FOURTH EDITION

PRINCIPLES OF TOXICOLOGY

ENVIRONMENTAL AND INDUSTRIAL APPLICATIONS

EDITED BY

STEPHEN M. ROBERTS | ROBERT C. JAMES
AND PHILLIP L. WILLIAMS

WILEY

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Environmental and Industrial Applications

Fourth Edition

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PREFACE

THIS BOOK'S ORIGIN AND HISTORY

This book initially resulted from a 1-week continuing education course organized and directed by Phillip L. Williams. The course was annually presented at Georgia Tech from 1981 to 1987. The course title was "Industrial Toxicology," and it provided an applied approach to occupational toxicology. In 1985, the first edition of this book was published entitled *Industrial Toxicology: Safety and Health Applications in the Workplace*. It was a compilation of the lectures from the 1983 program, and it was used as the text in the subsequent courses. The book's second edition was published in 2000. With the addition of two new editors (Robert C. James and Stephen M. Roberts) and many new contributors, the text was expanded to include environmental applications and a broader range of basic toxicological concepts. The book maintained an applied approach but, with these revisions, the book's title was changed to *Principles of Toxicology: Environmental and Industrial Applications, Second Edition*. A third edition was published in 2015, revising and updating the content from the second edition. The current book, the fourth edition, builds on the success of the prior editions and, again, has expanded and updated the content from the prior editions.

PURPOSE OF THIS BOOK

Principles of Toxicology: Environmental and Industrial Applications, Fourth Edition, presents compactly and efficiently the scientific basis to toxicology as it applies to the work place and the environment. The book covers the diverse chemical hazards encountered in the modern work and natural environment, and provides a practical understanding of these hazards for those concerned with protecting the health of humans and ecosystems. It provides the reader with an understanding of the processes used to develop allowable chemical exposure values and explains reasons why some chemicals have several different values from various regulatory agencies and other organizations.

INTENDED AUDIENCE

This book's fourth edition represents an update and expansion on the previous, very successful texts. The current edition retains the emphasis on applied aspects of toxicology, while extending its scope to cover new areas such as regulatory toxicology, alternative methods for toxicological testing, and a chapter on the variations occurring in environmental and occupational exposure limits and a discussion on how to select the appropriate allowable exposure value for a given chemical. The book was written for those health professionals who need toxicological information and assistance beyond that of an introductory text in general toxicology, yet more practical than that in advanced scientific works on toxicology. In particular, we have in mind industrial hygienists, occupational physicians, safety engineers, environmental health practitioners, occupational health nurses, safety directors, and environmental scientists.

ORGANIZATION OF THE BOOK

This volume consists of 25 chapters. The early chapters (1–3) establish the scientific basis to toxicology, which is then applied through the rest of the book. It discusses concepts such as using dose–response data, absorption, distribution, and elimination of toxic agents from the body. Chapters 4–6 present new material on the alternative testing methods, regulatory toxicology, and computational toxicology. Chapters 7–13 discuss the effects of toxic agents on specific physiological organs or systems, including the blood, liver, kidneys, nerves, skin, lungs, and the immune system.

The remainder of the book addresses specific areas of concern in toxicology as well as the adverse effects of

toxic agents and their toxic manifestations. Chapters 14–16 examine areas of great research interest – reproductive and developmental toxicology, mutagenesis, and carcinogenesis. Chapters 17–19 examine toxic effects of metals, pesticides, and organic solvents.

The final part of the book is devoted to specific areas and applications of the toxicological principles from both the environmental and occupational settings. Chapter 20 covers the emerging area of nanotoxicology. Chapters 21 and 22 discuss epidemiologic issues and occupational/environmental health. Chapters 23 and 24 describe the risk assessment process for both the human (Chapter 23) and the environmental settings (Chapter 24). The final chapter, Chapter 25, explains why exposure limits for a chemical vary across organizations promulgating them and discusses factors to consider when selecting an allowable chemical exposure value from the variety of sources available to the public.

FEATURES

The following features from *Principles of Toxicology: Environmental and Industrial Applications* will be especially useful to our readers:

- The book is compact and practical, and the information is structured for easy use by the health professional in both industry and government.
- The approach is scientific, but applied, rather than theoretical. In this, it differs from more general works in toxicology, which fail to emphasize the information pertinent to the industrial environment.
- The book consistently stresses evaluation and control of toxic hazards.
- Numerous illustrations and figures clarify and summarize key points.
- Case histories and examples demonstrate the application of toxicological principles.
- The reader is shown examples of how to select appropriate chemical exposure values and the reasons why these values can vary between sources, even for the same chemical.
- Chapters include annotated bibliographies to provide the reader with additional useful information.

Stephen M. Roberts
Robert C. James
Phillip L. Williams

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GENERAL PRINCIPLES OF TOXICOLOGY

Robert C. James, Stephen M. Roberts, and Phillip L. Williams

This chapter provides a concise description of the basic principles of toxicology and illustrates how these principles are used to make reasonable judgments concerning the potential health hazards and/or risks associated with chemical exposures. This chapter will explain:

- Some basic definitions and terminology used in toxicology and the area of risk assessment
- The general areas of study within toxicology, the scientific disciplines toxicologists draw upon, and specialized areas of interest within toxicology
- How whole animal studies and *in vitro* techniques provide the primary basis for hazard identification
- The importance of dose and the generation of dose–response relationships will be explained, and then, how dose–response data are used to predict the outcome of a particular chemical exposure
- Factors that may alter a chemical's toxicity or the dose–response relationship
- The basic methods and considerations used to develop exposure guidelines protective of public health

1.1 BASIC DEFINITIONS AND TERMINOLOGY

The literal meaning of the term *toxicology* is "the study of poisons." The root word toxic entered the English language around 1655 from the Late Latin word *toxicus* (which meant poisonous), which itself was derived from the earlier ancient Greek term for the poisons into which arrows were dipped, *toxikón*. The early history of toxicology focused on understanding the uses of different poisons, but today toxicology has evolved into a modern science whose interest has been expanded to encompass all adverse health effects produced by any chemical substance. The following definitions are provided to help the reader understand several basic terms that may be used in this and other chapters:

Toxic. Having the characteristic of being able to produce an undesirable or adverse health effect at some dose.

