CHAPTER 24

TOXICOLOGY

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CONTENTS

Geosciences and chemistry and the scientific discipline of toxicology itself have been on parallel, often intertwined paths for many years. Issues related to toxic substances from natural sources, such as arsenic, lead, and other metals, and from the contamination of soil and groundwater, have been recognized from the historical beginnings of the discipline. The Society of Toxicology groups areas of activity in toxicologic research and the biomedical fields with which they interact as seen in Table I. Other categories of specialties within toxicology exist and are equally valid.

Toxicology has a long and colorful history. Initially, it was developed as a forensic science. Later it became a subdiscipline of pharmacology as the mechanisms of drug effects (many of the drugs were derived from classical toxins) were elucidated. From its early preoccupation with particularly toxic chemicals, from which it gained its essential definition as the science of poisons, toxicology has expanded its scope to include biological mechanisms of toxicity and host defenses (or resistance) against toxicity. In the 20th century, momentum for its development as an independent discipline has come (in roughly historical order) from food safety, chemical warfare, defense, product safety (especially cosmetics and food additives but also industrial chemicals), radiation biology, pesticide research, concern for environmental quality, environmental medicine, recent refinements in methodology of epidemiology and risk assessment, materials science and biocompatibility, molecular genetics and carcinogenesis research, and immunology. Toxicology has become highly specialized in the area of risk assessment, which identifies the level of hazard peculiar to a particular chemical exposure and the limits of acceptably safe exposure. These issues go far beyond characterizing the effects of poisons, because most of the chemicals of modern concern are not classically poisons in the sense of being potentially lethal at low doses.

For convenience in terminology, all substances not normally present in the body and introduced from outside are referred to as "xenobiotics" (from the Greek xeno-, meaning foreign). Xenobiotics may be drugs, food constituents, natural chemical exposures, or anthropogenic environmental chemical exposures. Because the delineation of safe levels of exposures assumes a socially determined level of acceptable risk (implicit in the definition of safety), toxicology has been adapted in the form of risk assessment to provide guidance to regulatory bodies.

TABLE I. Scope of Toxicology

Field of specialization in toxicology	*Interdisciplinary with*
Clinical toxicology	
Drug adverse effects	Medicine, pharmacy, pediatrics, psychiatry
Drug abuse	Emergency medicine, pharmacy, forensic medicine, sports medicine
Natural products (venoms, toxins)	Pharmacology, pharmacy, pharmacognosy, emergency medicine
Suicide or accident prevention	Forensic medicine, pathology
Environmental and environmental toxicology	
Environmental toxicology	Medical geology, environmental medicine, epidemiology, agriculture, forestry
Environmental (media) toxicology	Medical geology, environmental health, epidemiology, agriculture, forestry
Risk assessment	Medical geology, political science, economics, law, public policy, epidemiology
Exposure assessment and biological monitoring	Medical geology, industrial hygiene, epidemiology
New product testing and product safety	Chemical engineering, cosmetology, food science, business, law, genetics, consumer protection, pharmaceuticals, agriculture, forestry
Basic toxicology	
Toxicokinetics	Pharmacology
Metabolism of xenobiotics	Pharmacology
Toxicodynamics	Biochemistry and molecular biology, carcinogenesis
Dermatotoxicology, ocular toxicology	Dermatology, ophthalmology, cosmetic
Target organ toxicology	(Following terminology of the Society of Toxicology)
Inhalation toxicology	Pulmonary medicine
Hematotoxicology	Hematology
Hepatotoxicity	Liver disease (hepatology)
Neurotoxicology	Neurology
Renal toxicology	Nephrology
Immunotoxicology	Immunology
Reproductive toxicology	Reproductive health
Carcinogenicity and genotoxicity	Oncology

Toxicology obviously plays a central role in medical geology. The scientific principles of toxicology are applied to medical geology in three broad areas: clinical toxicology, risk assessment, and hazard control and monitoring. Clinical toxicology is the recognition, diagnosis, and management of human toxicity, and in environmental medicine it reflects the outcome of environmental chemical exposures. Risk assessment, as the term is used here, is the identification and characterization of the level of risks resulting from exposure to hazards, including the uncertainties. Toxicology plays an essential role in risk assessment both in characterizing the potential toxicity of a chemical hazard, the first step in the process, and in providing the conceptual framework upon which quantitative risk assessment is based. The application of toxicology to risk assessment is particularly evident in the background to public policy and regulatory decisions.

Risk management is the general term for how the effects of these toxic substances are reduced. Hazard control is a term for measures that isolate, mitigate, or remove the toxic agent, and requires an understanding of the physiochemical characteristics of the chemical hazard. Here toxicology provides the essential information needed to design a control system and to set priorities for control.

In this chapter, the basic principles of toxicology will be briefly presented followed by a general framework for clinical toxicology and a general framework for toxicology as applied to risk assessment and to hazard control. The science of toxicology can be divided into toxicokinetics, the study and description of how xenobiotics enter and are handled by the body, and toxicodynamics, the study and description of what the xenobiotic does to the body (see also Chapters 8, 21, and 22, this volume).

I. Toxicokinetics

Regardless of their effect or origin, the behavior of xenobiotics in the body can be described by general terms and models reflecting the mechanisms by which exposure occurs and the body handles the chemical. From the standpoint of evolutionary biology, it is supposed that these mechanisms developed in response to selection pressures reflecting either of two biological needs: to detoxify and excrete harmful substances ingested in foods (especially in spoiled or putrefied foodstuffs) and to metabolize endogenous chemical compounds (such as steroid hormones).

Toxicokinetics is the toxicological analogy to pharmacokinetics and is based on identical concepts. It is therefore often useful to think of the disposition and metabolism of common drugs in thinking through the behavior of a toxic chemical or other xenobiotics. Four terms describe the disposition of xenobiotics: absorption, distribution, metabolism, and excretion. Modeled together, the terms describe the entry, local and overall accumulation, transformation, and removal from the body of the xenobiotic. Because tissue levels depend on transport of the xenobiotic to the target organ and the degree to which the xenobiotic partitions or is sequestered into the tissue, the kinetics of the xenobiotics determines the presentation of the xenobiotic to the target organ at the receptor level, where the toxic effect occurs. Figure 1 is an illustration of the principles of toxicokinetics.

A. Absorption

Xenobiotics may enter the body through any of several "portals" or routes of entry. In workplace situations, by far the most common opportunities for exposure are skin contact and breathing in the agent. In environmental medicine, the most significant portals of entry are therefore absorption through skin and inhalation. Ingestion, resulting from eating or placing objects such as cigarettes in the mouth in a situation where the object or the hands may have been contaminated, or in suicide attempts, is not a common problem in environmental medicine but appears from time to time. Splashes into the eyes are more often associated with local eye irritation and only rarely with absorption and systemic toxicity. Other routes of exposure, such as intravenous infusion or implantation of soluble agents, are artificial and seldom seen outside of medical care and experimental studies.

