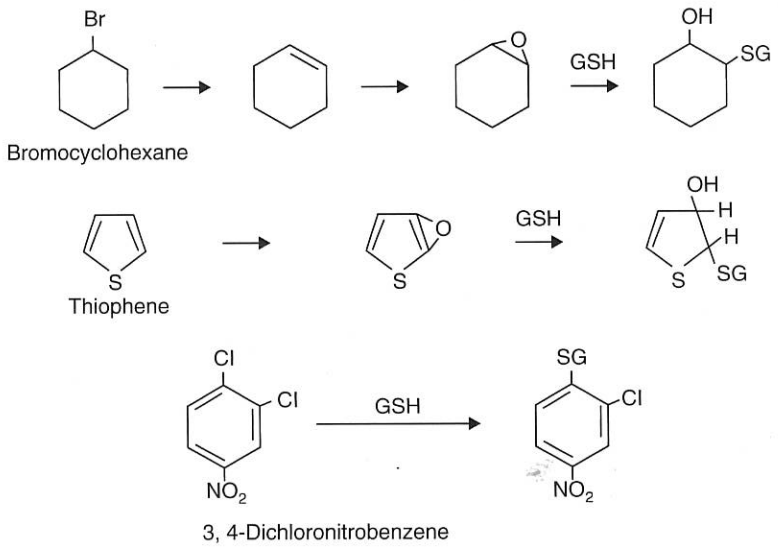

Figure 3.1 Conjugation of epoxide and dehalogenation catalyzed by glutathione (GSH) transferase. *Source*: From Timbrell (1991).

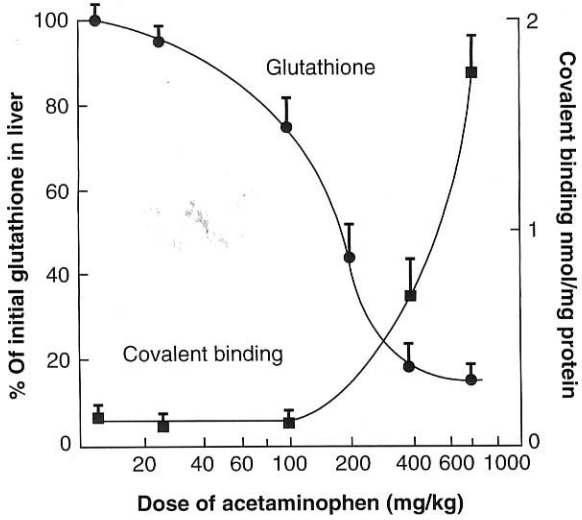

Figure 3.2 Protective effect of glutathione against covalent binding of acetaminophen to liver proteins. *Source*: From Wills (1981).

to macromolecules is shown in Figure 3.2. Similarly, 3-methylindole is bioactivated mainly in lungs and, after depleting the glutathione, will induce lung damages (Adams et al., 1988). Furthermore, certain glutathione conjugates may become toxic (Anders et al., 1988).

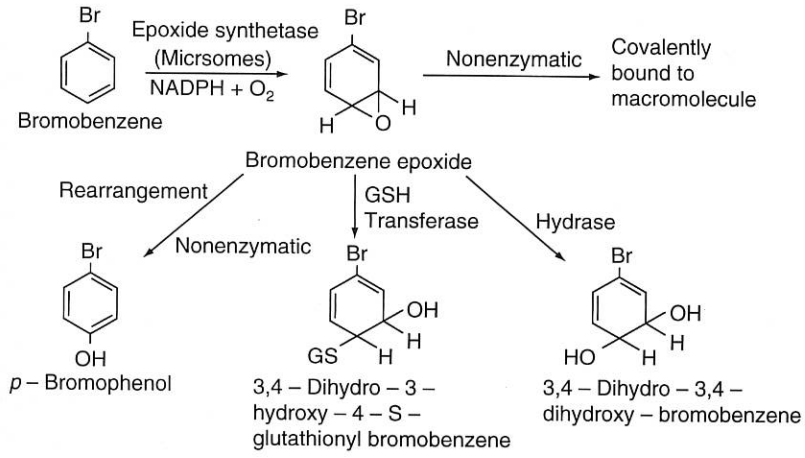

Figure 3.3 The bromobenzene epoxide formed from the parent chemical may covalently bind to macromolecules, but such binding may be minimal when the other metabolic routes are predominant. *Source*: From Gillette and Mitchell (1975).

