TOXICOLOGY

1. Introduction

Toxicology is the science that studies the adverse effects (toxicities) that chemical or physical agents (toxicants) induce in biologic systems. *Toxicology* is actually an old science; the English word toxicology is derived from the late Latin word *toxicus* which meant poisonous and the ancient Greek term for arrow poisons *toxikon*. Much like medicine, toxicology is a multidisciplinary field of study as it examines the physiologic, pathological, biochemical and molecular changes that chemical and physical agents initiate in organisms after having interacted with some extra- or intracellular molecular entity. In fact, the study of the toxic effects of chemicals in toxicology differs little from the manner in which the beneficial or therapeutic effects of drugs are studied in pharmacology; the main difference being a beneficial/therapeutic endpoint versus an undesirable/harmful endpoint is being investigated. Regardless of the types of toxicities a chemical

might induce, toxicologists perform two basic functions: (*1*) examine and characterize the specific set of adverse effects a chemical agent is capable of causing (the *hazard identification/characterization* function); and (*2*) use dose-response relationships to assess the probability these toxicities will or will not occur under specific conditions of exposure (the *safety* or *risk assessment* function). Given that in modern society a person may come in contact with thousands, if not tens of thousands, of different chemicals over a lifetime, it is the application of modern toxicological concepts and toxicity data that provides the rational basis for developing safe exposure guidelines to protect the health of workers against occupational toxicants found in the workplace and the health of the general population against the many chemicals now common to modern indoor and outdoor environments. The glossary at the end of the article gives brief definitions for the key terms used here.

2. Classification of Toxic Effects

Figure 1 provides a basis for the classification of toxic effects according to site and degree of exposure. In order to cause tissue injury, a substance must come into contact with an exposed body surface; this may be skin, eye, or the lining membranes of the respiratory and gastrointestinal tracts. An adverse effect that occurs at the site of contact with the organism is referred to as a local effect or local toxicity (eg, burning of the mucous membranes of eyes, nose, and throat after inhalation of high concentration of an irritant). However, an adverse effect can result from absorption and distribution of a toxicant to a site distant from its entry point (ie, the toxicant requires absorption and distribution within the organism to produce the toxic effect). This is known as a systemic effect or systemic toxicity. An example of this would be the adverse

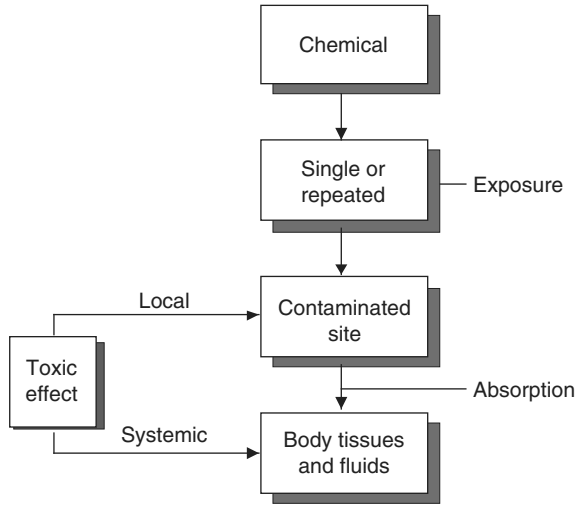


Fig. 1. Schematic representation showing the basis for classification of toxic effects into local and systemic by single or repeated exposure.

kidney or central nervous system effects resulting from chronic ingestion of sufficient doses of mercury. Systemic effects may be produced by the parent material that is absorbed, or by conversion products following absorption. The effects may be restricted to one organ or tissue system or may affect multiple organs and tissues. Some materials may cause both local and systemic toxicity.

The nature of a toxic effect and the probability of its occurring are often related to the number of exposures. The classification of toxic effects, and descriptions of toxicology tests, may be dictated by the number of exposures that elicit toxic effects. The following terms are convenient in this respect.

Acute exposure: exposures generally defined as those occurring for 24 hours or less consisting typically of a single exposure event (although they may consist of several repeated exposures during this short period of time).

Subacute exposures: exposures occurring for several days to one month.

Subchronic exposures: repeated exposures for an intermediate amount of time (about 1 to 3 months).

Chronic exposures: repeated exposures that occur for more than approximately 10% of the life-span for humans (equivalent to more than approximately 90 days to 2 years in laboratory animal species, eg, rodents).

The above terminology is useful in classifying toxic effects with respect to their development as a function of the number of exposures. For example, acute toxic effects are adverse effects that manifest within a relatively short period of time (immediately to within days after exposure); in contrast, chronic toxic effects are long-lasting, sometimes permanent effects manifested following exposure to a toxicant. It is important to remember that some materials of low acute toxicity may have a significant potential for producing harmful effects by repeated exposure, and vice versa. This stresses the need for a complete overview of the toxicity of a chemical by acute and repeated exposure in the process of hazard evaluation. The following additional descriptive terms are also useful for the classification of toxic effects.

Reversible toxicity: adverse effect which can be reversed once exposure has ceased; eg, recovery of burns on the skin after exposure to a caustic agent. Reversibility depends on factors such as extent of exposure and the capability of the tissue to repair or regenerate itself.

Delayed or latent toxicity: adverse effect occurring long after the initiation and cessation of exposure to a toxicant; eg, cervical cancer after infection from human papillomavirus.

Persistent effects: adverse effects that do not resolve after cessation of exposure. This type of effect can occur as a consequence of acute or repeated-exposure conditions. The use of the term persistent should be clearly differentiated from the implication of the use of the description of an effect as chronic. It should be noted, however, that some chronic effects may be persistent (for example, malignant neoplasia).

Table 1. **Examples of Differing Types of Toxic Effects Classified According to Time Scale for Development and Site Affected**

Time scale	Site	Effect	Chemical	Reference
acute	local	lung damage	hydrogen chloride	1
	systemic	hemolysis	arsine	2
	mixed	lung damage methemoglobinemia	oxides of nitrogen	3
short-term	local	sensitization	ethylenediamine	4
	systemic	peripheral neuropathy	methyl-*n*-butyl ketone	5
	mixed	respiratory irritation kidney injury	pyridine	6
chronic	local	bronchitis	sulfur dioxide	7
	systemic	liver angiosarcoma	vinyl chloride	8
	mixed	emphysema kidney damage	cadmium	9
latent	local	pulmonary edema	phosgene	10
	systemic	neuropathy	organophosphates	11
		lung fibrosis	paraquat	12

Some examples of toxic effects produced by different chemicals, and classified according to the preceding guidelines, are shown in Table 1.

Depending on the circumstances of exposure, any given material may produce more than one type of toxic effect. Therefore, when describing toxicity for a particular material, it is necessary to determine the following: whether the effect is local, systemic, or mixed; the nature of the injury; the organs and tissues affected; and the conditions of exposure, including route of exposure, number of exposures, and magnitude of exposure.

3. The Nature of Toxic Effects

The biological response to chemical insult may take numerous forms, depending on the physicochemical properties of the material and the conditions of exposure. Described below are some of the more significant and frequently encountered types of injury or toxic response; they may be defined in terms of tissue pathology, altered or aberrant biochemical processes, or extreme physiological responses. Because significant species-specific responses do exist, some examples of this phenomena are provided below to remind the reader that the extrapolation of animal toxicity data always contains uncertainty.

3.1. Inflammation. Inflammation describes the local and immediate biological response to tissue injury (6). There is increased blood flow, leak of blood plasma into the tissues, and migration of particular blood cells to the affected area; these have protective functions. A process of repair follows. Depending on the duration of the inflammatory response and the type of cells in the affected tissue; inflammation may be described as acute or chronic. Acute inflammation is rapid in onset with early and complete healing of the injured area, and is produced by locally irritant chemicals. In chronic inflammation, there is persistence of the aggravating agent, such as insoluble particles, or continual repetitive

exposure to an irritant material. A characteristic of chronic inflammation is that tissue destruction and the inflammatory process continue at the same time that healing processes are in operation. This may cause the development of excessive amounts of fibrous tissue (scar tissue), which may be sufficient to impair organ or tissue function; eg, in the lung there may be chronic, progressive fibrotic disease.

3.2. Degeneration. Degeneration is a generic description for a variety of abnormal changes, visible on microscopy, that occur in tissue cells as a response to toxic injury. Acutely induced degenerative changes may be reversible, but repetitive exposure can cause progression of the degenerative changes, resulting in cell malfunction and, ultimately, cell death.

3.3. Necrosis. Necrosis is used to describe the circumscribed death of tissue, and may be a consequence of many pathological processes induced by chemical injury.

3.4. Immune-Mediated Hypersensitivity Reaction. The immune system, as one of its primary functions, protects against invasion by foreign biological and other materials of potential harm. Such materials (antigens) stimulate various immune mechanisms in the host which cause functional elimination of the antigenic material. In some instances, there is an excess biological reactivity to the antigen, and a state of hypersensitivity develops (7). In the context of toxicology, the most important of such immune-mediated hypersensitivity reactions occurs in skin and lungs. In skin, following an appropriate period for the induction of immune defense mechanisms, the hypersensitivity reaction is recognized as an exaggerated inflammatory response at the site of application of the material; such materials are causes of allergic contact dermatitis (8). There is now an increasing awareness of the potential for immune-mediated hypersensitivity reactions by the inhalation of antigenic materials. Inhaling such materials results in the induction of a state of immunity against the antigen, which exhibits itself as a hypersensitivity reaction affecting the respiratory tract, and is clinically recognized as asthma (9,10). A classic cause of an immune-mediated hypersensitivity reaction affecting the respiratory tract caused by industrial chemicals is toluene diisocyanate (11).

3.5. Immunosuppression. Because a primary function of the immune system is protection against pathogenic foreign materials, any substance capable of producing a suppression of immune function will have a deleterious effect on such protective mechanisms, including defense against infective agents. Examples of immunosuppressants include glucocorticoids and drugs given following organ transplantation, eg, cyclosporins.

3.6. Neoplasia. Neoplasms are abnormal masses of cells in which growth control and divisional mechanisms are impaired, resulting in aberrant proliferation and growth. Neoplasms are basically classified as benign or malignant. Benign neoplasms grow locally without erosion of surrounding tissues. Adverse effects produced by benign neoplasms are due either to mechanical compressive effects, or to the liberation of biologically active materials from the tumor cells. Malignant neoplasms (cancers) may erosively invade surrounding tissues and become disseminated throughout the body, setting up secondary deposits of malignant-cell proliferation (*metastasis*). Induction of neoplasia is referred to as tumorigenesis or oncogenesis; the term carcinogenesis is used to describe the development of malignant neoplasms.

3.7. Mutagenesis/Genotoxicity. Chemically–induced genotoxicity involves an interaction between the agent and cellular constituents that results in heritable DNA damage. This change in the cell's DNA may then be reflected in some alteration of cell function, structure or activity. Genotoxic damage can be classified broadly into that which can be visualized by light microscopic examination of the chromosomes (cytogenetics), and that which occurs at a strictly molecular level and is not visible by microscopy. The former may be visible as chromosomal breaks and damage or rearrangement of segments of the chromosomes, such changes are referred to as *clastogenesis*. Any change in chromosomal number is referred to as *aneuploidy*. DNA damage restricted to focal molecular lesions of the nucleotides comprising strands of DNA is often specifically referred to as mutagenesis. There are several implications for genotoxic events. If mutagenesis occurs in rapidly proliferating tissue, there may be abnormalities in the differentiation and proliferation of cells leading to cancer. Cancer is viewed as a disease requiring a change in genetic expression with multiple steps, many of which may require a mutation or other genotoxic change. Thus, genotoxicity has long been considered as a part of the hazard identification process for chemical carcinogens. Similarly, if genotoxicity occurs in the embryonic tissues, the change in cellular function they may induce can result in teratogenic effects or death of the embryo. However, a variety of mechanisms may be involved in teratogenesis from differing materials, and a material which is devoid of mutagenic potential cannot necessarily be regarded as being devoid of teratogenic potential. The body has developed a variety of DNA repair defense systems to protect the cell against DNA lesions caused by ekogenous and endogenous mutations. Over 100 genes are devoted to DNA repair which respond to the average of 10,000 DNA modification events per hour that occur in every cell. Numerous excellent texts or chapters on genotoxicology are available that provide greater detail of test procedures and the role of genotoxicology in the hazard identification and risk assessment processes (12–21).

3.8. Enzyme Inhibition. Some materials produce toxic effects by the inhibition of biologically vital enzyme systems, leading to an impairment of normal biochemical pathways. Organophosphate insecticides, for example, inhibit the enzyme acetylcholinesterase. An important factor in the acute toxicity of organophosphate insecticides is the inhibition of acetylcholinesterase at neuromuscular junctions, resulting in an accumulation of the neurotransmitter material acetylcholine and resultant adverse effects that occur due to the accumulation of acetylcholine; eg, signs of increased salivation, lacrimation (tearing), urination, miosis, bronchoconstriction, sweating, diarrhea, and vomiting (see NEUROREGULATORS).

3.9. Biochemical Uncoupling. The energy liberated by normal biochemical processes is stored in high energy phosphate molecules (eg, adenosine triphosphate). Uncoupling agents, such as 2,4-dinitrophenol, interfere with the synthesis of these high energy phosphate molecules, resulting in the continual excess liberation of energy as heat.

3.10. Lethal Synthesis. This is a process in which the toxic substance has a close structural similarity to normal substrates in biochemical reactions. As a result, the material may be incorporated into the biochemical pathway and metabolized to an abnormal and toxic product. A classic example is fluoroacetic acid (eg, sodium flouoroacetate, the active ingredient in the rodenticide

'Compound 1080'), which is accepted in place of acetic acid in the Krebs tricarboxylic acid cycle. The result is formation of fluorocitric acid, which is an inhibitor of the enzyme aconitase and thus blocks energy production in the citric acid cycle.

3.11. Hepatotoxicity. The liver is the largest internal organ in the body and accounts for approximately 5% of our body mass. The liver performs many functions in the body, including nutrient homeostasis (eg, carbohydrate storage and metabolism); particulate filtration; synthesis of blood proteins; formation of urea; metabolism of hormones, endogenous wastes, and xenobiotics; fat metabolism; formation of bile; and biliary secretion. The liver receives about 30% of the total cardiac output, and about 10–15% of the body's blood is in the liver at any one time. Because of this, it is hard for most chemicals to not come into contact with the liver, an organ that is important in the detoxification and removal of foreign chemicals. Rapid and extensive removal of a compound by the liver can decrease the amount of the compound that is available to reach general circulation. This is known as the first-pass effect. Another factor that makes the liver susceptible to the effects of chemicals is that it is the main organ for biotransformation (eg, detoxification) of chemicals in the body. Several types of liver injury can occur following exposure to sufficient concentrations or after receiving sufficient doses of certain compounds that are hepatotoxic: hepatocellular degeneration and death (common organelles and structures that can be affected include the plasma membrane, mitochondria, the endoplasmic reticulum, the nucleus, and lysosomes); fatty liver (accumulation of lipids in the liver; eg, from alcohol abuse); vascular injury (eg, oral contraceptives); fibrosis and cirrhosis (eg, alcohol abuse); cholestasis (decreased or stoppage of bile flow; eg, paraquat); and tumors.

