

# Principles of Toxicology

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## KEY POINTS

- A poison is any agent capable of producing a deleterious response in a biological system.
- A mechanistic toxicologist identifies the cellular, biochemical, and molecular mechanisms by which chemicals exert toxic effects on living organisms.
- Toxicogenomics permits mechanistic toxicologists to identify and protect genetically susceptible individuals from harmful environmental exposures, and to customize drug therapies based on their individual genetic makeup.
- A descriptive toxicologist is concerned directly with toxicity testing, which provides information for safety evaluation and regulatory requirements.
- A regulatory toxicologist both determines from available data whether a chemical poses a sufficiently low risk to be marketed for a stated purpose and establishes standards for the amount of chemicals permitted in ambient air, industrial atmospheres, and drinking water.
- Selective toxicity means that a chemical produces injury to one kind of living matter without harming another form of life even though the two may exist in intimate contact.
- The individual or “graded” dose–response relationship describes the response of an individual organism to varying doses of a chemical.
- A quantal dose–response relationship characterizes the distribution of responses to different doses in a population of individual organisms.
- Hormesis, a “U-shaped” dose–response curve, results with some xenobiotics that impart beneficial or stimulatory effects at low doses but adverse effects at higher doses.
- Descriptive animal toxicity testing assumes that the effects produced by a compound in laboratory animals, when properly qualified, are applicable to humans, and that exposure of experimental animals to toxic agents in high doses is a necessary and valid method of discovering possible hazards in humans.

## INTRODUCTION TO TOXICOLOGY

Toxicology is the study of the adverse effects of chemicals on living organisms. A toxicologist is trained to examine the nature of those effects (including their cellular, biochemical, and molecular mechanisms of action) and assess the probability of their occurrence. Fundamental to this process is characterizing the relation of exposure (or dose) to the response. The variety of potential adverse effects from the abundant diversity of chemicals upon which our society depends often demands specialization in one area of toxicology.

## Different Areas of Toxicology

A mechanistic toxicologist identifies the cellular, biochemical, and molecular mechanisms by which chemicals exert toxic effects on living organisms (see Chapter 3 for a detailed discussion of mechanisms of toxicity). Mechanistic data may be useful in the design and production of safer chemicals and in rational therapy for chemical poisoning and treatment of disease. In risk assessment, mechanistic data may be very useful in demonstrating that an adverse outcome observed in laboratory animals is directly relevant to humans. Toxicogenomics permits the application of genomic, transcriptomic, proteomic, and metabolomic technologies to identify descriptive and mechanistic information that can protect genetically susceptible individuals

from harmful environmental exposures, and to customize drug therapies based on their individual genetic makeup. Numerous genetic tests can identify susceptible individuals in advance of pharmacological treatment.

A descriptive toxicologist is concerned directly with toxicity testing, which provides information for safety evaluation and regulatory requirements. Toxicity tests (described later in this chapter) in experimental animals are designed to yield information that can be used to evaluate risks posed to humans and the environment by exposure to specific chemicals.

A regulatory toxicologist has the responsibility for deciding, on the basis of data provided by descriptive and mechanistic toxicologists, whether a drug or another chemical poses a sufficiently low risk to be marketed for a stated purpose. Regulatory toxicologists are involved in the establishment of standards for the amount of chemicals permitted in foods, drugs, ambient air, industrial atmospheres, and drinking water (see Chapter 4).

Forensic toxicology is a hybrid of analytic chemistry and fundamental toxicologic principles that focuses primarily on the medicolegal aspects of the harmful effects of chemicals on humans and animals (see Chapter 31).

Clinical toxicology is concerned with disease caused by or uniquely associated with toxic substances (see Chapter 32).

Environmental toxicology focuses on the impacts of chemical pollutants in the environment on biological organisms,

specifically studying the impacts of chemicals on nonhuman organisms such as fish, birds, terrestrial animals, and plants. Ecotoxicology, a specialized area within environmental toxicology, focuses specifically on the impacts of toxic substances on population dynamics in an ecosystem (see Chapter 29).

Developmental toxicology is the study of adverse effects on the developing organism that may result from exposure to chemical or physical agents before conception (either parent), during prenatal development, or postnatally until the time of puberty. Teratology is the study of defects induced during development between conception and birth (see Chapter 10).

Reproductive toxicology is the study of the occurrence of adverse effects on the male or female reproductive system that may result from exposure to chemical or physical agents (see Chapter 20).

## Toxicology and Society

Knowledge about the toxicologic effect of a compound affects consumer products, drugs, manufacturing processes, waste cleanup, regulatory action, civil disputes, and broad policy decisions. The expanding influence of toxicology on societal issues is accompanied by the responsibility to be increasingly sensitive to the ethical, legal, and social implications of toxicologic research and testing.

There are several ethical dilemmas in toxicology. First, experience and new discoveries in the biological sciences have emphasized the need for well-articulated visions of human, animal, and environmental health. Second, experience with the health consequences of exposure to such things as lead, asbestos, and tobacco has precipitated many regulatory and legal actions and public policy decisions. Third, we have an increasingly well-defined framework for discussing our social and ethical responsibilities. Fourth, all research involving humans or animals must be conducted in a responsible and ethical manner. Fifth, the uncertainty and biological variability inherent in the biological sciences requires decision making with limited or uncertain information.

## General Characteristics of the Toxic Response

Virtually every known chemical has the potential to produce injury or death if it is present in a sufficient amount. Table 2–1 shows the wide spectrum of dosages needed to produce death in 50% of treated animals (lethal dose 50, LD<sub>50</sub>). Chemicals producing death in microgram doses are often considered extremely poisonous. Note that measures of acute lethality such as LD<sub>50</sub> may not accurately reflect the full spectrum of toxicity, or hazard, associated with exposure to a chemical. For example, some chemicals with low acute toxicity may have carcinogenic or teratogenic effects at doses that produce no evidence of acute toxicity. For a given chemical, each of the various effects that may occur in a given organism will have their own dose–response relationship.

**TABLE 2–1** Approximate acute LD<sub>50</sub> of some representative chemical agents.

Agent	LD <sub>50</sub> , mg/kg*
Ethyl alcohol	10 000
Sodium chloride	4 000
Ferrous sulfate	1 500
Morphine sulfate	900
Phenobarbital sodium	150
Picrotoxin	5
Strychnine sulfate	2
Nicotine	1
Tubocurarine	0.5
Hemicholinium-3	0.2
Tetrodotoxin	0.10
Dioxin (TCDD)	0.001
Botulinum toxin	0.00001

\*LD<sub>50</sub> is the dosage (mg/kg body weight) causing death in 50% of exposed animals.

## CLASSIFICATION OF TOXIC AGENTS

Toxic agents are classified depending on the interests and needs of the classifier. These agents may be discussed in terms of their target organs, use, source, and effects. The term toxin generally refers to toxic substances that are produced by biological systems such as plants, animals, fungi, or bacteria. The term toxicant is used in speaking of toxic substances that are produced by or are a by-product of human activities. Toxic agents may be classified in terms of their physical state, chemical stability or reactivity, general chemical structure, or poisoning potential. No single classification is applicable to the entire spectrum of toxic agents and, therefore, a combination of classifications is needed to provide the best characterization of a toxic substance.

## SPECTRUM OF UNDESIRABLE EFFECTS

The spectrum of undesirable effects of chemicals is broad. In therapeutics, e.g., each drug produces a number of effects, but usually only one effect is associated with the primary objective of the therapy; all the other effects are referred to as undesirable or side effects. However, some of these side effects may be desired for another therapeutic indication. Some side effects of drugs are always deleterious to the well-being of humans. These are referred to as the adverse, deleterious, or toxic effects of the drug.

## Allergic Reactions

Chemical allergy is an immunologically mediated adverse reaction to a chemical resulting from previous sensitization to that chemical or to a structurally similar one. The terms hypersensitivity, allergic reaction, and sensitization reaction are used to describe this situation (see Chapter 12). Once sensitization has occurred, allergic reactions may result from exposure to relatively very low doses of chemicals. Importantly, for a given allergic individual, allergic reactions are dose-related. Sensitization reactions are sometimes very severe and may be fatal.

Most chemicals and their metabolic products are not sufficiently large to be recognized by the immune system as a foreign substance and thus must first combine with an endogenous protein to form an antigen (or immunogen). Such a molecule is called a hapten. The hapten–protein complex (antigen) is then capable of eliciting the formation of antibodies. Subsequent exposure to the chemical results in an antigen–antibody interaction, which provokes the typical manifestations of an allergy that range in severity from minor skin disturbance to fatal anaphylactic shock.

## Idiosyncratic Reactions

Chemical idiosyncrasy refers to a genetically determined abnormal reactivity to a chemical. The response observed is usually qualitatively similar to that observed in all individuals but may take the form of extreme sensitivity to low doses or extreme insensitivity to high doses of the chemical. For example, some individuals are abnormally sensitive to nitrites and other substances capable of oxidizing the iron in hemoglobin. This produces methemoglobin, which is incapable of binding and transporting oxygen to tissues. Consequently, they may suffer from tissue hypoxia after exposure to doses of methemoglobin-producing chemicals, whereas normal individuals would be unaffected. It is now recognized that many idiosyncratic drug reactions are due to the interplay between an individual's ability to form a reactive intermediate, detoxify that intermediate, and/or mount an immune response to adducted proteins. Specific genetic polymorphisms in drug-metabolizing enzymes, transporters, or receptors are responsible for many of these observed differences.