For additional information on the biological functions of glutathione in the conjugation of electrophiles and as antioxidant, see DeLeve and Kaplowitz (1991) and Commandeur et al. (1995).

BIOACTIVATION

Certain chemically stable compounds can be converted into chemically reactive metabolites. The reactions are generally catalyzed by cytochrome P-450–dependent monooxygenase systems, but other enzymes, including those of the intestinal flora, are involved in certain cases. Furthermore, additional phase I or phase II reactions may be required. The reactive metabolites, such as epoxides, can become covalently bound to cellular macromolecules and cause necrosis and/or cancer. Others, such as free radicals, can cause lipid peroxidation resulting in tissue damage. Descriptions of the bioactivation of various classes of chemicals are available in the literature, such as the book edited by Anders (1985). The following are some notable examples. Appendix 1 lists a number of chemicals that are known to be bioactivated.

Epoxide Formation

Many aromatic compounds are converted to epoxides by microsomal mixed-function oxygenase systems. The biotransformation of bromobenzene to its epoxide and subsequent reactions serves as an interesting example of bioactivation and its consequences. These are depicted in Figure 3.3.

Although bromobenzene epoxide may become covalently bound to tissue macromolecules and cause injury, the alternative routes of metabolism may prevent or reduce the injury. The most important of these routes is the conjugation with glutathione. This reaction is important in that it serves as a protective mechanism. Only after the hepatic levels of reduced glutathione have been greatly depleted will the bromobenzene epoxide significantly bind to macromolecules and result in hepatic necrosis. Depletion of glutathione occurs when a huge dose of bromobenzene is present or when there has been an induction of microsomal enzymes; both conditions increase the amount of bromobenzene epoxide. Other reactions include a non-enzymatic arrangement to form *p*-bromophenol and the formation of 3,4-dihydro-3,4-dihydroxy-bromobenzene catalyzed by hydrase.

Other chemicals that undergo epoxidation include aflatoxin B_1, benzene, benzo(a)pyrene, furosemide, olefins, polychlorinated, and polybrominated biphenyls, trichloroethylene, and vinyl chloride. Bioactivation takes place mainly in the liver, and the resulting reactive metabolites induce toxicity through covalent binding with macromolecules in the tissue, resulting in necrosis or cancer formation.

N-Hydroxylation

Microsomal enzymes from many tissues can N-hydroxylate a variety of chemicals. Some of the *N*-hydroxy metabolites, such as those of acetaminophen, 2-acetylaminofluorene, urethane, and certain aminoazo dyes, can cause cancer or tissue necrosis through covalent binding, whereas others, such as certain aromatic amines, can induce hemolysis or methemoglobinemia.

N-hydroxy metabolites are also subject to conjugation reactions. Their conjugates with glucuronic acid are readily excreted; those formed with sulfuric or acetic acid, however, may be unstable and thus can be mutagenic, carcinogenic, and highly toxic (Weisburger and Weisburger, 1973).

Free Radical and Superoxide Formation

Free radicals are unstable and highly reactive molecules that are capable of independent existence, contain one or more unpaired electrons, have an extremely short half-life, and seek to gain additional electrons to have complete pairs or to bind to other molecules to increase their stability. Certain halogen-containing compounds undergo metabolism to form free radicals. For example, carbon tetrachloride forms trichloromethyl radical, which causes peroxidation of polyunsaturated lipid as well as covalently binds to protein and unsaturated lipid. These initial reactions are followed by disturbances of various cellular components, as described in chapter 12. Halothane and bromotrichloromethane are other examples of chemicals that may form free radicals. The herbicide paraquat is known to produce superoxide radicals (Halliwell et al., 1992).