It should be noted that while a variety of compounds have been shown to induce liver tumors in rodent species, only a very few have shown to be causally associated with liver tumors in humans (15–17).

3.12. Nephrotoxicity. The kidney is an organ that is important with regard to its role in the body of homeostasis, eg, excretion of wastes via the urine, electrolyte balance, acid–base balance, and regulation of extracellular volume. Similar to the liver, the kidneys receive a large volume of blood from the heart, 25% of total cardiac output, or about 1.2–1.3 liters of blood per minute. The three portions of the nephron, which is the functional unit of the kidney, are the blood-circulating portion, the glomerulus, and the tubules. Urine is normally produced at the rate of about 1 milliliter per minute. Types of adverse effects that can occur in the kidney as the result of exposure to nephrotoxic agents include: changes in kidney weight; changes in protein excretion; changes in urine volume; acute or chronic renal failure; and tumors. It should be noted that the kidney has compensatory capabilities after a loss in renal functional mass. One type of adverse effect in the kidney that is sex- and species-specific is $\alpha 2\mu$–globulin nephropathy or hyaline droplet nephropathy that occurs only in male rats. The $\alpha 2\mu$–globulin nephropathy can be seen in rats exposed to certain compounds (eg, d-limonene). This mechanism of action is not relevant to humans because humans do not make $\alpha 2\mu$–globulin (18).

3.13. Neurotoxicity/Neurobehavioral Effects. Neurotoxicity is characterized as adverse effects that occur in the structure and/or function of the central or peripheral nervous system resulting from exposure to chemical, physical,

or biologic agents. The onset may be immediate (eg, central nervous system effects from excessive exposure to ethyl alcohol) or delayed (eg, delayed peripheral neuropathy following high-dose exposure to selected organophosphate insecticides) in nature. Effects on the nervous system may be permanent or reversible. Specific types of neurotoxicity that can occur include: (*1*) Behavioral effects that include changes in motor coordination, learning and memory, altered states of consciousness. Examples would be the reversible central nervous system effects from excessive exposure to alcohol or chlorinated hydrocarbons (eg, dizziness, narcosis); (*2*) Physiological effects would include changes in nerve conduction, neuropathy (eg, following excessive exposure to *n*-hexane or tri-*ortho*-cresyl phosphate); and (*3*) Neurodevelopmental effects occurring in the young (eg, from excessive to methylmercury *in utero*). Mechanisms by which neurotoxic compounds may affect the nervous system include, but are not limited to: damage to neurons; disruption of the electrical impulse along the nerve axon (eg, damage to the myelin sheath); or interference with the action of neurotransmitters (19).

3.14. Pulmonary Toxicity. Pulmonary damage from exposure to chemicals may be manifested as irritation of either the lower or upper airways, fibrosis, pneumonoconiosis, silicosis, asbestosis, pulmonary edema, occupational asthma, and tumors. The lungs have protective mechanisms that can help guard against effects from toxins; eg, humidification and temperature control, mucociliary clearance, and the actions of alveolar macrophages (20,21).

3.15. Reproductive Toxicity. The gonads (ovaries in females and testes in males) serve two purposes, one being to secrete sex hormones, and the second non-endocrine function of producing germ cells via gametogenesis. The functions of these sex organs depends on secretion of follicle-stimulating hormone (FSH) and leutinizing hormone (LH) from the pituitary gland. Adverse effects in the female reproductive system from exposure to toxins can affect several areas and processes: oogenesis; ovulation; coitus; gamete and zygote transfer; fertilization; and implantation. With regard to the male, reproductive organ toxicity may be seen as interference in spermatogenesis (eg, effects on Sertoli cells or Leydig cells); androgen hormone secretion; or accessory organ function (eg, the prostate gland). The blood-testes barrier that is made up of Sertoli cells in the seminiferous tubules helps to prevent the exchange of chemicals and drugs between the blood and the fluid that is present in the seminiferous tubules. There are a number of drugs and chemicals that cause hyperplasia and/or neoplasia in Leydig cells in rodents (eg, cimetidine or Tagamet used for acid reflux). Leydig cell tumors in rodents are commonly occurring tumors (as opposed to humans, where the incidence of this tumor type is extremely low) and are associated typically with hormonal imbalances; this animal model is not appropriate for assessing human risk (22–24). See Refs. 25–27 for reviews on female and male reproductive toxicology are available.

3.16. Teratogenesis. Teratogenic effects are those resulting in the development of a structural or functional abnormality in the fetus or embryo. Depending on the nature of the material, teratogenic effects may be produced by a variety of mechanisms; these include mutagenesis, induction of chromosomal aberrations, interference with nucleic acid and protein synthesis, substrate deficiencies, and enzyme inhibition. With respect to the induction of structural

abnormalities in development, the most critical time for exposure is during the early stage of gestation when the greatest degree of cell differentiation and definitive organ formation are occurring. However, there is increasing interest and concern about the effects of exposure to foreign chemicals in the later stages of gestation, which may induce functional, including behavioral, abnormalities. Several groups of agents have been categorized as teratogenic in humans. For instance, radiation (eg, atomic weapons, radioiodine, and therapeutic radiation), several types of infections (eg, syphilis and toxoplasmosis), and maternal and metabolic imbalance conditions (eg, alcoholism, diabetes, and folic acid deficiency), and medications (eg, valproic acid, androgenic hormones, diethylstilbestrol, and thalidomide) have been classified as teratogenic agents in humans (28). Considering the vast number of chemicals, only a few compounds have been classified as known human teratogens (eg, organic mercury, toluene abuse, alcohol). Shepard's *Catalog of Teratogenic Agents* lists eight "Criteria for Proof of Human Teratogenicity" which he suggests are useful for evaluating a compound's teratogenic potential. Similarly, the U.S. Food and Drug Administration has a list of rankings for the developmental toxicity of medications (29). As with other toxicities, some test animal data for teratogenicity may have limited relevance to humans and might not represent a reliable extrapolation of a human hazard. For example, in some animal studies, it is possible that the fetal effects reported are actually not a direct result of toxicity of the test compound on the fetus (teratogenic effect), but rather an indirect effect that occurs only in the presence of maternal toxicity (eg, seen as reduction in body weight of the dam, clinical signs of toxicity) (30,31). In other words, these types of defects are not seen, or seen only rarely, in fetuses at dosages that were not toxic to the dam. The types of malformations that are due to maternal toxicity rather than a direct teratogenic effect on the fetus, for example in mice include: exencephaly; open eyes; atlas fused with occipital or axis; hemivertebrae; vertebrae with fused arches or centra in the thoracic and lumbar region; missing, forked, or fused ribs; supernumerary ribs; rib defects associated with defects in nearby vertebrae; and missing, fused, divided, or scrambled sternebrae (24,25,32).

3.17. Sensory Irritation. Some effects are undesirable rather than truly adverse, and may be bothersome and transient, but not an induction of a permanent, debilitating injury. Effects such as these can often be used as warning properties in occupational situations because they warn of exposure to a chemical and may be useful in exposure to conditions that are less than injurious. A good example of this is peripheral sensory irritation which is important in many occupational health exposure guidelines. Materials described as peripheral sensory irritants are capable of interacting with sensory nerve receptors in body surfaces, producing local discomfort and related reflex effects. For example, with the eye there is pain, excess lacrimation, and involuntary closure of the eyelids (blepharospasm); inhaled sensory-irritant materials cause respiratory tract discomfort, increased secretions, and cough. Although these effects may be regarded as protective because they warn of exposure to a potentially harmful material, they may become distracting and in some instances lead to physical accidents in the workplace. For this reason, sensory-irritant effects may be useful in the development of exposure guidelines for workplace environments where they provide good warning properties (eg, ketones, aldehydes, ammonia). Sensory irritation

can be measured in laboratory animal species (eg, rodents) and quantified based on the concentration in air required to produce a 50% decrease in respiratory rate, or RD_{50}. Reviews that deal with sensory irritation are available (33,34).

4. Factors Influencing Toxicity

During the design, conducting, and evaluation of toxicology studies, there is a constant need to be aware of the numerous factors that may influence the nature, severity, and probability of induction of the toxicity under observation. Some of the more important factors are listed below.

4.1. Number and Magnitude of Exposures. Some toxic effects are produced in response to a single exposure of sufficient magnitude, while others require multiple exposures for their development (Table 1). As discussed in detail later, the magnitude of the exposure will influence both the likelihood of an effect being produced and its severity.

4.2. Species Tested. In addition to the variation in susceptibility to chemically induced toxicity among members within a given population, there may be marked differences between species with respect to the relative potency of a given material to produce toxic injury. These species differences may reflect variations in physiological and biochemical systems, differences in distribution and metabolism, and differences in uptake and excretory capacity.

4.3. Route of Exposure. In order to induce a toxic effect, local or systemic, the material must first come into contact with an exposed body surface; these are the routes of exposure. In normal circumstances, and depending on the nature of the material, the major routes of exposure are the gastrointestinal tract (ingestion), lungs (inhalation), skin (dermal, topical, percutaneous) and eye (ocular) contact. In addition, eg, therapeutic purposes, it may be necessary to consider other routes of administration: intramuscular (IM), intravenous (IV), intraperitoneal (IP), or subcutaneous (SQ) injections. The water and lipid solubility of a compound affects its absorption across the lung, the skin, or the gastrointestinal tract. As the rate of absorption and the rate of elimination affects peak blood levels, and blood levels affect target tissue concentrations, the dose required to produce a toxicity may change with the route of exposure. In addition, the organ toxicity may vary based on which route of exposure is used. Table 2 shows the LD_{50} (dose that is lethal to 50% of test animals) for various routes of exposure for potassium cyanide.

Ingestion. The gastrointestinal tract is an important route by which toxic materials are absorbed, and absorption can occur all along the GI tract. If the liver has a great capacity to metabolize a compound in the bloodstream as it is

Table 2. **Example of the Influence of Route on the Acute Toxicity of Potassium Cyanide to the Female Rabbit**

Route	LD_{50} in mg/kg (95% CI)
intravenous (IV)	1.89 (1.66–2.13)
intraperitoneal (IP)	3.99 (3.40–4.60)
oral	5.82 (5.50–6.31)
percutaneous (SC)	22.3 (20.4–24.0)

absorbed and before it can be distributed to other tissue, this is known as the first-pass effect. The first-pass effect is frequently the reason why oral doses are larger than IV doses.

Inhalation. The potential for adverse effects from materials dispersed in the atmosphere depends on a variety of factors, including physical state, concentration, and time and frequency of exposure. Gases and vapors reach the alveoli. However, the solubility in water of a gas or vapor influences the depth of its penetration into the respiratory tract, with compounds with high water solubility typically affecting the eyes, nose, pharynx, and larynx, and with compounds with low water solubility impacting the lower respiratory tract (bronchioles, alveoli). For example, the differences in the water solubility of ammonia (high water solubility) versus phosgene (low water solubility) influence the depth of penetration or location and the irritant action of the two gases (35). The distribution of particles and fibers is also determined by their size. In general, particles of mass median aerodynamic diameter greater than 50 μm do not enter the respiratory system; those greater than 10 μm are deposited in the upper respiratory tract; those in the range of 2 to 10 μm are deposited progressively in the trachea, bronchi, and bronchioles. Only particles of $\leq$1–2 μm reach the alveoli. It follows that larger respirable particles are more likely to cause local reactions in the upper airway than in the gas-exchanging tissues. The potential for alveolar involvement is greater with small-diameter particles. Factors governing the deposition of particles in the lung have been reviewed extensively (36,37). The likelihood that materials will produce local effects in the respiratory tract depends on their physical and chemical properties, solubility, reactivity with fluid-lining layers of the respiratory tract, reactivity with local tissue components, and (in the case of particulates) the site of deposition. Depending on the nature of the material, and the conditions of the exposure, the types of local response produced include acute inflammation and damage, chronic inflammation, immune-mediated hypersensitivity reactions, and neoplasia. The degree to which inhaled gases, vapors, and particulates are absorbed, and hence their potential to produce systemic toxicity, depends on their solubility in tissue fluids, any metabolism by lung tissue, diffusion rates, and steady-state blood levels.

Skin. The skin may become contaminated accidentally or, in some cases, materials may be deliberately applied. For many chemicals, the skin provides a good barrier, but for other chemicals, the skin may also act as a significant route for the absorption of systemically toxic materials. Local effects that are produced include acute or chronic inflammation, allergic reactions, and neoplasia. Factors that can influence the amount of material absorbed include: the site of contamination; integrity of the skin; diffusivity and thickness of the skin; temperature; formulation of the material; and physicochemical characteristics including charge, molecular weight, and hydrophilic and lipophilic characteristics. For additional details, reviews of dermatotoxicology are available (25,38–41).

Eye. Adverse effects may be produced by splashes of liquids (eg, acids or alkalis) or solids, and by materials dispersed in the atmosphere. Toxic effects that may be induced include transient acute inflammation, persistent damage, and, occasionally, sensitivity reactions. Toxicologically significant amounts of material may be absorbed by the periocular blood vessels in cases of splash contamination of the eye with materials of high acute toxicity (33,42,43).

4.4. Metabolism. The metabolism of a material may result in the formation of a transformation product of lower intrinsic toxicity than the parent molecule, ie, detoxification. In other cases, the end result is a metabolite, or metabolites, of intrinsically greater toxicity than the parent molecule, ie, metabolic activation has occurred. A large group of enzymes present in the body are responsible for the biotransformation of foreign compounds and are classified as either phase I or phase II enzymes, depending on the specific reaction(s) catalyzed. Phase I enzymes (eg, mixed-function oxidases) are responsible for the metabolism of compounds, eg, via oxidation, hydrolysis, or reduction, creating compounds that are more water soluble and thus more readily excretable. Phase II enzymes (eg, glutathione-*S*-transferases) add polar biomolecules to the compound being metabolized or exposes polar functional groups (eg, glucuronidation, sulfonation, acetylation, methylation, amino acid conjugation, or glutathione conjugation) to create polar metabolites, again making them more easily excretable (in urine or bile). The liver has as one of its functions the metabolism of xenobiotics; some pathways result in detoxification and others in metabolic activation. Also, the liver may serve as a route of elimination of toxic materials by excretion in bile. Xenobiotic metabolizing enzymes are found throughout the body in locations in addition to the liver, eg, kidney, skin, lung, nasal mucosa, eye, and gastrointestinal tract. Some examples of detoxification and metabolic-activation processes are given in Table 3. References 24, 44, 45 are general reviews on metabolism.