Toxicity. Any adverse effect that a chemical or physical agent might produce within a living organism.

Toxicology. The science that deals with the study of the adverse effects (toxicities) that chemicals or physical agents may produce in living organisms under specific conditions of exposure. It is a science that attempts to qualitatively identify all the hazards (i.e., organ toxicities) associated with a substance, as well as to quantitatively determine the exposure conditions under which those hazards/toxicities are induced. Toxicology is the science that experimentally investigates the occurrence, nature, incidence, mechanism, and risk factors for the adverse effects of toxic substances.

As these definitions indicate, the toxicities of interest span a broad biologic and physiologic spectrum. The adverse effects of interest may range from something relatively minor like irritation or tearing, to a more serious response like acute but reversible liver or kidney damage, to an even more serious and permanent disability like cirrhosis of the liver or cancer in a specific tissue. Given this broad range of potentially adverse effects to consider, it is useful for those unfamiliar with toxicology to define additional terms that will be discussed in subsequent chapters of this book.

Principles of Toxicology: Environmental and Industrial Applications, Fourth Edition. Edited by Stephen M. Roberts, Robert C. James and Phillip L. Williams.

Exposure. A measure of the opportunity to have contact with a chemical present in one's environment. The presence of a chemical in an environmental medium of contact (e.g., in the air we breathe, the water we drink, on surfaces we touch, in foods we might eat). Exposure levels are typically expressed as the concentration of the chemical in the contact medium (e.g., as the ppm concentration in air, or in mg/l of water).

Dose. A dose is the total amount of a toxicant an organism receives as the result of some exposure. The most common use of the term dose refers to the *applied dose*, i.e., the amount of chemical present at the site of contact. But different definitions and terms arise for the concept of dose as we move from the site of contact on the body to the actual amount absorbed by the body and then the amount absorbed by various tissues within the body. So, more specific definitions for the term dose include the following:

- *Applied Dose*. Represents the total amount of the chemical that is directly applied to, or has direct contact with, body surfaces that are the portal(s) of entry (via absorption) into the body. The applied dose may be higher than the absorbed dose because not all of the chemical may get across the cell membranes at the site of contact.
- *Internal/Absorbed dose*. The quantity of a toxicant that is ultimately absorbed into the organism and distributed systemically throughout the body. The ratio of the absorbed dose/applied dose is referred to as the chemical's bioavailability.
- *Delivered/Effective/Target Organ Dose*. The amount of toxicant reaching the target organ (i.e., the specific organ adversely affected by the toxicant).
- *Exposure Concentration Response Curves (As a Surrogate Measure of Dose)*. In many ecological toxicity studies, the effects of a chemical on the test organism is measured as a function of the exposure concentration (i.e., the toxicant is usually added to an aquatic or soil medium and the response is measured as a function of the concentration in that medium. In these situations, exposure–response curves are generated instead of traditional dose–response plots because the applied dose is not known, and the response is a function of the organism's activity with the test medium.

Acute Exposure. Exposure that occurs only for a brief period of time (generally less than 24 h). Frequently, this term is applied to a single exposure (or dose), but it also may be an applicable term for repeated exposures that occur within a relatively short time period.

Subacute Exposure. Resembles acute exposure except that the exposure duration is greater, e.g., from several days to 1 month in animal studies.

Subchronic Exposure. Exposures repeated or spread over an intermediate time range. For animal testing, this time range is generally considered to be 1–3 months.

Chronic Exposure. Exposures (either repeated or continuous) over a long period of time. In animal testing, chronic exposures are those ranging between 90 days and the animal's lifetime. But it generally represents exposures that occur for a majority of that species' lifetime. For human exposures, it is defined as a long-term exposure measured in years.

Acute Toxicity. An adverse or undesirable effect that is manifested within a relatively short time interval ranging from almost immediately to within several days following exposure (or dosing). An example would be chemical asphyxiation from exposure to a high concentration of carbon monoxide (CO). Acute toxicities that are not fatal may be reversible.

Chronic Toxicity. A permanent or lasting adverse effect that is manifested after exposure to a toxicant. Examples would be the development of silicosis following a long-term exposure to silica in workplaces such as foundries or liver cirrhosis following chronic alcohol consumption.

Local Toxicity. An adverse or undesirable effect that is manifested at the toxicant's site of contact with the organism. Examples include an acid's ability to cause severe irritation, blistering or scarring of the eyes, upper respiratory tract, or skin.

Systemic Toxicity. An adverse or undesirable effect that can be seen anywhere within the organism. It typically involves an organ in the body with selective tissue vulnerability to a toxicity induced by the chemical. Systemic toxicities require the toxicant first be absorbed and distributed to the target organ and the organ adversely affected may be distant from the site at which absorption occurs. Examples include the adverse effects on the kidney or central nervous system (CNS) resulting from the acute or chronic ingestion or inhalation of mercury.