The toxicity of the xenobiotic may or may not involve the organ of first contact or site of entry. For example, carbon monoxide enters the body by the inhalation route but causes no toxicity to the lung. Other chemicals may cause local toxicity without significant absorption into the body, such as strong irritants applied to the skin. These routes of entry are not mutually exclusive. Inhalation of poorly soluble dusts such as silica, for example, may result in ingestion of the same material because of clearance from the lung bringing the material up the mucociliary escalator where it is swallowed or expectorated.

The rate at which a xenobiotic enters the bloodstream is determined by absorption across the barrier presented by the given route of exposure. Absorption of xenobiotics across membranes is determined for the most part by the chemical and physical properties of the agent. In general, lipid-soluble (lipophilic) substances are absorbed more readily than water-soluble substances across barriers such as skin. The rate of absorption is the most important determinant of the peak levels that will be reached in plasma. For many toxic substances, this is the prime determinant of acute toxicity.

The skin is sufficiently permeable to be a major route of entry of many chemicals into the body, particularly those that are readily lipid-soluble. Absorption across the skin is highly variable, depending on skin characteristics and the solubility of the xenobiotic in fat. Most transdermal absorption occurs directly across the superficial layers of the skin such as the stratum corneum, which consists of nonliving, keratinized cells, and the other living cell layers of the epidermis, where it is absorbed in the capillary bed of the dermis. Some chemicals applied to the skin may gain entry through a shortcut by passing more rapidly through hair follicles and sebaceous gland ducts. When the skin is injured with open wounds or abrasions, or in the presence of a skin rash, absorption across the skin is much faster. Transcutaneous absorption is generally a problem in the toxicology of pesticides, solvents, and halogenated hydrocarbons. Some agents may be significantly metabolized by enzyme systems in the skin, but most gain entry into the bloodstream unchanged.

Exposure by inhalation is relatively efficient absorption and the lung itself is vulnerable to damage from inhaled xenobiotics. The lungs are the organ of gas exchange and are in the circulation just before the heart. The organ receives venous blood from the body, oxygenates it, and returns it to the heart which pumps it out via arteries. Thus, blood reaching the lungs is initially low in oxygen and consists of mixed blood from

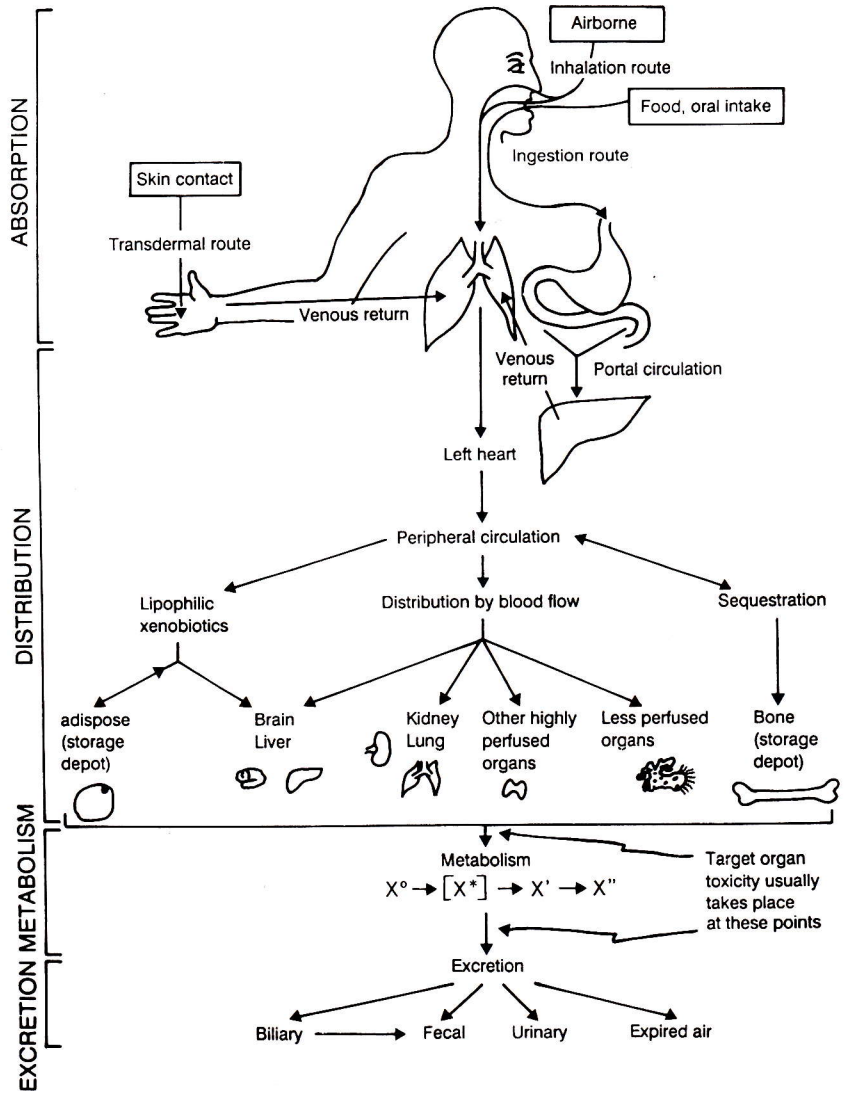

FIGURE 1 Principles of toxicokinetics: absorption, distribution, metabolism, and excretion. (Reproduced with permission from Guidotti, 1994.)

many tissues, but the oxygen tension in lung tissue itself is very high, which makes the organ susceptible to inflammatory chemicals derived from oxygen. Exposure by inhalation results in relatively efficient absorption of gases if the gas can penetrate the alveolar-capillary bed. Whether the gas will penetrate efficiently depends on its solubility in water, which reflects clearance rates in the bronchial tree. Once having penetrated the alveolar level, however, gases are readily absorbed across the alveolar-capillary bed by simple passive diffusion. Absorption across the alveolar membrane in the lung is usually very efficient and complete entry into the bloodstream is limited only by the xenobiotic's solubility in plasma, which is an aqueous medium. Particles, on the other hand, are subject to a number of host defense mechanisms in the respiratory tract, which limit the efficiency of penetration to the alveolar level. Once at the alveolar level, their size prevents them from passing directly into the bloodstream and they must dissolve or be digested by macrophages before their constituent chemical contents can be absorbed and enter the bloodstream. Particles may contribute to systemic toxicity if they are composed of a soluble material, such as lead or polycyclic aromatic hydrocarbons. For this reason, inhalation of toxic gases is usually associated with acute systemic toxicity or vascular injury to the lung (result-

ing in pulmonary edema), but particle deposition in the lung is usually associated with localized pulmonary effects and chronic systemic toxicity.

