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GENERAL PRINCIPLES OF TOXICOLOGY

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The intent of this chapter is to provide a concise description of the basic principles of toxicology and to illustrate how these principles are used to make reasonable judgments about the potential health hazards and the risks associated with chemical exposures. This chapter explains:

- Some basic definitions and terminology
- What toxicologists study, the scientific disciplines they draw upon, and the specialized areas of interest within toxicology
- Descriptive toxicology and the use of animal studies as the primary basis for hazard identification, the importance of dose, and the generation of dose–response relationships
- How dose–response data might be used to assess safety or risk
- Factors that might alter a chemical's toxicity or the dose–response relationship
- The basic methods for extrapolating dose–response data when developing exposure guidelines of public health interest

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), itself derived from *toxikón*, an ancient Greek term for poisons into which arrows were dipped. The early history of toxicology focused on the understanding and uses of different poisons, and perhaps even today most people tend to think of a chemical or products labeled as a "toxic" substance" as that group of chemicals for which minimal exposure inevitably leads to death or some serious long-term adverse effect like cancer. As toxicology has evolved into a modern science it has expanded to encompass all forms of adverse health effects that any substance might produce. 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 toxic (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 toxic responses that form the study of toxicology span a broad biological and physiological spectrum. Effects of interest may range from something relatively minor such as irritation or tearing to a more serious response like acute and reversible liver or kidney damage, to an even more serious and permanent disability such as cirrhosis of the liver or liver cancer. Given this broad range of potentially adverse effects to consider, it

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is perhaps useful for those unfamiliar with toxicology to define some additional terms, listed in order of relevance to topics that will be discussed in Chapters 2–24 of this book.

Exposure—a measure of the opportunity for contact with a chemical in one's environment. The presence of a chemical in an environmental media 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 water).

Dose—describes the total amount of a toxicant an organism receives as the result of some exposure. The definition of dose typically refers to the *applied dose*, but different definitions and terms arise for the concept of dose as we move from the site of contact on the body to that amount absorbed and then distributed to the various tissues of the body. For example:

Applied dose—this is the total amount of the chemical that is directly applied to or has direct contact with those body surfaces that represent a portal of entry (via absorption) into the body. The applied dose can be higher than the absorbed dose because all of the chemical does not necessarily get across the membranes or surfaces at the site of contact.

Internal/absorbed dose—the actual quantity of a toxicant that is ultimately absorbed into the organism and distributed systemically throughout the body.

Delivered/effective/target organ dose—the amount of toxicant reaching the organ (known as the *target organ*) that is adversely affected by the toxicant.

Acute exposure—exposure that occurs only for a brief period of time (generally <24 h). Often it is considered to be a single exposure (or dose) but may consist of repeated exposures within a short time period.

Subacute exposure—resembles acute exposure except that the exposure duration is greater, for example, 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 this exposure ranges between 90 days to a lifetime. It is generally any exposure that occurs for the majority of that species' lifetime. In occupational settings it is generally considered to be for a number of years or more and may include either a working lifetime or an entire lifetime of an individual.

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).

Chronic toxicity—a permanent or lasting adverse effect that is manifested after exposure to a toxicant. An example would be the development of silicosis following a long-term exposure to silica in workplaces such as foundries.

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 burning of the eyes, upper respiratory tract irritation, and skin burns.

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 the toxic effect of the chemical distant from the point of entry of the toxicant (i.e., toxicant requires absorption and distribution within the organism to produce a systemic effect). Examples would include the adverse effects on the kidney or central nervous system (CNS) resulting from the acute or chronic ingestion of mercury.

Reversible toxicity—an adverse or undesirable effect that can be reversed once exposure is stopped. Reversibility of toxicity depends on a number of factors, including the extent of exposure (time and amount of toxicant) and the ability of the affected tissue to repair or regenerate. An example includes hepatic toxicity from acute acetaminophen exposure and liver regeneration.

Delayed or latent toxicity—an adverse or undesirable effect appearing long after the initiation and/or cessation of exposure to the toxicant. An example is cervical cancer during adulthood resulting from in utero exposure to diethylstilbestrol (DES).

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 to a toxicant occurring at exposure levels much lower than those generally required to cause the same effect in most individuals within the population. This response is genetically determined, and a good example would be sensitivity to nitrates due to deficiency in NADH (reduced-form nicotinamide adenine dinucleotide phosphate)–methemoglobin reductase.

Mechanism of toxicity—the necessary biological interactions by which a toxicant exerts its toxic effect on an organism. A simple example is CO asphyxiation due to the binding of CO to hemoglobin, thus preventing the transport of oxygen within the blood.

Toxicant—any substance that causes a harmful (or adverse) effect when in contact with a living organism at a sufficiently high concentration.

Toxin—any toxicant produced by an organism (floral or faunal, including bacteria), that is, naturally produced toxicants. An example would be the pyrethrins, which are natural pesticides produced by pyrethrum flowers (i.e., certain chrysanthemums) that serve as the model for the man-made insecticide class 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 less dosage needed to induce the toxicity it produces. In general terms, the less potent a chemical is, the safer it is because the probability of achieving a dose sufficient to induce toxicity via a particular route of exposure is lessened. Similarly, more potent chemicals tend to be more dangerous because it takes a smaller dose from an exposure to be able to induced toxicity.

Hazard—the qualitative nature of the adverse or undesirable effect (i.e., the type of adverse effect or toxicity the chemical produces) resulting from exposure to a particular toxicant or physical agent. For example, asphyxiation is the hazard from acute exposures to CO. Cancer, liver toxicity, and immunotoxicity are other hazards (types of toxicities) a chemical exposure might potentially represent. A hazard typically refers to the kind(s) of toxic effect(s) the chemical can produce if 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, 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 of exposure are characterized. In risk assessment, a safe exposure concentration is extrapolated from the dose–response curve for an adverse effect produced by the chemical that is used to derive a safe exposure concentration. Alternatively, a risk assessment might determine the probability and/or acceptability of a toxicity occurring at a known or measured exposure level.

1.2 TOXICOLOGY: A DIVERSE SCIENCE WITH TWO BASIC GOALS

Toxicology has become a science that builds on and uses knowledge developed in many related medical sciences, such as physiology, biochemistry, pathology, pharmacology, medicine, and epidemiology, to name only a few. Toxicology has evolved from the study of poisons to the study of all adverse effects induced by all chemicals or substances. Although toxicology is a science where a number of areas of specialization have evolved, 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, and the studies performed at these facilities are designed to generate basic toxicity information that identifies the various organ toxicities (hazards) the test agent is capable of inducing over those exposure conditions 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 and distributed to tissues throughout the body, identify tissue accumulation or elimination, and ultimately determine how it is excreted from the body. Hopefully, appropriate dose–response test data are generated for those toxicities of greatest concern and that toxicity produced at the lowest dose during the completion of the descriptive studies so that the relative safety of any given exposure or dose level that humans might typically encounter can be predicted.

Basic research or mechanistic toxicologists are scientists who study the chemical or agent in depth for the purpose of gaining an understanding of how the chemical or agent initiates those biochemical or physiological changes within the cell or tissue that result in the toxicity (adverse effect). The goal of mechanistic studies is to understand the specific biological reactions (i.e., the adverse chain of events) within the affected organism that ultimately result in the toxic effect being studied. 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). Because animal species are generally used to identify chemical-induced hazards, and because there may be significant species-specific responses to a chemical, mechanistic studies help provide the information on those key changes required to induce toxicity, and help reduce the uncertainty of the animal-to-human extrapolation we need to make to develop a safe exposure guideline.

Applied toxicologists are scientists concerned with the use of chemicals in a "real world" or nonlaboratory setting. The primary goal of applied toxicologists is the control of chemical exposures in all work and nonwork environments by setting

safe exposure guidelines for each exposure pathway (e.g., air, skin, ingestion exposure to the chemical) in that environment. Toxicologists who work in this area of toxicology use descriptive and mechanistic toxicity studies to limit the dose received by each or all exposure pathways to a total dose of the chemical that is believed to be safe. 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 focuses on determining and understanding the toxic effects of medicines, simple over-the-counter (nonprescription) drugs, and other 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), regulated and unintentional industrial discharges into air or waterways, 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, the identification of the hazards or injuries that overexposure to an occupationally used chemical might represent, and the prevention of these exposures or the treatment of the injuries they might 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, which are to (1) examine the nature of the adverse effects produced by a chemical or physical agent (*hazard/toxicity identification* function) and (2) assess the probability of these toxicities occurring under specific conditions of exposure (*dose–response and risk assessment* function). Ultimately, the goal and basic purpose of toxicology is to understand the toxic properties of a chemical so that these adverse effects can be prevented by the development of appropriate handling or exposure guidelines.

1.3 HAZARD IDENTIFICATION FUNCTION

The hazard identification or the discovery of the toxicities a chemical produces requires the testing of chemicals at doses high enough to induce the full spectrum of toxicities a chemical can induce. Typically, the hazard identification process involves traditional animal testing to uncover the spectrum of adverse effects (hazards) the chemical is capable of producing at some dose. One way of characterizing and identifying the hazard is by examining toxicities as a function of exposure duration, as previously described for acute, subacute, subchronic, and chronic exposures.

Because each chemical induces a different spectrum of toxic effects and one does not know beforehand which set of toxicity tests to perform to adequately capture and identify the possible hazards posed by the chemical, the chemical is examined using as wide a range of test systems as possible to ensure that all potential hazards for that chemical have been identified. For a complete toxicological evaluation the typical hazard assessment would follow a scheme similar to that illustrated in Figure 1.1. Typically, one would perform these tests using a tiered approach that starts with short exposure interval testing such as acute and subacute exposure periods (tier 1) and subsequently moves through subchronic tests (tier 2) and then chronic tests (tier 3). At each tier, specialized tests are performed in addition to those assessing target organ toxicities by route of exposure. For example, during the acute testing phase, dermal and reparatory tract irritation may be necessary as well as tests for the development of sensitization by the chemical. During subchronic and chronic testing, target organ testing is augmented by reproductive and developmental studies, testing for immunotoxicity, genotoxicity and mutagenicity, and a chronic bioassay for possible carcinogenic responses.