Reversible Toxicity. Any adverse effect that can be reversed once exposure is stopped. The reversibility of toxic effect depends on a number of factors, including the duration and magnitude of the exposure, and the ability of the affected tissue to repair or regenerate once exposure ceases. Examples include liver regeneration following an acute overdose of acetaminophen, or the generation of new skin after an excessive exposure to sun has led to a sunburn, blistering, and the sloughing of dead skin.

Delayed or Latent Toxicity. An adverse or undesirable effect that appears long after the initiation and/or cessation of exposure to the toxicant. One example is the cervical cancer occurring during adulthood produced

by an *in utero* exposure to diethylstilbestrol (DES). Almost all chemical-induced cancers are examples of a latent toxicity.

Allergic Reaction. A reaction to a toxicant caused by an altered state of the normal immune response. The outcome of the exposure can be immediate (anaphylaxis) or delayed (cell-mediated).

Idiosyncratic Reaction. A response that occurs rarely and unpredictably. They typically cannot be explained by the known mechanism of toxicity of the causative agent.

Mechanism of Toxicity. Those necessary biologic interactions by which a toxicant exerts its adverse effect. A simple example would be CO asphyxiation; this mechanism involves the binding of CO to hemoglobin thereby preventing the binding of oxygen and reducing the amount of oxygen transported in the blood to other tissues.

Toxicant. Any substance that causes a harmful (or adverse) effect in a living organism at some defined, sufficient concentration.

Toxin. Any toxicant produced by another living organism (floral or faunal, including bacteria); i.e., toxins are naturally produced poisons. One example are the pyrethrins. These are natural pesticides produced by pyrethrum flowers (i.e., certain chrysanthemums), the natural biologicals that served as the model for the man-made insecticide class known as the pyrethroids.

Potency. A measure of the ability of a chemical to express its toxicity per unit of dose or dosage. The more potent a chemical, the smaller the dosage needed to induce the toxicity. Exposure to a less potent chemical is generally safer than exposure to a more potent chemical because a larger dose or exposure is needed to induce toxicity when potency is low. Similarly, more potent chemicals tend to be more dangerous because even small doses or exposures may induce toxicity.

Hazard. The qualitative nature of the adverse or undesirable effect; the type of adverse effect or toxicity the chemical produces at sufficient doses. For example, asphyxiation is the hazard from acute exposure to CO. Cancer, liver toxicity, and immunotoxicity are other hazards (types of toxicities) a chemical exposure might induce. A hazard typically refers to the kind(s) of toxic effect(s) the chemical is capable of inducing when the exposure/dose is sufficient.

Safety. The measure or mathematical probability that a specific exposure situation or dose will not produce a toxic effect.

Risk. As generally used in toxicology, is the measure or probability that a specific exposure situation or dose will produce a toxic effect.

Risk Assessment. The process by which the potential (or probability) of adverse health effects occurring is predicted for a specific dose or exposure level. In risk assessment, the typical goal is the setting of a safe exposure concentration that is extrapolated from the dose–response curve for an adverse effect the chemical is known to induce. Another product of a risk assessment might be the estimated probability of a toxicity occurring at a given level of exposure.

1.2 TOXICOLOGY: A DIVERSE SCIENCE WITH TWO BASIC GOALS

Toxicology has become a science that builds upon knowledge developed in other related medical sciences. Scientific disciplines that are incorporated into toxicology include physiology, biochemistry, pathology, pharmacology, medicine, and epidemiology, to name a few. Again, toxicology has evolved from the study of poisons to become the study of all adverse effects induced by all chemicals. Although a number of areas of specialization have evolved within toxicology, all toxicologists fall into three principal areas of endeavor: descriptive toxicology, research/mechanistic toxicology, and applied toxicology.

Descriptive toxicologists are scientists whose work focuses on the toxicity testing of chemicals. This work is done primarily at commercial and governmental toxicity testing laboratories. The studies performed at these facilities are designed to identify the various organ toxicities (hazards) the test agent is capable of inducing and the exposure conditions or doses necessary to induce each effect. A thorough description of a chemical's toxicology would identify all possible acute and chronic toxicities, including the genotoxic, reproductive, teratogenic (developmental), and carcinogenic potential of the test agent. It would identify important metabolites of the chemical that are generated as the body attempts to break down and eliminate the chemical, as well as understand how the chemical is absorbed into the body, distributed to tissues throughout the body, accumulated and eliminated from tissues, and ultimately how it is excreted from the body. Hopefully, with the completion of the descriptive studies, appropriate dose–response test data have been generated for those toxicities of greatest concern. Then, using either the highest dose producing no toxicity, or the lowest dose tested producing only limited toxicity, the relative safety might be predicted for those exposure levels or doses that humans typically encounter in their environment.

Basic research or mechanistic toxicologists are scientists who study the chemical or agent in-depth for the purpose of understanding how the chemical or agent initiates the biochemical or physiological changes within cells or tissues that result in a specific toxicity. The goal of mechanistic

studies is to understand which specific biological reactions (i.e., the adverse chain of events) within the affected organism that ultimately result in the toxic effect occurring. Mechanistic experiments are performed at the molecular, biochemical, cellular, and tissue level of the affected organism. So, mechanistic assessments may incorporate and apply the knowledge of a number of many other related scientific disciplines within the biological and medical sciences (e.g., physiology, biochemistry, genetics, molecular biology, pathology). The goal of mechanistic studies is to provide information that describes the key biochemical/physiologic changes that are necessary to induce toxicity. Once the mechanism (or "mode of action" evidence) for the key changes producing toxicity has been established in animal studies, the potential human hazard may be assessed via clinical tests or *in vitro* techniques such as cell cultures as discussed in subsequent chapters. This information, in turn, helps reduce the uncertainty of the animal-to-human extrapolation being used to develop the safe human exposure guidelines for the chemical.