Ingestion is an important route of exposure for water and food and sometimes soil. Absorption through the gastrointestinal (GI) tract for many organic compounds depends on pH (because passage is increased when they are in a non-ionized state) and therefore on location in the GI tract: the stomach is acid and the small intestine is basic. There are specialized transport mechanisms in the GI tract. Among them is facilitated diffusion to absorb glucose and a divalent-metal ion transporter that increases absorption of metals such as calcium and iron, as well as electrochemically similar ions. The GI route of exposure is unique in another important respect. Absorbed xenobiotics do not pass directly into the systemic circulation, as they do by transcutaneous and inhalation exposure, to be returned to the heart (via the lungs). Rather, veins draining the stomach and intestine conduct the blood to the liver by a specialized circuit (the portal circulation). The liver then metabolizes many xenobiotics before they pass into the systemic circulation and stores many xenobiotics. The veins draining the liver conduct blood to the main vein of the lower body and into the systemic circulation. Thus, when a xenobiotic is ingested it may produce a toxic effect on the GI tract; produce a toxic effect on the liver; be metabolized, sometimes to a more toxic product; and pass in an altered form into the general circulation (see also Chapters 5, 8, and 23, this volume).

B. Distribution

Once the xenobiotic is absorbed and enters the bloodstream, it is transported to the capillary level in tissues of the body where it becomes available for uptake by the target organ. After one pass through the circulation the xenobiotic is uniformly mixed in arterial blood regardless of its route of entry. When a bolus is absorbed, the peripheral tissues are therefore presented with an increasing concentration in the blood which peaks and then declines as the xenobiotic is distributed to tissues throughout the body and removed by metabolism, excretion, or storage.

When a xenobiotic is dissolved in plasma, some fraction of the total usually binds to circulating proteins, particularly albumin (which binds many organic compounds as well as calcium, copper, and zinc). Metals may also be bound to specialized proteins in the plasma, such as ceruloplasmin (copper) and transferrin (iron). Binding occurs quickly and an equilibrium is established between the fraction of the xenobiotic bound to plasma protein, which cannot leave the vascular space, and that dissolved in the plasma, which is free to diffuse or be taken up by tissues. As the concentration of free xenobiotic falls in plasma, some molecules will separate from their binding sites until a new equilibrium is reached. Binding therefore acts as both a storage and distribution mechanism. It maintains a more even blood concentration than would otherwise be the case and reduces the peak concentration that would otherwise be presented to tissues. Bound xenobiotics may be displaced by other xenobiotics. Some xenobiotics, such as barbiturates or sulfonamides, compete with others for binding sites and may increase the concentration of free xenobiotic in the plasma and therefore increase toxicity. As a practical matter, this is of greatest significance in drug-related toxicology as a mechanism of drug interaction and overdose and is seldom a consideration in environmental toxicology.

The persistence of a xenobiotic in the bloodstream is an important determinant of the duration of its action and the penetration that may occur into tissues less avid in their uptake of the particular agent. However, the most important determinant of uptake by the target organ is the uptake of the xenobiotic from plasma into the tissue.

Uptake of a xenobiotic by an organ from the plasma depends on the blood flow to the organ and the avidity of the tissue for the material. Special transport mechanisms exist at the cellular level for some xenobiotics. As mentioned above in the context of absorption into the body, absorption of a xenobiotic from the bloodstream into the tissue depends on the solubility of the xenobiotic in fat; lipophilic agents will be accumulated in adipose tissue or lipid-rich organs such as the nervous system or liver. Where the physicochemical properties of the organ attract and bind metals, as in bone, a metal xenobiotic will be sequestered and will accumulate over time.

Entry into some tissues is restricted by special barriers to passage, such as the blood-brain barrier and the placenta. In most cases, however, delivery of a xenobiotic depends on the blood supply to a tissue relative to its weight. When the xenobiotic is neither particularly lipophilic nor sequestered nor preferentially taken up by some organ-specific mechanism, it is largely distributed on the basis of blood flow to the target organ. Organs with greater perfusion will tend to accumulate the xenobiotic because of the increased total amount presented to it. The lung, a very lightweight organ, is the only organ of the body to receive 100% of the cardiac output

at a tissue level. (The heart, functioning as a pump, moves blood in bulk but is itself nourished by a much smaller coronary artery system.) Not surprisingly, the lung is a principal target organ for blood-borne as well as airborne xenobiotics. The liver and kidneys each receive massive fractions of the cardiac output and are therefore presented with circulating xenobiotics in quantity. The brain also receives a disproportionate fraction of the cardiac output but is partly protected by the blood-brain barrier; this barrier works well for most polar xenobiotics but is permeable to lipophilic compounds.

In the liver, the portal circulation also delivers ingested xenobiotics at high concentrations directly from the stomach and small intestine. This provides an opportunity for metabolism to take place before the xenobiotic enters the general circulation. The liver is the principal metabolic site for xenobiotics, as it is for nutrients. Xenobiotics metabolized in the liver may even be taken up and reprocessed through biliary excretion and reabsorption through the enterohepatic circulation, such as kepone. Some tissues have an affinity for xenobiotics with certain characteristics. Organs with a high adipose or lipid content accumulate much larger concentrations of highly lipophilic xenobiotics, such as the PCBs, than occurs in plasma or in other organs. This is useful scientifically as a means of measuring body burden, because subcutaneous fat biopsies are not difficult to perform and other fatty substances that can be easily recovered, such as cerumen, do reflect tissue levels. When an obese individual in whose adipose tissue is stored a high level of a fat-soluble toxic chemical rapidly loses weight as a result of dieting, food deprivation, unaccustomed exercise, or cachexia, the xenobiotic may be mobilized and a rapidly climbing circulating level of the agent may rise to toxic levels. In general, however, the principal significance of adipose and intracellular lipid is as a storage depot, in that the blood concentration comes into an equilibrium with release from the tissue where it is stored, remaining fairly constant for the remaining life of the individual. The xenobiotic can rarely be effectively purged from the body in this situation because of the extent of the storage, although strategies exist to steadily reduce the body burden over time by vigorous removal from plasma to force mobilization. Another important implication of storage in fatty tissues is accumulation in breast tissue and subsequent excretion into breast milk. This is the major route of exposure to a variety of xenobiotics for newborns who breastfeed. Metals such as lead are also sequestered in bone; mobilization from depots in bone by chelating agents may substantially increase blood levels and create a risk of renal toxicity (see also Chapter 23, this volume).