It should be noted that during the metabolic process some chemicals produce not only their reactive metabolites, but generate free radicals such as ROS (radical forms: superoxide anion, hydroxyl radical, peroxyl and alkoxyl radical; non-radical forms: O_3, H_2O_2, hypochlorous acid, and singlet oxygen) or chemical radicals. For example, benzo(a)pyrene (BaP) produces an ultimate metabolite [e.g., benzo(a)pyrene 7,8-diol 9,10-epoxide (BPDE)], ROS, and BaP radical cation (Jiang et al., 2007). Free radicals such as ROS, reactive nitrogen species (radical forms: nitric oxide and nitrogen dioxide; non-radical forms: peroxynitrite, nitroxyl anion, nitrosyl cation, nitronium anion, nitrous acid, etc.), and organic radicals can activate nonreactive toxicants to form biologically reactive intermediates or reactive toxicants without metabolizing enzymes. Free radicals play a central role in toxicological sciences and they are involved in various kinds of toxic manifestations and common mechanisms of toxic actions (Choi and Lee, 2004). Therefore, toxic effects may be ameliorated or prevented by scavenging free radicals with antioxidants, such as antioxidant vitamins, curcumin, isoflavonoids, resveratrol, and plant polysaccharides.

Other Pathways

Ethanol can be oxidized by a dehydrogenase to acetaldehyde, which has been implicated in some of the manifestations of alcohol toxicity. Pyrrolizidine alkaloids are dehydrogenated to reactive pyrrole derivatives, which are carcinogenic. There is evidence confirming that the acute toxicity of aliphatic nitriles is attributable to the cyanide released through hepatic microsomal enzyme activities (Willhite and Smith, 1981).

Activation in the GI Tract

Nitrites and certain amines can react in the acidic environment of the stomach to form nitrosamines, many of which have been shown to be potent carcinogens, and nitrates, which, under certain conditions, can be converted to nitrites that may induce methemoglobinemia. The artificial sweetener cyclamate is converted by intestinal bacteria to cyclohexylamine, which can induce testicular atrophy. Cycasin is converted to its aglycone, methylazoxymethanol, which is hepatotoxic and can induce tumors.

COMPLEX NATURE OF BIOTRANSFORMATION

Toxicants generally undergo several types of biotransformation, resulting in a variety of metabolites and conjugates. Some of the various metabolites and conjugates of bromobenzene are shown in Figure 3.3. Organophosphorous insecticides, such as fenitrothion, chlorofenvinphos, and omethoate, can be metabolized through dealkylation, oxidation, desulfuration, or hydrolysis, yielding 10 or more different metabolites.

Parathion, an organophosphorous pesticide, is bioactivated in the liver to paraoxon, which is a much more potent cholinesterase inhibitor. Infusion of parathion by way of the vena cava, bypassing the liver, therefore produced little

cholinesterase inhibition, but a moderate effect was induced following infusion by way of the portal vein. On the other hand, infusion of paraoxon via the vena cava nearly completely blocked the cholinesterase activity, whereas it had negligible effect after an infusion via the portal vein, since it is detoxicated in the liver (Westermann, 1961).

A reactive metabolite formed in a phase I reaction can be further metabolized, such as carbon tetrachloride and halothane. Such a metabolite may also be followed by a phase II reaction to produce another reactive metabolite. For example, 2-acetylaminofluorene after N-hydroxylation can undergo acetylation or form sulfate or glutathione conjugates, all of which are highly reactive.

The relative importance of various types of biotransformation of a toxicant depends on many host, environmental, and chemical factors as well as the dose of the toxicant. Since the metabolites resulting from different types of biotransformation are often markedly different in their effects, the toxicity of a chemical can be greatly altered by these factors, as will be discussed in chapter 5.

Some of the metabolic reactions take place in sequence; hence interference with the normal metabolic pathway may have considerable influence on the toxic effects. For example, ethanol is normally metabolized through the intermediary product acetaldehyde. In normal humans the acetaldehyde formed is rapidly further metabolized to acetate, which in turn is converted to carbon dioxide and water. However, if the aldehyde dehydrogenase is inhibited, such as after the administration of disulfiram, the level of acetaldehyde rises and results in distress symptoms such as nausea, vomiting, headache, and palpitations.

A toxicant may be transformed in one organ to a stable proximate metabolite, which is transported to another organ and metabolizes to the ultimate toxic metabolite (Cohen, 1986).

Reeves (1981) provides a number of examples of biotransformation of "typical" chemicals, as well as a limited generalization of the typical routes of foreign compound metabolism in humans (Fig. 3.4).