## Immediate versus Delayed Toxicity

Immediate toxic effects occur or develop rapidly after a single administration of a substance, whereas delayed toxic effects occur after the lapse of some time. Most substances produce immediate toxic effects. However, carcinogenic effects of chemicals usually have long latency periods, often 20 to 30 years after the initial exposure, before tumors are observed in humans.

## Reversible versus Irreversible Toxic Effects

Some toxic effects of chemicals are reversible, and others are irreversible. If a chemical produces pathological injury to a tissue, the ability of that tissue to regenerate largely determines

whether the effect is reversible or irreversible. Liver tissue has high regeneration ability and most injuries are, therefore, reversible. However, CNS injury is largely irreversible because its cells are differentiated and cannot be replaced. Carcinogenic and teratogenic effects of chemicals, once they occur, are usually considered irreversible toxic effects.

## Local versus Systemic Toxicity

Another distinction between types of effects is made on the basis of the general site of action. Local effects occur at the site of first contact between the biological system and the toxicant. In contrast, systemic effects require absorption and distribution of a toxicant from its entry point to a distant site, at which deleterious effects are produced. Most substances, except for highly reactive materials, produce systemic effects. Some materials can produce both effects.

Most chemicals that produce systemic toxicity usually elicit their major toxicity in only one or two organs, which are referred to as the target organs of toxicity of a particular chemical. Paradoxically, the target organ of toxicity is often not the site of the highest concentration of the chemical.

Target organs in order of frequency of involvement in systemic toxicity are the CNS; the circulatory system; the blood and hematopoietic system; visceral organs such as the liver, kidney, and lung; and the skin. Muscle and bone are seldom target tissues for systemic effects.

## Interaction of Chemicals

Chemical interactions can occur via various mechanisms, such as alterations in absorption, protein binding, and the biotransformation and excretion of one or both of the interacting toxicants. In addition to these modes of interaction, the response of the organism to combinations of toxicants may be increased or decreased because of toxicologic responses at the site of action.

An additive effect, most commonly observed when two chemicals are given together, occurs when the combined effect of two chemicals is equal to the sum of the effects of each agent given alone (e.g.:  $2 + 3 = 5$ ). A synergistic effect occurs when the combined effects of two chemicals are much greater than the sum of the effects of each agent given alone (e.g.:  $2 + 2 = 20$ ). Potentiation occurs when one substance does not have a toxic effect on a certain organ or system but when added to another chemical makes that chemical much more toxic (e.g.:  $0 + 2 = 10$ ). Isopropanol, e.g., is not hepatotoxic, but when it is administered in addition to carbon tetrachloride, the hepatotoxicity of carbon tetrachloride is much greater than that when it is given alone.

Antagonism occurs when two chemicals administered together interfere with each other's actions or one interferes with the action of the other (e.g.:  $4 + 6 = 8$ ;  $4 + (-4) = 0$ ;  $4 + 0 = 1$ ). There are four major types of antagonism: functional, chemical, dispositional, and receptor. Functional antagonism occurs when two chemicals counterbalance each other by producing opposite effects on the same physiologic function.

For example, the marked fall in blood pressure during severe barbiturate intoxication can be effectively antagonized by the intravenous administration of a vasopressor agent such as norepinephrine or metaraminol. Chemical antagonism or inactivation is simply a chemical reaction between two compounds that produces a less toxic product. For example, chelators of metal ions decrease metal toxicity and antitoxins antagonize the action of various animal toxins. Dispositional antagonism occurs when the absorption, biotransformation, distribution, or excretion of a chemical is altered so that the concentration and/or duration of the chemical at the target organ are diminished. Receptor antagonism occurs when two chemicals that bind to the same receptor produce less of an effect when given together than the addition of their separate effects (e.g.:  $4 + 6 = 8$ ) or when one chemical antagonizes the effect of the second chemical (e.g.:  $0 + 4 = 1$ ). Receptor antagonists are often termed blockers.

## Tolerance

Tolerance is a state of decreased responsiveness to a toxic effect of a chemical resulting from prior exposure to that chemical or to a structurally related chemical. Two major mechanisms are responsible for tolerance: one is due to a decreased amount of toxicant reaching the site where the toxic effect is produced (dispositional tolerance) and the other is due to a reduced responsiveness of a tissue to the chemical.

## CHARACTERISTICS OF EXPOSURE

Toxic effects in a biological system are not produced by a chemical agent unless that agent or its metabolic breakdown (biotransformation) products reach appropriate sites in the body at a concentration and for a length of time sufficient to produce a toxic manifestation. Whether a toxic response occurs is dependent on the chemical and physical properties of the agent, the exposure situation, how the agent is metabolized by the system, and the overall susceptibility of the biological system or subject.

## Route and Site of Exposure

The major routes (pathways) by which toxic agents gain access to the body are the gastrointestinal tract (ingestion), lungs (inhalation), skin (topical, percutaneous, or dermal), and other parenteral (other than intestinal canal) routes. Toxic agents generally produce the greatest effect and the most rapid response when given directly into the bloodstream (the intravenous route). An approximate descending order of effectiveness for the other routes would be inhalation, intraperitoneal, subcutaneous, intramuscular, intradermal, oral, and dermal. The “vehicle” (the material in which the chemical is dissolved) and other formulation factors can markedly alter absorption. In addition, the route of administration can influence the toxicity of agents. For example, an agent that acts on the CNS, but is efficiently

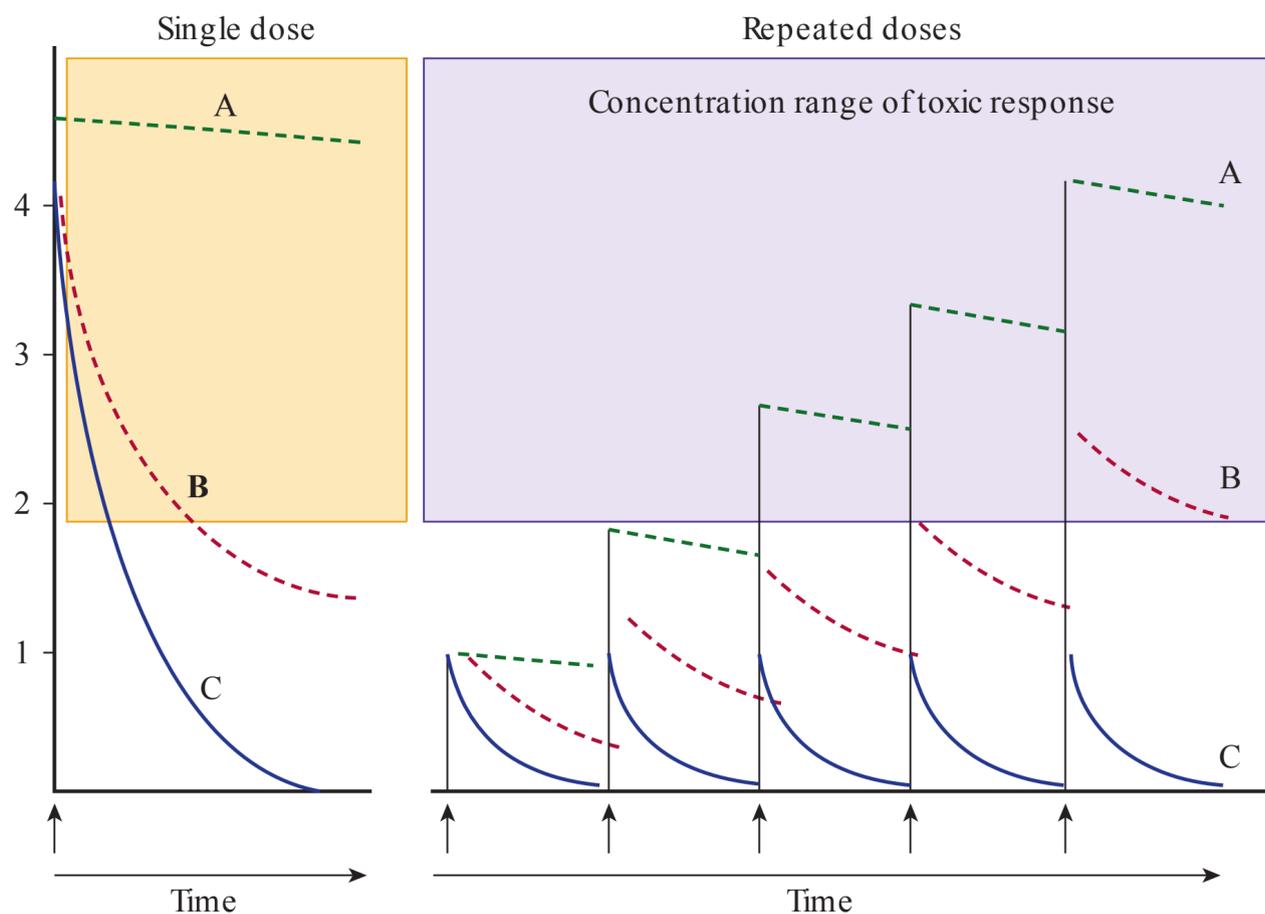
detoxified in the liver, would be expected to be less toxic when given orally than when inhaled, because the oral route requires that nearly all of the dose pass through the liver before reaching the systemic circulation and then the CNS.