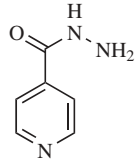
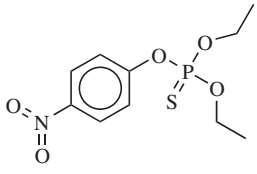
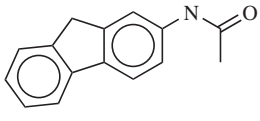
4.5. Sex and Age. The gender or sex or an organism may affect the toxicity of a substance. For instance, women have a greater proportion of body fat than do men. Important differences between sexes can exist for other factors, eg, metabolism.

Younger, as well as older, individuals may have differences in then metabolism and/or elimination of chemicals that can possibly affect the toxicity of a compound. For example, children have higher respiratory rates (breaths/minute) than do adults and are less sensitive to central nervous stimulants and are more sensitive to central nervous system depressants. As an example, the acute lethal dose of chloroform in a 14-day-old rat is 446 mg/kg, animal whereas it is higher in adult rats (1,188 mg/kg). Children also have different behaviors (more frequent hand-to-mouth activity) or different rates of absorption, eg, higher absorption of lead than adults (46).

4.6. Genetic Differences. Differences in the genetic makeup of individuals might influcence the toxic response of an individual to a particular agent. For instance, individuals who are deficient in glucose-6-phosphate dehydrogenase (G6PD deficiency) are more susceptible to the hemolytic effects of aspirin or certain antibiotics than other people (47).

4.7. Environmental Factors. The time of day or season may influence the toxic response (eg, diurnal effects). Other environmental factors that may play a role in the development of toxicity include: temperature conditions, humidity, housing conditions, repeated handling of laboratory animals, diet, or other environmental stressors such as noise. For example, in most chronic cancer bioassays rats are fed *ad libitum* (can eat whenever they want) and become obese as adults which increases the cancer rates of many chemical carcinogens.

Table 3. **Examples of Metabolic Detoxification and Metabolic Activation of Chemicals by Biological Systems**

Chemical	Transformation	Conversion
cyanide, CN^-	detoxification	enzyme conversion to less acutely toxic thiocyanate
benzoic acid, $C_6H_5CO_2H$	detoxification	conjugation with glycine to produce less toxic hippuric acid
isoniazid, 	detoxification	*N*-acetylation to less toxic acetyl derivative
parathion, 	activation	converted by oxidative desulfuration to paraoxon, a potent cholinesterase inhibitor
carbon tetrachloride, CCl_4	activation	microsomal enzyme-mediated metabolic activation to hepatotoxic trichloromethyl radical
2-acteylaminofluorene, 	activation	*N*-hydroxylation to the more potent carcinogen *N*-hydroxyacetylamino-fluorene

4.8. Formulation. The formulation of a material may have a significant influence on its potential to cause toxic injury. For example, solvents may facilitate or retard the penetration and absorption of a chemical, resulting in enhancement or suppression of a toxic response, respectively. The presence of impurities may modify the toxic response, particularly if they have high toxicity.

4.9. Chemical Interaction. *Effects.* Although toxicology testing is often performed with only a single material or a material in a relatively inert solvent or carrier, in some practical situations there can be simultaneous exposure to multiple chemicals and thus a potential for complex biological interactions. The following descriptive terms are useful in classifying such effects:

Independent: an effect in which each material exerts its own effect, irrespective of the presence of another compound.

Additivity: a situation in which the effects involve materials producing similar toxic effects where the magnitude of the response is numerically equal to

the sum of the effect produced by each individual material; ie, the combined effect of two chemicals is the same as the sum of the effects of each individual agent (eg, $3 + 4 = 7$). Organophosphate insecticides are examples of chemicals that can act in an additive fashion when co-exposure occurs.

Antagonism: where two chemicals, given together, interfere with each other's action or where one interferes with the action of the other (eg, $0 + 4 = 2$ or $5 + 5 = 7$), resulting in a less than additive or actual decrease in toxic injury. A special case of antagonism is in studies on antidotal action. Another example of antagonism would be exposure to *N*-acetylcysteine + acetaminophen.

Potentiation: where one material, of relatively low toxicity, enhances the expression of toxicity by another chemical. The result may be a larger response or more severe injury than that produced by the toxic chemical alone (eg, $2 + 0 = 10$). An example of potentiation is exposure to alcohol + carbon tetrachloride.

Synergism: where the effect of two or more chemicals that have common mechanisms of toxicity, given together is significantly greater than that expected from considerations on the toxicity of each material alone (eg, $2 + 4 = 20$). This differs from potentiation in that both materials contribute to the toxic injury, and the net effect is always greater than additive. Cigarette smoking + asbestos represents a good example of synergism.

Exposure to combinations of chemicals does not always necessarily produce clearly distinguishable interactions. Each situation must be considered in isolation with due regard to all the factors that are required to be analyzed in the process of hazard evaluation.

Modes. Chemical interactions can be increased or decreased by the following ways:

(1) *Functional*: when chemicals affect the same physiologic function.
(2) *Chemical*: chemical interaction between two compounds affecting the toxicity of one of the compounds.
(3) *Dispositional*: when the absorption, metabolism, distribution, or excretion of one of the chemicals is altered by the second chemical.
(4) *Receptor-mediated*: when two chemicals bind to the same receptor, the second chemical, which differs in activity, competes for the receptor and thereby alters the effect produced by the first chemical.

5. Testing Procedures

For descriptive purposes, toxicology testing procedures can be conveniently subdivided into general and specific forms. General toxicology studies are those in which animals are exposed to a test material under appropriate conditions and then examined for all types of toxic effects that the monitoring procedures employed allow. Specific toxicological studies are those in which exposed animals are monitored specifically for a defined toxic end point or effect. Several sets of

guidelines for performing toxicity tests are available (48–50). The following are some important considerations common to toxicity testing in general:

(1) There should be sufficiently large numbers of animals of each sex to allow a quantitative determination of the average response and the range of responses, including the demonstration of sensitive sub-populations. When objective procedures are undertaken, these should be sufficient to allow valid statistical comparisons to be made between treated and control groups.
(2) Sufficient numbers of control animals should be employed. The use of such controls allows a determination of normal values for features monitored in the study and background incidence of pathology in the population studied; detection of the onset of adverse conditions—eg, infections which are unrelated to, and detrimental to, the conduct of the study—and deviation of monitored features between controls and exposed animals, which may indicate a treatment-related effect.
(3) Vehicle-exposed control animals may be necessary to allow an assessment of the possible contribution of the vehicle to any effects observed in exposed animals.
(4) The route(s) of exposure should mimic the predominant route(s) of exposure for humans. The number of exposures tested and the magnitude of the exposures tested should span a range that includes the expected relevant human exposure level.
(5) Pharmacokinetic studies should be undertaken in order to investigate the factors that affect the toxicity of the chemicals as discussed above, eg, route of exposure, metabolism (and the profile of metabolites generated), and sex.

5.1. General Toxicology Studies. Studies may be conducted in live specimens (*in vivo*), or in test tubes (*in vitro*). For reasons inherent in both the toxicity assessment procedure and the design of studies, it is usual to proceed in sequence from single acute exposures to the various stages of multiple-exposure studies. Acute studies give information on the type of toxic injury produced by a single exposure, including the effects of massive overexposure. The fact that a particular type of toxic injury is not produced by an acute exposure does not necessarily imply the absence of potential for that type of injury by the chemical, because multiple exposures may be necessary to induce the effect. However, effects produced by acute or relatively short-term repeated exposure may also be produced by longer-term repeated exposures, and at lower concentrations. Hence, in addition to giving information on potential for toxicity, the acute and short-term repeated studies are used to give guidance on exposure conditions to be followed in longer-term repeated exposure studies. The type of monitoring to be employed will depend on a variety of considerations, including the chemistry of the material, its known or suspected toxicology, the degree of exposure, and the reason for conducting the test. In general, because the multiple exposure studies are more likely to produce the widest spectrum of toxic effects, the most extensive monitoring is typically employed in these studies.

The types of monitoring employed to assess the functional status of the living animal and for the detection of injury in dead animals may include the following:

General Observations/Clinical Signs. Animals are inspected at frequent intervals in order to discover any departure from normal appearance and function, the presence of abnormal patterns of behavior, and any other differences from the control animals. Simple observation of the animals may provide important information in assessing potential for toxicity and giving preliminary guidance on the nature of any injury (51). Emphasis should be placed on the proper training of staff or the individuals making the observations, eg, in administering the functional observational battery to assess neurotoxicity in animals (52).

Body Weight. The detection of a decrease in the rate of gain in body weight, in comparison with controls, may be one of the earliest indications of the onset of toxic effects, particularly if it follows a dose–response relationship (44).

Food and Water Consumption. Measurement of changes in food and water consumption may indicate a toxic potential, and can give guidance on the reason for abnormal body weight gains (44).

Hematology and Chemical Chemistry. This monitoring procedure involves the measurement of the concentration of certain materials in the blood, or of certain enzyme activities in serum or plasma. A variety of methods exist that allow (to variable degrees of specificity) the definition of a particular organ or tissue injury, the nature of the injurious process, and the severity of the effect. Types of measurements made include the following: (*1*) hematology: erythrocyte count; mean corpuscular volume; hemoglobin; packed cell volume; mean corpuscular hemoglobin; mean corpuscular hemoglobin concentration; erythrocyte morphologic assessment; leukocyte count with differential; reticulocyte count; platelet count and morphologic assessment; and (*2*) clinical chemistry that may indicate injury to the lever or other tissues in the body: sorbitol dehydrogenase (SDH); alkaline phosphatase (ALP); creatine kinase (CK); creatinine; total protein; albumin; urea nitrogen (BUN); total bile acids; alanine aminotransferase (ALT); glucose lactate dehydrogenase (LDH)(42,45).

Urinalysis. Urine is collected at various times and examined for various parameters eg., volume, osmolality, pH, electrolytes, ketones, glucose, protein, and sedimentation. The results may indicate kidney damage or suggest tissue injury at other sites (44,46).

Gross and Microscopic Pathology. Animals are examined macroscopically at autopsy following death during the study or at planned sacrifice. This may show features apparent to the naked eye which are abnormal and suggestive of tissue damage (gross pathology). Sections of tissue examined under the light microscope allow a detailed evaluation of the interrelationships and structural integrity, or otherwise, of cells and intercellular materials. In this way, normality of tissue may be confirmed, or a specific pathological diagnosis attached to induced, or coincidental tissue injury (histopathology) (44,47).

Organ Weight Determinations. Measurement of the weight of organs removed at autopsy is an integral part of most toxicology studies. This information may provide an indication of changes in these organs, although they have to be carefully related to the state of hydration and nutrition of the animal (44).

5.2. Types of Studies. Studies may be conducted in live specimens (*in vivo*), or in test tubes (*in vitro*). Studies may be carried out by single exposure or repeated exposure over variable periods of time. The design of any one study, including the monitoring procedures, is determined by a large number of factors, including the nature of the test material, route of exposure, known or suspected toxicity, practical use of the material, and the reason for conducting the study.

Acute Toxicity Studies. These studies should provide the following information: the nature of any local or systemic adverse effects occurring as a consequence of a single exposure to the test material; an indication of the exposure conditions producing the adverse effects; in particular, information on dose–response relationships, including minimum and no-effects exposure levels, and data of use in the design of short-term repeated exposure studies. Acute toxicity studies are often dominated by consideration of lethality, including calculation of the median lethal dose. By routes other than inhalation, this is expressed as the LD_{50} with 95% confidence limits. For inhalation experiments, it is convenient to calculate the atmospheric concentration of test material producing a 50% mortality over a specified period of time, usually 4 hours; ie, the 4 hour LC_{50}. It is desirable to know the nature, time to onset, dose–related severity, and reversibility of sublethal toxic effects. Recently, there has been a shift in the design of acute toxicity studies. For example, the USEPA emphasizes using the fewest number of animals necessary, simultaneous monitoring of sublethal as well as lethal endpoints, using data from structurally related substances, using alternative test protocols [eg, Fixed Dose Method (48) or the Up-and-Down Method (49)], or by using limit testing (50,51). The limit dose method is typically used for chemicals that are known or suspected to be of low toxicity. In this study type, a single high dose (eg, 5,000 mg/kg) of a compound is given to a small group of animals (eg, 5 males and 5 females). If no deaths are seen, no further testing is done. If the limit data are not sufficient for some reason, then a LD_{50} test can be conducted (52).

Short-Term Repeated Studies. These studies should give information about the potential for cumulative toxicity and allow the detection of toxicity, other than neoplasia, not detected in acute studies. Studies are generally carried out by exposing animals by an appropriate route for 5 days per week for 1 to 4 weeks. At a minimum, the conduct of these tests should include observations for signs of toxicity; measurement of body weight, food intake, and water consumption; autopsy; and gross pathology. Other monitoring requirements are dictated by the reasons for conducting the test.

Subchronic Studies. Although short-term repeated exposure studies provide valuable information about toxicity over this time span, they may not be relevant for assessment of hazard over a longer time period. For example, the minimum and no-effect levels determined by short-term exposure may be significantly lower if exposure to the test material is extended over several months. Also, certain toxic effects may have a latency which does not allow their expression or detection over a short-term repeated-exposure period; for example, kidney dysfunction or disturbances of the blood-forming tissues may not become apparent until subchronic exposure studies are undertaken.

Typically, subchronic inhalation studies involve exposing the animals for 6 hours per day, 5 days per week for about 3 months. For feeding studies, the

material is frequently included in the diet, provided palatability is not a problem. As with the shorter-term studies, several dose levels are used, together with a control group. Because of the potential for a wide spectrum of effects and the cost of conducting the basic test, a significant amount of relevant monitoring is employed in order to detect the nature, onset, progression, and severity of any toxic effects. Ideally, a small proportion of animals should be kept for several weeks after the end of the exposure period in order to determine the reversibility of any induced toxic effects. Subchronic exposure conditions usually detect all potential long-term repeated exposure toxicity, except for neoplasia.

Chronic Toxicity Studies. With the exception of tumorigenesis, most types of repeated exposure toxicity are detected by subchronic exposure conditions. Therefore, chronic exposure conditions are usually conducted for the following reasons: if there is a need to investigate the tumorigenic potential of a material; if it is necessary to determine a no-effect or threshold level of toxicity for lifetime exposure to a material; or if there is reason to suspect that particular forms of toxicity are exhibited only under chronic exposure conditions.