Developmental and Children Considerations

The ability to handle chemicals in the fetus, infant, and child varies distinctly from that in the adult. Makri et al. (2004) described the metabolic characteristics of neonates to the adolescent, which would ultimately affect the susceptibility of the subject to various chemicals. In general, a neonate, infant, or child is more susceptible to chemical exposure. The age categories are as follows: neonate is 0–1 month, infant from 1 month to 2 years, preschool from 2 to 6 years, the child from 6 to 12 years, and adolescent from 12 to 16 years. Cytochrome P-450 is low from zero to one year, which indicates that a chemical half-life is longer and elimination is prolonged. In essence, this manifests as a higher susceptibility to chemical effects. Alcohol dehydrogenase is barely detectable in the infant and is present at five years of age, indicating an inability to handle alcohol and thus toxic consequences to the infant. Glucuronidation pathways and acetylation capacity are not available until

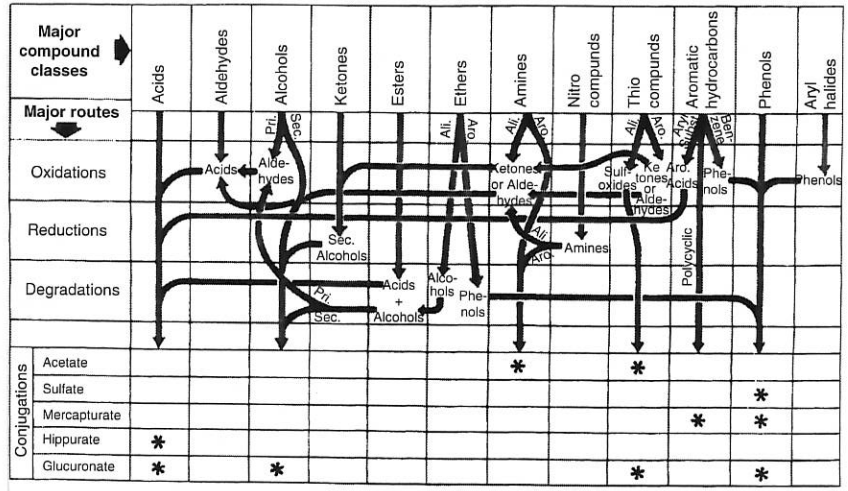

Figure 3.4 Typical routes of foreign compound metabolism in humans. *Source*: From Reeves (1981).

3 years of age, again indicating an enhanced susceptibility. Although sulfate conjugation is higher from 0 to 3 years, the capacity falls to the adult level 3 to 10 years. The difficulty here is that sulfation is not a predominant route for elimination of various compounds and thus results in enhanced chemical susceptibility.

The U.S. Environmental Protection Agency cancer risk assessment guidance recommends a default assumption that children are inherently up to 10-fold more sensitive than adults to carcinogen exposures. However, as pointed out by Pyatt et al. (2007), there is no evidence that children are at increased risk for chemically induced acute myelogenous leukemia development. In fact it would appear that the risk for drug-induced leukemia development is actually lower in younger children. These findings clearly demonstrate that generalized observations in toxicology can result in miscalculations of risk and unnecessary concerns. The need for extensive data in making decisions, especially as it pertains to sensitive subpopulations is warranted.

Elderly

It must also be borne in mind that the metabolic pathways are age dependent and less reliable in the elderly, which also manifests as increased susceptibility to toxicity in the elderly. The elderly are generally a population that is exposed to multidrug therapy, resulting in drug interactions and reduced capacity of the liver to handle these agents. With concurrent environmental chemical exposure and multidrug administration in a subject with compromised ability to eliminate chemicals, the occurrence of toxicity attributed to environmental chemicals can be additive or synergistic.

REFERENCES

Adams JD Jr, Laegreid WW, Huijzer JC, et al. (1988). Pathology and glutathione status in 3-methylindole-treated rodents. Res Commun Chem Pathol Pharmacol 60, 323–36.

Anders MW, ed (1985). Bioactivation of Foreign Compounds. New York, NY: Academic Press.

Anders MW, Lash L, Dekant W, et al. (1988). Biosynthesis and biotransformation of glutathione S-conjugates to toxic metabolites. CRC Crit Rev Toxicol 18, 311.

Choi SM, Lee BM (2004). An alternative mode of action of endocrine-disrupting chemicals and chemoprevention. J Toxicol Environ Health B Crit Rev 7, 451–63.