A tiered approach such as this allows the dose ranges to be set and as the duration of exposure increases, the dose needed to induce the effect is usually lowered (see Table 1.1). The shorter the duration of exposure the lower the cost of the test and the more time-efficient the study. So, trying to identify the end points of interest and toxic dose range is done more time and cost efficiently by seeking the toxicities a chemical induces by testing the chemical short-term tests first. However, both the types of hazards seen and the doses inducing these effects can change with the duration of exposure; and the hazards seen at shorter exposure durations cannot be assumed to be those that will be found after longer durations of exposure. For example, cancer is a latent disease that may require a lifetime of exposure to detect. The route of exposure may also impact the hazard because as the site of absorption is altered it may impact the occurrence of localized effects (like irritation or cellular necrosis at the site of contact) and it can change the tissue distribution as well as the target organ concentration per unit of absorbed dose. Either change may produce a different pattern of target organs affected with different routes of exposure. 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

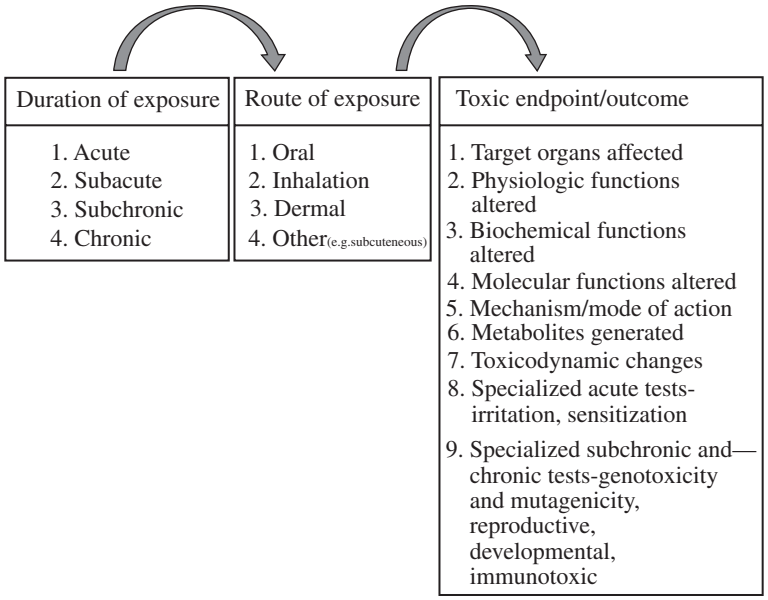

FIGURE 1.1 A generic toxicity testing scheme that shows the ways in which a toxicity test might differ because of the different choices to be made regarding the duration of exposure, the route of exposure, or the endpoint to be measured in the study.

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

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

oral administration induced liver tumors but not lung tumors. This kind of route-specific toxicity occurs frequently enough that regulatory agencies like the EPA no longer rely upon data gathered by one route of exposure to predict hazards or risk for another route of exposure, that is, there can be considerable uncertainty associated with route-to-route extrapolations without a mechanistic basis for doing so.

Since we are looking for adverse outcomes, the primary source of information for hazard identification comes for 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 end points of interest such as target organs, changes in physiological/biological/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 cellular macromolecules, isolated cell fractions, cellular organelles (e.g., mitochondria), tissue fractions, and isolated perfused whole organs as procedures for examining specific molecular, physiological, or biological 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 like nematodes, fruit flies, *Daphnia magna*, or fish, particularly when attempting to identify the ecological hazards or an environmental pollutant.

Some tests are easier and cheaper to perform and can better handle high-volume testing to screen candidate chemicals for further, more detailed toxicity testing or to predict toxicities in chemicals that have not been tested sufficiently via animal tests. One illustration of this approach is where toxicities are receptor-mediated and structure activity relationships may be used as a surrogate measure of subchronic and chronic hazards induced by structurally similar chemicals. The ever-expanding use of *in vitro* test systems may also be desirable in certain situations because they can isolate specific physiological or biochemical pathways in a way that better controls specific test conditions, doses, and outcomes besides being more time- and cost-efficient than whole organism testing. However, *in vitro* tests remove cell or target organism functions from the experimental *in vitro* concentrations (surrogate dose measure) used or the end point being measured may be modified in ways not easily extrapolated to whole organism responses. So, while *in vitro* tests may be undertaken more easily and repeated more consistently, they also have inherently greater uncertainty in comparison to what happens in a whole organism at specific exposure levels or exposure duration. For example, what metabolites are the chemical converted to in whole organisms that are not be seen when using certain *in vitro* test systems? Are toxic or nontoxic metabolites produced by the organism? How does the dose influence the metabolism and distribution throughout the body of the chemical and/or its metabolites? 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? In the end, *in vivo* or whole organism testing in a variety of species is generally necessary to identify the range of possible hazards the chemical might pose to humans.

In addition to animal methods, hazard information associated with human exposure to the chemical may also be available. As discussed in more detail elsewhere, there can be significant species differences in the both the beneficial and adverse responses induced by a chemical. So, in the final hazard assessment for a chemical, a toxicologist would like to review as much human data as are available. There are four basic categories of epidemiological information that can assist the hazard evaluation. These categories are 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 toxicological information and that of traditional *in vitro* and animal toxicity tests are summarized and compared in Table 1.2.

1.4 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 either a definition that one would apply to highly poisonous or very potently toxic chemicals or something that implies that only some chemicals produce adverse effects in humans and so can be described as toxic chemicals or those substances that we should all avoid. To help illustrate this point, and to begin to emphasize the fact that the toxicity is a function of dose, the reader is invited to take the following pop quiz. First, cross-match the doses shown in column A that produce lethality in 50% of the animals (lethal dose [LD_{50}]) 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 unsafe or dangerous you perceive the chemical to be, the higher the numerical ranking you should give it. After testing yourself with the chemicals

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. 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 (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 Epidemiological 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, and so on, may weaken the ability to discriminate real disease 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, that is, 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 that 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, that is, 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, that is, 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, and so on.
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.

Source: Adapted from James et al. (2000).

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 it 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.

listed in Tables 1.3, review the correct answers in tables found at the end of this chapter.)

According to the ranking scheme that you selected for these chemicals, were the least potent chemicals 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 pain relief medication you can buy at a local drugstore? What were the three most potentially toxic chemicals (most dangerous at the lowest single dose) in your opinion? Were they "natural" or the "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

TABLE 1.3 Cross-Matching Exercise: Comparative Acutely Lethal Doses

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.

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

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

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.

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	75	Trichloroethylene (solvent/degreaser)	_____
7	147	1,1,1-Trichloroethane (solvent/degreaser)	_____
8	170	1,1,2-Trichloroethane (solvent/degreaser)	_____
9	890	Toluene (organic solvent)	_____
10	1910	Vegetable oil mists (cooking oil)	_____

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 lower (numerically) the allowable air concentration, the more potently toxic the substance is per unit of exposure. Review the correct answers for tables recreated at the end of this chapter.

Hopefully, the preceding quiz helped illustrate the perceived toxicity or perceived hazard a chemical is thought to pose may mislead one regarding the actual toxic dose or potency of that chemical. As we have defined toxicants (toxic chemicals) as agents 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?" or "Which chemicals do we consider safe?" The short answer to both questions is 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 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 chemicals express toxicity became as important as, if not more important than, the kind of adverse effect 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

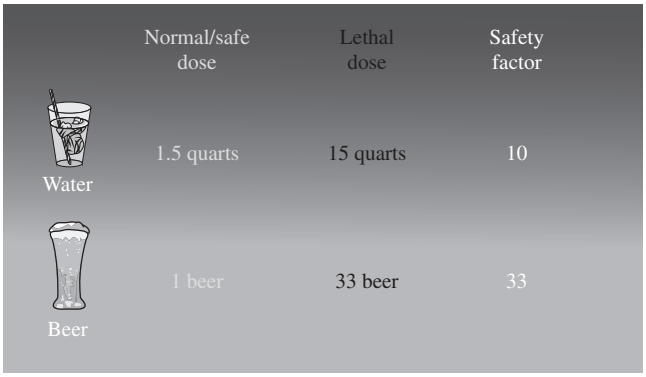

FIGURE 1.2 Acute lethal dose comparisons of two substances commonly used by human populations. *Source:* Adapted from James et al. (2000).

poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy." This statement serves to emphasize the 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. A simple illustration of Paracelsus's admonition and how it applies to all substances is seen Figure 1.2. This figure 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 is fatal. Normally this toxicity is limited to persons with a serious psychological disorder, but it was also recently illustrated during a radio station–sponsored contest to see who could drink the most water to win a new video game system. One of the contestants vying for the game system unfortunately died the day of the contest from water intoxication. In short, even safe substances are toxic if the dose is high enough. Consequently, another way of viewing the importance of the dose as being key to the toxicity of substances was that provided by Emil Mrak, who sated the concept first attributed to Paracelsus in the following manner—There are no harmless substances, only harmless ways of using substances. An illustration of this principle is exemplified in Figure 1.3 showing that the dose of aspirin increases as one moves through several different desirable target organ effects into those doses that are toxic to other target organs and finally lethality. So, the evaluation of those circumstances under which an adverse effect can be produced is the key to considering whether the exposure is safe or is hazardous. All chemicals are toxic at some dose and may produce harm if the exposure is sufficient (e.g., water or aspirin). Similarly, all chemicals may be used safely under prescribed conditions of dose or usage (e.g., the occupational

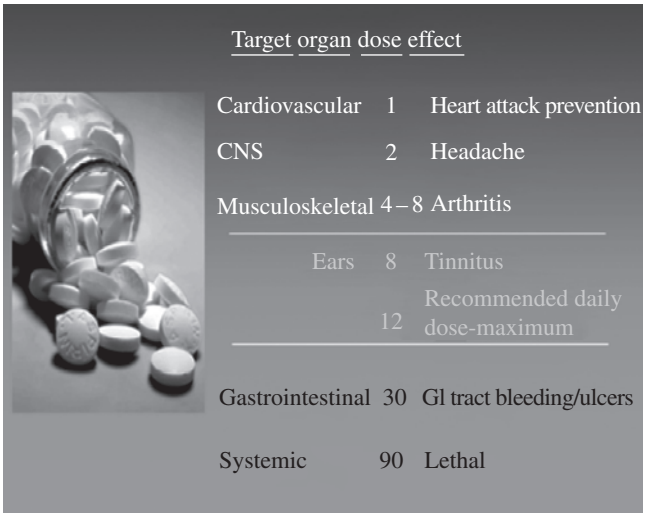

FIGURE 1.3 The dose–response curve for the therapeutic and toxic effects of aspirin.

handling of toxic chemicals during the manufacture of different products). Both quotations serve to remind us that describing a chemical exposure as being either harmless or hazardous is a function of the magnitude of the exposure (dose), and not necessarily the types of toxicities that a chemical might be capable of producing at some dose. Two additional illustrations of this concept are (1) the fact that the vitamins that we consciously take to improve our health and well-being continue to rank as a major cause of accidental poisoning among children; and (2) essentially all the types of toxicities that we associate with the term "hazardous chemicals" are produced by prescription and over-the-counter medication used today. In fact, a number of highly prescribed lipid-lowering drugs produce cancer in certain test animals at high doses but are safely used by many individuals on a daily basis.

Defining Dose and Response

Because all chemicals are toxic at some dose, what judgments determine their use? To answer this, one must first understand the use of the dose–response relationship because this provides the basis for estimating safe and hazardous exposure levels for all chemicals. A dose–response relationship is said to exist when a change in dose produces a consistent, nonrandom change in effect. This effect change can be either in the magnitude of effect or in the percentage of individuals responding at a particular level of effect. For example, the number of animals dying increases as the dose of strychnine is increased, or with therapeutic agents the number of patients recovering from an infection increases as the dosage is increased. In other instances, the severity of the response seen in each animal increases with an increase in dose once the threshold for toxicity has been exceeded.