For the above reasons, chronic exposure studies are frequently designed in such a way that it is possible to combine observations for tumorigenesis and nonneoplastic tissue injury. Chronic studies are usually extensively monitored. It is common practice to sacrifice animals at intervals during the study in order to detect the onset of any tissue injury. For two-year exposure studies, it is most meaningful to have interim sacrifices at 12 and 18 months.

Guidelines are available for conducting acute or repeated exposure studies by inhalation (51,53,54), application to the skin (55,56), or perorally (57,58).

5.3. Specific Toxicology Studies. Many procedures, both *in vivo* and *in vitro*, are available to detect specific organ toxicity or quantitatively monitor for particular end points or effects. Although many of these studies are directed at measuring a particular toxic effect for hazard-evaluation purposes, some are employed as screening or short-term tests to determine the potential of a material to induce chronic toxic effects or those with a long latency period. In this context, screening means an experimental approach that allows the rapid and cost-effective prediction of the likelihood that a material exerts a particular type of adverse biological activity. Such approaches should be based on studies showing the method gives a high degree of correlation with conventional and credible methods for detecting the particular toxic end point. Some of the most commonly employed special toxicology methods and approaches are listed below.

Primary Irritancy Studies. These studies are employed to determine the potential of materials to cause local inflammatory effects in exposed body surfaces, notably skin and eye, following acute or short-term repeated exposure. In general, the approach involves applying the test material to the surface of the skin or eye, and observing for signs of inflammation, their duration, and resolution. Reviews have been written about the conduct of primary eye irritation (25,59,60) and primary skin irritation studies (61,62).

Studies for Immune-Mediated Hypersensitivity. Allergenic materials may produce hypersensitivity reactions by skin contact or by inhalation. In conventional tests for determining allergenic potential by skin contact, the basic approach involves repeatedly applying test material to the skin, or under the skin, in order to induce a state of hypersensitivity. After a latent period, the

skin is challenged with test material to determine if an exaggerated local response, typical of delayed hypersensitivity contact dermatitis, has been produced. Details of the test procedures are available (42,63–65). Experimental methods for determining the potential of materials to produce hypersensitivity reactions by inhalation use procedures to detect hyperreactivity of the airways as demonstrated by marked changes in resistance to air flow, and the detection of antibodies in blood serum (66).

Neurological and Behavioral Toxicology. Observations on animals in general toxicology studies may indicate a potential for injury to the nervous system, particularly if there are abnormalities of movement, gait, and reaction to the environment. Where it is known or suspected that a material may produce structural or functional damage to the nervous system, special methods should be incorporated into general toxicology studies in order to determine the nature and extent of any neurological injury. This may include the use of simple observational test batteries in order to better assess the clinical status of the animal (67,68) (eg, the Functional Observational Battery (69)), more detailed examination of the potentially affected areas of the nervous system by light and electron microscopy (70,71) and/or the use of selective biochemical procedures (72).

However, in order to precisely define the nature of a neurotoxic process, its mechanism of production, and the quantitative determinants for the effect, it may be necessary to conduct specific studies. These may involve the use of electrophysiological, pharmacological, tissue culture, and metabolism techniques (73–76). Special observational methods are available for behavioral studies (77–79).

Teratology. At present, most studies that are conducted to determine the teratogenicity of materials are aimed primarily at assessing structural defects of development. Basically, these studies involve administering the test material to the pregnant animal during the period of maximum organogenesis; for rats, it is usual to expose on days 6–15, and for rabbits on days 6–18. The day before anticipated normal parturition, fetuses are delivered by Caesarian section, to prevent the cannibalization of any deformed fetuses by the mother. Resorbed and dead fetuses are counted. The viable fetuses are sexed, weighed, measured (crown–rump length); some are used for examination of the integrity of the skeleton; and the remainder are dissected to determine the presence of any soft-tissue abnormalities. Additionally, observations are made for pathology in the maternal reproductive system. To allow for dose–response considerations, several exposure levels are used. Control groups should include animals that are untreated and others given the vehicle alone. The design and conduct of conventional teratology studies for the detection of structural abnormalities of development have been extensively reviewed (80–83).

Reproductive Toxicology Tests. In contrast to teratology studies, which are aimed at assessing adverse effects on the developing fetus, reproductive studies cover a much wider spectrum of developmental biology. They are designed to assess the potential for adverse effects on gonads, fertility, gestation, fetuses, lactation, and general reproductive performance. Exposure to the chemical may be over one or several generations. Tests for reproductive toxicity have been reviewed (42,84–87).

Metabolism and Pharmacokinetics. Because the potential for systemic toxicity of a material may be highly dependent on its distribution, residence time, and bioconversion, studies on metabolism and pharmacokinetics can be of fundamental importance with respect to interpretation of the significance of conventional toxicology studies, determination of mechanisms of toxicity, relation of environmental exposure conditions to target organ toxicity, selection of dosages, and the design of further toxicology studies (see PHARMACODYNAMICS).

Metabolism is concerned with a determination of the biotransformation of the parent material, the sites at which this occurs, and the mechanism of the biotransformation.

Pharmacokinetic studies are designed for the following: to measure quantitatively the rate of uptake and metabolism of a material and determine the absorbed dose; to determine the distribution of absorbed material and its metabolites among body fluids and tissues, and their rate of accumulation and efflux from the tissues and body fluids; to determine the routes and relative rates of excretion of test material and metabolites; and to determine the potential for binding to macromolecular and cellular structures.

Pharmacokinetic studies do allow an assessment of the relationship between the environmental-exposure conditions and the absorbed dose, and how these influence the doses of test material and metabolites received by various body tissues and fluids. Bioavailability is the term used to reflect the extent to which a chemical is absorbed, it is simply a ratio of the applied dose (dose given to an organism) divided by the absorbed dose (the dose that gains entry into the systemic circulation and tissues). Numerous publications are available on the design and conduct of metabolism and pharmacokinetic studies (42,88–90).

Genotoxicity. Studies to determine the potential for materials to produce genotoxic events may be conducted *in vitro* and *in vivo*. The most widely used test system for genotoxic potential has been the Ames procedure (91). This test is based on the ability of mutagenic chemicals to cause certain bacteria to regain their ability to grow in media deficient in an essential amino acid. Other tests of the *in vitro* type make use of various end points, indicating a genotoxic event, in mammalian cells grown in culture. *In vivo* studies involve the exposure of animals to the test chemical after which cells are removed, usually from blood or bone marrow, and examined for chromosomal abnormalities or for focal mutagenic events using a biochemical or morphological marker. The tests for assessing genotoxic potential of chemicals have been published (9,43).

A positive result in a genotoxicity test system is not necessarily a directly usable end point in toxicity evaluation. There is general agreement that materials exhibiting a genotoxic potential, particularly by an *in vivo* approach, need to be reviewed in particular with respect to their possible genetic, teratogenic, and direct carcinogenic activity. The relationship between genotoxicity tests, both *in vivo* and *in vitro*, and the ability of a chemical to produce genetically transmitted adverse effects in the progeny of exposed individuals is unclear and the subject of much debate and research. Perhaps the most common application of genotoxicity studies is to assess the carcinogenic potential of materials. There are correlative relations between mammalian carcinogens and their genotoxic potential. However, in the last decade, it has become increasingly clear that many carcinogens

may induce cancer via non-genotoxic mechanisms, and that for some chemicals, the genotoxic potential *in vivo* requires higher concentrations than those necessary to induce cancer. Alternatively, a number of genotoxic chemicals have been shown to be devoid of carcinogenic activity. Thus, genotoxicity measurements may add to our understanding to the mechanism by which the chemical induces cancer, but this endpoint is much less useful for predicting the carcinogenic potential of a chemical than was originally thought.

Because the major regulatory interest in genotoxic chemicals was initially based on the potential for this type of toxicity to induce carcinogenesis, the use of a mutagenicity test battery as a screening method for the detection of potentially carcinogenic materials has long been of interest. But not all mutagens are carcinogens, and not all carcinogens are mutagens. For example, based on an analysis of chemicals listed in the Carcinogenic Potency Database (92), 79% of the mutagenic chemicals tested were determined to animal carcinogens, but only 57% of all chemicals identified as carcinogens were also mutagens. Similarly, many carcinogens may induce cancer by processes in which genotoxic activity may be a necessary but not a sufficient effect for cancer induction (93–99). Other features of a chemical's genotoxicity, eg, occurring only at doses higher than those required to induce carcinogenicity, or the *in vivo* genotoxicity of a chemical is limited to one species, may also determine whether the genotoxicity of a specific chemical is or is not a relevant mechanistic consideration for a particular chemical carcinogen (100). Finally, increasing evidence that thresholds exist for chemical-induced genotoxic damage and chemical carcinogens thought to be acting via a genotoxic mode of action continues to emerge (101–107). All of this evidence contradicts the long held regulatory assumption that toxicities produced via a genotoxic mode of action (like cancer) should always be presumed to have a no-threshold, linear dose-response curve. Thus, in the future genotoxicity data may become more relevant for risk assessment purposes than as a suggestive hazard indicator of the chemical's carcinogenic potential.

Carcinogenicity. The carcinogenic potential of a chemical is typically tested using lifetime exposures in two rodents species. In a typical National Toxicology Program investigation of a chemical, long-term toxicology and carcinogenesis studies are generally performed in both sexes of rats (usually the Fischer 344/N strain) and mice (usually the B6C3F1 hybrid), with three exposure levels plus untreated controls in groups of 50 animals for two years. The highest dose selected for testing is known as the "Maximally Tolerated Dose" (MTD). The MTD is supposed to represent the highest dosage rate the animal can be given without causing excessive systemic toxicity during the completion of the bioassay. As currently defined excessive systemic toxicity is somewhat crudely determined when selecting an MTD as a decrease in animal body weight of no more than about 10% or an excessive increase in the early mortality of the animals. Generally speaking, chemicals providing positive responses in two species, or in two different tests, or in multiple organs of one species are classified by regulatory agencies as known animal carcinogens. Depending then on the strength and weight of the epidemiologic and/or mechanistic data that is also available, agencies may then classify a chemical's carcinogenic potential into one of following categories: (*1*) it is not likely to be carcinogenic in humans; (*2*) there is

suggestive evidence of carcinogenic potential; (*3*) it is likely to be carcinogenic in humans; or (*4*) that it is carcinogenic to humans (108).

While the default regulatory assumption is that an animal carcinogen should be quantitatively modeled as though it were a human carcinogenic substance, there are a number of features of the chronic animal bioassay that raise uncertainty regarding this assumption and the regulatory use of linear dose-response modeling as has been typically applied to animal carcinogens in the past. One feature of particular interest and controversy is the use of the MTD in chronic animal cancer bioassays because it represents a compromise between two desired goals, sensitivity (avoiding false negatives) and specificity (avoiding false positives). The first goal is to ensure the carcinogenic potential of the chemical has been adequately tested; ie, the test is a sensitive measure of a chemicals carcinogenic potential so that all human carcinogens will be identified. Here, testing the highest dose possible is desirable because it reduces the chance a false negative will be generated simply because the doses tested were too low to generate an observable response in a study with an adequate number of test animals. In addition, the higher the dose tested, the greater the likelihood a 100% response rate will be achieved if the chemical is in fact a carcinogen. This in turn means that fewer animals are required to be able to detect a statistically change in the observed tumor rate for each organ. This increases the ease of observing a positive response as well as reduces cost and space needs which in turn, increases the ability to test more chemicals over a shorter interval of time. For these two reasons, testing the highest dose possible is highly desirable for regulatory purposes. The second desired goal, that the test paradigm not be one that generates a high percentage of false positives, and thereby potentially eliminate or severely restrict the use of chemicals that may benefit society. However, the use of very high doses of some chemicals can create cellular, biochemical, and physiologic changes that can produce carcinogenicity only under these altered cellular conditions (109–115). Lower doses do not induce these same changes and so carcinogenicity is not induced or expected at lower doses. Thus, a number of scientists have argued that continued use of the MTD, as has been defined for decades, can create a situation where the biochemical changes in the test animal, and the resulting positive carcinogenic response for the chemical being tested, simply do not exist at lower doses that may still far exceed the known or intended human exposure level to that chemical. For example, Gold (92) noted that 44% of the chemical carcinogens they reviewed were not capable of inducing a carcinogenic response at doses as high as one-half the MTD. In this situation concern for the hazard (carcinogenicity) and conservative dose-response modeling (linear extrapolation) may be inappropriately applied to a chemical exposure situation which may in fact carries zero cancer risk in the exposed human population (ie, extrapolation of the high-dose animal test response in essence generates a "false positive" for the intended human use). Other features of the chronic rodent bioassay may compound the extrapolations problems already created by the use of the MTD include the fact that certain mechanisms of tumorigenicity and/or the responses in species/strains with high background tumors rates may be of limited human relevance (12,18,116–122), and the fact that feeding animals *ad libitum* promotes the tumorigenic responses of test animals for some chemical carcinogens (123,124). Example chemicals where additional studies have

shown the animal carcinogenic response is induced via a mechanism that is not believed to translate into a corresponding human cancer risk under typical human use conditions of that chemical include - saccharin (bladder tumors), gasoline (kidney tumors), cimetidine (Tagamet, reflux treatment) and clofibrate (antilipidemic) (Leydig cell tumors), and simvastatin (antilipidemic drug) (thyroid tumors). Thus, like systemic toxicities identified via animal studies, considerable uncertainty may be associated with the human extrapolation of animal carcinogenicity data, especially where both negative and positive responses have been observed. Future studies will better delineate which toxic effects and modes of action carry either significant or no human carcinogenic risk.

6. Review of Toxicology Studies

The review and interpretation of toxicology studies is a professional matter, requiring experience in both the laboratory conduct of such studies and the practice of applied toxicology. Although all studies should be reviewed on a case-by-case basis, there are some general considerations to be kept in mind during the review process, described below.

Where the data are from an unpublished study, the reviewer should establish that the laboratory reporting the study has the necessary professional reputation, scientific experience, and expertise in the area investigated. It should be confirmed that adequate quality-control facilities are in place and good laboratory practices (GLP) and procedures followed. Sources exist that provide more detailed information on GLP practices, regulatory requirement of agencies, animal care requirements, and Association of Assessment and Accreditation of Laboratory Animal Care (AAALAC) accreditation (44,125–127).

The objectives of the study should be precisely stated and the work presented in a clear and coherent matter, with all the detail necessary to allow the reviewer to make his or her own assessment of the study. It should be confirmed that the overall design of the protocol satisfies the needs of the objectives of the study. It is of the utmost importance that meticulous detail be given in the planning of a study and preparing the protocol for that study (128).

The material tested should be specified, including nature, relative proportions of any impurities, and stability over the test period. All details of the conduct of the study should be presented. It must be established that the methods employed for exposing and monitoring the animals are appropriate and sufficiently specific for the end points or effects planned to be studied.