Applied toxicologists are scientists concerned with the use of chemicals in the "real world" or nonlaboratory setting. The primary goal of applied toxicologists is the control of chemical exposures in work and nonwork environments by setting safe exposure guidelines for each exposure pathway (inhalation, dermal, or oral) in that environment. Toxicologists who work in this area of toxicology use descriptive and mechanistic toxicity studies to limit the doses received by each exposure pathway so that the total dose of the chemical an individual receives will hopefully be a safe one. The process whereby this safe dose or level of exposure is derived is generally referred to as the area of *risk assessment*. Within applied toxicology, a number of subspecialties occur. *Forensic toxicology* is that unique combination of analytical chemistry, pharmacology, and toxicology concerned with the medical and legal aspects of drugs and poisons. It is concerned with the determination of which chemicals are present and responsible in exposure situations of abuse, overdose, poisoning, and death that become of interest to the police, medical examiners, and coroners. *Clinical toxicology* specializes in ways to treat poisoned individuals and normally focuses on determining and understanding the toxic effects of medicines, simple over-the-counter (nonprescription) drugs, and common household products. *Environmental toxicology* is the subdiscipline concerned with those chemical exposure situations found in our general living environment. These exposures may stem from the agricultural application of chemicals, the release of chemicals during modern-day living (e.g., chemicals released by household products), and the regulated or unintentional industrial discharges of industrial chemicals into air, water, soils, and various nonpoint emission sources (e.g., the combustion by-products of cars). Within this area, there may be even further subspecialization (e.g., ecotoxicology, aquatic toxicology, mammalian toxicology, avian toxicology). *Occupational toxicology* is the subdiscipline concerned with the chemical exposures and diseases found in the workplace. It is the identification of the hazards or injuries produced by over-exposure to the chemicals used within an occupation, the prevention of adverse/toxic exposures to these chemicals, and the treatment of injuries these chemicals produce.

Regardless of the specialization within toxicology, or the types of toxicities of major interest to the toxicologist, essentially every toxicologist performs one or both of the two basic functions of toxicology. The two basic functions of toxicology are as follows: (i) to identify and elucidate the toxicities (adverse effects) a chemical or physical agent is capable of inducing at some dose (i.e., the *hazard/toxicity identification* function); and/or (ii) assess the specific conditions of exposure/dose under which these toxicities will occur or can be prevented (*dose–response and risk assessment* function). Or, stated another way, the fundamental purpose of toxicology is to identify the toxicities a chemical is capable of producing so that these adverse effects can be prevented in humans via the development of safe exposure guidelines for both occupational and nonoccupational environments.

1.3 THE HAZARD IDENTIFICATION FUNCTION

The hazard identification process, i.e., discovering the toxicities chemicals produce, requires the testing of chemicals at doses high enough to induce the full spectrum of toxicities a chemical might induce. Traditionally, the hazard identification process involved exposing animal test species to a range of doses for different durations of exposure (previously described as acute, subacute, subchronic, and chronic exposure intervals). Different exposure intervals are tested because the toxicity induced by a chemical may vary with the dose administered and the duration of exposure. Frequently, as the duration of exposure lengthens, the dose needed to induce a particular toxicity decreases, and the severity or irreversibility of the damage seen to an organ increases. Because each chemical induces a different spectrum of toxic effects that the investigator does not know beforehand, the toxicant is examined using as wide a range of test systems as possible to ensure all hazards have been identified. For a complete toxicologic evaluation, the hazard assessment would typically follow a testing scheme similar to the one illustrated in Figure 1.1 Tier 1 testing is designed to identify the acute and subacute toxicities of the chemical. Testing starts with short duration tests because the cost of testing is less and these tests typically identify the target organs affected during longer exposure periods as well. The doses producing toxicity at a lower tier sets the upper range of exposure or doses to test when the exposure interval is extended. Once the acute toxicities are established, testing moves through subchronic tests (Tier 2) and then chronic tests (Tier 3). At each

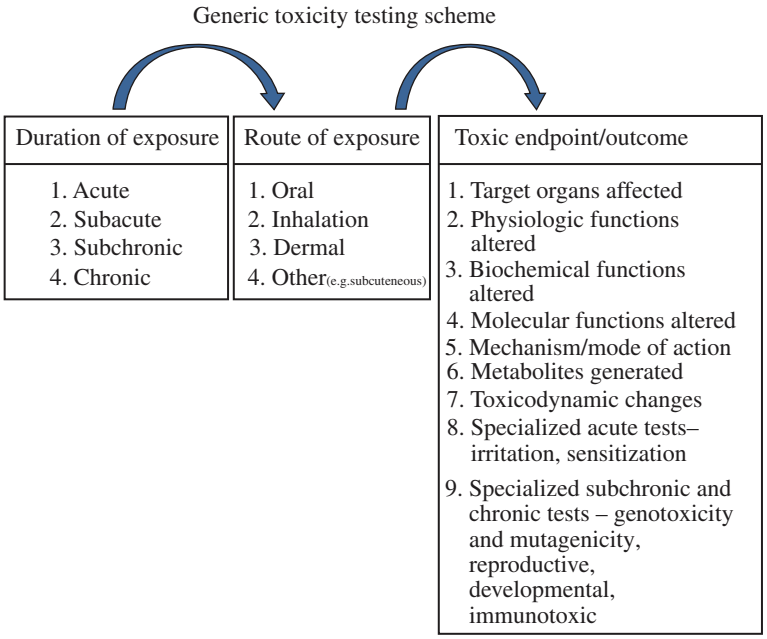

FIGURE 1.1 A generic toxicity testing scheme that shows the ways in which a toxicity test might differ because of the different choices made regarding the duration of exposure, the route of exposure, or the endpoint to be measured in the study.

tier, route specific testing and the use of specialized toxicity tests might be performed in addition to those investigating the target organs affected. For example, if the use of the chemical will likely result in skin contact and/or the inhalation of the chemical, as would be expected during its manufacture, then testing for respiratory tract irritation and dermal sensitization might be required. During subchronic and chronic testing, the initial target organ testing might be augmented by reproductive and developmental studies and specialized testing for immunotoxicity, genotoxicity/mutagenicity, and carcinogenicity. While the testing scheme depends on the use of the chemical and the likelihood of human exposure, part or all of the following testing scheme might be required in a descriptive toxicology testing program.