Dose-Response Graphs

Not only does response to a chemical vary among different species; response also varies within a group of test subjects of the same species. Experience has shown that typically this intraspecies variation follows a normal (Gaussian) distribution when a plot is made relating the frequency of response of the organisms and the magnitude of the response for a given dose. Well-established statistical techniques exist for this distribution and reveal that two-thirds of the test population will exhibit a response within one standard deviation of the mean response, while approximately 95 and 99%, respectively, lie within two and three standard deviations of the mean. Thus, after testing a relatively small number of animals at a specific dose, statistical techniques can be used to define the most probable response (the mean) of that animal species to that dose and the likely range of responses one would see if all animals were tested at that dose (about one or two standard deviations about the mean.) Knowing this for each dose, one can then plot doses, with the standard deviations for each dose, and characterize the dose–response curve and the dose range over which toxicity affects all test organisms (see Figure 1.4).

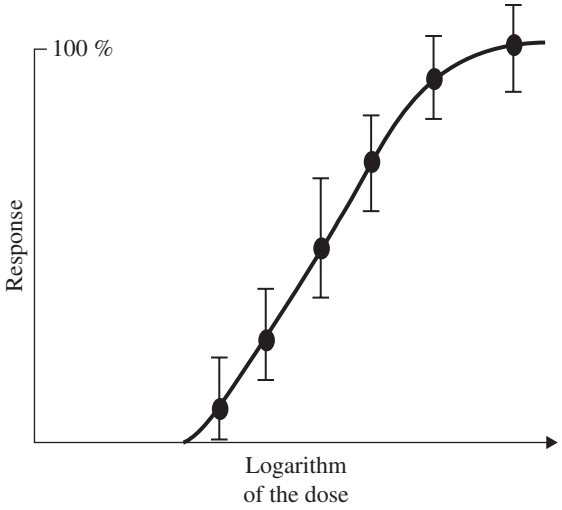

FIGURE 1.4 A simple graphic presentation showing a basic way to portray the dose–response data by plotting the mean responses at each dose and the variation (e.g., standard deviation) about the mean response observed at each dose over the range of doses tested.

In Figure 1.5, a cumulative dose–response curve is featured with a dotted line falling through the highest dose that produces no response in the test animals. Because this dose, and all doses lower than it, fail to produce a toxic response, each of these doses might be referred to as no observable adverse effect levels (NAOELs), which are useful to identify because they represent safe doses of the chemical. The highest of these NAOELs is commonly referred to as the *threshold dose*, which may simply be defined as the dose below which no toxicity is observed (or occurs). For all doses that are larger than the threshold dose, the response increases with an increase in the dose until the dose is high enough to produce a 100% response

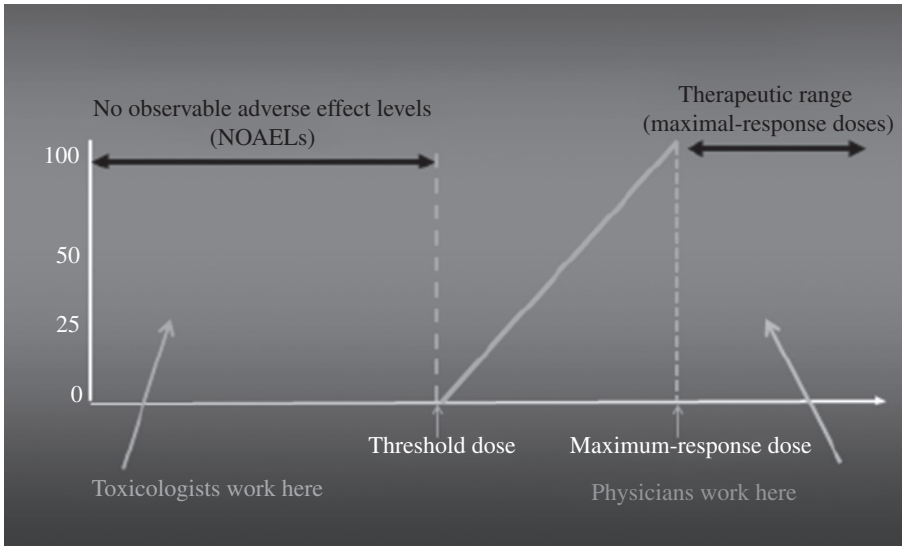

FIGURE 1.5 A schematic representation showing how physicians and toxicologists focus on different responses and areas of the available dose–response curves for a specific chemical. Physicians, because they are interested in producing a beneficial effect from the chemical (drug) in all persons exposed, select those doses in the dose–response range where a maximal response is always achieved. In contrast, toxicologists want to prevent any harmful effects from occurring, and so they select exposures that lead to doses below the threshold of the toxicity so that the harmful response will not occur.

rate (i.e., all subjects respond), and this dose is sometimes referred to as the *maximal-response dose*. All doses larger than the maximal-response dose produce a 100% response, and so the dose–response curve becomes flat again as increasing the dose no longer affects the response rate. For therapeutic effects, this region of the dose–response curve is typically the region physicians seek when they prescribe medicines. Because physicians are seeking a beneficial (therapeutic) effect, typically they would select a dose in this region that is just large enough so that individual variations in response to the dose would still result in a 100% response so as to ensure the efficacy of the drug. In contrast, a toxicologist is generally seeking those doses that produce no response because the effect induced by the chemical is an undesirable one. Thus, toxicologists seek the threshold dose and no-effect region of the dose–response curve.

Before discussing other ways in which dose-response data can be used to assess safety, it will be useful to briefly discuss the various shapes a dose–response curve might take. Although the schematic shape illustrated in Figure 1.6 is the most common shape, the dose–response curve could have either a *supralinear* or *sublinear* shape to it. In Figure 1.6a, the normal linear sigmoid curve is illustrated by line 1; line 2 is an example of a sublinear relationship, and line 3 depicts a supralinear relationship. In addition, some chemicals, while toxic at high doses, produce beneficial effects at low doses. Figure 1.6b–e provides illustrations of the shape of other dose–response relationships. For example, Figure 1.6b 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 1.6c 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 1.6d is a general representation of the most typical dose response curve, the curve for a threshold-dependent toxicity (sometimes referred to as the "hockey stick" 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 1.6e depicts hormesis, which typically has a j-shaped or even a U-shaped curve because at low doses the

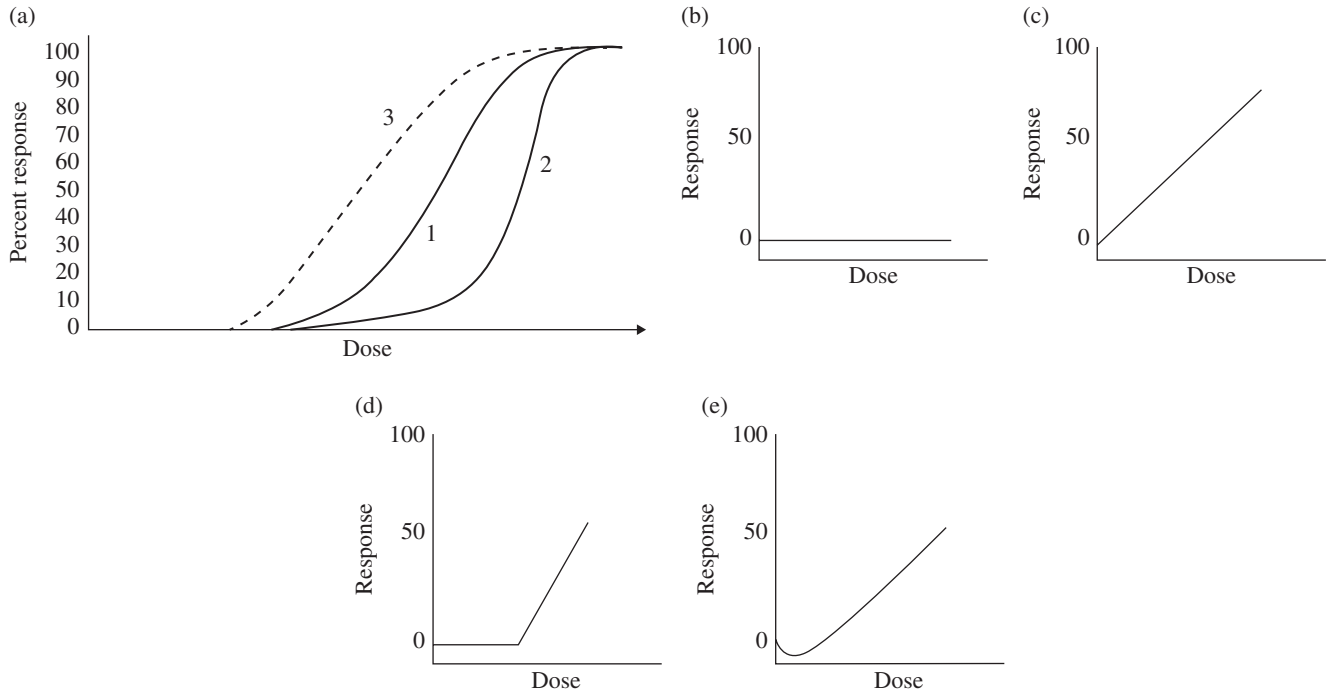

FIGURE 1.6 (a) The dose–response curves with log-linear (1), sublinear (2), and supralinear (3) shapes. (b) The dose–response curve where no effect is seen in the range of doses tested. (c) A graphical depiction of a linear, nonthreshold type of dose–response curve; this shape is typically assumed for carcinogenic substances by regulatory agencies. (d) A graphic representation of a nonlinear, threshold-dependent (toxicity generally seen with noncancer effects; this is commonly referred to as a "hockey stick" shaped dose–response. (e) The "J-shaped" dose–response curve seen with hormesis, a condition where low doses reduce toxicity or represent a beneficial effect that is lost as the dose increases and changes to a toxic responses at even higher doses. Dose–response curves for vitamins, hormones, and medicines frequently express this dose–response curve shape as the desired or beneficial effects are replaced by toxic effects at higher doses.

presence of the chemical benefits the organism and decreases the background response rate of a particular adverse effect. The phenomenon of low-dose stimulation (e.g., growth, reproduction, survival, or longevity) and high-dose inhibition is termed *hormesis*, and the most obvious examples of chemicals that exhibit this phenomenon are vitamins, essential nutrients, and drugs where low doses produce a beneficial effect while higher doses produce toxicity. However, there are other agents that display hormesis for which the benefit of low doses is less intuitive. For example, a number of studies on animals and humans have suggested that low doses of ionizing radiation decrease cancer incidence and mortality, possibly by increasing the presence of DNA repair enzymes, while high doses lead to increased cancer risk. It has been suggested that over time more evidence will show hormesis may be applicable to most, if not all, types of chemical toxicities, but a careful assessment of the extent to which this represents a generalized phenomenon has tended to be hampered by the limited availability of dose–response data below the toxic range for most chemicals. As evidence for hormesis continues to grow, a much clearer understanding of its role will emerge.