## Duration and Frequency of Exposure

Toxicologists usually divide the exposure of experimental animals to chemicals into four categories: acute, subacute, subchronic, and chronic. Acute exposure is defined as exposure to a chemical for less than 24 h. While acute exposure usually refers to a single administration, repeated exposures may be given within a 24-h period for some slightly toxic or practically nontoxic chemicals. Acute exposure by inhalation refers to continuous exposure for less than 24 h, most frequently for 4 h. Repeated exposure is divided into three categories: subacute, subchronic, and chronic. Subacute exposure refers to repeated exposure to a chemical for 1 month or less, subchronic for 1 to 3 months, and chronic for more than 3 months.

In human exposure situations, the frequency and duration of exposure are usually not as clearly defined as in controlled animal studies, but many of the same terms are used to describe general exposure situations. Thus, workplace or environmental exposures may be described as acute (occurring from a single incident or episode), subchronic (occurring repeatedly over several weeks or months), or chronic (occurring repeatedly for many months or years).

For many agents, the toxic effects that follow a single exposure are quite different from those produced by repeated exposure. Acute exposure to agents that are rapidly absorbed is likely to produce immediate toxic effects but also can produce delayed toxicity that may or may not be similar to the toxic effects of chronic exposure. Conversely, chronic exposure to a toxic agent may produce some immediate (acute) effects after each administration in addition to the long-term, low-level, or chronic effects of the toxic substance. The other time-related factor that is important in the temporal characterization of repeated exposures is the frequency of exposure. The relationship between elimination rate and frequency of exposure is shown in Figure 2–1. A chemical that produces severe effects with a single dose may have no effect if the same total dose is given in several intervals. For the chemical depicted by line B in Figure 2–1, in which the half-life for elimination (time necessary for 50% of the chemical to be removed from the bloodstream) is approximately equal to the dosing frequency, a theoretical toxic concentration of  $2U$  is not reached until the fourth dose, whereas that toxic concentration is nearly reached with only two doses for chemical A, which has an elimination rate much slower than the dosing interval (time between each repeated dose). Conversely, for chemical C, where the elimination rate is much shorter than the dosing interval, a toxic concentration at the site of toxic effect will never be reached regardless of how many doses are administered. Of course, it is possible that residual cell or tissue damage occurs with each dose even though the chemical itself is not accumulating. The important consideration, then, is whether the interval between



**FIGURE 2–1** Diagrammatic view of the relationship between dose and concentration at the target site under different conditions of dose frequency and elimination rate. Line A. A chemical with very slow elimination (e.g., half-life of 1 year). Line B. A chemical with a rate of elimination equal to frequency of dosing (e.g., 1 day). Line C. Rate of elimination faster than the dosing frequency (e.g., 5 h). Purple shaded area is representative of the concentration of chemical at the target site necessary to elicit a toxic response.

doses is sufficient to allow for complete repair of tissue damage. Chronic toxic effects may occur, therefore, if the chemical accumulates in the biological system (rate of absorption exceeds the rate of biotransformation and/or excretion), if it produces irreversible toxic effects, or if there is insufficient time for the system to recover from the toxic damage within the exposure frequency interval. For additional discussion of these relationships, consult Chapters 5 and 7.

## DOSE–RESPONSE RELATIONSHIP

The characteristics of exposure and the spectrum of effects come together in a correlative relationship customarily referred to as the dose–response relationship. Whatever response is selected for measurement, the relationship between the degree of response of the biological system and the amount of toxicant administered assumes a form that occurs so consistently as to be considered the most fundamental and pervasive concept in toxicology.

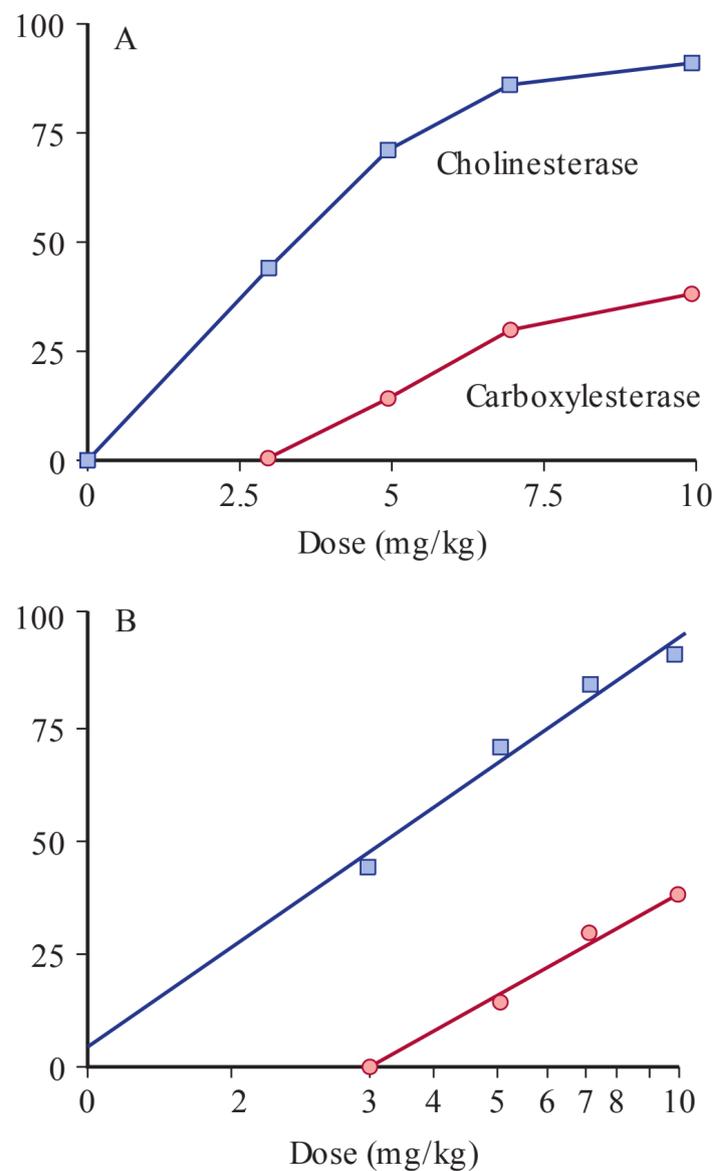
From a practical perspective, there are two types of dose–response relationships: (1) the individual dose–response relationship, which describes the response of an individual organism to varying doses of a chemical, often referred to as a “graded” response because the measured effect is continuous over a range of doses, and (2) a quantal dose–response relationship, which characterizes the distribution of responses to different doses in a population of individual organisms.

## Individual, or Graded, Dose–Response Relationships

Individual dose–response relationships are characterized by a dose-related increase in the severity of the response. For example, Figure 2–2 shows the dose–response relationship between different dietary doses of the organophosphate insecticide chlorpyrifos and the extent of inhibition of two different enzymes in the brain and liver: acetylcholinesterase and carboxylesterase. In the brain, the degree of inhibition of both enzymes is clearly dose-related and spans a wide range, although the amount of inhibition per unit dose is different for the two enzymes. From the shapes of these two dose–response curves, it is evident that, in the brain, cholinesterase is more easily inhibited than carboxylesterase. The toxicologic response that results is directly related to the degree of cholinesterase enzyme inhibition in the brain. It should be noted that most toxic substances have multiple sites or mechanisms of toxicity, each with its own “dose–response” relationship and subsequent adverse effect. When these dose–response data are plotted using a logarithmic scale for the dose, the data “fit” a straight line.