C. Metabolism

Many xenobiotics are substrates for intracellular enzyme systems, most of which appear to have evolved as mechanisms for clearing endogenous, mainly steroid, hormones or foreign substances taken in with food. These enzyme systems transform the xenobiotic in a series of steps from the original compound to a series of stable metabolites, often through intermediate unstable compounds. For many xenobiotics there are many pathways of metabolism, which result in numerous metabolites. These transformations may have the effect of either "detoxifying," by rendering the agent toxicologically inactive, or of "activation," by converting the native agent into a metabolite that is more active in producing the same or another toxic effect. An active xenobiotic may be transformed into an inactive metabolite, which effectively removes the agent from the body in its toxicologically active form. However, an inactive precursor may also be transformed into an active metabolite.

In general, the enzyme systems available for the metabolism of xenobiotics tend to convert non-polar, lipid-soluble compounds into polar water-soluble products that are more easily excreted in urine or bile. The general pattern consists of two phases. These are illustrated in Figure 2.

Phase I of the metabolic process involves the attachment of functional chemical groups to the original molecule. This usually results in activation, especially in the very important "mixed function oxidase" (MFO) system, and results in a metabolite capable of interacting with macromolecules, such as DNA in the early steps of carcinogenesis. The mixed function oxidase system requires a great deal of metabolic energy and is closely linked with the cytochrome oxidase system, which provides it. Because the particular cytochrome most closely linked with the system has a spectral absorption peak at 450 nm, there is frequent reference in the literature to P-450 as an indicator of MFO activity.

Most important of the metabolizing systems, the MFO system also is known by other names: aryl hydrocarbon hydroxylase, arene oxidase, epoxide hydroxylase, and cytochrome oxidase. It is a complex of membrane-associated enzymes closely linked to the cytochrome P-450 system (and other cytochromes) that acts on

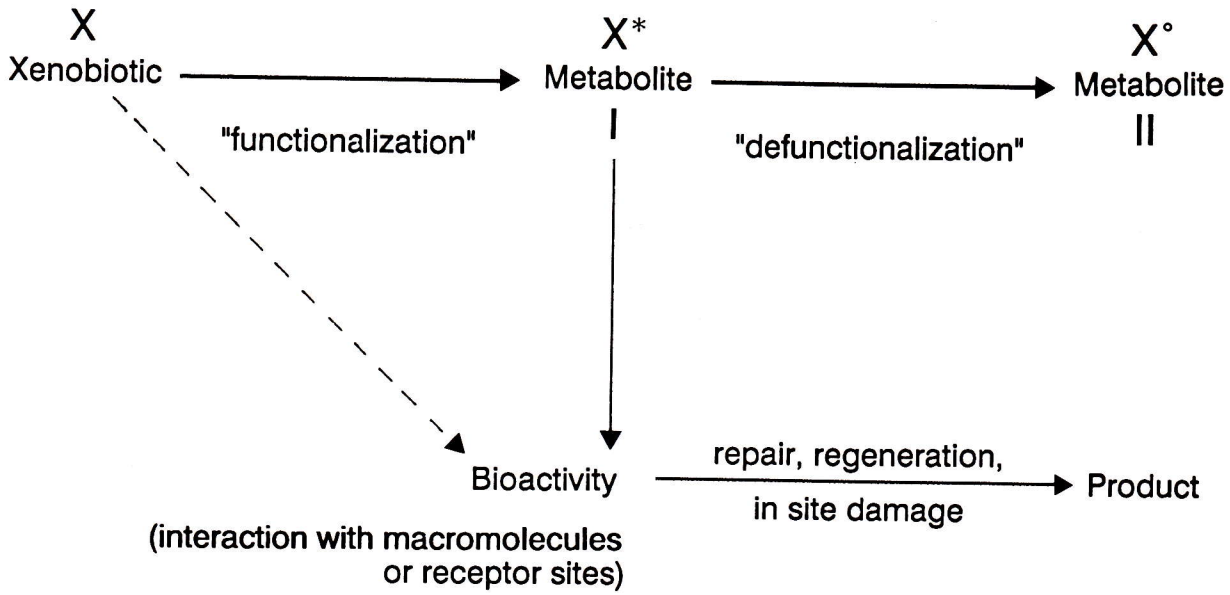

FIGURE 2 Metabolism of organic xenobiotics often follows a pattern of chemical "functionalization" in which the intermediate product may be more reactive and toxic than the original xenobiotic. This is followed by de-activation and defunctionalization or conjugation to make the product more water soluble.

organic compounds with aromatic or double bonds. The system attacks these bonds, which creates first an epoxide and then an alcohol, and in the process it first activates the compound and then deactivates it and renders it more easily excreted. The MFO system is virtually ubiquitous in the body but activity is particularly concentrated in liver and lung, and it can be found and conveniently studied in circulating lymphocytes. The MFO system has a huge capacity and acts on a wide variety of substrates. It is also inducible; when presented with suitable substrate, the cell synthesizes more MFO enzymes, which increases the capacity of the system and prepares itself for a greater load. The degree of inducibility and the level of baseline activity in a given tissue is genetically determined, so that at any one time MFO activity in a particular tissue reflects heredity combined with exposure in recent past.

Phase II involves the removal or conversion of chemical groups in such a way as to render the molecule more polar and therefore more easily excreted by the kidney (and less easily diffused back across the renal tubular epithelium after filtration). In the process, the activated xenobiotic metabolite from phase I usually becomes inactivated. This process frequently involves "conjugation," the attachment of a functional group such as sulfonate or glucoronic acid that makes the molecule much more hydrophilic.

The most complicated metabolic pathways are those for organic compounds. Metals may also be metabolized, however. The methylation of mercury and arsenic, especially, plays a major role in their toxicity. The methylation pathway of arsenic is species specific and this is thought to be the reason why arsenic is a carcinogen in humans but not in animals.

D. Excretion

The xenobiotic or its metabolites would accumulate and remain within the body if there were no mechanisms for excretion. Elimination is the term used for removal of the xenobiotic from the bloodstream, whether by excretion, metabolism, or sequestration (storage).

The kidney is the major route of excretion for most xenobiotics. Those that are water soluble may be filtered or excreted unchanged. The reserve capacity of the kidney is very great and this mechanism is rarely saturated in healthy people, but individuals with renal insufficiency may show accumulation and persistence of the xenobiotic and, consequently, prolonged and more severe toxicity. Other xenobiotics may be metabolically transformed into more water-soluble metabolites before renal clearance occurs. Xenobiotics that are themselves

nephrotoxic may injure the kidney and reduce their own clearance thereby enhancing their own toxicity by further accumulation.

The liver, besides being an important metabolizing organ, secretes some xenobiotics into bile. These include heavy metals such as lead and mercury. These may recirculate by the enterohepatic circulation, persisting in the body much longer than otherwise, or they may pass out of the body in feces. Forced biliary excretion is not presently possible but interruption of the enterohepatic circulation by binding agents such as cholestyramine is a practical clinical intervention to hasten excretion and reduce the body burden of xenobiotics excreted in the bile and reabsorbed in the gut. This was first demonstrated for kepone. Although hepatotoxic agents may interfere with their own excretion by the liver, they are more likely to interfere with metabolism and as a practical matter this effect is rarely significant.