1.5 HOW DOSE–RESPONSE DATA CAN BE USED

Dosages are often described as *lethal doses* (LD), where the response being measured is mortality; *toxic doses* (TD), where the response is a serious adverse effect other than lethality; and *sentinel doses* (SD), where the response being measured is a nonadverse or minimally adverse effect. Sentinel effects (e.g., minor irritation, headaches, drowsiness) serve as a warning that greater exposure may result in more serious effects. Construction of the cumulative dose–response curve enables one to identify doses that affect a specific percentage of the exposed population. For example, LD_{50} is the dosage lethal to 50% of the test organisms (see Figure 1.7), or one may choose to identify a less hazardous dose, such as LD_{10} or LD_{01}.

Dose–response data allow the toxicologist to make several useful comparisons or calculations. As Figure 1.7 shows, comparisons of the LD_{50} doses of toxicants A, B, and C indicate the potency (toxicity relative to the dose used) of each chemical. Knowing this difference in potency may allow comparisons among chemicals to determine which is the least toxic per unit of dose (least potent) and therefore the safest of the chemicals for a given dose. This type of comparison may be particularly informative when there is familiarity with at least one of the substances being compared. In this way, the relative human risk or safety of a specific exposure may be approximated by comparing the relative potency of the unknown chemical to the familiar one, and in this manner one may approximate a safe exposure level for humans to the new chemical. For toxic effects, it is typically assumed that humans are as sensitive to the toxicity as the test species. Given this assumption, the test dose producing the response of interest (in units of milligrams per kilogram of body weight (mg/kg)), when multiplied by the average human weight (about 70 kg for a man and 60 kg for a woman), will give an approximation of the toxic human dose.

A relative ranking system developed years ago used this approach to categorize the acute toxicity of a chemical, and is shown in Table 1.5. In this ranking system, the potency of the oral lethal dose of a chemical is used to provide a relative ranking system that characterizes how the toxicity of the chemical is viewed. Again, the least potent category of chemicals (a dose of >15,000 mg/kg for lethality) requires a

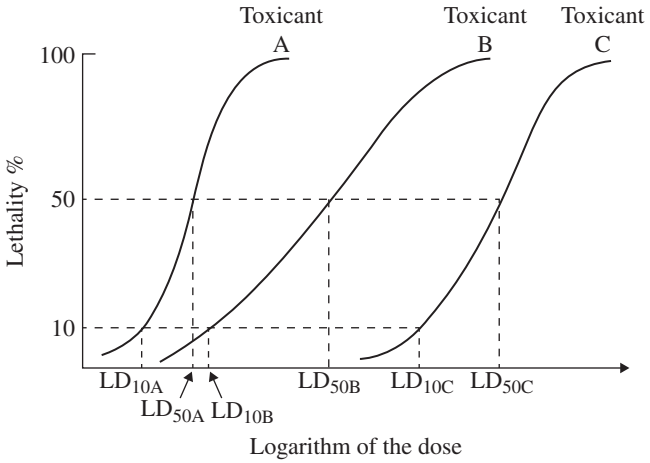

FIGURE 1.7 By plotting the cumulative dose–response curves (log dose), one can identify those doses of a toxicant or toxicants that affect a given percentage of the exposed population. Comparing the values of LD_{50A} to LD_{50B} or LD_{50C} ranks the toxicants according to relative potency for the response monitored.

TABLE 1.5 A Relative Ranking System for Categorization of the Acute Toxicity of a Chemical in Humans

	Probable Oral Lethal Dose	
Toxicity Rating or Class	Dose (mg/kg)	For Average Adult
1. Essentially nontoxic	>15,000	>1 quart
2. Slightly toxic	5,000–15,000	2 cups to 1 quart
3. Moderately toxic	50–5,000	1 ounce to 2 cups
4. Highly toxic	50–500	1 teaspoon to 1 ounce
5. Extremely toxic	1–50	7 drops to 1 teaspoon
6. Supertoxic	<1	<7 drops

Source: Adapted from Canadian Centre for Occupational Health and Safety (CCOHS) (2014).

large oral exposure (e.g., one quart or more) before the substance is lethal. Chemicals like this are considered relatively safe because lethality is unlikely to occur unless a person should ingest a quart or more. As the lethal dose decreases (i.e., becomes more potent at producing lethality), the toxicity rating of the chemical increases because the amount of the dose that may be ingested to incur lethality becomes smaller. Using this ranking system, an industrial hygienist within a work setting might obtain some insight into the acute danger posed by workplace exposure. Similarly, if chronic toxicity is the greatest concern, that is, if the toxicity occurring at the lowest average daily dose is chronic in nature, combining a measure of this toxic dose (e.g., TD_{50}) and appropriate safety factors might generate an acceptable workplace air concentration for the chemical.

Often the dose–response curve for a relatively minor acute toxicity such as odor, tearing, or irritation involves lower doses than more severe toxicities such as coma or liver injury, and much lower doses than fatal exposures. This situation is shown in Figure 1.8, and it can be easily seen that understanding the relationship of the three dose–response curves might allow the use of sentinel effects (represented in Figure 1.8 by the SD curve, the safe dose–response curve) to prevent overexposure and the occurrence of more serious toxicities.

The difference in dose between the toxicity curve and a sentinel effect represents the *margin of safety* (see safety factor expressed in Figure 1.2). In the past, the margin of safety was calculated from data like that shown in Figure 1.8 by dividing TD_{50} by the SD_{50}. This value represents a calculation similar to what physicians refer to as the therapeutic index. The higher the margin of safety, the safer the chemical is to use (i.e., greater room for error). However, if the dose–response curves are complete enough to characterize both the low-response and maximal-response range of doses for both curves then one would generally want to use a more protective definition for the margin of safety (e.g., TD_{01}/SD_{100}). Changing the definition of what the margin of safety represents to include a higher percentile of the sentinel dose–response curve (e.g., the SD_{100}) and correspondingly lower percentile of the toxic dose–response curve (e.g., the TD_{01}) forces the margin of safety to be defined as something protective for the vast majority, if not all, of a population, and so represents an improved method for defining the margin of safety.

Probably the most common use of dose–response data is to use the threshold (or highest NOAEL) dose from an animal toxicity test to extrapolate a corresponding safe dose in humans. Because all exposures producing doses less than the threshold dose (or a NOAEL) should be devoid of toxicity, all exposure below these points will 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 and qualitatively 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 a total safety/uncertainty factor from a combination of different uncertainty factors that each reflects the uncertainty of the dose–response data being used in the extrapolation (this is explained in more detail in the risk assessment chapter).

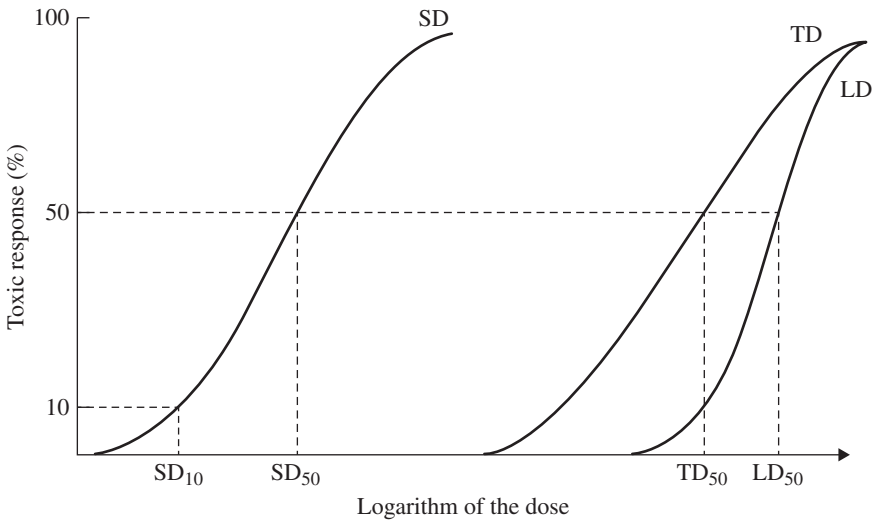

FIGURE 1.8 By plotting or comparing several dose–response curves for a toxicant, one can see the relationship that exists for several responses the chemical might produce. For example, the sentinel response (SD curve) might represent a relatively safe acute toxicity, such as odor or minor irritation to the eyes or nose. The toxic response (TD curve) might represent a serious toxicity, such as organ injury or coma. The lethal response (LD curve), of course, represents the doses producing death. Thus finding symptoms of minor toxicity in a few people at sentinel response (SD_{10}) would be sufficient warning to prevent a serious or hazardous exposure from occurring.

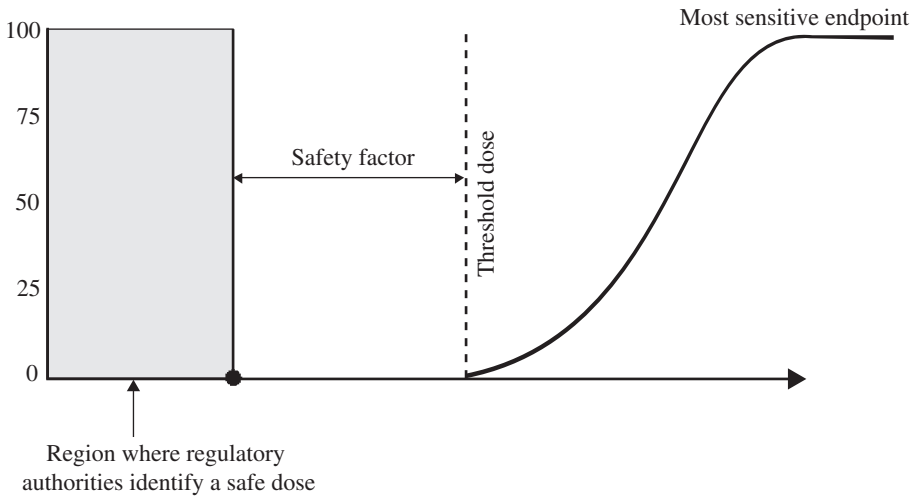

FIGURE 1.9 Graphically demonstrates how the regulatory community develops safe exposure levels for chemical-induced toxicities. Starting at a dose where the adverse effect is not likely by induced (the threshold or NOAEL) dose, the allowable dosage rate is then reduced further by the adoption of safety (uncertainty) factors based on the strength of the available evidence. Reduction by a factor of 10-fold is typically adopted based on the presence of characteristics of the dose–response curve under consideration such as the following: (1) use of animal (nonhuman) data, (2) use of less than chronic exposure duration data, and (3) limited toxicity testing and similar characteristics than contribute to the uncertainty of the extrapolation being made.

After accounting for the potential uncertainty associated with the threshold/NOAEL dose, the final dose selected is considered to be a "safe dosage" that can be used in the development of human exposure guidelines for that chemical (see Figure 1.9). As can be seen in Figure 1.9, the net effect of dividing the threshold or NOAEL dose by some total safety/uncertainty factor is that it is equivalent to selecting a substantially lower dose from the no-effect region of the dose–response curve. This approach essentially adds an additional margin of safety that ensures that the animal data used for the extrapolation has not understated the potency of the chemical in humans.