Tier 1: Toxicity testing for acute and subacute exposure conditions

a. Plot dose–response curves for lethality and possible organ injuries.
b. Test eyes and skin for irritation.
c. Make a first screen for mutagenic activity.
d. Investigate how changing the route of exposure alters the target organs affected and dose–response relationship

Tier 2: Testing for subchronic exposure

a. Plot dose–response curves (for 90-days exposure) in two species; the test should use the expected human route of exposure.
b. Test organ toxicity; note mortality, body weight changes, hematology, and clinical chemistry; make microscopic examinations for tissue injury.
c. Conduct a second screen for mutagenic activity.
d. Test for reproductive problems and birth defects (teratology).
e. Examine the pharmacokinetics of the chemical, i.e., the absorption, distribution, metabolism, and elimination of the chemical in the test organism.
f. Conduct behavioral tests.
g. Test for synergism, potentiation, and antagonism when other chemicals are present.

Tier 3: Test for chronic exposure

a. Conduct mammalian mutagenicity tests.
b. Conduct a 2-year carcinogenesis test in rodents.
c. Examine pharmacokinetics in humans.
d. Conduct human clinical trials (for drugs and therapeutics).
e. Compile the epidemiologic data of acute and chronic exposure.

Establishing the safety and hazard of a chemical can be a very costly and time-consuming effort because the necessary testing scheme is extensive. For example, a rodent bioassay to investigate the chemical's carcinogenic potential will cost several million dollars and takes 2–3 years to complete. When completed, the results, if positive, may severely limit or even prohibit the use of the chemical. So, this final toxicity

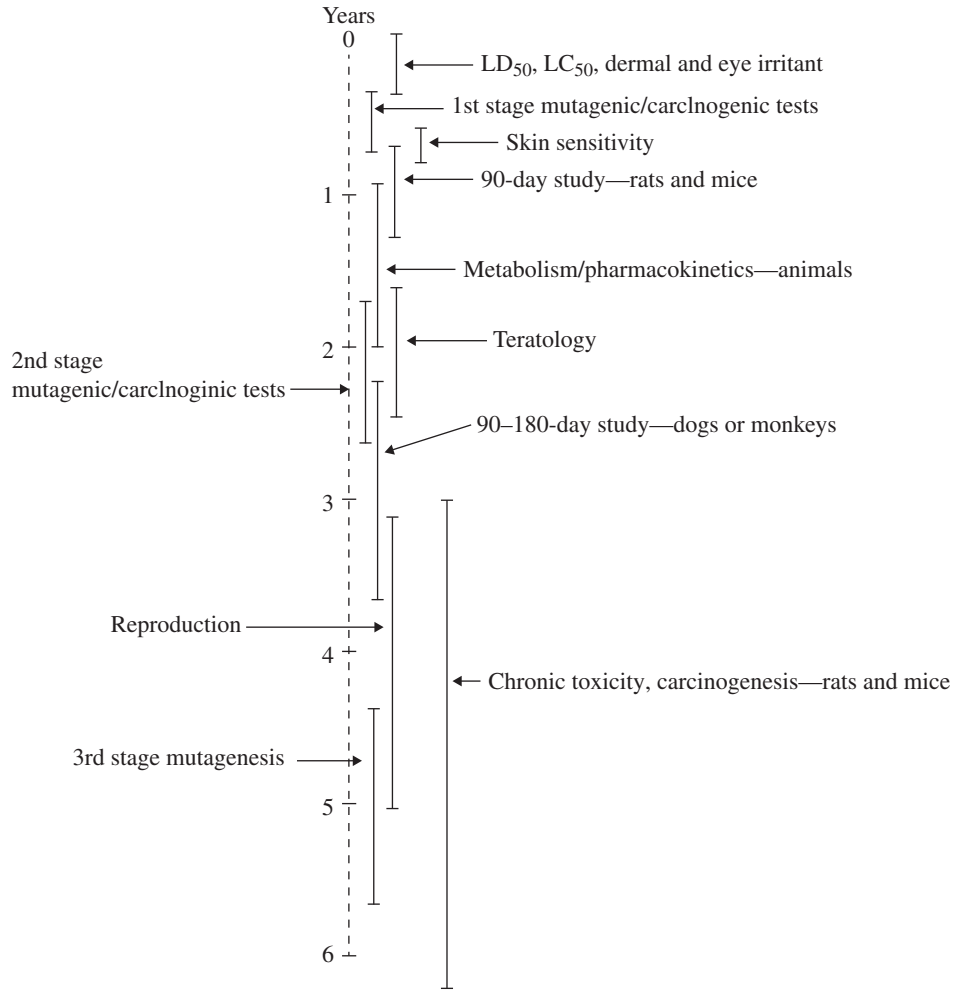

FIGURE 1.2 A timeline showing the progression of toxicity testing for a chemical having a broad exposure to the human population. The bars represent the approximate time required to complete the tests and suggest when testing might be initiated and completed.

test may engender additional costs if the manufacturer must now find a replacement chemical that does not have significant carcinogenic activity. Figure 1.2 outlines the approximate time required to test and develop the toxicity data for chemicals assumed to have widespread human impact. However, as discussed in Chapters 4–6, there is currently a push to reduce the use of animal testing by developing reliable surrogate testing that relies upon the results provided by *in vitro* tests rather than whole organisms with the goal being a reduction of animal suffering as well as reducing the time and cost of the chemical's hazard assessment.