## Quantal Dose–Response Relationships

In contrast to the “graded” or continuous-scale dose–response relationship that occurs in individuals, the dose–response relationships in a population are by definition quantal—or “all or none”—in nature; that is, at any given dose, an individual in the

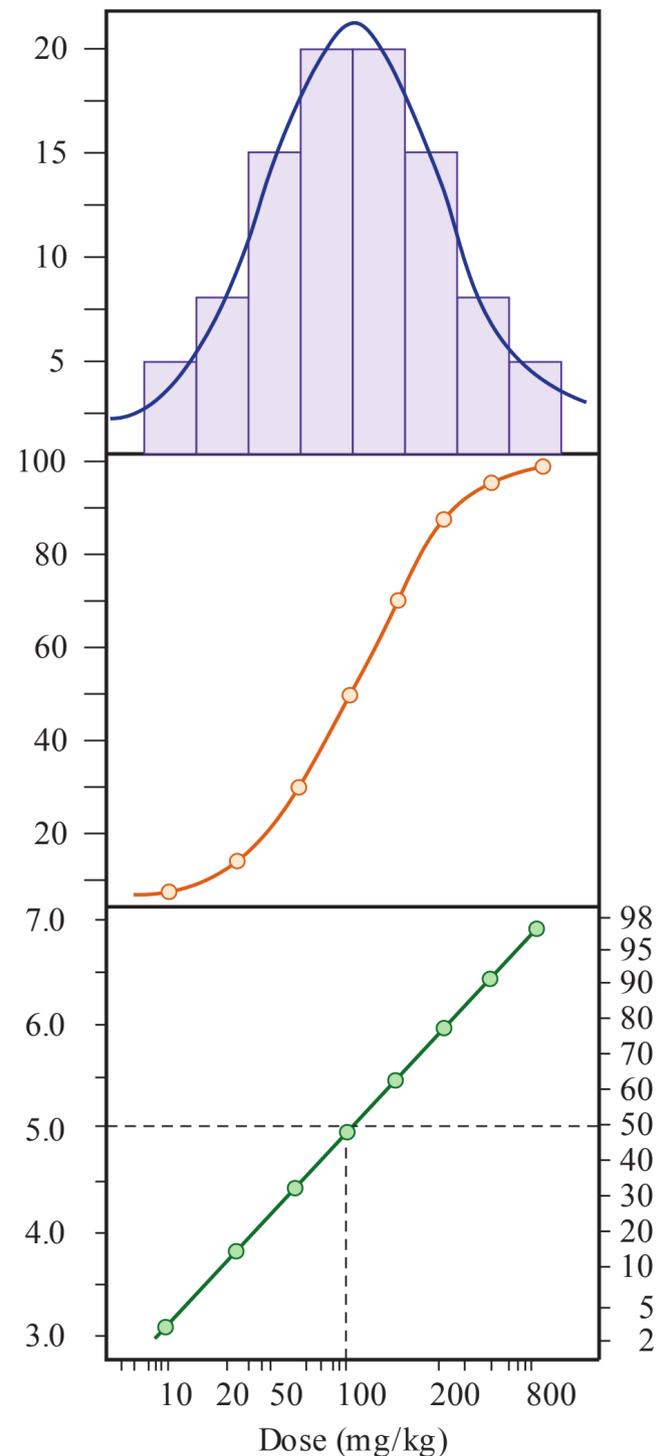


**FIGURE 2–2** Dose–response relationship between different doses of the organophosphate insecticide chlorpyrifos and esterase enzyme inhibition in the brain. Open circles and blue lines represent acetylcholinesterase activity and closed circles represent carboxylesterase activity in the brains of pregnant female Long–Evans rats given 5 daily doses of chlorpyrifos. A. Dose–response curve plotted on an arithmetic scale. B. Same data plotted on a semi-log scale. (Data from Lassiter TL, et al.: Gestational exposure to chlorpyrifos: dose response profiles for cholinesterase and carboxylesterase activity, *Toxicol Sci*, 1999 Nov;52(1):92–100.)

population is classified as either a “responder” or a “nonresponder.” Although these distinctions of “quantal population” and “graded individual” dose–response relationships are useful, the two types of responses are conceptually identical. The ordinate in both cases is simply labeled the response, which may be the degree of response in an individual or system or the fraction of a population responding, and the abscissa is the administered dose range.

The effective dose (ED) is a widely used statistical approach for estimating the response of a population to a toxic exposure. Generally, the 50% response level is used ( $ED_{50}$ ), although any response level, such as an  $ED_{01}$ ,  $ED_{10}$ , or  $ED_{30}$ , could be chosen.

The top panel of Figure 2–3 shows that quantal dose–responses exhibit a normal or Gaussian distribution. The frequency histogram in this panel also shows the relationship between dose and effect. The bars represent the percentage of



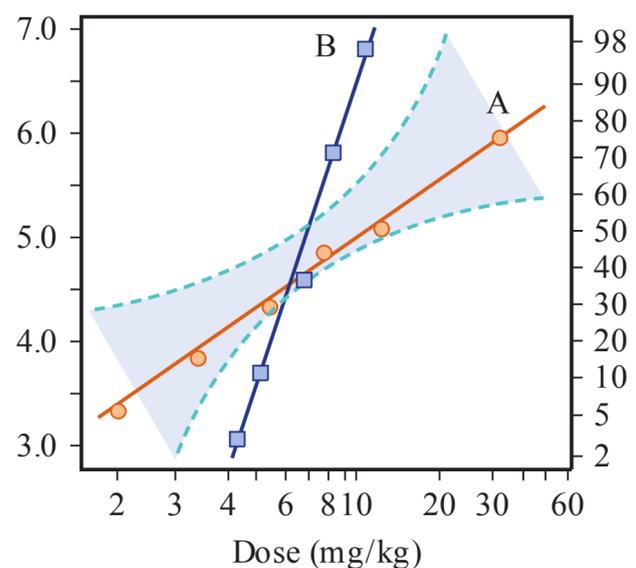
**FIGURE 2–3** Diagram of a quantal dose–response relationship. The abscissa is a log dosage of the chemical. In the top panel the ordinate is response frequency, in the middle panel the ordinate is percent response, and in the bottom panel the response is in probit units (see text).

animals that responded at each dose minus the percentage that responded at the immediately lower dose. One can clearly see that only a few animals responded to the lowest dose and the highest dose. Larger numbers of animals responded to doses intermediate between these two extremes, and the maximum frequency of response occurred in the middle portion of the dose range. Thus, we have a bell-shaped curve known as a normal frequency distribution. The reason for this normal distribution is that there are differences in susceptibility to chemicals among individuals. Animals responding at the left end of the curve are referred to as hypersusceptible, and those at the right end of the curve are called resistant. If the numbers of individuals responding at each consecutive dose are added together, a cumulative, quantal dose–response relationship is obtained. When sufficient doses are used with a large number of animals per

dose, a sigmoid dose–response curve is observed, as depicted in the middle panel of Figure 2–3. With the lowest dose (6 mg/kg), 1% of the animals respond. A normally distributed sigmoid curve such as this one approaches a response of 0% as the dose is decreased and approaches 100% as the dose is increased, but—theoretically—it never passes through 0% and 100%. However, the minimally ED of any chemical that evokes a stated all-or-none response is called the threshold dose even though it cannot be determined experimentally.

The sigmoid curve has a relatively linear portion between 16% and 84%. These values represent the limits of 1 standard deviation (SD) of the mean (and the median) in a population with truly normal distribution. Thus, the mean  $\pm$  1 SD represents 68.3% of the population, the mean  $\pm$  2 SD represents 95.5% of the population, and the mean  $\pm$  3 SD equals 99.7% of the population. One can convert the percent response to units of deviation from the mean or normal equivalent deviations (NEDs). Thus, the NED for a 50% response is 0; an NED of + 1 is equated with an 84.1% response. Units of NED can be converted by the addition of 5 to the value to avoid negative numbers and be called probit units (from the contraction of probability unit). In this transformation, a 50% response becomes a probit of 5, a + 1 deviation becomes a probit of 6, and a – 1 deviation is a probit of 4.

The data given in the top two panels of Figure 2–3 are replotted in the bottom panel with the mortality plotted in probit units to form a straight line. In essence, what is accomplished in a probit transformation is an adjustment of quantal data to an assumed normal population distribution, resulting in a straight line. The  $ED_{50}$  is obtained by drawing a horizontal line from the probit unit 5, which is the 50% response point, to the dose–effect line. At the point of intersection, a vertical line is drawn, and this line intersects the abscissa at the  $ED_{50}$  point. In addition to the  $ED_{50}$ , the slope of the dose–response curve can also be obtained. Figure 2–4 demonstrates the dose–response curves of two compounds. Compound A exhibits a “fat” dose–response curve, showing that a large change in dosage is required before a



**FIGURE 2–4** Comparison of dose–response relationship for two different chemicals, plotted on a log dose–probit scale.

Note that the slope of the dose–response relationship is steeper for chemical B than for chemical A. Dotted lines represent the confidence limits for chemical A.

significant change in response will be observed. However, compound B exhibits a “steep” dose–response curve, where a relatively small change in dosage will cause a large change in response. The  $ED_{50}$  for both compounds is the same (8 mg/kg); however, the slopes of the dose–response curves are quite different. At one-half of  $ED_{50}$  of the compounds (4 mg/kg), less than 1% of the animals exposed to compound B would respond but 20% of the animals given compound A would respond.