Volatile gases are readily excreted by the lungs through passive diffusion from the blood while crossing the alveolar-capillary barrier in "reverse" direction. Gases that are poorly soluble in blood, such as ethylene, are rapidly and efficiently eliminated by this route. Those that are readily soluble in blood, such as chloroform, are less efficiently eliminated and may be detectable in expired air for days or even weeks.

Xenobiotics and their metabolites are also eliminated by various minor routes that matter little with respect to reduction of the total body burden but may have toxicological implications. Lipid-soluble agents may be secreted in breast milk; this is a major route of exposure of neonates and young children to substances such as the organohalides, which include PCBs. Water-soluble agents are excreted in saliva and tears and are filtered through sweat glands; the latter function much like miniature nephrons. Lipid-soluble agents may also be found in cerumen and sebum. These minor elimination pathways permit non-invasive monitoring techniques for the detection of the agent but are rarely reliable enough to quantify exposure.

E. Kinetics

Metabolism and excretion define the rates of elimination and the change in the concentration of the xenobiotic in the plasma with time. Elimination may occur either because the xenobiotic is excreted, because it is converted to something else by metabolism, or because

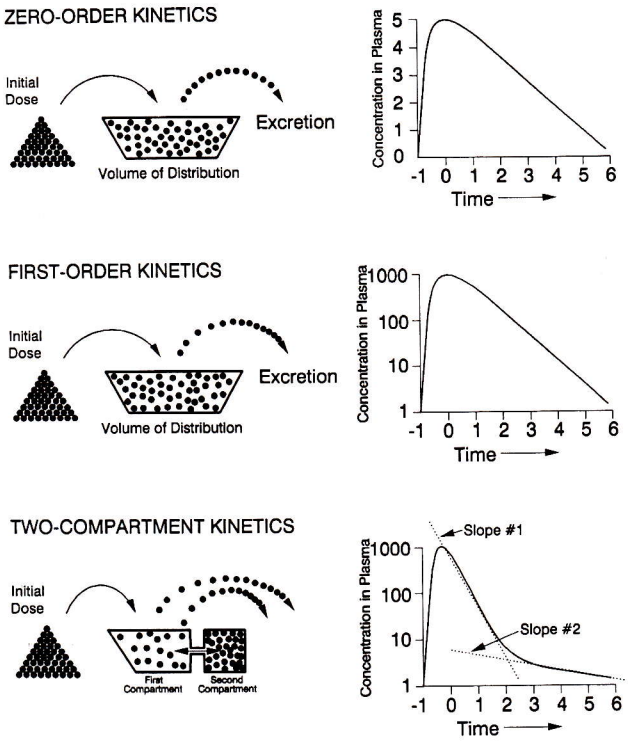

FIGURE 3 Kinetics of elimination are determined by the behavior of the xenobiotic in different toxicokinetic phases and can be modeled.

it is stored somewhere inaccessible to the bloodstream. The description of the rates of elimination of the agent is an important tool in understanding its behavior in the body. Each phase of the kinetics of a xenobiotic is governed by rates determined by properties of the agent and characteristics of the biological system, as illustrated in Figure 3. Each rate is described by a rate constant (k) that determines how rapidly the process proceeds.

Rate constants are described by their "order." A zero-order rate constant describes an elimination curve in which the rate is limited intrinsically by the fixed ability of the body to eliminate the agent, regardless of its concentration. In practice, the only important example of this is, ironically, the most common metabolizing system of toxicological concern: alcohol dehydrogenase, which metabolizes ethanol and other alcohols. Regardless of how much alcohol a person ingests, elimination will occur at the same rate and the rate of elimination will occur at a fixed rate regardless of the dose or plasma concentration.

A first-order rate constant describes a process in which the rate of elimination is independent of the dose

and the concentration of the xenobiotic in plasma is proportional to the concentration of the agent in tissue. This is the most common situation. The concentration of the xenobiotic in plasma decreases over time by a constant proportion per unit time. This is called a "one-compartment" model, because the agent behaves as if it is restricted to one compartment of the body, the vascular space. In reality, the agent may not remain in the vascular space but it may equilibrate freely with it from its tissue depots. Water-soluble xenobiotics usually show first-order kinetics, except for alcohols.

A multicompartment, or "multiexponential," function of elimination suggests that the agent equilibrates in more than one compartment and is eliminated at different rates from each. The rate of elimination varies with the concentration in plasma and the initial dose and is biphasic. The elimination will not fit a simple exponential decay (or straight line on a logarithmic scale), but it will be described by a more complex equation with two rate constants: a "fast" rate constant and a "slow" one. Organohalides typically show two-compartment kinetics because of their storage and slow release from fatty tissue. (The term second order is not used because it would imply that elimination rate is a function of the square of the concentration, which is not the case.) Increasingly, the behavior of such xenobiotics is modeled using "physiologically based pharmacokinetic" (PBPK) models, so-named because they were first worked out for drugs. Metals often have multiple compartments and complicated toxicokinetics.

First-order kinetics are most common for water-soluble xenobiotics. In such systems elimination of the agent that is proportional to concentration results in an exponential decay or reduction in plasma concentration over time. The period required for the plasma concentration to drop by half is called the half-life ($t_{1/2}$). The $t_{1/2}$ can be calculated easily and accurately and is related to the elimination rate for first-order systems by the following equation:

$$t_{1/2} = 0.693/k_{el}.$$

II. TOXICODYNAMICS

The mechanisms of toxic injury are too numerous to accommodate simple classification or generalizations. There are, however, a few general principles that are useful in understanding toxic effects.

A. Mechanisms of Toxicity

Xenobiotics exert toxic effects by interfering with the normal functions of the body. These effects occur at the molecular and cellular level. Thus, an understanding of normal function and biochemistry is essential for understanding toxicodynamics. The toxic effect is an interaction between the xenobiotic and the cellular and biochemical mechanism. This usually involves the interaction between the xenobiotic and a macromolecule, as illustrated in Figure 2. This has not always been understood. For centuries, poisons were considered to be a special class of chemicals and the toxicity of poisons were understood to be intrinsic properties of the chemical, or magic (see also Chapter 5, this volume).

The liver, kidney, lungs, skin, and bladder are particularly susceptible to toxic effects. They are in harm's way because they may be the first to encounter a toxic exposure. These organs are highly metabolically active, actively metabolize xenobiotics themselves, concentrate toxic substances or their metabolites, or have biochemical characteristics that render them vulnerable.

Although there are as many potential mechanisms of toxic effects as there are reactions in biochemistry and functions in physiology, there are a few processes that play special roles. These process are listed below.