Attention should be paid to the sufficiency of the study with respect to determining significance and assessing hazard, eg, whether the number of control and test animals is sufficient to allow detection of biological variability in response and for comparative statistical procedures.

There should be sufficient dose–response information to allow decisions on causal relationships and relevance.

The results of the study should allow decisions on whether injury is a direct result of toxicity or secondary to other events. In addition to confirming a causal relationship between exposure to the test material and development of an injury,

Table 4. **Guidelines for Evaluating Studies**

Has the test used an unusual, new, or unproven procedure?
Does the test measure a toxicity directly, or is it a measure of a response purported to indicate an eventual change (a pre-toxic manifestation)?
Have the experiments been performed in a scientifically valid manner?
Are the observed effects statistically significant against an appropriate control group?
Has the test been reproduced by other researchers?
Is the test considered to be more or less reliable than other types of tests in which the chemical has also been tested but has yielded different results?
Is the species a relevant or reliable human surrogate, or does this test conflict with other test data in species phylogenetically closer to humans?
Are the conclusions from the experiment justified by the data, and are they consistent with the current scientific understanding of the test or area of toxicology?
Is the outcome of the experiment dependent on the test conditions, or is it influenced by competing toxicities?
Does the study indicate causality or merely suggest a correlation that could be due to chance?

the study should be reviewed in order to assess whether information is available to determine if the effect is traceable to parent material or metabolite.

In evaluating numerical information, it is important to remember that, although an effect may be statistically significant, this does not necessarily imply that the effect is of adverse biological significance. Conversely, a change or trend which is determined not to be statistically significant may be of biological consequence. Quantitative information, particularly when this involves dose–response considerations, should be reviewed against the background of the study as a whole and the perspective of normal biological variations. In addition to this, Table 4 contains guidelines that may be considered when evaluating studies (47).

7. Dose–Response Relationships

7.1. Basic Concepts. A cornerstone of toxicology is the dose-response relationship. The importance of understanding dose-response relationships is generally attributed to Paracelsus (Philippus Aureolus Theophrastus Bombastus von Hohenheim-Paracelsus; 1493–1541) who so accurately noted - *All substances are poisonous, there is none which is not a poison. The right dose differentiates a poison from a remedy.* In recent times Emil Mrak restated this concept as – *There are no harmless substances, only harmless ways of using substances*. Both statements serve to remind us that there are no safe or unsafe chemicals, and that describing a safe or unsafe exposure is more a function of the magnitude of the exposure (dose) as opposed to the types of toxicities that a chemical might be capable of producing at some dose. For example, vitamins and over-the-counter medications continue to rank as a major cause of accidental poisoning among children. Similarly, all of the types of toxic effects that are associated with "hazardous chemicals" are also produced by prescription medicines, the chlorination by-products formed in drinking water during the disinfection process, the "natural pesticides" in many foods, the heat-derived toxicants formed when certain foods are cooked, and of course, alcoholic beverages or certain

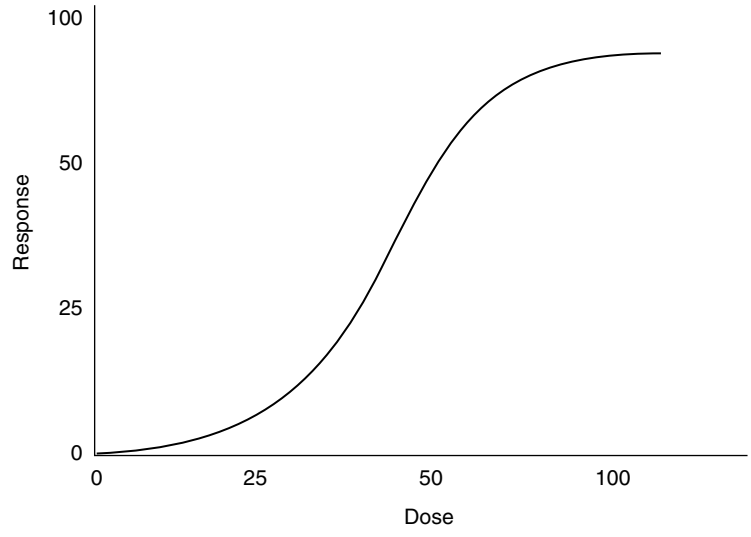


Fig. 2. Typical dose–response relationship.

components of cigarette smoke (129). So, consciously or unconsciously, a number of lifestyle choices alter the numbers and kinds of "toxic chemicals" to which all are exposed to each day.

Because all chemicals are toxic, how do we set safe exposure guidelines? The answer utilizing dose-response relationships to set exposure guidelines at levels where safe, or virtually safe, doses result from each exposure. Across a population of individuals or test animals there is a natural variation in the dose to which a particular member of the group responds. For this and other reasons, the most typical dose-response relationship is represented by a sigmoid curve (Fig. 2). This figure shows that at some dose the most susceptible individuals within a population begin to exhibit the toxicity. Then as the dose is increased more individuals become affected until at some dose even the more resistant individuals respond and 100% of the exposed populations has now developed the toxicity. A dose-response relationship like that depicted in Fig. 2 might also be developed for an exposed population where the *severity* of the response is being measured rather than the *rate* of response.

While Fig. 2 depicts the classic dose-response relationship, Fig. 3 provides illustrations of other types of dose-response relationships. For example, Fig. 3**a** depicts the dose–response curve where the doses are not high enough to induce the toxic response being measured. Here no adverse effect is seen regardless of dose. Figure 3**b** depicts a toxicity where the adverse response is a linear function of any dose greater than zero and represents the assumed dose-response relationship that regulatory agencies typically apply to, and model for, carcinogenic substances. Figure 3**c** is another representation of the most typical dose response curve, showing that at lower doses the chemical is not capable of inducing an adverse response; then, above a specific dose, toxicity increases as the dose increases. Figure 3**d** depicts hormesis, a situation where at low doses the presence of the chemical benefits the organism and decreases the background

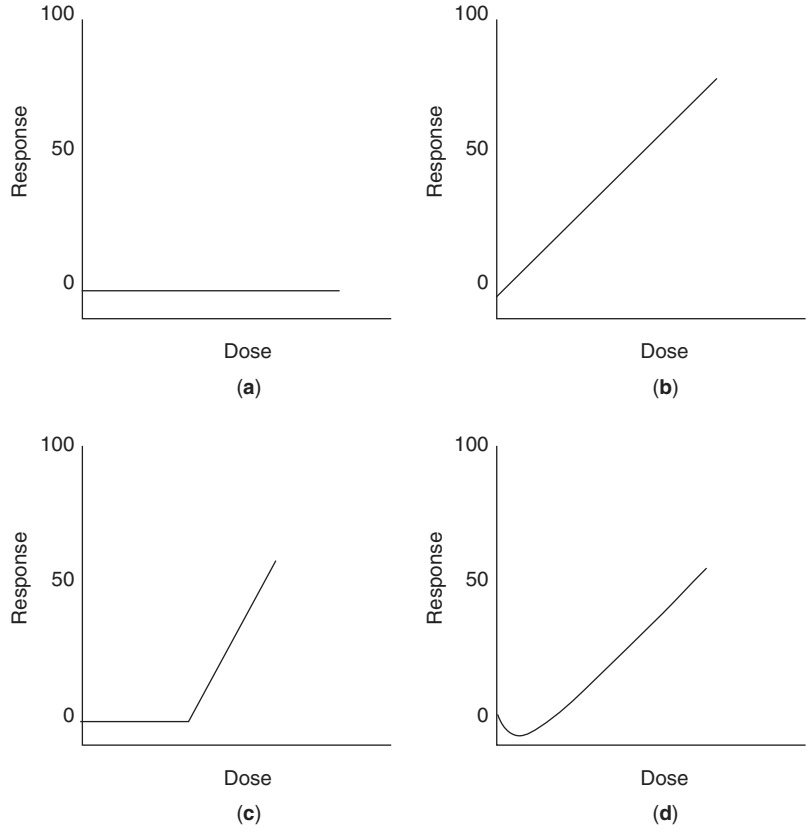


Fig. 3. Other dose-response relationship. See text for explanation.

response rate for the adverse effect under observation. At subsequent high doses however, the biologic presence of too much chemical again results in toxicity and an increase in the adverse effect being observed. Hormesis was initially observed with chemicals that had both beneficial and toxic effects, chemicals like vitamins or medications. However, in recent years interest in this type of dose-response relationship has increased and it has been found that many chemicals considered as "hazardous" or "toxic" produce hormetic dose-response curves when lower doses are examined more rigorously (130).

7.2. Test Conditions May Impact the Dose–Response Relationship. Because toxicology is the study of the adverse responses that a chemical or agent induces, it relies heavily upon test systems that identify and characterize the responses seen in nonhuman organisms or, *in vitro* test systems using isolated organ or cellular components. While a basic premise of toxicology is that the responses measured in these tests systems are representative of what can happen in humans, many experimental design features of the specific testing paradigm being used can affect the reliability of the intended extrapolation to a predicted human response. For example, the design of any toxicity test (testing paradigm) incorporates the following five basic test components: (*1*) The selection of a test organism; (*2*) the selection of a response to measure; (*3*) an exposure period over

which the chemical is given; (*4*) the test duration (the observation period over which responses are measured); and (*5*) a series of doses (dose range) to test.

For example, (*1*) the "test organism" may range from isolated cellular material to strains of bacteria to higher-order plants and animals; (*2*) the biological endpoint being measured can range from subtle changes in organism physiology, biochemistry or behavior up to severe organ pathology or even to death of the organism; (*3*) the exposure and or observation periods may vary from a few hours to weeks to months to lifetime; and (*4*) a vast range of different doses might be selected for testing – the dose-response relationship may vary with a change in any one of the five basic test components. For this reason, testing a chemical under different test conditions and in different test systems produces a number of different dose-response relationships, and each of these relationships may or may not be unique to the exact test components selected. Furthermore, the less comparable the test conditions are to the human exposure situation of interest, the greater the potential uncertainty that will exist in the safety/risk extrapolation being attempted with the dose–response data for that test system. Clearly, tests are sought for which the response being measured is not subjective and can be consistently determined; that the results are conclusive even when the exposure period is relatively short; and the test organism/system responds in a manner that mimics, or is predictive of, the likely human response. However, because some degree of uncertainty exists with the human extrapolation of most types of toxicity data that is collected (eg, individual variation uncertainty, species-extrapolation uncertainty, *in vitro* to *in vivo* extrapolation uncertainty, etc) the total uncertainty associated with the dose–response relationship being used to extrapolate a safe human dosage from may not be known with quantifiable accuracy. To handle this issue safety/risk assessments made using non-human data typically incorporate a number of safety/uncertainty factor considerations, the intent of which is to ensure the final dosage being extrapolated is indeed a safe one upon which to base exposure guidelines (112,131,132,134–136).

As but one example of how dose–response data can change as one changes just one of the test components, Table 5 provides dose–response data for one

Table 5. **Chloroform Toxicity: Inhalation Studies**[a]

Species	Toxicity of interest	Duration of exposure	Exposure/Dose, ppm
mouse	no effect - liver	6 h/day for 7 days	3
mouse	mild liver damage	6 h/day for 7 days	10
mouse	severe liver damage	6 h/day for 7 days	100
mouse	no effect - kidneys	6 h/day for 7 days	100
mouse	mild kidney injury	6 h/day for 7 days	300
mouse	no effect - respiratory	6 h/day for 7 days	300
rat	no effect - respiratory	6 h/day for 7 days	3
rat	nasal injury	6 h/day for 7 days	10
rat	no effect - kidneys	6 h/day for 7 days	10
rat	mild kidney injury	6 h/day for 7 days	30
rat	no effect - liver	6 h/day for 7 days	100
rat	mild liver damage	6 h/day for 7 days	300

[a]Adapted from Ref. 137.

Table 6. **LD_{50} of Chloroform for Various Species**[a]

Species	LD_{50} (mg/kg/day)
rabbit (Dutch Belted)	100[b]
mouse (CD-1)	250
human	**602**
rat (Sprague-Dawley)	908
mouse (Swiss)	1,100
mouse (ICR-Swiss)	1,400[c]
rat (Wistar)	2,180

[a]Adapted from Ref. 137.
[b]Based on 13 days of dosing.
[c]Female mice.

chemical, chloroform, after testing it in just two different but closely related and commonly used rodent test species, the mouse and the rat. Notice that rank order of organ sensitivity to chloroform-induced toxicity in the mouse is liver > kidney > respiratory system. In the rat it is respiratory system > kidney > liver. Note also that the No Observable Adverse Effect Level (NOAEL) for each toxicity is different in both species, and at the highest dose tested, 300 ppm, three different types of toxic effects are observed in the rat but only two are seen in the mouse. These differences raise a common question: which species best represents the human response and why? Note also that selecting 3 ppm as the NOAEL prevents any toxicity that is ultimately observed at higher doses in both species.

As another example of how changing a test component changes the resulting data, the LD_{50} (the dose that induces death in 50% of the tested population) of chloroform is listed for the rabbit, three strains of mice, and two rat strains, along with the estimated human dosage likely taken from an accidental poisoning in Table 6. If one were to assume humans respond like each test organism, then the Dutch Belted rabbit strain and CD-1 mouse strain test results would suggest chloroform is lethal at smaller doses that actual data indicate. In contrast, assuming any one of the remaining four rodent strains mimics the human response would lead one to predict chloroform is not lethal at doses where it actually is. In this situation, one can see that if only one rodent strain had been tested, the possibility that the animal to human extrapolations under- or over-predicted the human response would depend upon the rodent strain test data being extrapolated and the size of the safety/uncertainty factor attached to the extrapolated lethal dose.

When characterizing and/or attempting to extrapolate both qualitatively and quantitatively the hazards associated with a particular chemical exposure, a toxicologist may review the findings of five different categories of toxicity testing (dose–response) information for the safety evaluation of a particular chemical. These categories are: occupational epidemiology (mortality and morbidity studies), clinical exposure studies, accidental acute poisonings, chronic environmental epidemiology studies, basic animal toxicology tests (see Table 7), and the less traditional alternative testing data (eg, invertebrates, *in vitro* data).

7.3. How Dose–Response Data Might be Used. Dose–response data may allow the toxicologist to make several useful comparisons or calculations.