Allometry studies the relationship of body size to shape, and allometry is often expressed as a scaling exponent based on body mass or body length. If allometric principles are considered in dosage determination, then viewing dosage on the basis of body weight would be considered less appropriate than if based on surface area, which is approximately proportional to  $10.5 \times (\text{body weight})^x$ , where  $x = 2/3$  or  $3/4$ . In Table 2–2, selected values are given to compare the differences in dosage

**TABLE 2–2** Allometric scaling of dose across different species.

Species	Weight (kg)	Surface Area (cm <sup>2</sup> )*	Fold Difference, Relative to Humans, Normalized by Body Weight		
			mg/kg	(BW) <sup>2/3</sup>	(BW) <sup>3/4</sup>
Mouse	0.02	103	1	13.0	7.0
Rat	0.2	365	1	6.9	4.3
Guinea pig	0.4	582	1	5.5	3.6
Rabbit	1.5	1410	1	3.5	2.6
Cat	2	1710	1	3.2	2.4
Monkey	4	2720	1	2.6	2.0
Dog	12	5680	1	1.8	1.5
Human	70	18500	1	1.0	1.0

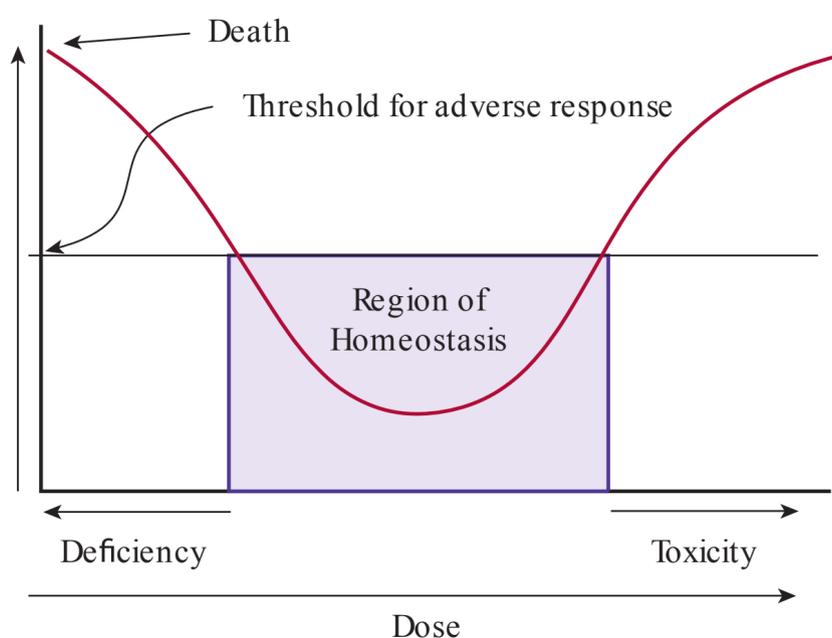
\*Surface area of animals is closely approximated by the formula  $SA = 10.5 \times (\text{body weight [in grams]})^{2/3}$ .

by the two alternatives. If a scaling factor of  $(\text{body weight})^{2/3}$  is used, then the dose would be approximately 13 times greater in mice than if that dosage were expressed per surface area ( $\text{mg}/\text{cm}^2$ ). However, not all toxic responses will necessarily scale across species in the same way.

### Shape of the Dose–Response Curve

**Essential Nutrients**—The shape of the dose–response relationship has many important implications in toxicity assessment, e.g., for substances that are required for normal physiologic function and survival (e.g., vitamins and essential trace elements such as chromium, cobalt, and selenium), the shape of the “graded” dose–response relationship in an individual over the entire dose range is actually U-shaped (Figure 2–5). That is, at very low doses (or deficiency), there is a high level of adverse effect, which decreases with an increasing dose. As the dose is increased to a point where the deficiency no longer exists, no adverse response is detected and the organism is in a state of homeostasis. However, as the dose is increased to abnormally high levels, an adverse response (usually qualitatively different from that observed at deficient doses) appears and increases in magnitude with increasing dose.

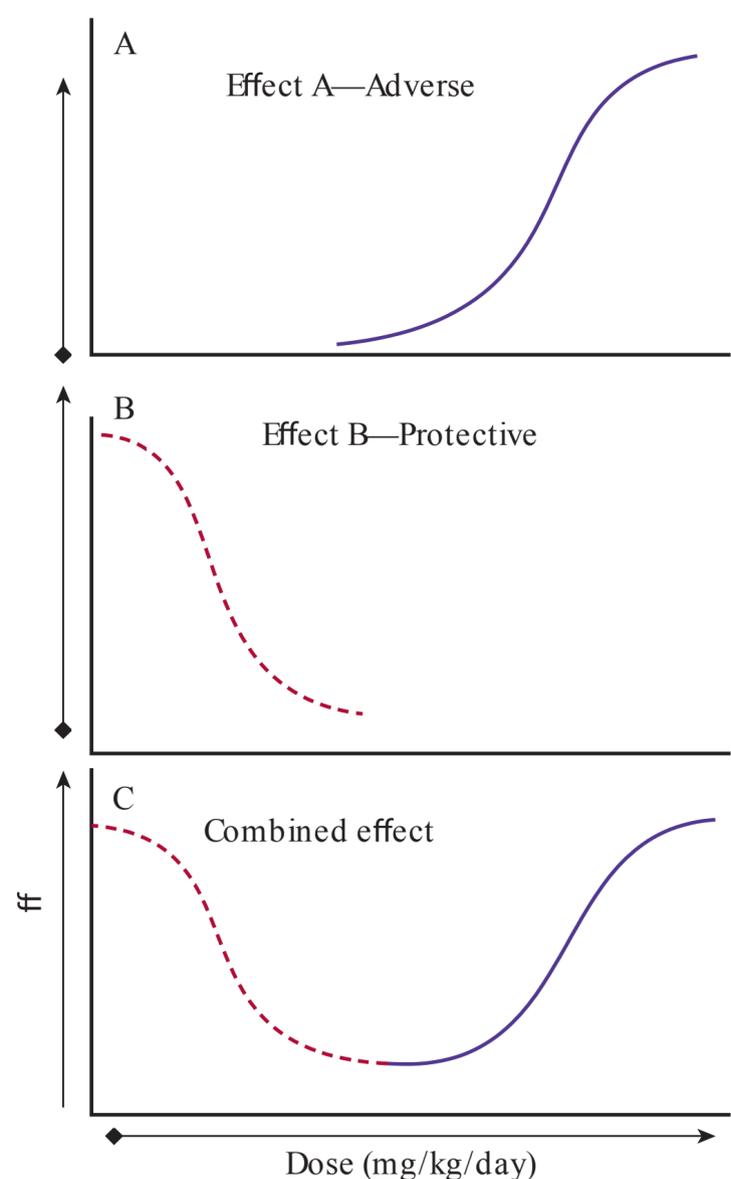
**Hormesis**—Some nonnutritional toxic substances may also impart beneficial or stimulatory effects at low doses but, at higher doses, they produce adverse effects. This concept of “hormesis” may also result in a U-shaped dose–response curve. For example, chronic alcohol consumption is well recognized to increase the risk of esophageal cancer, liver cancer, and cirrhosis of the liver at relatively high doses, and this response is



**FIGURE 2–5 Individual dose–response relationship for an essential substance such as a vitamin or trace element.** It is generally recognized that, for most types of toxic responses, a threshold exists such that at doses below the threshold, no toxicity is evident. For essential substances, doses below the minimum daily requirement, as well as those above the threshold for safety, may be associated with toxic effects. The purple-shaded region represents the “region of homeostasis”—the dose range that results in neither deficiency nor toxicity.

dose-related (curve A, Figure 2–6). However, there is substantial clinical and epidemiologic evidence that low to moderate consumption of alcohol reduces the incidence of coronary heart disease and stroke (curve B, Figure 2–6). Thus, when all responses are plotted on the ordinate, a U-shaped dose–response curve is obtained (curve C, Figure 2–6).

**Threshold**—Another important aspect of the dose–response relationship at low doses is the concept of the threshold, that is some dose below which the probability of an individual responding is zero. For the individual dose–response relationship, thresholds for most toxic effects certainly exist, although interindividual variability in response and qualitative changes in response pattern with dose make it difficult to establish a true “no effects” threshold for any chemical. In the identification of



**FIGURE 2–6 Hypothetical dose–response relationship depicting characteristics of hormesis.** Hormetic effects of a substance are hypothesized to occur when relatively low doses result in the stimulation of a beneficial or protective response (B), such as induction of enzymatic pathways that protect against oxidative stress. Although low doses provide a potential beneficial effect, a threshold is exceeded as the dose increases and the net effects will be detrimental (A), resulting in a typical dose-related increase in toxicity. The complete dose–response curve (C) is conceptually similar to the individual dose–response relationship for essential nutrients shown in Figure 2–5.

“safe” levels of exposure to a substance, it is important to determine the absence or presence of a threshold.

In evaluating the shape of the dose–response relationship in populations, it is realistic to consider inflections in the shape of the dose–response curve rather than absolute thresholds. That is, the slope of the dose–response relationship at high doses may be substantially different from the slope at low doses, usually because of dispositional differences in the chemical. Saturation of biotransformation pathways, protein-binding sites or receptors, and depletion of intracellular cofactors represent some reasons why sharp inflections in the dose–response relationship may occur.

**Nonmonotonic Dose–Response Curves**—Some chemicals, especially the endocrine disruptors, may exert effects at low doses that are not evident at high doses. These agents produce the so-called nonmonotonic dose–response curves. These curves may result from upregulation of some receptors at low doses with downregulation of those receptors at higher doses. The chemical may also act on different molecular pathways with common endpoints but opposite effects. Bisphenol A is one chemical that shows nonmonotonic dose response curves.

## Assumptions in Deriving the Dose–Response Relationship

A number of assumptions must be considered before dose–response relationships can be used appropriately. The first is that the response is due to the chemical administered, a cause-and-effect relationship.

The second assumption is that the magnitude of the response is in fact related to the dose. This assumes that there is a molecular target site (or sites) with which the chemical interacts to initiate the response, which is related to the concentration of the agent at the target site, which, in turn, is related to the dose administered.