Cohen GM (1986). Basic principles of target organ toxicity. In: Cohen GM, ed Target Organ Toxicity. Boca Raton, FL: CRC Press.

Commandeur JNML, Stijntjes GJ, Vermeulen NPE (1995). Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. Pharmacol Revs 47, 271–330.

DeLeve LD, Kaplowitz N (1991). Glutathione metabolism and its role in hepatotoxicity. Pharmacol Ther 52, 287–305.

Gillette JR, Mitchell JR (1975). Drug actions and interactions: theoretical considerations. In: Eichler O, Farah A, Herken H, Welch AD, eds. Handbook of Experimental Pharmacology. New Series, vol. 28. New York, NY: Springer-Verlag, 359–82.

Ginsberg G, Smolenski S, Hattis D, et al. (2009). Genetic Polymorphism in Glutathione Transferases (GST): Population distribution of GSTM1, T1, and P1 conjugating activity. J Toxicol Environ Health B Crit Rev 12, 389–439.

Halliwell B, Gutteridge JMC, Cross CE (1992). Free radicals, antioxidants and human diseases—Where are we now? J Lab Clin Med 119, 598–620.

Jiang H, Gelhaus SL, Mangal D, et al. (2007). Metabolism of benzo[a]pyrene in human bronchoalveolar H358 cells using liquid chromatography-mass spectrometry. Chem Res Toxicol 20, 1331–41.

Johansson I, Ingelman-Sundberg M (2011). Genetic polymorphism and toxicology—with emphasis on cytochrome p450. Toxicol Sci 120, 1–13.

Makri A, Goveia M, Balbus J, et al. (2004). Children's susceptibility to chemicals: A review by developmental stage. J Toxicol Environ Health B 7, 417–35.

Pyatt DW, Aylward LL, Hays, SM (2007). Is age an independent risk factor for chemically induced acute myelogenous leukemia in children? J Toxicol Environ Health B 10, 379–400.

Reeves AL (1981). The metabolism of foreign compounds. In: Reeves AL, ed Toxicology: Principles and Practices, Vol 1. New York, NY: John Wiley.

Timbrell JA (1991). Principles of Biochemical Toxicology. London, UK: Taylor & Francis.

Westermann EO (1961). Bioactivation of parathion. Proceedings of International Pharmacology Meeting 6: 205. In: Ariens EJ, Simmonis AM, Offermeier J, eds Introduction to General Toxicology. New York, NY: Academic Press,147.

Weisburger JH, Weisburger EK (1973). Biochemical formation and pharmacological, toxicological and pathological properties of hydroxylamines and hydroxyamic acids. Pharmacol Rev 25, 166.

Willhite CC, Smith RP (1981). The role of cyanide liberation in the acute toxicity of aliphatic nitriles. Toxicol Appl Pharmacol 59, 589–602.

Wills ED (1981). The role of glutathione in drug metabolism and the protection of the liver against toxic metabolites. In: Gorrod JW, ed Testing for Toxicity. London, UK: Taylor & Francis.

Appendix 1 Examples of Bioactivation[a]

Parent Compound	Toxic Metabolite	Mechanism of Toxicity	Toxic Effect
Acetaminophen	*N*-acetyl-*p*-benzoquinone imine	Covalent binding	Hepatic necrosis, GI bleeding, nephrotoxicity
2-Acetylaminofluorene (AAF)	*N*-hydroxy-AAF, sulfate ester	Covalent binding	Cancers (liver, kidney, bladder)
Aflatoxin B_1	Aflatoxin-8,9-epoxide	Covalent binding	Hepatic cancer
Allyl formate	Acrolein	Covalent binding	Hepatic necrosis, lung toxicity
Amiodarone	Des ethyl amiodarone	Ethylation	Pulmonary fibrosis, thyroid abnormalities, ophthalmological effects, neurological changes
Amygdalin	Mandelo nitrile, hydrogen cyanide	Cyanide formation	Cytotoxic hypoxia, toxicity of nervous, cardiovascular, and respiratory systems
N-arylsuccinimide	3,5-Dichlorophenyl-2-hydroxysuccinimide	Oxidation and hydrolysis	Nephrotoxicity
Benzene	Benzene epoxide→ hydroquinone, 1,2,4-trihydroxybenzene	Covalent binding	Bone marrow depression, leukemia
Benzo (a)pyrene (BaP)	BaP-7,8-epoxide → (+)BaP-7,8-diol-9, 10-epoxide [(+)BPDE-I]	Covalent binding	Cancers (lung, stomach, cervix, skin)
Bromobenzene	2-Bromoquinone, 4-bromoquinone, bromobenzene epoxides	Covalent binding	Hepatic, renal, bronchiolar toxicity
1,3-Butadiene	3,4-Epoxy-1-butene (EB) 1,2:3,4-diepoxybutane (DEB), 3,4-epoxy-1,2-butanediol (EB diol)	Covalent binding	Cancer (stomach, blood, lymphatic system), hepatic necrosis, narcosis, reproductive toxicity
2-Butoxyethanol	2-Butoxyacetic acid	Oxidation	Hemolytic disorders
Carbon tetrachloride	Trichloro methane free radical	Covalent binding	Hepatic necrosis, hepatic cancer, neurotoxicity