Table 7. **Some of the Advantages and Disadvantages of Toxicity Data by Category**[a]

Advantages	Disadvantages
Occupational Epidemiology (Human) Studies	
May have relevant exposure conditions for the intended use of the chemical.	Exposures (especially past exposures) may have been poorly documented.
As these exposure levels are usually far higher than those found in the general environment, even low or frank effect levels may allow for a realistic extrapolation of a safe level for environmental exposures.	Difficult to properly control; many potential confounding influences (lifestyle, concurrent diseases, genetic, etc) are inherent to most work populations. These potential confounders are often difficult to identify.
The chance to study the interactive effects of other chemicals that might be present. Again at high doses relative to most environmental situations.	*Post facto* – not necessarily designed to be protective of health.
	Separating interactive effects resulting from combinations of chemical exposures may be difficult or impossible.
Avoid uncertainties inherent in extrapolating toxicities and dose-response relationships across species.	The increase in disease incidence may have to be large or the measured response severe to be able to demonstrate the existence of the effect being monitored (eg, cancer). The power to detect risk may be limited.
The full range of human susceptibility (sensitivity) may be measurable if large enough, and diverse enough, populations can be examined.	The full range of human sensitivity for the toxicity of interest may not be measurable because some potentially sensitive populations (young, elderly, infirm) are not represented.
May help identify gender, race or genetically controlled differences in responses.	Effects must be confirmed by multiple studies as heterogeneous populations are examined and confounders cannot always be excluded.
The potential to study human effects is inherent to almost all industrial uses of chemicals. Thus, a large number of different possible exposure/chemical regimens are available to study.	Often costly and time consuming. Cost-benefit may be low if confounders or other factors limit the range of exposures, toxicities, confounders, or population variations that might occur with the chemical's toxicity.
Clinical (Human) Exposure Studies	
The toxicities identified and the dose-response relationship measured are reported for the most relevant species to study (humans).	The most sensitive group (eg, young, elderly, infirm) may often be inappropriate for study.
Typically the components of these studies are better defined and controlled than occupational epidemiology studies. Prospective study design, rather than retrospective design, is used.	Moderately costly to costly to perform.
The chance to study the interactive effects of other chemicals.	Usually limited to shorter exposure intervals than epidemiological studies.

Table 7. (*Continued*)

Advantages	Disadvantages
The dose-response relationship is measured in humans. Exposure conditions may be altered during the exposure interval in response to the presence or lack of an effect making NOAELs or LOAELs easier to obtain.	Only NOAELs are targeted for study. These studies are primarily limited to examining safe exposure levels or effects of minimal severity. More serious effects caused by the chemical cannot intentionally be examined by this type of study.
Better than occupational studies for detecting relatively subtle effects. Greater chance to control for the many confounding factors that might be found in occupational studies.	Chronic effects are generally not identifiable by this type of study.
Allows the investigator to test for and identify possible confounders or potential treatments.	Requires study participant compliance.
Allows one to test the specific subpopulations of interest.	May require confirmation by another study.
May help identify gender, race or genetically controlled differences in responses.	May raise ethical questions about intentionally exposing humans to toxicants.
May be the best method for allowing initial human exposure to the chemical, particularly if medical monitoring is a prominent feature of the study.	Unexpected human toxicities may occur as animal extrapolations are not perfect.
Use of randomization improves the study design and provides best causal inference.	The change being monitored may be statistically significant but still of unknown biological/clinical relevance, leaving the interpretation of results open to question.
Environmentally Exposed Epidemiology Studies	
The toxicities identified and the dose-response relationship measured are reported for the most relevant species to study (humans).	Exposures to the chemical are typically low relative to other types of human exposures to the chemical in question, or to chemicals causing related toxicities (eg, exposure to other environmental carcinogens). Thus, attributing the effects observed in a large population may be difficult if many confounding risk factors are present and uncontrolled for in the exposed population.
Exposure conditions are relevant to understanding or preventing significant environmentally caused health effects from occurring.	The exposure of interest may be so low that it is nontoxic and only acting as a surrogate indicator for another risk factor that is present but not identified by the study.
The chance to study the effects of interactive chemicals may be possible.	The number of chemicals with interactive effects may be numerous and their exposures large relative to the chemical of interest. This will confound interpretations of the data.
The full range of human susceptibility may be present.	The full range of human susceptibility may not be present.

Table 7. (*Continued*)

Advantages	Disadvantages
May allow one to test specific subpopulations of interest for differences in thresholds, response rates, and other important features of the dose-response relationship.	The full complement of relevant environmental exposure that is associated with the population are not necessarily identified or considered.
May help identify gender, race or genetically controlled differences in responses.	Large populations may be so heterogeneous in their makeup that when compared to control responses that differences in confounders, gender, age, race, etc, may weaken the ability to discriminate real diseases associations of the chemical exposure from other causes of the disease.
	There may be too many potential confounders to identify and control for and the correlation may be coordinated rather than causal, ie, the problem of the 'Ecological Fallacy.'
	Exposures are frequently not quantified at the individual level.
Acute Accidental Poisonings	
Exposure conditions are realistic for this particular safety extrapolation. In most instances, poisonings are limited to acute exposure situations.	Because the exposure is either accidental or related to a suicide attempt, accurate exposure/dose information is frequently lacking.
These studies often provide a temporal description indicating how the disease will develop in an exposed individual.	This knowledge gained from these studies may be of limited relevance to all other human exposure situations.
Identifies the target organs affected by high, acute exposures. These organs may become candidate targets for chronic toxicity studies.	Confounding factors affecting the magnitude of the response may be difficult to identify as exposure conditions will not be recreated to identify modifying factors.
The clinical response requires no planning as the information gathering typically consists of responding to and treating the organ injuries present as they develop.	Acute toxicities may not mimic those seen with chronic exposure. This may mislead efforts to characterize the effects seen under chronic exposure situations.
	These studies are typically case reports or a small case series and so measures of individual variations in response may be difficult to estimate.
	These chance observations develop without warning, a feature which prevents the development of a systematic study by interested scientists who are knowledgeable about the chemical.
	Because these typically occur as emergency situations, important clinical data may not always be collected.

Table 7. (*Continued*)

Advantages	Disadvantages
Animal Toxicity Tests	
Easily manipulated and controlled.	Test species response is of uncertain human relevance. Thus, the predictive value is lower than that of human studies.
Best ability to measure subtle responses.	Species/strain/sex/age responses may vary significantly both qualitatively and quantitatively. Thus, a number of different species/strains (both sexes) should ideally be tested.
Widest range of potential toxicities to study.	Exposures levels may not be relevant to (they may far exceed) the human exposure level. The restricted environment of the animal study may not be representative of the complex and variable environment of humans. For example, the practice of allowing animals to eat at will (*ad libitum* feeding) in bioassays has been shown to increase response rates of certain carcinogens.
Chance to identify and elucidate mechanisms of toxicity that allow for more accurate risk extrapolations to be made using all five categories of toxicity test data.	Selecting the best animal species to study, ie, the species with the most accurate surrogate responses, is always unknown and is difficult to determine *a priori* (without a certain amount of human test data). Thus, animal data poses somewhat of a *Catch 22* situation, ie, you are testing animals to predict human responses to the chemical but must know the human response to that chemical to accurately select the proper animal test species. Mechanisms that are developed may be unique to that species/strain/sex being tested.
Cheaper to perform than full scale epidemiology studies.	May be a poor measure of the variability inherent to human exposures because animal studies are so well controlled for genetics, doses, observation periods, etc.
No risk of producing adverse human health effects during the study.	The reproducibility of the animal response may create a false sense of precision when attempting human extrapolations.
The introduction of more *in vitro* and alternative animal models that use simpler organisms may lessen the need for larger animals in toxicity testing for regulatory, mechanistic, and descriptive purposes.	The desire to add *in vitro* studies as a means of lessening the use of whole animals adds even greater uncertainty to human extrapolation.

[a]Adapted from Ref. 133

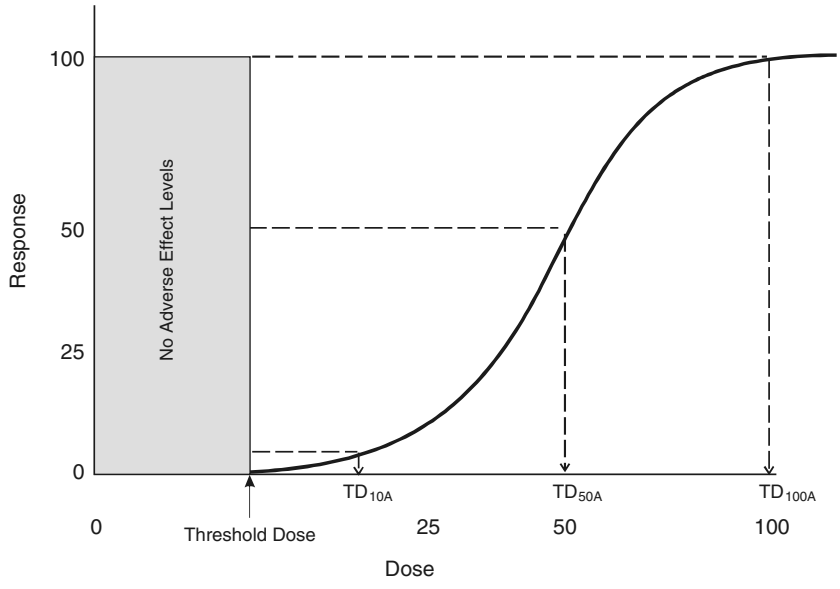


Fig. 4. Dose–response curve showing threshold dose, TD_{10}, TD_{50}, and TD_{100}.

For example, depending upon the situation the toxicologist may want to know the threshold dose, the dose toxic to 10% of the population (TD_{10}), the dose toxic to 50% of the population (TD_{50}) or the entire population (TD_{100}). Figure 4 shows all of these points on a single dose response curve. At all doses below this point no one in the population exhibits toxicity. This dose, below which there is no toxicity and above which toxicity begins to increase with increasing dose is known as the *threshold dose*.

Knowledge of these basic characteristics of a particular dose-response curve might be used in the following manner. For example, as in Figure 5 one might choose to compare three different dose-response curves – dose-response curves that represent either hypothetical toxicants A, B, and C, or the hypothetical toxicities-1, -2, and -3 of chemical X. Looking at either the NOAEL (or threshold dose) or the TD_{50} dose, one can make a relative potency comparison (toxicity relative to the dose used) of the three chemicals A/B/C, or for the three toxicities-1/2/3 that is produced by a chemical ("X"). Knowing this difference in potency for chemicals A, B, and C may allow one to select the least toxic chemical (least potent chemical) for a particular use. In this situation, one would select chemical C because individuals would have to be exposed to greater doses of chemical C before toxicity is inadvertently induced. Likewise, knowing the relative potencies of adverse toxicites-1, -2, and -3 might help one focus on which toxicity is most likely to be seen in human studies at the lowest dose, ie, this would indicate which toxicity to look for first in a population where the exposure has been increased above safe levels. Alternatively, if one were attempting to set a safe exposure limit then by keeping all doses below the threshold dose for toxicity-1 will prevent all three toxicities that chemical induces. For example, assume toxicity-1 is a reversible, nondebilitating adverse effect (eg, eye/nose irritation)

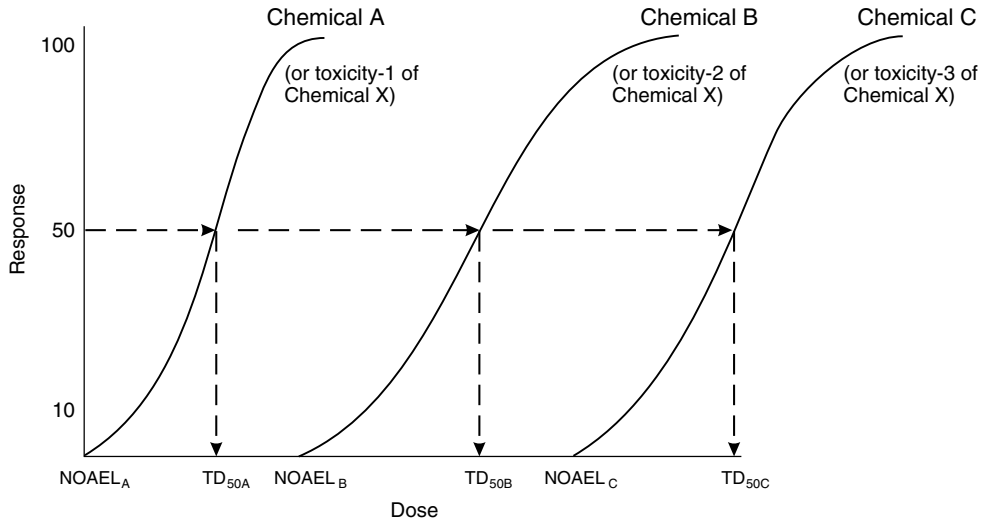


Fig. 5. Comparison of three dose–response curves.

whereas toxicity-2 represents chemical-induced asthma and toxicity-3 represents chemical-induced bronchiolar damage. By determining the dose range of the irritant effects of chemical X in humans one could strive to set the allowable exposure level such that no one or almost no one incurs even slight irritation may be a way of setting an occupational exposure guideline for chemical X that prevents it from causing more serious pulmonary toxicities in the workplace.

Another way in which dose–response data can be used to make a relative safety comparison is to perform a *margin of safety* determination. Here one determines the relative magnitude of the differences in dose required to induce a toxicity one is trying to prevent relative to the dose that induces a mild, safely monitored effect. For example, margins of safety are typically derived for chemicals which have either innocuous or desirable effects at low doses, but have serious side effects or toxicities at higher doses, such as might be seen with the use of vitamins or a medication. The margin of safety was originally calculated from data like that shown in Figure 6, by dividing the TD_{50} (the dose associated with the 50th percentile response for some adverse effect) by the ED_{50} (the dose associated with the 50th percentile response for some "safe" or "harmless" effect). This difference is shown by arrow A in Figure 6. This calculation is frequently considered with chemicals like medications/drugs where one wants to know the magnitude of difference between the therapeutic effects the drug is used for and any toxicities it might induce at higher dosages. The higher the margin of safety, the safer the chemical is to use (ie, the greater the room for usage error). However, one might want to use a more protective definition of the margin of safety (for example, TD_{10}/ED_{100}), the margin of safety might express the relative difference in magnitude of dose between the dose inducing a safe but observable response in 100% of the population versus that dose causing an adverse affect in sensitive subpopulations. This difference is shown by arrow B in Figure 6. By changing the definition to include a higher percentile of the nontoxic

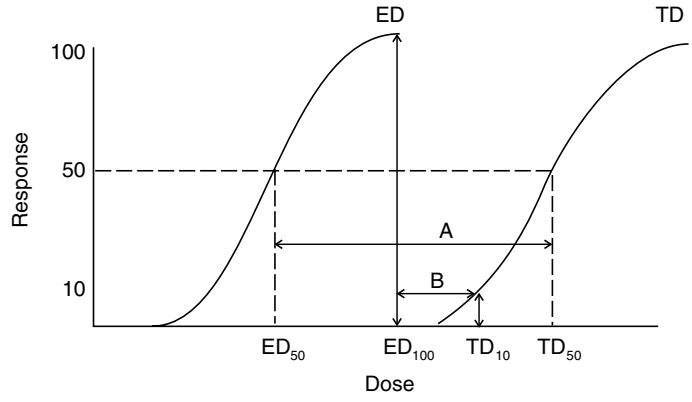


Fig. 6. Dose–response curve, as used to determine margin of safety.

dose-response curve (eg, the ED_{100}) and correspondingly lower percentile of the toxic dose–response curve (eg, the TD_{10} or the TD_{01}) the calculated margin of safety, becomes the difference between the completely effective dose for all individuals versus the dose toxic to any individual of a population. (Margin of safety $= TD_{50}/ED_{50}$ or redefine it as $= TD_{01}/ED_{100}$).