The third assumption in using the dose–response relationship is that there exists both a quantifiable method of measuring and a precise means of expressing the toxicity. A given chemical may have a family of dose–response relationships, one for each toxic endpoint. For example, a chemical that produces cancer through genotoxic effects, liver damage through inhibition of a specific enzyme, and CNS effects via a different mechanism may have three distinct dose–response relationships, one for each endpoint.

With a new substance, the customary starting point is a single dose acute toxicity test designed to provide preliminary identification of target organ toxicity. Studies specifically designed with lethality as an endpoint are no longer recommended by U.S. or international agencies. Data from acute studies provide essential information for choosing doses for repeated dosing studies, as well as choosing specific toxicologic endpoints for further study. From these studies, clues as to the direction of further studies come about in two important ways. Detailed physiologic measurements and behavioral

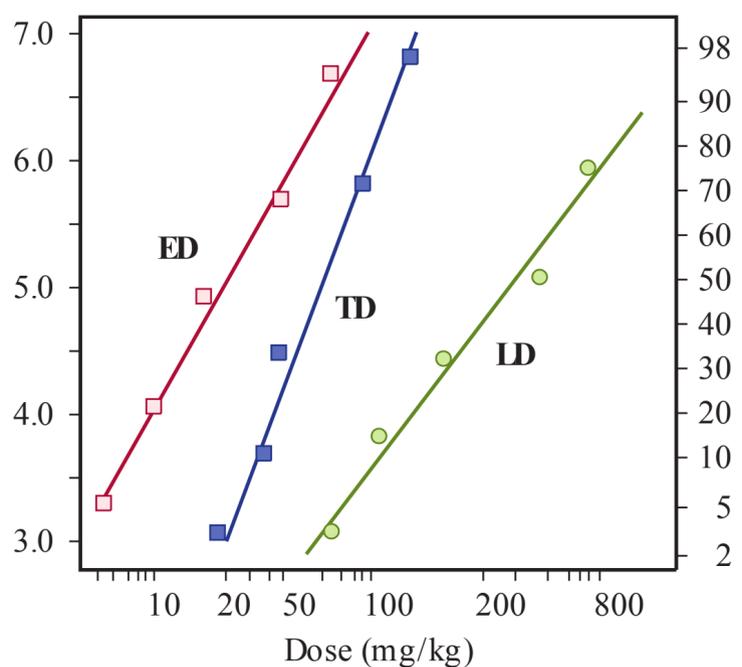
observations are collected from onset of exposure to the toxicant to the end of the observation period. An acute toxicity study ordinarily is supported by histologic examination of major tissues and organs for abnormalities. From these observations, one can usually obtain more specific information about the events leading to the lethal effect, the target organs involved, and often a suggestion about the possible mechanism of toxicity.

## Evaluating the Dose–Response Relationship

**Comparison of Dose–Responses**—Figure 2–7 illustrates a hypothetical quantal dose–response curve for a desirable effect of a chemical (ED) such as anesthesia, a toxic dose (TD) effect such as liver injury, and the lethal dose (LD). Even though the curves for ED and LD are parallel, the mechanism by which the drug works is not necessarily that by which the lethal effects are caused. The same admonition applies to any pair of parallel “effect” curves or any other pair of toxicity or lethality curves.

**Therapeutic Index**—The hypothetical curves in Figure 2–7 illustrate two other interrelated points: the importance of the selection of the toxic criterion and the interpretation of comparative effect. The therapeutic index (TI) is defined as the ratio of the dose required to produce a toxic effect and the dose needed to elicit the desired therapeutic response. Similarly, an index of comparative toxicity is obtained by the ratio of doses of two different materials to produce an identical response or the ratio of doses of the same material necessary to yield different toxic effects.

The most commonly used index of effect, whether beneficial or toxic, is the median dose—that is, the dose required to result in a response in 50% of a population (or to produce 50% of a



**FIGURE 2–7** Comparison of effective dose (ED), toxic dose (TD), and lethal dose (LD). The plot is of log dosage versus percentage of population responding in probit units.

maximal response). The TI of a drug is an approximate statement about the relative safety of a drug expressed as the ratio of the TD (historically the LD) to the therapeutic dose:

$$TI = \frac{TD_{50}}{ED_{50}}$$

From Figure 2–7, one can approximate a TI by using these median doses. The larger the ratio is, the greater the relative safety. The  $ED_{50}$  is approximately 20, and the  $TD_{50}$  is about 60; thus, the TI is 3, a number indicating that reasonable care in exposure to the drug is necessary to avoid toxicity. However, median doses tell nothing about the slopes of the dose–response curves for therapeutic and toxic effects.

**Margins of Safety and Exposure**—One way to overcome this deficiency is to use the  $ED_{99}$  for the desired effect and the  $LD_1$  for the undesired effect. These parameters are used to calculate the margin of safety:

$$\text{Margin of safety} = \frac{LD_1}{ED_{99}}$$

For nondrug chemicals, the term *margin of safety* is an indicator of the magnitude of the difference between an estimated “exposed dose” to a human population and the no observable adverse effect level (NOAEL) determined in experimental animals.

**Potency versus Efficacy**—To compare the toxic effects of two or more chemicals, the dose–response to the toxic effects of each chemical must be established. The potency and maximal efficacy of the two chemicals to produce a toxic effect can be explained by reference to Figure 2–8. Chemical A is said to be more potent than chemical B, and C is more potent than D, because of their relative positions along the dosage axis. Potency

thus refers to the range of doses over which a chemical produces increasing responses. Maximal efficacy reflects the limit of the dose–response relationship on the response axis to a certain chemical. Chemicals A and B have equal maximal efficacy, whereas the maximal efficacy of C is less than that of D.

## VARIATION IN TOXIC RESPONSES

### Selective Toxicity

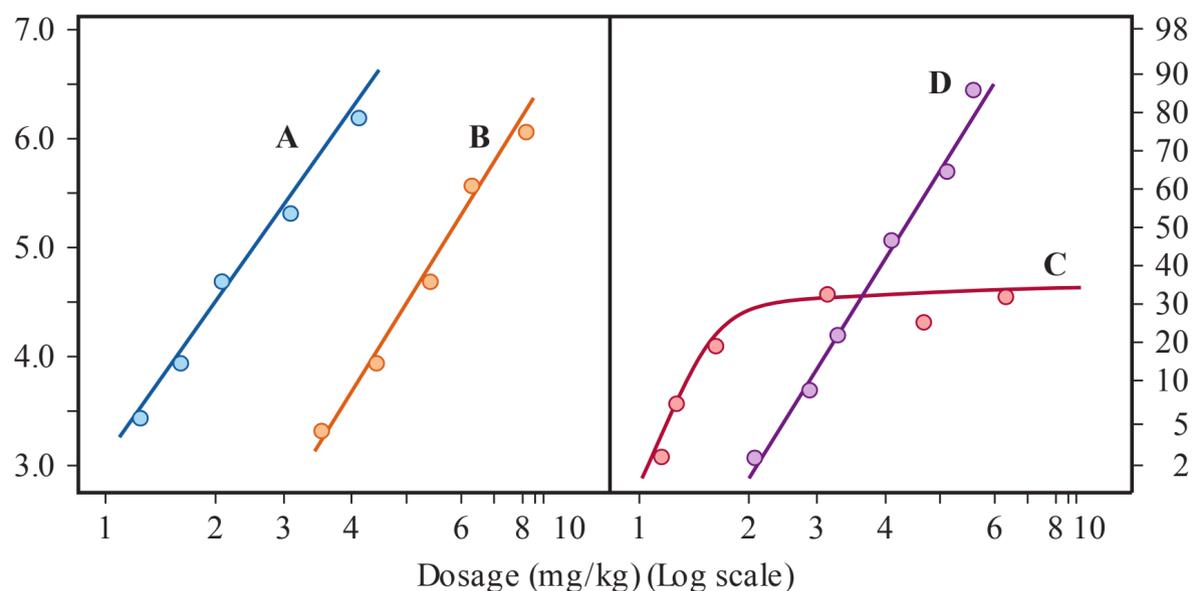
Selective toxicity means that a chemical produces injury to one kind of living matter without harming another form of life even though the two may exist in intimate contact. By taking advantage of biological diversity, it is possible to develop agents that are lethal for an undesired species and harmless for other species. Such selective toxicity can be due to differences in distribution (absorption, biotransformation, or excretion) or to differing biochemical processing of the toxicant by different organisms.

### Species Differences

Although a basic tenet of toxicology is that “experimental results in animals, when properly qualified, are applicable to humans,” it is important to recognize that both quantitative and qualitative differences in response to toxic substances may occur among different species. Identifying the mechanistic basis for species differences in response to chemicals establishes the relevance of animal data to human response.

### Individual Differences in Response

Even within a species, large interindividual differences in response to a chemical can occur because of subtle genetic differences referred to as genetic polymorphisms. These may be responsible for idiosyncratic reactions to chemicals and for interindividual differences in toxic responses.



**FIGURE 2–8** Schematic representation of the difference in the dose–response curves for four chemicals (A–D), illustrating the difference between potency and efficacy (see text).