1.6 AVOIDING INCORRECT CONCLUSIONS FROM DOSE–RESPONSE AND HAZARD IDENTIFICATION DATA

While the dose–response relationship can be determined for each adverse health effect of a toxicant, one must be cognizant of certain limitations when using dose–response data:

1. If only single values from the dose–response curves are available (e.g., the LD_{50}), it must be kept in mind that those values will not provide any information about the shape of the curve. So, while toxicant A in Figure 1.10 would appear to be more toxic than toxicant B chemical at higher doses, this is not true at lower doses. Toxicant B has a lower threshold and actually begins to cause adverse effects at lower doses than toxicant A. Once someone is exposed to a toxicant, the shape of the dose–response curve may be as important as the dose at which toxicity first begins (the threshold dose). Actually, in this regard, toxicant A is a greater concern, not necessarily because of its lower LD_{50} and LD_{100}, but rather because of its steeper dose–response curve. Once individuals become overexposed (exceed the threshold or safe dose), the increase in response occurs with much smaller increases in dose, and more persons are affected with subsequent increases in dose. In other words, once the toxic level is reached, the margin of error for substance A decreases more rapidly than for substance B, because each incremental increase in exposure greatly increases the percentage of individuals affected.
2. Acute toxicity, which is typically generated first because of the savings in time and expense, may not accurately reflect chronic toxicity dose–response relationships. Evidence of this was provided in Table 1.1, which showed the dosage representing the NOAEL for a specific effect decreased as the duration of exposure was increased to subchronic or chronic length of exposure. The type of adverse response generated by a substance may also differ significantly as the exposure duration increases in time and chronic toxicities are sometimes not the same as acute adverse responses. For example, both toluene and benzene cause depression of the CNS, and for this acute effect toluene is the more potently toxic of the two compounds. However, benzene is of greater concern to those with chronic, long-term exposure, because it is carcinogenic while toluene is not. Likewise, the acute hazard of many

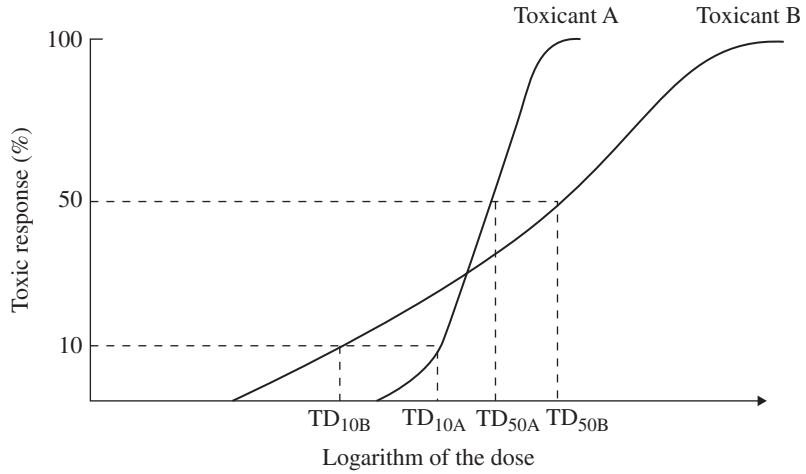

FIGURE 1.10 The shape of the dose–response curve is important. By finding the LD_{50} values for toxicants A and B from a table, one would erroneously assume that A is (always) more toxic than B. The figure demonstrates that this is not true at low doses.

TABLE 1.6 Oral LD_{50} Data for Chloroform

Species	LD_{50} (mg/kg/day)
Rabbit (Dutch Belted)	100[a]
Mouse (CD-1)	250
Human	602
Rat (Sprague-Dawley)	908
Mouse (Swiss)	1100
Mouse (ICR-Swiss)	1400[b]
Rat (Wistar)	2180

Source: Adapted from ATSDR (1996), *Toxicant Profile for Chloroform*.
[a]Based on 13 days of dosing.
[b]Female mice.

TABLE 1.7 Chloroform Toxicity: Inhalation Studies

Mouse as Test Species	Exposure (ppm)	Rat as Test Species
NOAEL—liver	3	NOAEL—respiratory tract
LOAEL—liver	10	LOAEL—respiratory tract (nasal)
	10	NOAEL—kidney
	30	LOAEL—kidney
AEL—liver	100	
NOAEL—kidney	100	NOAEL—liver
LOAEL—kidney	300	LOAEL—liver
NOAEL—respiratory tract	300	

Source: Adapted from ATSDR (1996), *Toxicant Profile for Chloroform.* The duration of exposure for all tests were 6 h/day for 7 days. NOAEL (no observable adverse effect level), LOAEL (low observable effect level—mild organ injury), AEL (adverse effect level—severe/frank organ injury).

chlorinated solvents is generally limited to the CNS depressant properties of the chemical, while for chronic exposure liver and/or kidney effects and possibly cancer may become the primary concern.

3. There is usually little information for guidance in deciding what animal data will best mimic the human response. For example, a question that often arises initially in the study of a chemical is the following: is the test species less sensitive or more sensitive than humans? As shown in Table 1.6, the dose of chloroform that is lethal to 50% of the test animals (i.e., the LD_{50}) varies depending on the species and strain of animal tested. Estimation of the fatal human dose based on the animal results (shown in Table 1.6) would overstate the toxicity of chloroform when using the rabbit or CD-1 mouse data, and underestimate the toxicity of chloroform if projecting lethality using data from the two remaining mouse strains or the two rat strains tested. Another example was illustrated in Table 1.7, where the pattern of toxicity as exposure increased was significantly different when comparing results for the mouse versus those for the rat. In short, dose–response curves for a species less sensitive to the toxicity of interest than humans will understate or underpredict the potential for harm (the risk), while dose–response curves for a species more sensitive or uniquely sensitive (incurs a toxicity not seen in other species) to a specific toxicity will overstate or exaggerate the potential for harm (the risk). The only way to know which species provides the most accurate dose and hazard extrapolations is if you have reliable mechanistic data to be able to pick the correct species to use.
4. In subchronic and chronic testing, generally the highest dose to test is defined as the *maximally tolerated dose* (MTD). This dose is generally defined as the highest dose the test species can be given without generating excessive systemic toxicity (it is usually defined as a dose not inducing a greater than 10%

decrease in body weight or sufficient lethality that adversely impacts completion of the test). Use of the MTD in subchronic and chronic animal tests represents a compromise between two desired goals, sensitivity (avoiding false negatives) and specificity (avoiding false positives). The first goal is to ensure all possible hazards of the chemical have been 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 incidence of the response (usually a function of dose), the smaller the number of animals that have to be used to be able to see a statistically significant change. So, using the MTD 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 desirable, especially for regulatory purposes. The second desired goal is 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, bio-chemical, and physiological changes that can produce chronic organ toxicity or carcinogenicity, but do so only under these altered cellular conditions created by the high dose. Where lower doses do not induce these cellular or biochemical changes toxicity is not induced or expected. For example, Gold noted that 44% of the chemical carcinogens she reviewed were not capable of inducing a carcinogenic response at doses as high as ¼–½ the MTD. So, while the MTD might induce all possible toxicities and identify the complete list of potential human hazards, concern for and consideration of these hazards may exaggerate the potential human harm if they are only seen at exposures and doses that substantially exceed the worst-case human exposure scenario.

Species-Related Differences in Hazard and Dose–Response Information: A Frequent Problem that Raises Uncertainty for Animal-to-Human Extrapolations

The basic premise for using animal data to try and predict safe or unsafe human exposure situations is that the effects seen in animal tests are applicable to, and predictive of, the human response. That is, the premise of animal toxicity testing is that the animal response is qualitatively and quantitatively the same, or very similar to, that found in humans. Unfortunately, species-specific differences in the pattern of toxicities observed or in the potency of the chemical are a relatively common phenomenon in toxicology. There are numerous reasons for these species-specific differences, but in the end one can state that there are genetically controlled differences among species that produce anatomical, physiological, biological, and biochemical differences across animal species, or between rodent species (the most frequently used test species in toxicity testing) and man. These differences may confound the animal-to-human extrapolation by increasing the uncertainty and concern we have for the accuracy of both the hazard extrapolation and the dose–response extrapolation being made.

For example, some laboratory animals possess certain anatomical features that humans lack, such as the Zymbal gland and a forestomach. So, when a chemical produces organ toxicity or cancer within these structures, the human relevance for such findings is unknown. Similarly, male rats produce a protein known as α-2microglobulin, which has been shown to interact with the metabolites of certain chemicals in a manner that results in repeated cellular injury within the kidney. This reaction is believed to be responsible for the kidney tumors seen in the male rat after chronic exposure to a number of chemicals (e.g., gasoline). Because this unique protein from these animals does not occur to any appreciable extent in female rats or in both sexes of mice, kidney tumors are not seen in female rats or male and female mice. From these important sex and species differences, scientific groups and regulatory agencies have concluded that the male rat kidney tumors are not relevant to humans, a species that is also deficient in α-2microglobulin.

Certain animal strains are uniquely sensitive to certain types of cancer. For example, a large proportion of B6C3F1 mice develop liver tumors before they die, and this sensitivity appears to be due in part to the fact that the H-*ras* oncogene in this mouse strain is hypomethylated, allowing this oncogene to be expressed more easily, especially during recurrent hepatocellular injury. Similarly, 100% of strain A mice typically develop lung tumors before these animals die, and so a chemical that promotes the early development of lung tumors in this strain of mice may not produce any lung tumors in other strains that have lower lung cancer rates. The Fischer 344 rat, the rat strain commonly used in chronic cancer bioassay testing, have higher background tumors rates in certain tissues, and this difference in background tumors rates in specific organs differs from those organs with the highest background tumor rates in the B6C3F1 mouse strain, the mouse strain most commonly used in cancer bioassays. Thus, the target organs frequently sensitive to the carcinogenic effects of a particular chemical differ between the rat and the mouse. So, when tumors are induced only in those organs with high spontaneous background rates in these two rodent test species, the uncertainty regarding the human relevance of the observation increases because humans have a

TABLE 1.8 Target Organ Comparison of Tumors Induced in Chronic Cancer Bioassays

Correlations in Site-Specific Carcinogenicity[a]		
Site of Cancer	Rat Response Predicts Mouse Response	Mouse Response Predicts Rat Response
Liver	75% (25/33)	32% (25/78)
Lung	29% (2/7)	11% (2/18)
Hematopoietic system	21% (3/14)	27% (3/11)
Kidney (tubular cells)	14% (3/21)	75% (3/4)
Mammary gland	22% (4/18)	57% (4/7)
Forestomach	57% (8/14)	53% (8/15)
Thyroid gland	44% (7/16)	78% (7/9)
Zymbal gland	17% (2/12)	100% (2/2)
Urinary bladder	17% (2/12)	67% (2/3)
Skin	27% (3/11)	100% (3/3)
Circulatory system	50% (2/4)	20% (2/10)
Overall totals (all data[a])	35% (61/173)	37% (61/167)

Source: Adapted from Haseman and Lockhart (1993) and based on an examination of the results provided in 379 cancer bioassays performed by the NTP.

[a]Proportion of chemicals carcinogenic in the first species that are also carcinogenic in the second species. (Example: of the 33 chemicals inducing liver cancer in rats, 25 of these also induced liver cancer in the mouse; in contrast, of the 78 chemicals that induced liver cancer in the mouse only 25 of these also produced liver cancer in the rat).

third pattern of organ tumor incidence rates that differ from these two rodent species. The concern for human relevance may be further heightened where humans will typically be exposed at doses that are orders of magnitude lower than those required to induce tumors in either rodent species.