Perhaps the most common use of dose-response curves is to estimate the threshold dose as shown in Figure 7. The threshold dose is defined as that dose on the dose-response curve as the point above which toxicity begins to be observed and below which no toxicity is observed. Where a limited number of doses are tested, one might not identify the exact dose representing the true threshold dose but still identify a dose below it representing a No Observable Adverse Effect Level (NOAEL). In these situations the NOAEL is used in place of the threshold dose. Because all exposures producing doses less than

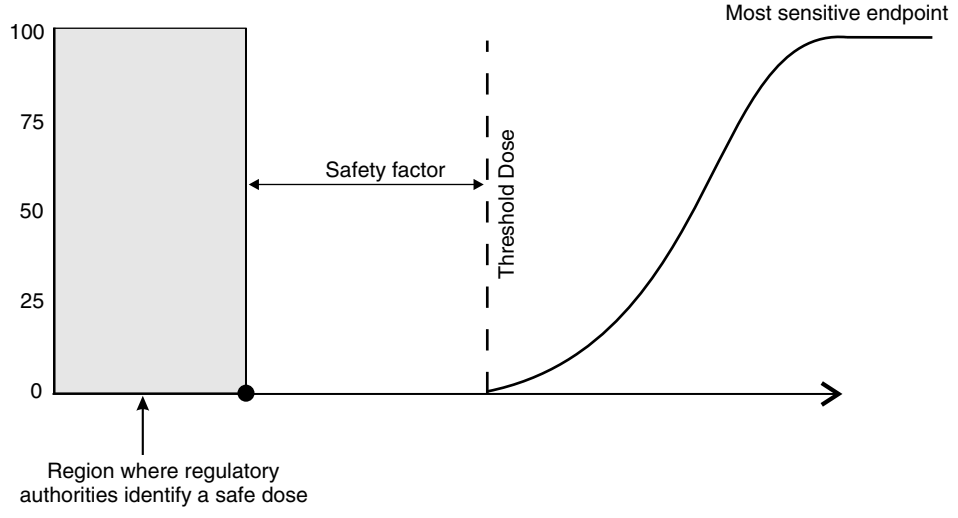


Fig. 7. Regulatory use of dose-response curves to set safe levels.

the threshold dose (or NOAEL) should be devoid of observable toxicity, they all represent safe exposure levels. However, when extrapolating from animal data, as must typically be done in toxicology, there is always some uncertainty as to how closely the animal dose–response data quantitatively mimics the actual human dose-response curve. As a precautionary approach then, safety/uncertainty factors are selected and the NOAEL/threshold dose is divided by the combined safety/uncertainty factor to lower the final dose selected as the safe dosage for the development of any intended exposure guideline. As can be seen in Fig. 7, the net effect of dividing the threshold or NOAEL dose by some total safety/uncertainty factor is that it is equivalent to selecting a lower dose from the no-effect region of the dose-response curve we are utilizing. This, in turn, helps ensure that the data which we have extrapolated from has not understated the potency of the chemical in humans, and provides an additional margin of safety to our estimate of the safe human dose.

7.4. Risk Assessment. *Regulatory Applications of Dose–Response Data.* Regulatory risk assessments treat and model the hazards posed by the noncarcinogenic, systemic toxicities of chemicals differently from the carcinogenic effects of chemicals. For noncarcinogenic substances, the method for setting the safe human dosage is graphically like that illustrated in Figure 7 and is mathematically derived by a process similar to the equation shown below. In short, it is believed that for all systemic toxicities there exists a dose below which no observable or statistically measurable response exists. This dose, the threshold dose, exists because the body possesses a variety of detoxification and cell defense and repair mechanisms which for doses below the threshold dose render the magnitude of effect of the chemical so small that it is undetectable and biologically meaningless. By limiting exposure to doses where no effect is expected, the risk of sustaining a systemic toxicity is effectively zero. For carcinogenic substances a different dose-response relationship has long been assumed by regulatory agencies that is graphically represented in Fig. 3**c**. In this model there is no threshold and no zero response range; instead it is assumed that any finite, nonzero dose possesses some potential for producing an adverse effect. Based on this presumption, defining safe exposures becomes probability-based as zero risk occurs only at zero dose. In contrast to non-cancer health effects, where a determination is made of the dose at which no toxic effect will occur, cancer risk assessment is a matter of limiting exposure to a dose where the chance of developing cancer over a lifetime is so small that it can be regarded as a *de minimis* or inconsequential risk relative to the total background risk one incurs from everyday life. Thus, the presumed dose–response relationships, and the use of these relationships to provide estimates of risk or safety, are very different for non-carcinogenic and carcinogenic effects as described in further detail below.

Calculating Safety for Threshold (Systemic) Toxicities

$$SHD = \left(NOAEL \text{ or } \frac{LOAEL}{UF_L}\right)^* \frac{1}{UF_H^* UF_A^* UF_{SC}^* UF_{DB}} \quad (1)$$

Where:

SHD = safe human dosage

$NOAEL$ = denotes the threshold dose or some other no-observable-adverse-effect-level, which is selected from the no-effect region of the dose-response curve for the most sensitive endpoint (the toxicity occurring at the lowest doses).

$LOAEL$ = denotes a lowest-observable-adverse-effect-level, which is selected from the dose-response region of the most sensitive endpoint, and is only used when a NOAEL or threshold cannot be determined from the available data.

The total uncertainty factor is the multiplication of the values for each kind of uncertainty factor as reflected in the quality of the data. The different kinds of uncertainty factors considered include:

UF_H = an uncertainty factor that may range in value between 1 and 10 to account for variations/uncertainty in the susceptibility of individuals within a human population.

UF_A = an uncertainty factor that may range in value between 1 and 10 to account for the uncertainty introduced when extrapolating from animal data. This uncertainty factor is based on the conservative assumption that humans may be more sensitive than the animal species tested. It helps ensure that the potency of the chemical in humans has not been underestimated by the animal species that was tested.

UF_{SC} = an uncertainty factor that may range in value between 1 and 10 to account for the uncertainty introduced when using dose-response data that was derived from studies of less than a chronic or lifetime exposure period. It is conservatively assumed that as the exposure period increases, smaller daily dosages may produce the same toxicity observed with shorter dosing intervals. It is typically applied when subchronic data are used because a chronic study was not available in the database.

UF_L = an uncertainty factor that may range in value between 1 and 10 to account for the uncertainty introduced when using a LOAEL rather than a NOAEL or threshold dose as the dose-response-point upon which the calculation is based. It is assumed that at a dose ten-fold lower than the LOAEL a no-effect response would be seen.

UF_{DB} = an uncertainty factor that may range in value between 1 and 10 to account for the uncertainty introduced when the available database is not considered robust. This uncertainty factor is based on the assumption that where certain kinds of toxicities have not been studied, the most sensitive endpoint and dose-response curve might not have been identified yet.

Where the database does not suffer from one or more of these uncertainty factors a value of 1 is selected. Because of concerns that the multiplication of all five uncertainty factors (100,000) is likely to yield an unrealistically low number, regulatory agencies like the USEPA tend to limit the total uncertainty factor to 3,000 when uncertainty exists in four areas and to 10,000 when uncertainty

exists in all five areas. Consistent with this concern studies indicate that when several uncertainty factors are applied the perceived NOAEL so generated is lower than that which would actually be observed in animal studies if a more rigorous database were to be developed (12,92,138–142).

The most common quantitative means of expressing hazards for systemic or noncancer health effects is through a hazard quotient (HQ). Agencies such as the U.S. EPA calculate a HQ as the estimated dose from exposure divided by their form of the SHD, the agency's reference dose (RfD):

$$HQ = \frac{D}{RfD} \tag{2a}$$

$$\left[or\ HQ = \frac{D}{SHD} \right] \tag{2b}$$

Where:

HQ = hazard quotient
D = dosage (mg/kg-day) estimated to result from exposure via the relevant route
RfD = reference dose (mg/kg-day)

When an exposure situation results in an estimated human dosage where the HQ is less than one, the exposure is considered safe (no adverse effects would be expected under the exposure circumstances). An interpretation of HQ values greater than one is more complicated and is dependent upon how the safe human dosage or RfD was calculated. While a value greater than one indicates that the estimated exposure exceeds the Agency's version of the safe human dose, the SHD includes a number of uncertainty factors that may still impart a substantial margin of safety. So, even exposures that exceed the SHD, but lie well within this total margin of safety, may still be considered relatively safe exposures if the remaining margin of exposure between the actual human dose and original NOAEL selected for the safe dose calculation remains large. In this situation, an adverse effect might still not be anticipated.

Typically, the safe human dosage (or RfD) is calculated for each chemical based on the toxic endpoint that occurs over the lowest dose range. By preventing this toxicity, all other systemic toxicities are prevented because the remaining endpoints require higher doses. However, a safe human dosage could also be calculated for each type of systemic toxicity that the chemical induces, and systemic organ-specific safe human doses could be used to evaluate potential human exposures where desired.

Typically, the uncertainty factor used varies from 10 to 10,000 and is dependent upon the confidence placed in the animal database as well as whether or not there are human data to substantiate the reliability of the animal no-effect levels that have been reported. The safe human dosage calculated should, where possible, use chronic exposure data if chronic exposures are expected and acute data where acute exposures are expected.

This type of model calculates one value, the expected safe human dosage, which regulatory agencies have variously referred to using terms like the Acceptable

Daily Intake (or ADI; U.S. Food & Drug Administration), the Reference Dose (or RfD; U.S. Environmental Protection Agency), or the Minimal Risk Level (or MRL; Agency for Toxic Substances and Disease Registry). When the exposure assessment develops an expected human exposure that produces a human dose/dosages that is(are) at or below these safe human dosages (ADIs or RfDs), then the exposure is considered safe. It would presumably seem that the ADIs and RfDs for each chemical would be the same, but frequently they are not. This is because they are derived by different regulatory agencies and scientific groups, may be derived at different times when different data are available, occasionally emphasize different endpoints or dose-response data, and/or may reach different decisions regarding the uncertainty and modifying factors considered to be sufficient. Thus, both the perceived safe daily human dosage rate and the biologic endpoint that an ADI, RfD, or MRL is designed to prevent may differ among the regulatory agencies.

Information on acute exposure guidelines, reference doses, reference concentrations, cancer categories, etc, are available from various sites (Table 8).

Once the safe human dose has been estimated, it may be necessary to convert the dose into a concentration of the chemical in a specific environmental medium (eg, air, water, food, soil, etc) that corresponds to a safe exposure level for that particular route of exposure. Because the volume of air people inhale each day differs from the volume of water they drink, the volumes of food they eat, the surface area they might have dermal contact with, or the soil they might incidentally ingest, the final medium-specific concentration or medium-specific safe exposure level of a chemical will differ depending upon the anticipated route of exposure and corresponding environmental medium the chemical is found in. In short, different equations are used to derive the exposure concentration for each medium that represent the same, safe daily dose. As inhalation is usually a major route for occupational exposures, a formula for converting the safe human dosage into its corresponding air concentration is shown below:

$$Dosage = \frac{(\alpha)(BR)(C)(t)}{BW} = \#\,mg/kg$$

$$\text{if} \quad HQ = \frac{ADD}{SHD} \quad \text{(eq. 2b)} \quad \text{and} \quad ADD = \frac{C^{*}\alpha^{*}BR^{*}t}{BW}, \quad \text{then} \qquad (3)$$

$$HQ = \frac{\dfrac{C^{*}\alpha^{*}BR^{*}t}{BW}}{SHD}$$

Where:

$\alpha =$ percent of the chemical absorbed by the lungs (if not known, considered to be 100 percent);

$BR =$ breathing rate of the individual (which, for a normal worker, can be estimated as two hours of heavy breathing at 1.47 m^3/hr or as six

Table 8. **Guidelines Sites**

Source	Information provided	Reference
American Industrial Hygiene Association's (AIHA) Emergency Response Planning Guidelines (ERPGs)	provides acute emergency response guidelines	www.aiha.org/1documents/Committees/ERP-erpglevels.pdf
USEPA's Acute Exposure Guideline Levels (AEGLs)	provides acute threshold exposure limits for the general public for exposure durations ranging from 10 minutes to eight hours	www.epa.gov/oppt/aegl/index.htm
USEPA's National Ambient Air Quality Standards (NAAQS)	provides air standards for six 'criteria' pollutants (ozone, particulate matter, carbon monoxide, sulfur oxides, nitrogen oxides, and lead) for the general public	www.epa.gov/air/criteria.html
Toxicology Excellence for Risk Assessment's (TERA) International Toxicity Estimates for Risk (ITER) Database	provides cancer and noncancer risk values from various agencies	toxnet.nlm.nih.gov/cgibin/sis/htmlgen?iter or iter.ctcnet.net/publicure/pub_search_list.cfm
USEPA's Integrated Risk Information System (IRIS) Database	provides cancer and noncancer risk values along with cancer categories for chemicals	www.epa.gov/iris
USEPA's List of Drinking Water Contaminants and Maximum Contaminant Levels (MCLs)	provides allowable concentrations for various chemicals, biological, and radiological constituents in drinking water	www.epa.gov/OGWDW/mcl.html
Agency for Toxic Substances and Disease Registry's (ATSDR) Minimal Risk Levels for Hazardous Substances (MRLs)	provides risk levels for acute, intermediate, and chronic exposure situations for oral and/or inhalation exposure routes	www.atsdr.cdc.gov/mrls.html
American Conference of Governmental Industrial Hygienists (ACGIH) TLVs (Threshold Limit Values) and BEIs (Biological Exposure Indices)	provides occupational exposure value for air and biological exposure limits for workers	American Conference of Governmental Industrial Hygienists. Cincinnati, Ohio (513)742-2020 or www.acgih.org
Occupational Safety and Health Administration's (OSHA) PELs (Permissible Exposure Levels)	provides occupational exposure limits for workers	www.osha.gov/SLTC/pel

Table 8. (*Continued*)

Source	Information provided	Reference
National Toxicology Program's (NTP) Report on Carcinogens	cancer classifications for compounds and mixtures	ehis.niehs.nih.gov/roc
International Agency for Research on Cancer (IARC) Monographs	discussion of carcinogenicity of compounds, mixtures, and exposure circumstances and cancer classification	monographs.iarc.fr

hours of moderate breathing at 0.98 m^3/hr), depending upon the size and physical activity of the individual;

$C =$ concentration of the chemical in the air (mg/m^3);

$t =$ time of exposure in hours (usually considered to be 8 h);

$BW =$ body weight in kilograms (usually considered to be 70 kg for men and 60 kg for women).