1. Inflammation: The body has natural mechanisms to repair and limit damage. Many xenobiotics are irritating to human tissues and induce local inflammation. For others, inflammatory reactions may contribute to systemic toxicity. A particularly important phenomenon associated with inflammation is the production of reactive oxygen and nitrogen free radicals, which cause intracellular damage as a by-product of inflammation.
2. Immune responses: The body also has natural mechanisms to respond to specific foreign substances (antigens) or cells by producing antibodies or by mobilizing special cells that destroy the foreign material. In the process the body sets off inflammatory responses. These immune responses require that the body sees the antigen first or that it is persistent, and they are triggered by subsequent exposure to low levels of the antigen. When the immune response is dysfunctional, it may cause allergies, diseases collectively called "immunopathies" and self-directed autoimmune diseases.
3. Carcinogenesis: Cancer is the prototype for "stochastic" or probabilistic toxic effects, in which

the response depends on the probability of an interaction rather than the magnitude of exposure and degree of response. This is discussed below. There is some evidence that certain other classes of toxic response, such as neurotoxicity, follow similar patterns.

4. Endocrine mimics: Many xenobiotics interact with hormonal receptors, sometimes by simulating the effect of hormones and sometimes by inhibiting them.

B. Exposure-Response Relationships

The exposure-response relationship is a concept fundamental to an understanding of toxicology. Paracelsus, the great medieval toxicologist, first said "it is the dose that makes the poison" and thereby established that poisons were not a mystically benighted form of matter but that all chemicals had toxic properties that become apparent as increasing quantities are consumed or absorbed. It follows from this simple observation that there may be "safe" levels of exposure to even the most toxic substances, which is a much more controversial assertion. Obviously, there are several dimensions to this seemingly straightforward concept. There are three distinct varieties of the exposure-response relationship that need to be distinguished conceptually. These are the toxicological dose-response relationship, the clinical dose- or exposure-response relationship, and the epidemiological exposure-response relationship. These are illustrated in Figure 1.

Dose is generally understood to mean the total quantity of a toxic substance administered; exposure is generally considered to be the level of concentration available for absorption by any or all routes at or over a given period of time. Thus, dose is best understood as total or cumulative exposure over a relevant time period. If the dose is given all at once, the dose-response relationship is most meaningful, as it is when the toxic substance is accumulated in the body. If the exposure takes place over a prolonged period of time, the internal dose at any given time tends to vary and it is more useful to think of an exposure-response relationship. When a xenobiotic accumulates and persists in the body, such as lead over a period of weeks or dioxin and pesticides over a period of months and years, cumulative exposure approximates dose in toxicological terms. When a xenobiotic does not readily accumulate and is quickly eliminated, cumulative exposure over a long period of time does not equate to effective dose in toxicological terms, although there may be cumulative effects if each exposure produces permanent injury.

The most fundamental building block of toxicology is the dose-response relationship demonstrable in the laboratory, often called the "toxicological" dose- or exposure-response relationship. The fundamental principle is that the physiological response depends on the amount of the agent presented to the tissue. In a given individual, exposure to an increasing amount of a toxic substance leads to the progressive appearance of new and usually more severe health problems leading to death, a sort of stepladder to lethality.

This gives rise to another type of dose- or exposure-response relationship, which might be termed the "clinical" exposure-response relationship. At a given level of exposure, often referred to clinically (if colloquially) as a "threshold," one can usually expect a given constellation of symptoms and signs. This clinical exposure-response relationship depends on the strength of the host defenses of the individual (which can be very variable) and whether the individual has an acquired condition or genetically determined phenotype that renders him or her more vulnerable than others. In a given exposure situation, one person may show one symptom and another a different symptom, based on personal susceptibility. At relatively low levels of lead toxicity, some patients show elevated uric acid levels because of reduced renal clearance, but most do not. As well, the detection of the expected clinical response depends on the sensitivity of clinical examination and laboratory tests. Clinical tests are often inadequate for early detection of equivocal cases because they were designed for making specific diagnoses in people known to be sick in a way that strongly suggested a particular type of disease.

The third type of exposure-response relationship relates exposure levels to the frequency of the response in a population. If one is interested in what personal characteristics of those exposed render them vulnerable to a toxic effect or in how frequently a response is associated with a given level of exposure in a population, one may do a "nose-count" of the observed response among individuals exposed. This is the essential method of epidemiology and yields what is usually called the "epidemiological" exposure-response relationship. To be meaningful, however, the outcome must be experimentally or clinically detectable. This removes from study many types of response that cannot be directly measured and which are usually considered "subclinical" or "adaptive responses." In this system, a threshold means the level of exposure associated with the first appearance of an excess of the health outcome representing the toxic response. It is this threshold for

response that generates the most controversy in regulatory policy. However, interpretation of this type of threshold depends on understanding the basis for selecting and detecting the health outcome.

At higher levels of exposure, the exact shape of these exposure-response relationships are not critical and the general relationship is usually obvious. At lower levels of exposure, however, interpretation of the population response is very dependent on an interpretation of the general mechanism of the toxic effect and extrapolation to low exposures is very sensitive to the biological model applied.

A particularly important, if confusing, term in toxicology is threshold. This means the level of exposure at which an effect is first observed. The existence of thresholds for certain types of response (particularly carcinogenicity) are controversial and arguments surrounding identification of a threshold for response frequently neglect to specify the type of threshold under consideration.

C. Interaction

Some xenobiotics interact with others to produce disproportionate effects. For example, exposure to sulfur oxides in the presence of particulate air pollution in combination causes worse lung irritation than would be predicted by the individual effects of each added together. This is because the sulfur oxide adsorbs onto the surface of the particle and is carried deeper into the lung than it would be as a gas.

Interaction may be positive (often called synergy) or negative (often called inhibition). Different models of interaction are applied to the interpretation of data. When the effects of two or more are additive, no interaction occurs. This may suggest that both are acting by the same pathway or mechanism and are simply adding their proportionate share to the total magnitude of the effect. When the effects multiply, this is strong evidence for extensive interaction, and suggests that the xenobiotics are acting by different pathways that potentiate each other's effects. When the effects are less than additive, it is evidence that in some way the effects of one exposure are reducing or blocking the effects of the other or are acting by a similar pathway that has only limited capacity.

Toxicologists are very concerned that exposure to mixtures, such as cigarette smoke or heavily contaminated groundwater, could present the potential for numerous interactions and unpredictable effects. In practice, relatively few examples of interaction producing significant health effects have been documented. Some of the most important involve cigarette smoking and persistent carcinogens, which include asbestos, silica, and radon daughters. These greatly increase the risk of lung cancer compared to the sum of the separate risks of either smoking alone or exposure to the other carcinogen alone.