As shown in Table 1.1, increasing the duration of exposure generally results in a lowering of the dose necessary to induce an adverse effect in the organ affected. The shorter the duration of exposure the lower the cost of the testing. When trying to identify the endpoints of interest the highest dose range that still allows for the completion of the test is typically done as this approach is generally the most time and cost efficient when seeking to identify the toxicities a chemical induces. But because both the toxicities observed and the doses necessary to induce these toxicities may change with the duration of exposure, the hazards seen with shorter exposure duration cannot be assumed to be the same as those occurring after longer duration of exposure. For example, cancer is a latent disease that may require a lifetime of exposure to detect. It cannot be reliably predicted with complete accuracy using short-term tests, nor is the organ affected always those identified in short-term tests. The route of exposure can also change the hazard. As the site of absorption is altered, the occurrence of localized effects (like irritation or cellular necrosis at the site of contact) may no longer be relevant. And as explained in more detail in Chapter 2, if the site of absorption (route of exposure) is changed the bioavailability of the chemical (percent absorbed) may change, and this may in turn change the tissue distribution of the chemical and the target organ concentration per unit of absorbed dose. Because changes in tissue distribution

TABLE 1.1 Examples Showing a NOAEL or LOAEL May Change with Exposure Duration

Exposure Duration	Species (Strain)	Organ/Endpoint	Dose (mg/kg/day)
	A. NOAEL Comparisons		
1,4-Dioxane			
Acute (2 wk)	Rat (Fischer-344)	Hepatic	1040
Intermediate (13 wk)			60
Chronic (2 yr)			16
Acute (2 wk)	Rat (Fischer-344)	Renal	1040
Intermediate (13 wk)			330
Chronic (2 yr)			21
Di(2-ethylhexyl)phthalate			
Acute (once)	Rat (Fischer-344)	Renal	5000
Intermediate (90 d)	Rat (Wistar)		1900
Chronic (1 yr)	Rat (Sherman)		200
	B. LOAEL Comparisons		
1,4-Dioxane			
Acute (2 wk)	Rat (Fischer-344)	Hepatic	2750
Intermediate (13 wk)			150
Chronic (2 yr)			81
Acute (2 wk)	Rat (Fischer-344)	Renal	2750
Intermediate (13 wk)			760
Chronic (2 yr)			103
Di(2-ethylhexyl)phthalate			
Acute (7 d)	Rat (Wistar)	Hepatic	2000
Intermediate (21 d)			1730
Chronic (79 wk)			1000

and tissue concentration may result in a different pattern of organ toxicities, changing the route of exposure can alter the toxicities of greatest concern as well as the applied doses required to induce these toxicities. For example, after testing trichloroethylene (TCE) for carcinogenicity using the mouse as the test organism, it was observed that inhalation exposure induced lung tumors but not liver tumors while oral administration induced liver tumors but not lung tumors. This kind of route-specific toxicity occurs frequently enough that regulatory agencies like the US EPA no longer rely upon data gathered by one route of exposure to predict the hazards or risks for another route of exposure; and route-to-route extrapolations are only considered acceptable when there is a mechanistic basis for doing so.

Since we are looking for adverse outcomes, the primary source of information for hazard identification comes from animal toxicity tests using nonhuman species. Over the years, we have developed an extensive array of different toxicity test systems. These test systems are designed to examine endpoints of interest such as target organs, changes in physiologic/biologic/molecular function, the different chemical metabolites generated by enzymes whose function is the conversion of both endogenous and exogenous substances into chemical forms more easily eliminated from the body, the mechanism or modes of action, and chemical reactions with key cellular macromolecules (e.g., enzymes, proteins, RNA, DNA).

For example, besides animal or whole organism test results, a toxicologist might use a specialized *in vitro* test system that involves test-tube or cell culture methods to examine effects on specific cellular macromolecules, isolated cell fractions, or isolated cellular organelles (e.g., mitochondria). When examining a specific target toxicity, a toxicologist might utilize an isolated perfused whole organ to investigate the chemical's effects on specific molecular, physiologic, or biologic functions. A toxicologist might also perform *in vivo* tests in a variety of nonmammalian organisms ranging from simple, single cell organisms (e.g., bacteria, algae) to larger and more complex nonmammalian organisms such as nematodes, fruit flies, *Daphnia magna*, or fish, particularly when attempting to identify the ecologic hazards of an environmental pollutant.