## DESCRIPTIVE ANIMAL TOXICITY TESTS

Two main principles underlie all descriptive animal toxicity testing. The first is that the effects produced by a compound in laboratory animals, when properly qualified, are applicable to humans. The second principle is that exposure of experimental animals to toxic agents in high doses is a necessary and valid method of discovering possible hazards in humans because the incidence of an effect in a population is greater as the dose or exposure increases. Obtaining statistically valid results from the small groups of animals used in toxicity testing requires the use of relatively large doses so that the effect will occur frequently enough to be detected. However, the use of high doses can create problems in interpretation if the response(s) obtained at high doses does not occur at low doses.

Toxicity tests are not designed to demonstrate that a chemical is safe but to characterize the toxic effects a chemical can produce. There are no set toxicology tests that have to be performed on every chemical intended for commerce. Depending on the eventual use of the chemical, the toxic effects produced by structural analogs of the chemical, as well as the toxic effects produced by the chemical itself, contribute to the determination of the toxicology tests that should be performed.

### Acute Toxicity Testing

The first toxicity test performed on a new chemical is acute toxicity, which is determined from the administration of a single exposure. The  $LD_{50}$  and other acute toxic effects are determined after one or more routes of administration (one route being oral or the intended route of exposure) in one or more species, usually the mouse and rat, but sometimes the rabbit and dog. Daily examination of the animals for signs of intoxication, lethargy, behavioral modifications, food consumption, etc., and tabulation of the number of animals that die in a 14-day period after a single dosage occurs. Acute toxicity tests (1) give a quantitative estimate of acute toxicity ( $LD_{50}$ ), (2) identify target organs and other clinical manifestations of acute toxicity, (3) identify species differences and susceptible species, (4) establish the reversibility of the toxic response, and (5) provide dose-ranging guidance for other studies.

Determination of the  $LD_{50}$  has become a public issue because of increasing concern for the welfare and protection of laboratory animals. Because  $LD_{50}$  is not a constant and many variables influence its estimation, for most purposes it is only necessary to characterize the  $LD_{50}$  within an order of magnitude range (e.g., 5 to 50 and 50 to 500 mg/kg).

If there is a reasonable likelihood of substantial exposure to the material by dermal or inhalation exposure, acute dermal and acute inhalation studies are performed. When animals are exposed acutely to chemicals in the air they breathe or the water they (fish) live in, the lethal concentration 50 ( $LC_{50}$ ) is usually determined for a known time of exposure, that is, the concentration of chemical in the air or water that causes death to 50% of the animals. The acute dermal toxicity test is usually performed in rabbits. The site of application is shaved, and the

substance is applied and covered for 24 h, and then removed. The skin is cleaned and the animals observed for 14 days to calculate  $LD_{50}$ . Acute inhalation studies are performed that are similar to other acute toxicity studies except that the route of exposure is inhalation for 4 h.

Acute lethality studies are essential for characterizing the toxic effects of chemicals and their hazard to humans. The most meaningful scientific information derived from acute lethality tests comes from clinical observations and postmortem examination of animals rather than from the specific  $LD_{50}$  value.

### Skin and Eye Irritations

For the dermal irritation test (Draize test), the skin of rabbits is shaved, the chemical applied to one intact and two abraded sites and covered for 4 h. The degree of skin irritation is scored for erythema (redness), eschar (scab), edema (swelling) formation, and corrosive action. These dermal irritation observations are repeated at various intervals after the covered patch has been removed. To determine the degree of ocular irritation, the chemical is instilled into one eye of each test rabbit. The contralateral eye is used as the control. The eyes of the rabbits are then examined at various times after application.

Alternative *in vitro* models, including epidermal keratinocyte and corneal epithelial cell culture models, have been developed for evaluating cutaneous and ocular toxicity of substances.

### Sensitization

Information about the potential of a chemical to sensitize skin is needed in addition to irritation testing for all materials that may repeatedly come into contact with the skin. In general, the test chemical is administered to the shaved skin of guinea pigs topically, intradermally, or both, over a period of 2 to 4 weeks. About 2 to 3 weeks after the last treatment, the animals are challenged with a nonirritating concentration of the test substance and the development of erythema is evaluated.

### Subacute (Repeated-dose Study)

Subacute toxicity tests are performed to obtain information on the toxicity of a chemical after repeated administration for typically 14 days and as an aid to establish doses for subchronic studies.

### Subchronic

Subchronic exposure usually lasts for 90 days. The principal goals of the subchronic study are to establish a “lowest observed adverse effect level” (LOAEL) and a NOAEL, and to further identify and characterize the specific organ or organs affected by the test compound after repeated administration.

A subchronic study is usually conducted in two species (rat and dog for FDA; mouse and rat for EPA) by the route of intended exposure. At least three doses are employed (a high

dose that produces toxicity but less than 10% fatalities, a low dose that produces no apparent toxic effects, and an intermediate dose). Animals should be observed once or twice daily for signs of toxicity. All premature deaths should be recorded and necropsied. Severely moribund animals should be terminated immediately to preserve tissues and reduce unnecessary suffering. At the end of the 90-day study, all the remaining animals should be terminated and blood and tissues should be collected for further analysis. The gross and microscopic conditions of the organs and tissues are recorded and evaluated. Hematology, blood chemistry, and urinalysis measurements are usually done before, in the middle of, and at the termination of exposure. Hematology measurements usually include hemoglobin concentration, hematocrit, erythrocyte counts, total and differential leukocyte counts, platelet count, clotting time, and prothrombin time. Clinical chemistry determinations commonly include glucose, calcium, potassium, urea nitrogen, alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), gamma-glutamyltranspeptidase (GGT), sorbitol dehydrogenase, lactic dehydrogenase, alkaline phosphatase, creatinine, bilirubin, triglycerides, cholesterol, albumin, globulin, and total protein. Urinalysis includes determination of specific gravity or osmolarity, pH, proteins, glucose, ketones, bilirubin, and urobilinogen as well as microscopic examination of formed elements. If humans are likely to have significant exposure to the chemical by dermal contact or inhalation, subchronic dermal and/or inhalation experiments may also be required.

## Chronic

Long-term or chronic exposure studies are performed similarly to subchronic studies except that the period of exposure is usually for 6 months to 2 years. Chronic toxicity tests are often designed to assess both the cumulative toxicity and the carcinogenic potential of chemicals. Both gross and microscopic pathological examinations are made not only on animals that survive the chronic exposure, but also on those that die prematurely.

Dose selection is critical to ensure that premature mortality from chronic toxicity does not limit the number of animals that survive to a normal life expectancy. Most regulatory guidelines require that the highest administered dose be the estimated maximum tolerable dose (MTD), that is, the dose that suppresses body weight gain slightly in a 90-day subchronic study. Generally, one or two additional doses, usually one-half and one-quarter MTD, and a control group are tested.

Chronic toxicity assays commonly evaluate the potential oncogenicity of test substances. Both benign and malignant tumors must be reported. Properly designed chronic oncogenicity studies require a concurrent control group matched for variables such as age, diet, and housing conditions.

## Other Tests

The effects of chemicals on reproduction and development are discussed in Chapters 10 and 20. Oncogenicity bioassays are

introduced in Chapter 8. Mutagenicity is discussed in detail in Chapter 9. Information on methods, concepts, and problems associated with inhalation toxicology is provided in Chapters 15 and 28. A discussion of neurotoxicity and behavioral toxicology can be found in Chapter 16. Immunotoxicity assessment is mentioned in Chapter 12.

## TOXICOGENOMICS

Toxicogenomics defines the interaction between genes and toxicants in toxicity etiology. Transcript, protein, and metabolite profiling is combined with conventional toxicology. The human genome consists of approximately 3 billion base pairs of deoxyribonucleotides. The differential expression of genes in a given cell is largely responsible for the diverse function of the thousands of different cells, tissues, and organs that constitute an individual organism. Experimental data on how a toxicant affects gene expression (transcriptomics), protein production (proteomics), and small molecule metabolism and function (metabolomics) from a test species (rat/mouse, etc.) can be combined with those of humans and analyzed with the computational tools of bioinformatics to ascertain unique or predictive patterns of toxicity.