D. Carcinogenesis

Much of environmental toxicology is oriented toward the etiology and prevention of cancer. Carcinogenesis is not a straightforward, deterministic process. Rather, at each step in the sequence there is a finite probability of events leading to the next step. Chemical carcinogenesis is thus a stochastic, or probabilistic process, like a roulette wheel or radioactive decay, not a certain prediction based on chemical structure and properties. In any one individual, an exposure may increase the odds of getting cancer but does not make it certain in absolute terms that this will happen.

Chemical carcinogens are demonstrable by their effect in increasing the frequency of cancers observed in exposed subjects as compared to unexposed. They may produce malignant tumors that are often different in tissue type and wider in diversity than those usually observed among unexposed subjects, produce malignant tumors at characteristic or unusual sites in the body, and produce these malignant tumors earlier in the life span of subjects than they would otherwise be seen. Often, however, chemical carcinogens produce malignancies identical in tissue type, location, and onset to those seen in unexposed populations. The only clue is an increased frequency of cancers in exposed groups.

Recent advances in research on carcinogenesis, especially the identification of the oncogene, may have identified new and rather complicated mechanisms, but the effect has been to simplify our understanding by providing common pathways and unitary, comprehensible mechanisms by which many causes may act. The principal categories of causes of cancer are fairly conclusively identified as heredity, chemical exposure, viral infection, and radiation exposure. Other categories of causes may be identified, but these appear to be primary. Specific causes within each category may act by similar mechanisms such as by activation of oncogenes.

As understanding of the basic mechanisms of cancer improves, concepts of chemical carcinogenesis have also grown more refined. A deep understanding of the biology of cancer helps to explain many of the phenomena critical to regulation and control, such as latency

periods and cancer promotion. For example, low-dose radiation and radiomimetic health effects have been difficult to unravel because three competing theoretical models exist for low-dose extrapolation (linear, quadratic, and linear-quadratic). The divergence in goodness-of-fit to available response data results from differences in the underlying assumptions involving adaptive mechanisms, threshold effects, receptor behavior, and transport to the target organ. Similarly, the population response to exposure to chemical carcinogens at low exposure levels depends on whether a "one-hit" model or an interactive model is operative. (One-hit refers to a single interaction with DNA in a single cell as theoretically sufficient to cause cancer, no matter how improbable; an interactive model assumes that more than one hit is required to sustain the carcinogenic process.) The discovery of the various oncogenes and emerging evidence as to their distribution in the genome among individuals in the general population and, perhaps, high-risk subgroups has led to a rethinking of our concepts of cancer-risk and susceptibility.

The contemporary model for induction of cancer by chemicals that is most consistent with available evidence for most chemicals and for radiation is the "two-stage model of carcinogenesis." (The model is insufficient to account for some other types of cancer induction and these are discussed below.) The two-stage model assumes the introduction of a carcinogen into the body (or the metabolic activation of a pro-carcinogen) and its distribution in the body in such a way as to be presented to a tissue at levels in which it is likely to be taken up intracellularly and to react with cellular constituents, most specifically nuclear DNA. This process may result in activation of proto-oncogenes into oncogenes or may, by other means, redirect the cell's set of instructions. Transformation of the cell may override normal regulation of cell growth and mutual adherence instead activates more primitive or fetal-like genes that result in less contact inhibition, greater migratory potential, and less surface adhesion, which are the basis for tumor growth, invasion, and metastasis. This does not occur with every interaction between a carcinogen and DNA, however. Only in a relatively small fraction of such interactions will the critical sites on DNA be affected, which results in a probabilistic phenomenon. When it occurs, the process is called "initiation" because those cancers that may ultimately result are initiated at this step. In many cases, things presumably go no further than initiation. The mechanism for much, if not most, initiation activity is oncogene activation.

Among these interactions with DNA are a handful that may cause the cell to behave in a manner more appropriate to a primitive, embryo-like state, and these are thought to be the mechanism for transforming normal cells into neoplastic cells. Oncogenes are capable of being activated by chemical exposure. They are latent within the genetic structure of all humans (and probably all advanced life) and at least some probably play a physiological role in normal embryonic and fetal growth and development. Activated in the absence of regulation; however, the oncogenes trigger malignant transformation of the cell, which causes a previously differentiated cell to regress to a more primitive state abnormal for that stage of the life of the organism.

The derepressed oncogene comes to life, and expresses itself by the production of proteins (many of them enzymes, others messenger molecules or receptors) for which it codes and that have the effects required to transform the cell. These "oncogene products" are not only important in the transforming process, but may serve as very early markers that initiation has occurred. Theoretically, this would allow early identification of workers at risk for subsequent development of cancer. This raises the possibility of prophylactic treatment such as chemoprevention or other interventions designed to avoid promotion or reduce cancer risk generally.

Next in sequence is the growth of a clone of transformed cells from a single cell altered in its growth characteristics to a small focus *in situ*. The transformed cell, having been altered in its DNA blueprint, does not necessarily begin to multiply at once. Rather, it may be held in check by host factors or cell-specific factors, such as those needed for further DNA reorganization or oncogene activation to take place. The abnormal cell may rest for a very long time contributing to the greater part of the latency period before appearance of the clinically evident tumor. Additional exposures may trigger the conversion of the initially abnormal cell into a transformed or pre-neoplastic cell capable of giving rise to a tumor. This process may be facilitated by exposure to chemicals that also have genotoxic potential, either simultaneously or after the action of the primary carcinogen. This ancillary process is called "co-carcinogenesis," which implies that the second or combination exposure may not be the initiator but it may participate in the genotoxic cell events that either lead to expression of the critical event, resulting in oncogene activation, or override mechanisms that would otherwise inhibit oncogene activation and cell transformation. In general, the same chemicals that are primary carcinogens are likely also to be co-carcinogens. The distinguishing feature is not which chemical reaches the DNA first or which exposure preceded which, but

which chemical actually participated in the critical event that specifically altered the DNA in such a way as to activate the oncogene.

At this stage, exposure may occur to chemicals that are capable of triggering proliferation by removing the inhibitory factors that are suppressing the transformed cell. This is called "promotion" and it is the second stage in the two-stage model of carcinogenesis. Promoters are sometimes primary carcinogens themselves and probably act through genetic mechanisms, such as the polycyclic aromatic hydrocarbons, but others are either weakly or not carcinogenic and presumably act by nongenetic mechanisms. The most well known are the phorbol esters (specifically tetradecanoyl phorbol acetate, TPA), which are constituents of croton oil that are chemically extremely complex and seem to act at least in part pharmacologically by activating certain specific receptors on the cell surface. Chlorinated hydrocarbon species are often potent promoters, including the PCBs, DDT, PBBs, and certain dioxins. They seem to act by nongenetic means and have variable primary carcinogenic activity, depending on the species.

By whatever mechanism, promotion results in deregulation and progression of the neoplasm by proliferation into a clone of cells. The transformed cell has now become a cancer cell with the essential features of a malignancy: unresponsiveness to regulation, loss of contact inhibition, potential for sloughing and migration of cells, and the potential for inducing growth of new nutrient blood vessels.