Some tests are easier and cheaper to perform and can better handle high volume testing; these tests can serve to screen candidate chemicals for further, more detailed toxicity testing, or to predict certain toxicities to look for when the chemical is tested in whole animals. A classic example of this approach is seen with those toxicities that are receptor mediated. For this type of toxicity, the

receptor-based structure activity relationships are frequently used to extrapolate a surrogate measure of relative potency for the subchronic and chronic hazards induced by the chemicals acting at the receptor. The ever-expanding use of *in vitro* test systems may also be desirable in certain situations because the investigator can isolate and test specific physiologic or biochemical pathways in a manner that better controls specific test conditions, doses, and outcomes as well as being more time and cost efficient than whole organism testing. However, *in vitro* tests may remove key cell or target organism functions from the experimental conditions and can easily allow for the test concentrations (the surrogate measure of dose) that cannot be achieved in the whole animal (e.g., lethality might occur at lower blood concentrations). Thus, the endpoint being measured using *in vitro* test systems might be modified in ways not easily or accurately extrapolated to whole organism responses. As will be explained further in Chapter 3, isolated *in vitro* tests also eliminate important toxicokinetic parameters that impact or control the induction of toxicity in the whole organism. So, while the results of *in vitro* tests can be produced more easily and cost effectively, and often with greater consistency, there tends to be greater uncertainty associated with the human extrapolation of *in vitro* data than there is for data derived by testing the whole organism at specific exposure levels and for a specific exposure duration. For example, what metabolites are the chemical converted to in whole organisms that are not seen when using an *in vitro* test system? Are both toxic or nontoxic metabolites produced by the organism? How does the dose of the chemical influence its metabolism and/or tissue distribution? Are the exposure conditions of an *in vitro* system much higher than those that occur in tissues when the chemical is administered in whole animal experiments? So, currently *in vivo* or whole organism testing using a variety of mammalian species is still necessary to identify the range of possible hazards the chemical might pose to humans, and the extent to which this can be changed in the future with better alternative *in vitro* test methods remains to be seen and will likely depend on the extent to which these new methods can be validated as accurate surrogates for the responses identified with whole organism testing (see Chapters 4–6 for more discussion on this issue).

In addition to animal tests, hazard information gathered from different human exposure scenarios to the chemical may also be available. Accidental poisonings and occupational exposures in industries that use the chemical may generate epidemiology studies describing the chemical's acute and chronic toxicities in humans. As discussed in more detail later in this chapter, there can be significant species differences in the both the beneficial and adverse responses induced by a chemical. Therefore, in the final hazard assessment for any chemical, a toxicologist would like to be able to review all of the human data that is available. There are four basic categories of epidemiologic information that can assist the hazard evaluation. These categories are as follows: occupational epidemiology (mortality and morbidity studies), clinical exposure studies, accidental acute poisonings, and chronic environmental epidemiology studies. The advantages and disadvantages of the hazard information typically provided by these four categories of human toxicologic information and that of traditional *in vitro* and animal toxicity tests are summarized and compared in Table 1.2.

1.4 THE DOSE–RESPONSE/RISK ASSESSMENT FUNCTION

It is probably safe to say that among lay individuals there exists considerable confusion about the term "toxic." If asked, most lay individuals would probably define a toxic substance using a description that would apply to highly poisonous or very potently toxic chemicals. For most of the general public, the word "toxic" is assumed to be used to identify "highly hazardous" chemicals, chemicals for which almost no level of exposure is safe. To help illustrate the fallacy of this misunderstanding and help the reader recognize the fact that all chemicals are toxic at some dose, whether natural or synthetic, and that the occurrence of any chemical's adverse effects is a function of dose, the reader is invited to take the following pop quiz. First, cross-match the LD_{50} doses shown in column A (does producing lethality in 50% of the animals tested) with the chemicals listed in column B. These chemicals are a collection of food additives, medicines, drugs of abuse, poisons, pesticides, and hazardous substances for which the correct LD_{50} is listed somewhere in column A. To perform this cross-matching, first photocopy Table 1.3 and simply mark the ranking of the dose (i.e., the number corresponding next to the dose in column A) you believe correctly corresponds to the chemical it has been measured for in column B. (*Note:* The doses are listed in descending order, and the chemicals have been listed alphabetically. So, the three chemicals you believe to be the safest, should have the three largest doses [you should rank them as 1, 2, and 3], and the more dangerous or potently toxic you perceive the chemical to be, the higher the numerical ranking you should give it. After testing yourself with the chemicals listed in Table 1.3, you can find the correct answers in a table found at the end of this chapter.)

According to the ranking scheme that you selected for these chemicals, were the safest or least potent chemicals in your opinion common table salt, vitamin K (which is required for normal blood clotting times), the iron supplement dosage added to vitamins for individuals that might be slightly anemic, or a common over-the-counter pain relief medication you can buy at any local drugstore? What were the three most potentially toxic chemicals (most

TABLE 1.2 Some of the Advantages and Disadvantages of Toxicity Data by Category

Advantages	Disadvantages
a. 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.
Avoid uncertainties inherent in extrapolating toxicities and dose–response relationships across species	Separating interactive effects resulting from combinations of chemical exposures may be difficult or impossible
	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 (e.g., 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
b. 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 (e.g., 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
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

(*continued*)

TABLE 1.2 (*Continued*)

Advantages	Disadvantages
	c. Environmentally Exposed Epidemiologic 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 (e.g., 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
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, i.e., the problem of the "Ecological Fallacy."
	Exposures are frequently not quantified at the individual level
	d. 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
	e. 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

(*Continued*)

TABLE 1.2 (*Continued*)

Advantages	Disadvantages
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, i.e., 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, i.e., 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

f. Alternatives to Traditional Animal Testing

Type of Toxicity Test	Advantages	Disadvantages
Structure–activity relationships (SARs)	Does not require the use of any experimental animals Quick to perform	Many toxicants with very similar chemical properties have very different toxicities
In vitro testing	Reduces the number of experimental animals needed Allows for better control of the toxicant concentration at the target site Allows for the study of isolated functions such as nerve-muscle interaction and release of neurotransmitter Easier to control for host factors such as age dependency, nutritional status, and concurrent disease Possible to use human tissue	Cannot fully approximate the complexities that take place in whole organisms (i.e., absorption, distribution, biotransformation, and elimination)
Alternative animal testing (nonmammalian and nonavian species)	Less expensive and quicker (due to shorter lifespans) than using higher animals Since a whole organism is used allows for absorption, distribution, biotransformation, and elimination of the toxicant	Since the animal is far removed from humans, the effect of a toxicant can be very different from that found with higher animals

Source: (a–e) Adapted from James et al. (2015).

dangerous at the lowest single dose) in your opinion? Were the most dangerous substances in your opinion "natural" or "synthetic" (human-made) chemicals? How toxic did you rate the nicotine that provides the stimulant properties of tobacco products? How did the potency ranking of prescription medicines like the sedative phenobarbital or the pain killer morphine compare to the acutely lethal potency of a poison such as strychnine or the pesticide malathion?