An illustration showing that mice and rats typically respond differently in chronic cancer bioassays is provided in Table 1.8. This table contains most of the organ comparisons made for 379 animal cancer studies undertaken by the National Toxicology Program (NTP). It shows how consistently a chemical that induced cancer in an organ of either the mouse or the rat produced the same response in the other rodent species. There are a few species-specific target organ findings where the response in one rodent species does reliably indicate where cancer might occur in the other rodent species (e.g., chemicals that produce liver cancer in rats also produce liver cancer in mice 75% of the time); however, overall there is limited predictability as to which organ might develop cancer in a particular rodent species based on the test results provided by another rodent species.

Because species differences do exist, part of any hazard or dose assessment might also be a consideration of how reproducible a specific animal response is when other studies are performed to confirm a specific toxicity. As one example of this potential problem, a group of scientists compared the results of cancer bioassays generated in the NTP to that of chemicals evaluated in other protocols as reported in the Carcinogenic Potency Database (CPDB). This comparison allowed for a determination of the percentage of replicate responses seen when a chemical was tested again for a chronic hazard of considerable public interest, cancer. Some 121 chemicals were identified that had results in both databases. Of these 121 comparisons, only 69 (57%) had concordant conclusions (i.e., the chemical had the same classification as being either a carcinogen or a noncarcinogen in the replicate test). Thus, 43% of the time 52 chemicals had discordant classifications between the two experiments. The mouse test results proved to be the least consistent between the two species with only 49% of the 70 mouse experiments showing concordant results. Of 71 rat experiments, the results were concordant 62% of the time. When evaluated by sex and species, the concordance was 46% for male mice, 36% for female mice, 55% for male rats, and 69% for female rats. Because the test comparisons involved strain differences within a species, the results were also broken down into tests using the same rat (Fischer-344) and mouse (B6C3F1) strains. This comparison resulted in the test concordance being slightly lower, with 57% concordant results for male rats, 64% for female rats, 39% for male mice, and 33% for female mice. So, the poor reproducibility of carcinogenic responses seen in the overall analysis was not caused by strain differences and variations in the rat or mouse strain being tested for a particular chemical. Less than a 100% concordance for repeated testing is not limited to cancer and may be seen for other types of toxicity tests. Failure to confirm a specific result may stem from strain, species, or test protocol differences between two or more tests of the same end point. The response differences across test species increases the uncertainty as to which result provides the most reliable reflection of the actual human response.

A similar analysis of concordant responses compared test animal responses used to predict drug safety to the human toxicities ultimately seen later during early clinical trials. This study was limited to those drug-induced toxicities that were severe enough to either terminate the development of the drug or limit the dosage used, to restrict drug use to required monitoring or restrict the targeted population. In this manner, the confusing complication of addressing the myriad of minor side effects typically associated with almost all drugs was avoided. Still, 221 examples of human toxicity for 150 different drugs were ultimately available for analysis using these selection criteria. The toxicity correlations between the human response and the animal test species were loosely defined as any effect that involved the same target organ, a choice that essentially inflates the true concordance. Still, the overall true positive concordance was stated to be only 70% when one or more species could be compared to the human response (i.e., did any test species identify the correct target organ). However, when concordance was broken down by the specific species being tested, it was found that nonrodent species had a higher concordance of 63% (primarily the dog); rodent species were concordant only 43% of the time

(here concordance was primarily from the rat). Only 36% of the time was the human toxicity concordant with both a rodent and a nonrodent species. The total animal concordance was highest among Phase 1 clinical trials (75%), but much lower in Phase 2 (58%) and Phase 3 (52%) clinical trials. Analyzing the false-negative animal findings, the authors found that when an adverse human response was not predicted, it was not the result of an inadequately low dose being tested in the animal species. Some 91% of the rodent and 90% of the nonrodent toxicology tests were judged to have been performed at a dose approximating the MTD for that species. Similarly, the animal metabolism profile correlated with that of man in 86% of the false-negative animal responses that were analyzed. Therefore, differences in metabolism were not a likely explanation for the key species-specific response differences. In fact, 89% of the time the animal and human metabolites formed are similar in both concordant and nonconcordant tests. It should be noted that the animal species that are the mainstay of toxicity studies performed on industrially and environmentally important chemicals are primarily the rat and the mouse rodent species. But these two test species resulted in poor predictions of human toxicity even though concordance was exaggerated by defining it as any effect in the same target organ, and not defined as inducing the same organ toxicity or end point.

Given these findings, it should be clear to the reader why mechanistic studies are an important component of the hazard and dose–response assessments performed in toxicology. By investigating the physiological, biochemical, and molecular changes induced in a *responsive* species/strain (one that develops the toxicity) and a *nonresponsive* species/strain (one that does not develop the toxicity), key findings may be developed that help us understand the basis for the observed species/strain differences we observe during animal testing. When this same mechanistic data is generated in human studies, the toxicologist has a better basis for predicting (or later explaining) which animal response is more likely to be relevant to humans, and thereby this leads to better species extrapolations when attempting to predict the risk or safety of human exposure to a specific chemical. Examples of this are some studies by Green examining the lung tumors induced in mice or rats by TCE. These studies provided a mechanism of action involving key biochemical and cellular responses that predicted the rank order of the tumorigenic risk would be mouse > rat >> humans. Given that the rat was the nonresponsive species even at high exposure levels, the proposed mechanism suggested TCE would not induce lung cancer even in high-dose TCE-exposed workers, a conclusion that was consistent with the available epidemiology studies.

To summarize, there are a number of important genetically driven species differences that may cause changes in the:

1. Basal metabolic rate of the test species
2. Anatomy and organ structure of the test species
3. Physiology of the test species
4. Cellular biochemistry of the test species
5. Metabolism, bioactivation, and detoxification of the chemical (see Chapter 2)
6. Toxicokinetics of the chemical (see Chapter 3)
7. One or more of these species differences may ultimately produce cellular, tissue, or organ response differences between different test species or between a test species and man

Because these differences can produce significant differences in the potency for an effect and/or in the pattern of adverse effects seen across the animal species tested, they add uncertainty to the hazard and dose–response assessment processes. Selection of the right animal to study requires a prior knowledge of the fate and effects of the chemical in humans (the goal of the animal testing), as well as its fate and effects in various animals. If data generated in only one or few test species is available, there is always uncertainty about which data will most accurately predict the human response; and there are numerous examples where either the dose–response curve or the effect are exaggerated, understated, or completely missed by the results produced in a test animal species. Determining or choosing which species best represents the human response has a great impact on the perceived and estimated risk or safety of any human exposure guideline developed from animal data. While such extrapolations may be improved where mode-of-action or mechanism-of-action data are available, or by developing information from those high-dose human exposure situations, many times these kinds of additional information simply do not exist.

1.7 ADDITIONAL FACTORS INFLUENCING HAZARD IDENTIFICATION AND DOSE–RESPONSE DATA

Route of Exposure

The exposure pathway by which a substance comes in contact with the body determines how much of it enters (rate and extent of absorption) and which organs are initially exposed to the largest concentration of the substance. For example, the water and lipid solubility characteristics of a chemical affect its absorption across the lungs (after inhalation), the skin (after dermal application), or the gastrointestinal (GI) tract (after oral ingestion), and the effect differs for each organ. The rate and site of absorption (organ) may also in turn determine the rate of metabolism and excretion of the chemical. So, changing the route of exposure may alter the dose required to produce toxicity. It may also alter the organ toxicity that is observed. For example, the organ with generally the greatest capacity for the metabolism and breakdown of chemicals is the liver. Therefore, a chemical

may be more or less toxic per unit of dosage when the chemical is given orally or peritoneally, routes of administration that ensure the chemical absorbed into the bloodstream passes through the liver before it perfuses other organs within the animal. If the capacity of the liver to metabolize the chemical within the bloodstream is great, this leads to what is referred to as a *first-pass effect*, in which the liver metabolizes a large proportion of the chemical as it is absorbed and before it can be distributed to other tissues. If the metabolism of this chemical is strictly a detoxification process, then the toxic potency of the chemical (i.e., toxicity observed per unit of dose administered) may be reduced relative to its potency when administered by other routes (e.g., intravenously). On the other hand, if the metabolism of that dose generates toxic, reactive metabolites, then a greater toxic potency may be observed when the chemical is given orally relative to inhalation, dermal, or intramuscular administrations of the chemical. (See also discussion in Chapters 2 and 3.)

As an illustration that the route of exposure may or may not affect the toxic potency of the chemical, Table 1.9 lists LD_{50} data for various routes of exposure for three different chemicals. All of these chemicals were administered to the same test species so that differences relating to the route of exposure may be compared. As this table shows, in some instances the potency changes very little with a change in the route of administration (e.g., potency is similar for the pesticide DFP: for all routes except dermal); in other instances—DDT, for example—the potency decreases 10-fold when changing the route of administration from intravenous to oral, and another 10-fold when moving from oral to dermal.

Sex

Gender characteristics may affect the toxicity of some substances. Women have a larger percentage of fat in their total body weight than men, and women also have different susceptibilities to reproduction system disorders and teratogenic effects. Some cancers and disease states are sex-linked. Large sex-linked differences are also present in animal data. One well-known pathway for sex-related differences occurs in rodents where the male animals of many rodent strains have a significantly greater capacity for the liver metabolism and breakdown of chemicals. This greater capacity for oxidative metabolism can cause the male animals of certain rodent strains to be more or less susceptible to toxicity from a chemical depending on whether oxidative metabolism represents a bioactivation or detoxification pathway for a chemical at the dose it is administered. For example, in the rat, strychnine is less toxic to male rats when administered orally because their greater liver metabolism allows them to break down and clear more of this poison before it reaches the systemic circulation. This allows them to survive a dose that is lethal to their female counterparts. Alternatively, this greater capacity for oxidative metabolism renders male rodents more susceptible to the liver toxicity and carcinogenicity of a number of chemicals that are bioactivated to a toxic, reactive intermediate during oxidative metabolism.

Age

Older people have differences in their musculature and metabolism, which change the disposition of chemicals within the body and therefore the levels required to induce toxicity. At the other end of the spectrum, children have higher respiration rates and different organ susceptibilities (generally they are less sensitive to CNS stimulants and more sensitive to CNS depressants), differences in the metabolism and elimination of chemicals, and many other biological characteristics that distinguish them from adults in the consideration of risks or chemical hazards. For example, the acute LD_{50} dose of chloroform is 446 mg/kg in 14-day-old Sprague-Dawley rats, but this dose increases to 1188 mg/kg in the adult animal.

TABLE 1.9 Effect of Route of Administration on Response (LD_{50})

Route of Administration	Methadone[a]	Strychnine[a]	DDT[a]	DFP[b]
Oral	90	16.2	420	4
Subcutaneous	48	3	1500	1
Intramuscular	—	4	—	0.75
Intraperitoneal	33	1.4	100	1
Intravenous	10	1.1	40	0.3
Intraocular	—		—	1.15
Dermal	—		3000	117

Source: Adapted from *Handbook of Toxicology*, 1956, Vol. 1.
All doses are in units of mg/kg.
[a]Rat.
[b]Rabbit.