To metastasize, malignant cells must digest or displace the matrix binding them (especially basement membranes), migrate through the degraded tissue, gain access to blood or lymphatic vessels for transport, and be deposited in a tissue favorable to growth. This does not occur until most tumors have reached a size of at least 1 cm^3, representing a population of 10^9 cells. This takes time, since the doubling time of a cancer is rarely less than six months and cells are being continually killed by host factors (especially natural killer, or NK, lymphocytes) or local nutrient inadequacy.

Because this all takes time at each step, there is a delay between the initiation (commonly assumed to be at first exposure) and earliest clinical presentation of a tumor. This is called the "latency period." For most chemically induced cancers it is on the order of 20–30 years but may be as long as 50 or more (in the case of mesothelioma and asbestos) or as short as 5 years (for radiation or radiomimetic exposures and some bladder carcinogens).

Meanwhile, back at the primary, local invasion and mechanical effects that lead to clinical detection are largely a function of tumor size and, therefore, are not obvious until the mass of the primary cancer has passed through some number of doubling times. Thus, there is a further delay between malignant proliferation and detection of the tumor clinically, which contributes to the latency period at the end of the process. The latency period is also influenced by the intensity of exposure and can be shortened by intense exposure at initiation or during promotion.

It is only at this late phase that screening programs for clinically apparent cancers have a role. Coming so late, cancers that are usually aggressive and metastasize early (such as lung cancer and melanoma) or that are difficult to detect because of their location (pancreas and ovary) do not lend themselves to effective management by early detection and treatment, because it is already too late in the great majority of cases by the time the tumor is detected. Less aggressive and more accessible malignancies, such as breast and cervical cancer, are more readily dealt with by these means.

In time, earlier screening methods for the detection of antigens reflecting oncogene expression may allow identification of persons at risk for cancer following exposure to carcinogens. The past interest in using cancer-related embryonic antigen (CEA) as a screening test may be revived with the introduction of more specific oncogene products that can be determined in urine or blood. How oncogenes and their products will become incorporated into occupational and environmental medicine in practice is not yet apparent, but it is clear that they will someday become a part of population health monitoring and part of the mainstream.

Not all chemically induced cancers act by this genetic mechanism. "Epigenetic" refers to the actions of cancer-inducing agents and exposures that do not directly interact with DNA. At least some probably act by inducing intracellular free radicals that damage DNA in a nonspecific manner, however. Others are more obscure in their mechanisms. None are adequately explained by the conventional two-stage model of carcinogenesis, but subsequent refinements in theory will almost certainly result in a unitary model demonstrating a final common mechanism for most cancers.

Because epigenetic mechanisms are associated with important occupational exposures (benzene), laboratory reagents (dioxane), consumer products (nitriloacetic acid, NTA), medical devices (foreign body), and pharmaceuticals (hormones), epigenetic carcinogens are of particular concern to occupational physicians. They represent a class of carcinogens of particular concern in risk assessment because they do not behave as typically genotoxic agents in the usual *in vitro* assays and are

therefore more difficult to anticipate. With respect to new products, the risk of foreign body and hormonal induction of cancer demands particular attention because of, respectively, the development of new biomedical technology and the weakly estrogenic effects of many substituted hydrocarbon compounds, including some pesticides. Metal-induced carcinogenesis occurs by a variety of mechanisms and often strongly depends on the chemical composition, redox state, and solubility: arsenic (lung, bladder, and skin), beryllium (lung), cadmium (lung), chromium (hexavalent ion: lung), and nickel (subsulfide; lung).

Armed with a better comprehension of the process, it should become possible for society to set standards of exposure that provide greater assurance of protection and to anticipate problems with newly synthesized or introduced chemicals. It may even be possible, using interventions on the horizon or presently available, to reduce the risk of cancer once exposure to a carcinogen has occurred. When this is done by the administration of a drug it is called chemoprevention. There is a great deal of interest in applying chemoprevention in populations of workers occupationally exposed to carcinogens. Clinical trials were conducted in the United States to determine the efficacy of cis-retinoic acid (a vitamin A derivative) in blocking steps in the sequence of events leading to certain cancers. Unfortunately, they were not only unsuccessful but for reasons that are not clear there was actually an increased incidence of cancer in the treated group, which forced the study to be terminated. A successful strategy of chemoprevention could help thousands in high-risk groups that have already sustained exposure, but to date there is no good model.

III. TOXICITY TESTING

The usual approach to assessing the toxicity of a new chemical or an agent that has recently come under suspicion is to conduct a sequence of studies, each level of which is called a "tier." A tier-one study, for example, may involve the use of *in vitro* studies, such as the Ames assay (described below), in an effort to identify potential carcinogens early and to exclude them from further consideration as a possible product. A tier-two study might involve determination of LD_{50} or LC_{50} in animals. Higher tiers may involve "subchronic" studies (90-day exposures, which result in sacrifice of the animals to examine sublethal effects), "chronic" studies of 6 months or a year, lifetime studies (to evaluate carcinogenicity over two to three years), and special studies to examine teratology, reproductive effects, toxicokinetics and metabolism, allergenicity, phototoxicity, and behavioral effects. As the tests become more sophisticated and the outcomes become more difficult to detect, they become much more expensive. Alternatives to animal studies are becoming available for specific purposes, but they cannot replace *in vivo* testing for all needs.

A major issue in selecting any kind of animal model is the biological relevance of the model to the application intended. The experiment must be at least comparable to human routes of exposure, metabolic pathways (if applicable), and the potential for expression of the effect. Strain differences within species are as important as species differences. Inbreeding has resulted in considerable differences among rat strains in response to longer term effects. The longevity of animals species places constraints on what can be studied. Animals that survive less than two years in confinement, such as mice, are difficult to use for long-term exposure studies. Rats do survive this long but full expression of the effects of exposure may require the animal to live out its life span rather than be sacrificed after an arbitrary time period. The age and sex of the animals are also important considerations. Although it is difficult to generalize, females are sometimes more susceptible to the effects of toxic exposures involving metabolism of the agent, especially if there is a possible parallel with hormonal effects as in the case of certain aromatic hydrocarbons. Young animals may differ from older animals in their degree of resistance to toxic effects; for example, neonate mice are relatively resistant to oxidant gases compared to older animals. (The dose or concentration, respectively, is sufficient to kill 50% of test animals as calculated from the dose-responsive curve.)

SEE ALSO THE FOLLOWING CHAPTERS

Chapter 5 (Uptake of Elements from a Biological Point of View) · Chapter 8 (Biological Responses of Elements) · Chapter 21 (Environmental Epidemiology) · Chapter 22 (Environmental Medicine) · Chapter 23 (Environmental Pathology)