$ADD =$ average daily occupational dose (mg/kg-day)

$HQ =$ hazard quotient (unitless)

Rearranging the preceding formula to be able to calculate the safe air concentration corresponding to the safe daily dosage of the chemical and assuming an HQ of 1 represents a safe dosage, equation 3 becomes:

$$C = \frac{1^{*}SHD^{*}BW}{\alpha^{*}BR^{*}t} \tag{4}$$

Where:

$SHD =$ (Threshold dosage divided by the uncertainty factor)

This type of calculation can be used in two ways: (*1*) to predict a safe occupational airborne concentration for a chemical when there are no established airborne standards; and (*2*) to compare an established occupational airborne standard (such as the threshold limit value (TLV) established by the ACGIH–or an OSHA standard) to newly derived animal toxicity data.

For many environmental exposures the USEPA assumes that $\alpha = 100$ percent, and that for adults, the daily inhalation volume, equal to $(BR)(t)$, is 20–24 m^3 for a 24-hour period. To calculate the corresponding safe daily environmental air concentration for a chemical, the safe human daily dose (in units of mg/day) is divided by this total inhalation volume (in units of m^3/day). So, the acceptable air concentration (C) mg/m^3 = SHD ÷ 20 m^3/day (or 24 m^3/day). Should it be desirable to express the safe air concentration in parts of toxicant per million parts of air, the value of C (where the air concentration is in units of milligrams per cubic meter of air [mg/m^3]) may be converted to a ppm using the following equation:

$$ppm = \frac{C(\mathrm{mg/m^3}) \times 24.5}{MW} \tag{5}$$

where *MW* is the molecular weight of the chemical (g/mol), and 24.45 is the amount (L) of vapor per mole of contaminant at 25°C and 760 mm Hg.

The same logic as employed for equations 3 and 4 can be applied to other exposure routes; and, safe exposure levels can be derived for other exposure media (eg, drinking water, food, and incidental soil ingestion). One simply substitutes the intake rate assumptions (*BR*t* in eq. 3) for appropriate route-specific assumptions for other media of interest. For instance, if incidental soil ingestion is the route of interest then the intake rate is the amount of soil ingested per day (usually assumed for an adult to be 50 mg [or 0.00005 kg] per day). Another route-specific intake rate assumption is 2 L/day drinking water. For crops, or homegrown vegetables, the average daily intake for that food group could be inserted. As an example, the calculation for a safe soil concentration of a non-carcinogenic contaminant in soil involves the following substitutions in equation 4:

$$C = \frac{1^{*}SHD^{*}BW}{\alpha^{*}IR_{\text{soil}}} \tag{6}$$

Where IR_{soil} = soil ingestion rate (kg soil per day)

One should always be careful when using equations such as 4 and 6 that the dimensional units of the equation's parameters will result in the correct units in the solution. That is, incidental soil ingestion rates usually have units of mg soil per day. Because soil concentrations usually have units of mg chemical per kg soil, either the soil ingestion rate has to be converted to kg per day (as was done above) or a unit correction factor of 1,000,000 mg soil per kg soil has to be used in the equation. Also equation 6 presumes that exposure is continual (ie, occurs seven days per week, 52 weeks per year) which may not be the usual case; families may go on vacation for two weeks out of the year. Terms for Exposure Frequency (EF, days per year), Exposure Duration (years), and Averaging Time (AT, ED * 365 days per year) can be included in equation 6 to account for discontinuous exposure.

Calculating Safety for Exposures to Carcinogens. As stated above, the modeling of safe exposure levels to carcinogenic substances differs in two fundamental ways. Because the long-held regulatory assumption has been that carcinogenic effects are a linear function of dose for all doses above zero (see Fig. 3**b**), the likelihood of the response being zero does not become zero until the dose is zero. So, unlike non-carcinogenic systemic effects, some risk is assumed to be associated with even small doses. This in turn means that at some small dose, the probability of an effect will be so small that the attendant probability the effect will occur is so small it can be considered to be a safe exposure. This dose has frequently been termed the virtually safe dose, and is a lifetime average daily dosage where the risk of getting cancer typically ranges between one in a million persons (1/1,000,000 or 1×10^{-6}) so exposed to one in ten thousand persons so exposed (1/10,000 or 1×10^{-4}).

Because the lifetime risk of the effect occurring is a linear function of the lifetime average daily dosage (LADD), the calculation of the cancer risk associated with a particular exposure is relatively simple. Typically, agencies like

the USEPA provide cancer slope factors for many carcinogens (referred to simply as slope factors), and these slope factors are provided in inverse units of dosage (they are essentially the slope of the modeled dose-response curve of a particular carcinogen). When the *slope factor* for a chemical is multiplied by the LADD (mg/kg-day) resulting from exposure to that chemical, the resulting dimensionless number is the probability that the exposed person (or fraction of the exposed population) will develop cancer during their lifetime as a result of that exposure. So, estimating the lifetime cancer risk associated with a particular dose is a relatively simple mathematical process, and is essentially calculated in the following manner:

$$\mathrm{R} = \mathrm{D} \times \mathrm{SF} \tag{7a}$$

or

$$\mathrm{R} = \mathrm{LADD} \times \mathrm{SF} \tag{7b}$$

Where:

$R =$ risk
$D =$ dose [Normally expressed as the Lifetime Average Daily Dose or LADD (mg/kg-day)]
$SF =$ the chemical-specific cancer slope factor [the slope of the dose–response curve in units of $(\mathrm{mg/kg\text{-}day})^{-1}$]

In equation 7b, the total cumulative dose the individual or population has accumulated during their entire exposure interval is first converted into a lifetime average daily dose (LADD), a dose that if received everyday for a lifetime would be equivalent to the total dose accumulated during the actual exposure period. For example, if the exposure assessment projected a daily dosage of 0.3 mg/kg-day for a 7-year exposure interval, then the LADD (assuming a 70-year lifespan), would be 0.030 mg/kg-day (ie, 0.3 mg/kg-day $\times$ 7 years $+$ 70 years $=$ 0.03 mg/kg-day [or 3.0E-2 in scientific notation]). The dose is expressed in units of mg/kg-day and the CSF is in units of reciprocal mg/kg-day or $(\mathrm{mg/kg\text{-}day})^{-1}$. Using this example LADD calculation and assuming the *slope factor* for a carcinogen is 0.0015 $(\mathrm{mg/kg\text{-}day})^{-1}$ (in scientific notation a value of 1.5E-3 $(\mathrm{mg/kg\text{-}day})^{-1}$), the corresponding lifetime risk would be (eq. 8):

$$\mathrm{Risk} = 0.0015 \times 0.03 = 0.000045 \tag{8}$$

which can also be written as

$$\mathrm{Risk} = 4.5/100,000 \quad \text{or as} \quad \mathrm{R} = 4.5 \times 10^{-5}$$

In eq. 8 the risk estimate that represents a 4.5/100,000 chance, or mathematical probability, that a cancer will develop sometime during their lifetime for each person so exposed. It should also be noted, however, that because regulatory agencies strive for conservative, health-protective risk calculations, the SF used is statistically an upper-bound estimate of the dose-cancer relationship. Thus, the calculated risk is an upper bound estimate of the risk. The true cancer risk of the chemical at this dose may be much less than that calculated, and in fact, could be as low as zero because of the conservative assumptions typically used by regulatory agencies to derive the slope factor as well as the conservative assumptions used in exposure assessment used to derive the daily dosage during the exposure interval.

In recent years, controversy involving the actual shape of the dose-response curve for carcinogens in the low-dose region and the possible existence of thresholds for certain carcinogenic substances has caused regulatory agencies to evaluate other risk assessment methodologies. Out of this re-evaluation has come a movement to adopt two major policy changes in the cancer risk assessment methodologies employed by regulatory agencies.

One proposed change is to use risk extrapolation models that make fewer assumptions about the shape of the dose–response curve in that portion of the dose-response region where the responses are too small to accurately measure. One approach, the benchmark dose and margin-of-exposure method, makes no assumptions about the shape of the dose–response curve in the low-dose region and merely models the shape of the observable portion of the dose–response region. Data within the observable dose-response range can be used to estimate the doses associated with small response rates like 1%, 5% or 10%, and the lower confidence limit of one of these response rates on the dose-response curve is then selected as the "point of departure." For noncarcinogenic effects then, the point of departure is used (NOAEL, LOAEL or threshold dose) and safety is determined by selecting a dose that is 100 to 10,000-fold lower than the point of departure. This difference is referred to as the "margin of exposure" that exists between these two doses. Rather than assuming this dose so selected is now assuredly in the no-response region it is merely assumed that the exposure is safe because a sufficient margin exists between the dose associated with the allowed exposure and the point of departure, a dose where a low rate of toxicity exists.

The second proposed change is to begin to allow for the consideration and use of nonlinear and threshold models for carcinogens where empirical and mechanistic evidence argues strongly which type of dose-response model may be most appropriate for a particular chemical. For carcinogenic substances where the mechanistic data suggests the chemical may induce cancer via a genotoxic mode of action, a nonthreshold extrapolation is assumed and a straight line drawn between the point of departure and zero is assumed to define the dose-response curve for doses below the point of departure. Where the mechanistic data suggests the chemical does not induce cancer via a genotoxic mode of action, a dose-response curve like that shown in Fig. 3**c** is assumed and the risk of cancer would be evaluated in a manner entirely analogous to non-cancer health effects, such as through a calculation deriving a threshold dose with a sufficient margin of safety.

8. Glossary

Brief definitions for keys terms that are of interest in toxicology are as follows (3–5,133):

Toxicology: the study of the interactions between chemicals and biological systems in order to determine quantitatively their potential to produce injury that results in adverse health effects in intact living organisms; and, to investigate the nature, incidence, mechanism of production, and reversibility of such adverse effects. Different sub disciplines of toxicology exist and include: basic research or mechanistic toxicology; regulatory toxicology; forensic toxicology; clinical toxicology; veterinary toxicology; occupational toxicology; environmental toxicology; food toxicology; ecotoxicology; aquatic toxicology; and analytic toxicology.

Toxicant: chemical, physical, or biological agent that has the potential to cause an adverse effect to a living organism at sufficient doses.

Toxin: a toxin is a toxicant that is naturally produced by organisms, including plants or animals (eg, cardiac glycosides from the oleander plants).

Hazard: the qualitative nature of the adverse effect (ie, the type of adverse effect) resulting from exposure to a particular toxicant (eg, asphyxiation is the hazard from acute exposure to carbon monoxide).

Safety: a measure or mathematical probability that a specific exposure or dose will not result in an adverse effect.

Exposure: when a toxicant comes into contact with an organism; various routes of exposure include air (inhalation exposure), water, soil, oral, or skin (dermal exposure).

Dose: the total amount of a toxicant given to an organism at specific time intervals (dose is commonly expressed in units of milligrams per kilogram per day, which is abbreviated as mg/kg-day).

Absorbed dose, or internal dose: the actual amount of a toxicant that is absorbed into the organism and distributed throughout the body.

No observable effect level (NOEL): the highest dose or exposure level at which there are no statistically or biologically significant increases in observable effects in an exposed population when compared to a control population.

No observable adverse effect level (NOAEL): the highest dose or exposure level at which there are no biologically significant increases in observable adverse effects in an exposed population compared to a control population; while some effects may be produced at this exposure level, they are not considered adverse or precursors of adverse health effects.

Lowest observable effect level (LOEL): the lowest dose or exposure level at which a statistically or biologically significant effect is observed in the exposed population when compared to a control population.

Lowest observable adverse effect level (LOAEL): the lowest dose or exposure level at which there is a biologically significant increase in adverse effects in exposed versus control groups.

Reference dose (RfD): an estimate of daily oral exposure to the human population that is likely to be without an appreciable risk of adverse effects over a lifetime. The RfD can be based on a NOAEL, LOAEL, or benchmark dose, with uncertainty factors generally applied.

Reference concentration (RfC): an estimate of a continuous inhalation exposure to the human population that is likely to be without an appreciable risk of an adverse effect during a lifetime. As with the RfD, the RfC can be derived from a NOAEL, LOAEL, or benchmark concentration, typically with uncertainty factors applied.

Benchmark dose (BMD): an exposure level determined from a dose of a substance which is associated with a specified low incidence of risk, eg, in the range of 1% to 10%, of a health effect; or the dose associated with a specified measure of, or change in, a biological effect.

Risk: the measure or mathematical probability that an adverse effect will result from a specific dose or exposure situation.

Risk assessment: the process by which the potential or probability of an adverse health effect of exposure is characterized.

Risk management: essentially, the final regulatory decision-making process that attempts to balance various political, social, economic, and engineering factors against the theoretical risk estimates derived during the risk assessment process. In a sense, it is an attempt to balance society's need and desire for the use of the chemical against the possible hazards represented by the exposures that may result from the use, disposal, and cleanup of the chemical.

Toxicogenomics: a newly emerging subdiscipline of toxicology that uses information regarding the changes in gene expression and the resulting alteration of protein activity in response to chemical exposure as a means of identifying mechanisms/modes of actions of suspected toxic compounds. Detailed information on this issue is available (143–147).

Hormesis: a dose-response phenomenon characterized by a low-dose stimulation or high-dose inhibition, resulting in either a J-shaped or an inverted U-shaped dose response; ie, a toxicant that has a hormetic effect has the opposite effect in small doses than in large doses. For example, in rodents, arsenic is believe to have a hormetic effect.

Allergic response: a reaction to a toxicant caused by a change in the normal immune response. This can include a reaction such as immediate hypersensitivity (eg, anaphylaxis) or a more delayed response (cell-mediated).

Idiosyncratic reaction: a genetically determined abnormal response to a toxicant that occurs at a level of exposure much lower than those generally required to cause the same effect for most individuals [an example is a sensitivity to nitrate due to nicotinamide adenine dinucleotide phosphate (NADH)-methemoglobin reductase deficiency].

Tolerance: a state of decreased effects of a particular dose of a toxicant resulting from previous exposure to the chemical.

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