Effects of Chemical Interaction (Synergism, Potentiation, and Antagonism)

Mixtures represent a challenge because the response of one chemical might be altered by the presence of another chemical in the mixture. A synergistic reaction between two chemicals occurs when both chemicals produce the toxicity of interest, and when combined, the presence of both chemicals causes a greater-than-additive effect in the anticipated response. Potentiation describes that situation when a chemical that does not produce a specific toxicity, nevertheless, increases the toxicity caused by another chemical when both are present. Antagonists are chemicals that diminish another chemical's measured effect. Figure 1.11 provides simple mathematical illustrations of how the effect of one or two chemicals changes if their combination causes synergism, potentiation, additivity, or antagonism, and gives a well-known example of a chemical combination that produces each type of interaction.

Modes of Chemical Interaction

Chemical interactions can be increased or decreased in one of four ways:

1. *Functional*—both chemicals affect the same physiological function.
2. *Chemical*—a chemical interaction between the two compounds affects the toxicity of one of the chemicals.
3. *Dispositional*—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 tissue receptor, the second chemical, which differs in activity, competes for the receptor and thereby alters the effect produced by the first chemical.

To help illustrate the ways in which chemical interactions are increased (additive, potentiation, synergism) or decreased (antagonism), Table 1.10, adapted from a textbook on chemical interactions by Edward Calabrese, is provided. This table summarizes a few of the chemical interactions identified for drinking alcohol (ethanol) and other chemical agents that might be found in home or occupational environments.

Like alcohol, smoking may also alter the effects of other chemicals, and the incidence of some minor drug-induced side effects have been reported to be lower in individuals who smoke. For example, smoking seems to diminish the effectiveness of propoxyphene (Darvon) to relieve pain, and it lowers the CNS depressant effects of sedatives from the benzodiazepine and barbiturate families. Smoking also increases certain metabolic pathways in the liver and so enhances the metabolism of a number of drugs. Examples of drugs whose metabolism is increased by smoking include antipyrine, imipramine, nicotine, pentazocine, and theophylline. Table 1.11 summarizes a few of the chemical interactions that have been reported in aquatic toxicity studies.

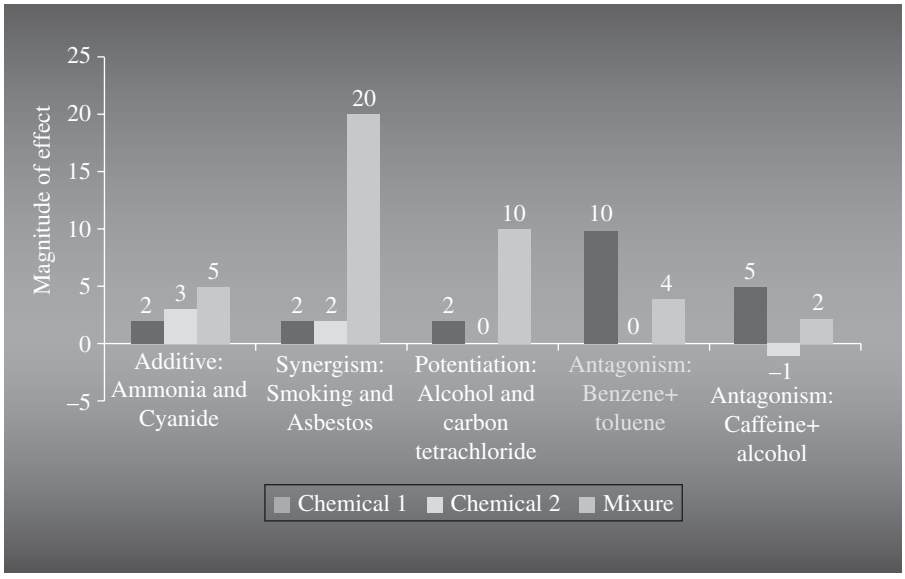

FIGURE 1.11 A simple schematic showing how a mixture formed by combining two chemicals alters the specific response of interest that is seen when the chemicals are given alone. These mixtures of chemicals the reader is likely familiar with show how the response may show additivity, synergism, potentiation, or antagonism depending on the type of interaction that occurs when the exposure contains both chemicals.

TABLE 1.10 Chemical Interactions with Ethanol

Agent	Toxic Interaction	Mode: Mechanism
Aspirin	Increased gastritis	Functional—both agents irritate the GI tract
Barbituates	Increased barbiturate toxicity	Functional/dispositional—both agents are CNS depressants; altered pharmacokinetics and pharmacodynamics of the barbiturates
Benzene	Increased benzene-induced hematotoxicity	Dispositional—enhanced benzene bioactivation to toxic metabolites
Caffeine	Caffeine antagonizes the CNS depressant effects of ethanol	Functional—both agents affect the CNS, but one is a stimulant and one is a depressant
Carbon disulfide	Enhanced CS_2 toxicity	Dispositional—increased CS_2 bioactivation and retention in critical tissues
Chloral hydrate	Increased CNS sedative effects of chloral hydrate	Functional/dispositional—both agents are CNS depressants; ethanol also alters the metabolism of chloral hydrate, leading to greater trichloroethanol accumulation
Ethylene glycol	Decreased ethylene glycol toxicity	Dispositional—ethanol inhibits the metabolism of ethylene glycol to its toxic metabolites
Nitrosamines	Increase in formation of extrahepatic tumors induced by nitrosamines	Dispositional—ethanol alters the tissue distribution of nitrosamines by inhibiting hepatic metabolism

Source: Adapted from Calabrese (1991).

TABLE 1.11 Aquatic Toxicity Interactions between Ammonia and Other Chemicals

Chemicals	Toxic End Point	Ratio of Chemical EC_{50s}	Interaction
Ammonia + cyanide	96-h LC_{50}	1:1	Additive
Ammonia + sulfide	24-h LC_{50}	1:2.2	Antagonism
Ammonia + copper	48-h LC_{50}	1:1	Additive
	48-h LC_{25}	1:1	Synergism
	48-h LC_{10}	1:1	Synergism
Ammonia + phenol	24-h LC_{50}	1:0.1	Antagonism
		1:0.7	Additive
Ammonia + phenol + zinc	48-h LC_{50}	1:1:0.5	Additive
		1:7:1	Synergism
		1:1:6	Antagonism

Source: Adapted from Calabrese (1991).

Note that when the same chemicals are present but the ratio of components present in the mixture is changed, the type of interaction observed may change. So, the interaction observed can be dose-dependent just like the toxicity is.

Genetic Makeup

We are not all born physiologically equal, and this provides both advantages and disadvantages. For example, people deficient in glucose-6-phosphate dehydrogenase (G6PD deficiency) are more susceptible than others to the hemolysis of blood by aspirin or certain antibiotics, and people who are genetically slow acetylators are more susceptible to neuropathy and hepatotoxicity from isoniazid. Table 1.12 lists some of the genetic differences that have been identified in humans and some of the agents that may trigger an abnormal response in an affected individual.

Health Status

In addition to the genetic status, the general well-being of an individual, specifically their immunological status, nutritional status, hormonal status, and the absence or presence of concurrent diseases, are features that may alter the dose–response relationship.

Chemical-Specific Factors

We have seen that a number of factors inherent in the organism may affect the predicted response; certain chemical and physical factors associated with the form of the chemical or the exposure conditions also may influence toxic potency (i.e., toxicity per unit of dose) of a chemical.

Chemical Composition The physical (particle size, liquid or solid, etc.) and chemical (volatility, solubility, etc.)

TABLE 1.12 Pharmacogenetic Differences in Humans

Condition	Enzyme Affected	Some Chemicals Provoking Abnormal Responses
Acatalasia	Catalase—red blood cells	Hydrogen peroxoide
Atypical cholinesterase	Plasma cholinesterase	Succinyl choline
Acetylation deficiency	Isoniazid acetylase	Isoniazid, sulfamethazine, procainamide, dapsone, hydralazine
Acetophenetidin-induced methemaglobinemia	Cytochrome P450	Acetophenetidin
Polymorphic hydroxylation of debrisoquine	Cytochrome P450	Encainide, metoprolol, debrisoquine, perphenazine
Polymorphic hydroxylation of mephenytoin	CYP 2C19	Mephenytoin
Glucose-6-phosphate dehydrogenase deficiency	Glucose-6-phosphate dehydrogenase	*Hemolytic anemia:* aspirin, acetanilide, aminosalicylic acid, antipyrine, aminopyrine, chloroquine, dapsone, dimercaprol, Gantrasin, methylene blue, naphthalene, nitrofurantoin, probenecid, pamaquin, primaquine, phenacetin, phenylhydrazine, potassium perchlorate, quinacrine, quinine, quinidine, sulfanilamide, sulfapyridine, sulfacetamide, trinitrotoluene

Source: Adapted from Vesell (1987).

properties of the toxic substance may affect its absorption or alter the probability of exposure. For example, the lead pigments that were used in paints decades ago were not an inhalation hazard when applied because they were encapsulated in the paints. However, as the paint aged, peeled, and chipped, the lead became a hazard when the paint chips were ingested by small children. Similarly, the hazards of certain dusts can be reduced in the workplace with the use of water to keep finely granulated solids clumped together.

Exposure Conditions The conditions under which exposure occurs may affect the applied dose of the toxicant and, as a result, the absorbed and target organ doses of the chemical. For example, chemicals bound to soils may be absorbed through the skin poorly compared to absorption when a neat solution is applied because the chemical may have affinity for, and be bound by, the organic materials in soil. The water solubility of an environmental contaminant impacts its transport through the environment and the concentrations that might be found in groundwater discharged to local waterways or used as a source of potable water. So, concentration, type of exposure (dermal, oral, inhalation, etc.), exposure pathway (soil, water, air, food, surfaces, etc.), and exposure duration (acute or chronic) are all factors associated with the exposure assessment that might alter the applied or absorbed dose of chemical.

1.8 DESCRIPTIVE TOXICOLOGY: TESTING ADVERSE EFFECTS OF CHEMICALS AND GENERATING DOSE–RESPONSE DATA

Since the dose–response relationship aids both basic tasks of toxicologists—namely, identifying the hazards associated with a toxicant and assessing the conditions of its usage—it is appropriate to summarize toxicity testing, or descriptive toxicology. While a number of tests may be used to assess toxic responses, each toxicity test rests on two assumptions:

1. *The Hazard Is Qualitatively the Same*. The effects produced by the toxicant in the laboratory test are assumed to be the same effects that the chemical will produce in humans. Therefore, the test species or organisms are useful surrogates for identifying the hazards (qualitative toxicities) in humans.
2. *The Hazard Is Quantitatively the Same*. The dose producing toxicity in animal test is assumed to be the same as the dose required to produce toxicity in humans. Therefore, animal dose–response data provide a reliable surrogate for evaluating the risks associated with different dose or exposure levels in humans.