(*continued*)

Appendix 1 Examples of Bioactivation[a] (*continued*)

Parent Compound	Toxic Metabolite	Mechanism of Toxicity	Toxic Effect
Carcinogenic alkyl nitrosamines	α-Hydroxylation	Alkylation	Cancer (liver, esophagus, many organs)
Carcinogenic aminoazo dyes	*N*-hydroxy derivatives	Covalent binding	Hepatic cancer, bladder cancer
Carcinogenic Polycyclic aromatic hydrocarbons (PAHs)	PAH-diolepoxide	Covalent binding	Cancers (lung, skin, stomach)
Chloroform	Phosgene	Covalent binding	Hepatic, renal necrosis
Cycasin	Methyl azoxy methanol, gut flora	Alkylation	Cancers (liver, kidney, intestine), hepatic necrosis
Cyclophosphamide	Phosphoramide	Alkylation	Cytotoxic, nephrotoxicity
Formaldehyde	—	Covalent binding	Nasal cancer, leukemia?
Fluoroacetate	Fluoro citrate	Enzyme inhibition	General toxicity
Furosemide	Epoxide?	Covalent binding	Hepatic, renal necrosis
Halothane	Free radical	Covalent binding	Hepatic necrosis
Isoniazid	Acetyl hydrazine	Covalent binding	Hepatic necrosis
Metam sodium	(Metabolite of) methyl isothiocyanate	Reduction	Immunosuppression, spontaneous abortion with increased frequency, asthma
Methemoglobin-producing aromatic amines and nitro compounds	*N*-hydroxy metabolites	Cyclic oxidoreduction	Methemoglobinemia
Methoxyflurane	Inorganic fluoride	Enzyme inhibition	Renal failure
Monocrotaline	Monocrotaline pyrrole	Electrophile monocrotaline pyrrole → formation of free radical	Lung and liver damage
Naphthylamine	*N*-hydroxy naphthylamine	Covalent binding	Bladder cancer

(*continued*)

Appendix 1 Examples of Bioactivation[a] (*continued*)

Parent Compound	Toxic Metabolite	Mechanism of Toxicity	Toxic Effect
Nitrates	Nitrites	Hemoglobin oxidation	Methemoglobinemia
Nitrites plus secondary or tertiary amines	Nitrosamines	Alkylation	Hepatic, pulmonary cancers
Parathion	Paraoxon	Covalent binding to cholinesterase	Neuromuscular paralysis
Purine and pyrimidine base analogues	Mononucleotides, nucleotide triphosphates	Lethal synthesis, lethal Incorporation	Cytotoxicity
Safrole (also known as shikimol)	1′-Hydroxy safrole	Covalent binding	Cancers
Styrene	Styrene oxide	Covalent binding	Lung tumors
Urethane (ethyl carbamate)	*N*-hydroxy urethane, vinyl carbamate?	Alkylation	Cancers (liver, lung), cytotoxicity
Vinyl chloride	Chloroethylene epoxide	Covalent binding	Liver cancer

[a]The site of bioactivation is the liver, except as otherwise noted. Covalent binding refers to DNA, protein, or lipid adduct formation with reactive metabolites of chemicals or reactive chemicals themselves.