Now, take the allowable workplace chronic exposure levels for the following chemicals—aspirin, gasoline, iodine, several different organic solvents, and vegetable oil mists—and again rank these substances going from the highest to lowest allowable workplace air concentration (listed in Table 1.4). Remember that the chemicals with the lower numerical allowable air concentration, the more potently toxic that substance is per unit of exposure. Review the correct answers for tables recreated at the end of this chapter.

TABLE 1.3 Cross-Matching Exercise: Comparative Acutely Lethal Doses

N	*A* LD_{50} (mg/kg)	B Toxic Chemical	Correct Order
1	15,000	Alcohol (ethanol)	_____
2	1,375	Botulinum toxin (food poison)	_____
3	10,000	Curare—arrow poison	_____
4	4,000	Dioxin or 2,3,7,8-TCDD	_____
5	900	Iron supplement (ferrous sulfate)	_____
6	2	Malathion—Insecticide (malathion)	_____
7	150	Morphine	_____
8	142	Nicotine	_____
9	1,500	PCBs—an electrical insulation fluid	_____
10	1	Rat poison (strychnine)	_____
11	0.5	Sedative/sleep aid (phenobarbital)	_____
12	0.00001	Table salt (sodium chloride)	_____
13	0.001	Tylenol (acetaminophen)	_____

The chemicals listed in this table are *not* correctly matched with their acute median lethal doses (LD_{50}'s). Rearrange the list so that they correctly match. The correct order can be found in the answer table at the end of the chapter.

TABLE 1.4 Cross-Matching Exercise: Occupational Exposure Limits—Aspirin and Vegetable Oil Versus Industrial Solvents

N	Allowable Workplace Exposure Level (mg/m^3)	Chemical (Use)	Correct Order
1	0.05	Aspirin (pain reliever)	_____
2	5	Gasoline (fuel)	_____
3	10	Iodine (antiseptic)	_____
4	54	Perchloroethylene (dry-cleaning fluid)	_____
5	55	Tetrahydrofuran (organic solvent)	_____
6	890	Toluene (organic solvent)	_____
7	147	1,1,1-Trichloroethane (solvent/degreaser)	_____
8	170	1,1,2-Trichloroethane (solvent/degreaser)	_____
9	75	Trichloroethylene (solvent/degreaser)	_____
10	1910	Vegetable oil mists (cooking oil)	_____

The chemicals listed in this table are *not* correctly matched with their allowable workplace exposure levels. Rearrange the list so that they correctly match. The correct order can be found in the answer table at the end of the chapter. Source: Data from American Industrial Hygiene Association Journal 1948.

Hopefully, the preceding quiz helped illustrate the fact that one's perceived risk for a chemical exposure may not correspond to the actual potency of that chemical's toxicity. As we have defined toxicants as a chemical capable of producing an adverse effect in a biological system, a reasonable question for one to ask becomes "Which group of chemicals do we consider to be toxic?" and "Which chemicals do we consider safe?" The short answer to both questions is actually: *all chemicals*. For even relatively safe chemicals can become toxic if the dose is high enough, and even potent, highly toxic chemicals may be used safely if the exposure is kept low enough. As toxicology evolved from the study of substances that were poisonous to a more general study of the adverse effects of all chemicals, the conditions under which the chemical expresses its toxicity became as important as knowing the kind of adverse effect it produced. The importance of understanding the dose at which a chemical becomes toxic (harmful) was recognized centuries ago by Paracelsus (1493–1541), who essentially stated this concept as—*All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy*. This statement serves to emphasize the two basic functions of toxicology. With the first sentence, Paracelsus tells us that all chemicals express one or more toxicities (hazard identification). However, whether these toxicities are induced or seen is expressed in the second sentence and underscores the second function toxicology—*under what dose or exposure conditions is the toxicity expressed*. In more recent times, Emil Mrak paraphrased the admonition of Paracelsus more simply by stating—*There are no harmless substances, only harmless ways of using substances*. This simple phrase emphasizes that while knowing what toxicities a chemical induces is important, it is even more important to understand the dose or exposure conditions under which these toxicities will occur or are prevented.

A simple illustration of admonitions by Paracelsus and Mrak showing how their statements apply to all substances is seen in Figure 1.3. Figure 1.3 lists the lethal doses for two substances that most or all adults have been exposed to water and beer. While some might find it surprising to think that a dose of something as simple and necessary for life as water can be fatal, the ingestion of about 15 quarts of water within a 24-h period may be fatal. Normally, this toxicity is limited to persons with a serious psychological disorder, but it was also illustrated once during a radio station sponsored contest in which the person who could drink the most water in a limited amount of time would win a new video game system. One of the contestant's vying for the game system actually died the day of the contest from acute water intoxication. This unfortunate event once again emphasized the fact that even safe substances can be unsafe under some conditions of exposure because everything becomes toxic if the dose is