Which tests or testing scheme to follow depends on the use of the chemical and the likelihood of human exposure. In general, part or all of the following scheme might be required in a descriptive toxicology testing program.

Level 1: Testing for acute exposure

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.

Level 2: Testing for subchronic exposure

a. Plot dose–response curves (for 90-day 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 test species: the absorption, distribution, metabolism, and elimination of chemicals from the body.
f. Conduct behavioral tests.
g. Test for synergism, potentiation, and antagonism.

Level 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.
e. Compile the epidemiological data of acute and chronic exposure.

Establishing the safety and hazard of a chemical is a costly and time-consuming effort. For example, the rodent bioassay for carcinogenic potential requires 2–3 years to obtain results at a cost between $3,000,000 and $7,000,000 and when completed the results, if positive, may in the end severely limit or prohibit the use of the chemical in question. Thus, this final test may entail additional costs if now a replacement chemical must be sought that does not have significant carcinogenic activity. Figure 1.12 outlines the approximate time required to test and develop the safety of chemicals assumed to have widespread human impact.

1.9 EVIDENCE-BASED TOXICOLOGY

Toxicologists often rely heavily (and sometimes exclusively) on nonhuman studies to predict safe exposure concentrations in human populations. This type of extrapolation has many inherent issues that create uncertainty. For example, how appropriately do the *in vitro* studies reflect or predict the outcome of exposure in a whole living organism? Does the strain and/or species used in the animal toxicity studies reflect the human response qualitatively (same specific hazards and toxicities) and/or quantitatively (same dose–response data)?

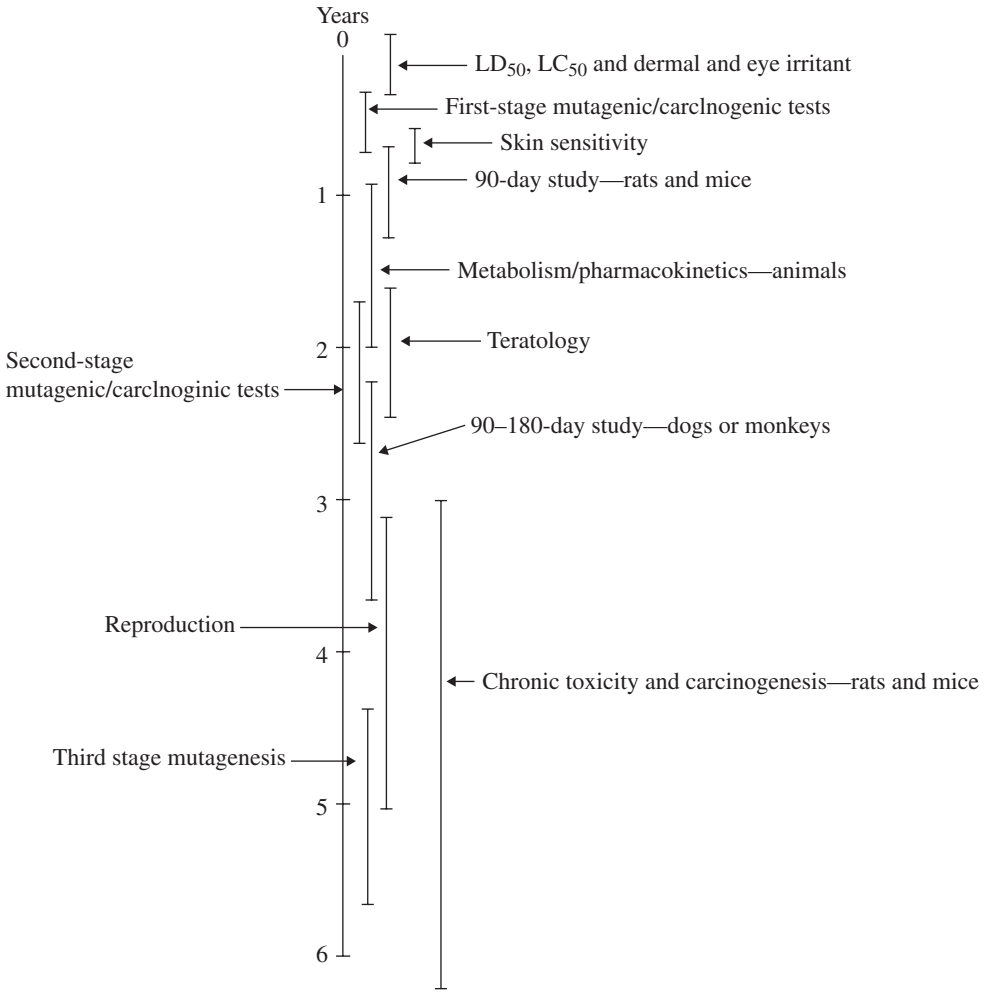

FIGURE 1.12 A timeline showing the approximate time that it might take to test a chemical having a broad exposure to the human population. The bars represent the approximate time required to complete the tests and suggest when testing might be initiated and completed.

Subsequent chapters in this book will discuss these issues in much more detail. The chapter on risk assessment will explain the deliberations required to identify the range of uncertainties with data and describe the methods used to select safety/uncertainty factors to ensure that the true human risk is not underestimated. In other chapters the reader will learn ways to limit the need for traditional animal studies and the ways that *in vitro* procedures can be performed more quickly and economically than whole animal studies. The chapters on "omics" and "computational toxicology" discusses two relatively recent subdisciplines within toxicology whose findings are driven by *in vitro* data. Other specialized disciplines within toxicology also rely heavily on *in vitro* methodologies and computer modeling. So, as animal testing becomes replaced by these approaches, the uncertainty will increase for making the correct extrapolation to human exposure.

Similar considerations occur when epidemiological data are available for a given chemical. Human exposures that occur in the workplace or community following chemical releases may afford scientists the chance to gather actual human data but this kind of information still presents challenges in extrapolation to broader populations. Epidemiology is largely an observational science and generally relies on studies where there can be limited ability to control for variables that may affect the outcome. As a result, qualitative or quantitative uncertainties may impact the use of the data in establishing acceptable human exposure values. In short, there has long been a need to better understand how to evaluate and interpret both human and animal toxicological data.

Recently two groups sought to address this issue and the term "evidence-based toxicology" (EBT) was coined to describe methods for improving the extrapolation and/or interpretation of the available epidemiology and animal toxicity data. One group has focused on improving causation analyses using human data and has proposed that toxicologists adopt the same evidence-based procedures currently used in medicine to evaluate the adverse outcomes or efficacies of different therapeutic treatments. The second group has called for the development of methods and procedures that would better characterize the utility of animal test data in a manner that ultimately could be used to improve and refine the human extrapolations/predictions from the animal data.

While this is a developing area within toxicology, it is receiving widespread interest. The initial key step in EBT is the attempt to ensure all relevant studies were considered in the hazard evaluation and subsequent risk characterization. History has shown that different "expert-based" panel evaluations for a specific chemical may reach different conclusions regarding the characterization of that chemical's hazards and possible risks. Some of these differences can arise because the data sets of studies being evaluated are not the same, and other differences ostensibly result from philosophical differences as to how specific pieces of information should be interpreted. In an attempt to reduce these problems and improve the consistency of evaluations by different scientific groups and panels, the proponents of EBT have pushed for the use of established or agreed-upon methodologies that would help improve the interpretation of test data in a manner driven more objectively by a data rating system and less by the experience and indiscernible philosophies of the panel of experts performing the evaluation. Or, stated another way, one goal of EBT is to promote a better integration of expert judgment by providing more transparent consensus methods for evaluating the evidence. In this manner differences in interpretation that arise between groups evaluating the same chemical will be more readily identified. In some instances this might help identify the key studies yet to be performed and lead to development of data critical for more accurately characterizing a chemical's hazards. For example, which mechanistic studies might be needed to understand where the threshold exists or to identify biomarkers of the disease that could be measured in an exposed population.

There are too many aspects of EBT to provide here an adequate discussion of this topic. However, the interested reader may learn more about this developing area by reading the related-articles listed in the suggested reading list at the end of this chapter.

1.10 SUMMARY

Toxicology is a scientific discipline that utilizes basic knowledge from many different but related disciplines (biology, physiology, genetics, biochemistry, etc.). The two goals or basic functions of toxicology are (1) identification of the toxicities (hazards) a chemical produces and (2) a determination of the dose range over which these hazards will be observed. Information in these two areas helps one predict what human exposures should be acceptably safe or potentially harmful, and if harmful what injuries to the exposed individual's health might be anticipated. To provide the information needed to complete these two basic functions generally necessitates the testing of animals, or the use of simpler *in vitro* tests, to predict both the hazard and dose–response outcomes in humans. The basic assumption in performing toxicological testing is that there are animal species or *in vitro* tests available that reliably and accurately provide the hazard and dose–response information we seek. Unfortunately, genetic differences across species produce differences in the anatomy, physiology, biology, and biochemistry and these species-specific differences introduce uncertainty in the premise that test animal species will respond like humans. In addition, the hazard and dose responses we observe can be changed by changes in the

testing conditions and protocol; the sex, strain, or species tested; whether exposure is to just a single chemical or that chemical as part of a mixture of chemicals or chemical exposures; the route or pathway of exposure; the genetic makeup of the human receptor exposed; and whether other chemicals the individual is also exposed to interact with and alter the toxicity of the chemical of interest. Overall, there may be considerable uncertainty in the animal-to-man extrapolation being attempted. In short, by its very nature toxicology is forced to develop data upon which uncertain extrapolations or predictions must be made. This in turn impacts how the hazards and potential risks are to be communicated to those experiencing the exposure or responsible for regulating the exposure.

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Answers to TABLE 1.3A Comparative Acutely Lethal Doses

Actual Ranking No.	LD_{50} (mg/kg)	Toxic Chemical
1	15,000	PCBs
2	10,000	Alcohol (ethanol)
3	4,000	Table salt—sodium chloride
4	1,500	Ferrous sulfate—an iron supplement
5	1,375	Malathion—a pesticide
6	900	Morphine
7	150	Phenobarbitol—a sedative
8	142	Tylenol (acetaminophen)
9	2	Strychnine—a rat poison
10	1	Nicotine
11	0.5	Curare—an arrow poison
12	0.001	2,3,7,8-TCDD (dioxin)
13	0.00001	Botulinum toxin (food poison)

Source: Adapted from Loomis and Hayes (1996).

Answers to TABLE 1.4 Occupational Exposure Limits: Aspirin and Vegetable Oil Versus Industrial Solvents

No.	Allowable Workplace Exposure Level (mg/m^3)	Chemical (Use)
1	0.05	Iodine
2	5	Aspirin (acetylsalicyclic acid)
3	10	Vegetable oil mists (cooking oil)
4	54	Trichloroethylene (solvent/degreaser)
5	55	1,1,2-Trichloroethane (solvent/degreaser)
6	75	Toluene (organic solvent)
7	147	Tetrahydrofuran (organic solvent)
8	170	Perchloroethylene (dry-cleaning fluid)
9	890	Gasoline (fuel)
10	1910	1,1,1-Trichloroethane (solvent/degreaser)

Source: American Conference of Government Industrial Hygienists (ACGIH) (2012).