### Genomics

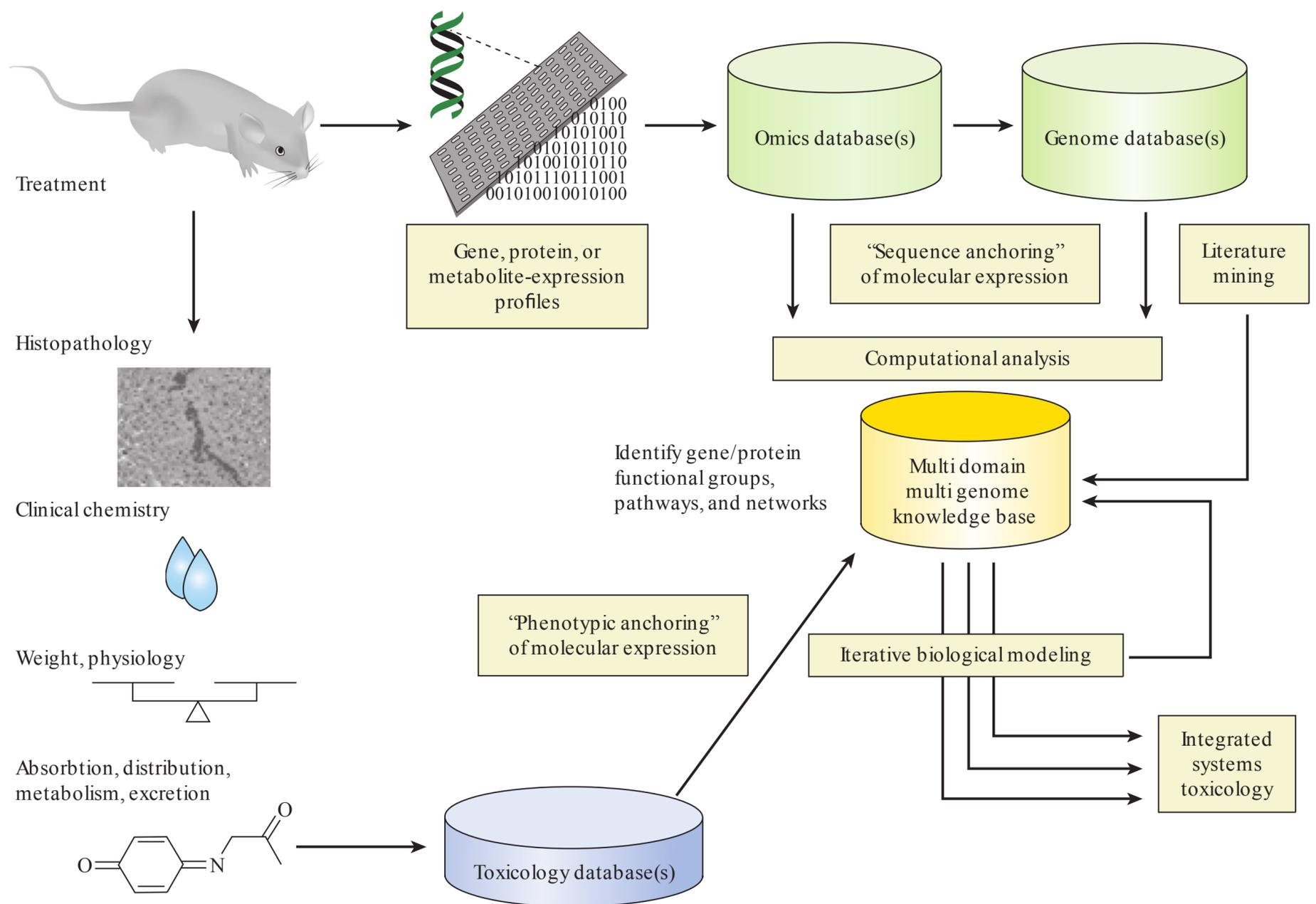
The identification and characterization of various genetic variants will aid understanding of interindividual differences in susceptibility to chemicals or other environmental factors and the complex interactions between the human genome and the environment. How chemicals affect genomic DNA, mRNA, small interfering RNA (siRNA), etc. is of particular importance to toxicogenomics.

### Epigenetics

Toxicants may also act on areas “above or in addition” to genes. Epigenetics concerns a mitotically or meiotically heritable change in gene expression that occurs independently of an alteration in DNA sequence. Changes in DNA methylation or histone acetylation may suppress, silence, or activate gene expression without altering the DNA sequence. There is evidence in some animal models that epigenetic changes may be transgenerational thereby influencing toxicological assessment. Subtle epigenetic changes resulting from environmental exposures may not produce cytotoxicity or mutation or may lead to cancer, neurodevelopment disorders, autoimmune diseases, metabolic disorders, asthma, or neurologic/behavioral disorders (Figure 2–9).

### Transcriptomics and Proteomics

The transcriptome contains all mature mRNA species in the cell at a given time. It is dynamic and represents the steady state



**FIGURE 2–9 Conceptual approach for incorporating “omics” technologies and resulting large databases into toxicological evaluation.** Data from experiments that evaluate the effects of a chemical on global patterns of gene expression (transcriptomics), protein content (proteomics), and small molecules/metabolites (metabonomics/metabolomics), combined with genomic information from both the test species (e.g., rats, mice) and the target species of interest (e.g., humans), are analyzed by computational tools (bioinformatics) for unique or potentially predictive patterns of toxicity. Essential to the use of omics data for predictive toxicology/safety assessment is the ability to reliably tie observed omics patterns to traditional measures of toxicity, such as histopathology and clinical chemistry (phenotypic anchoring). (Reproduced with permission from Waters MD and Fostel JM. Toxicogenomics and systems toxicology: aims and prospects. *Nat Rev Genet*, 2004 Dec;5(12):936–948.)

between synthesis (transcription) and degradation of mRNA. Northern blots, reverse transcriptase polymerase chain reaction, and microarray technologies permit determination of effects of chemical exposure on gene expression. One of the major challenges in toxicogenomics is the recognition that transcriptional regulation is highly dynamic as gene expression profiles can change dramatically with both dose and time. Alterations in gene expression often contribute to phenotypic changes that occur, but the transcriptome is somewhat removed from the ultimate biochemical functions that dictate the actual biologic function of the cell.

The proteome is the entire complement of proteins that are present in a cell or tissue at a specific time point. Unambiguous protein identification is difficult and generally requires separation techniques (gel electrophoresis or high pressure liquid chromatography) followed by tandem mass spectrometry. Proteomics can potentially identify unique patterns of protein

expression that may be predictive of early toxicity or subsequent disease development.

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## QUESTIONS

1. Five identical experimental animals are treated with 1 mg of one of the following toxins. The animal treated with which toxin is most likely to die?
  - a. ethyl alcohol ( $LD_{50} = 10,000$  mg/kg).
  - b. botulinum toxin ( $LD_{50} = 0.00001$  mg/kg).
  - c. nicotine ( $LD_{50} = 1$  mg/kg).
  - d. ferrous sulfate ( $LD_{50} = 1500$  mg/kg).
  - e. picrotoxin ( $LD_{50} = 5$  mg/kg).
2. Place the following mechanisms of toxin delivery in order from most effective to least effective—1: intravenous; 2: subcutaneous; 3: oral; 4: inhalation; 5: dermal.
  - a. 1, 5, 2, 4, 3.
  - b. 4, 1, 2, 3, 5.
  - c. 1, 4, 2, 3, 5.
  - d. 4, 2, 1, 5, 3.
  - e. 1, 4, 3, 2, 5.
3. A toxin with a half-life of 12 h is administered every 12 h. Which of the following is true?
  - a. The chemical is eliminated from the body before the next dose is administered.
  - b. The concentration of the chemical in the body will slowly increase until the toxic concentration is attained.
  - c. A toxic level will not be reached, regardless of how many doses are administered.
  - d. Acute exposure to the chemical will produce immediate toxic effects.
  - e. The elimination rate of the toxin is much shorter than the dosing interval.
4. Urushiol is the toxin found in poison ivy. It must first react and combine with proteins in the skin in order for the immune system to recognize and mount a response against it. Urushiol is an example of which of the following?
  - a. antigen.
  - b. auto-antibody.
  - c. superantigen.
  - d. hapten.
  - e. cytokine.
5. Toxic chemicals are most likely to be biotransformed in which of the following organs?
  - a. central nervous system.
  - b. heart.
  - c. lung.
  - d. pancreas.
  - e. liver.
6. When chemicals A and B are administered simultaneously, their combined effects are far greater than the sum of their effects when given alone. The chemical interaction between chemicals A and B can be described as which of the following?
  - a. potentiative.
  - b. additive.
  - c. antagonistic.
  - d. functionally antagonistic.
  - e. synergistic.
7. With respect to dose–response relationships, which of the following is true?
  - a. Graded dose–response relationships are often referred to as “all or nothing” responses.
  - b. Quantal dose–response relationships allow for the analysis of a population’s response to varying dosage.
  - c. Quantal relationships characterize the response of an individual to varying dosages.
  - d. A quantal dose–response describes the response of an individual organism to varying doses of a chemical.
  - e. The dose–response always increases as the dosage is increased.
8. When considering the dose–response relationship for an essential substance:
  - a. there are rarely negative effects of ingesting too much.
  - b. the curve is the same for all people.
  - c. adverse responses increase in severity with increasing or decreasing dosages outside of the homeostatic range.
  - d. the relationship is linear.
  - e. deficiency will never cause more harm than over-ingestion.

9. The therapeutic index of a drug:
- is the amount of a drug needed to cure an illness.
  - is lower in drugs that are relatively safer.
  - describes the potency of a chemical in eliciting a desired response.
  - describes the ratio of the toxic dose to the therapeutic dose of a drug.
  - explains the change in response to a drug as the dose is increased.
10. Penicillin interferes with the formation of peptidoglycan cross-links in bacterial cell walls, thus weakening the cell wall and eventually causing osmotic death of the bacterium. Which of the following is true?
- Treatment with penicillin is a good example of selective toxicity.
  - Penicillin interferes with human plasma membrane structure.
  - Penicillin is a good example of a drug with a low therapeutic index.
  - Penicillin is also effective in treating viral infections.
  - Penicillin is completely harmless to humans.