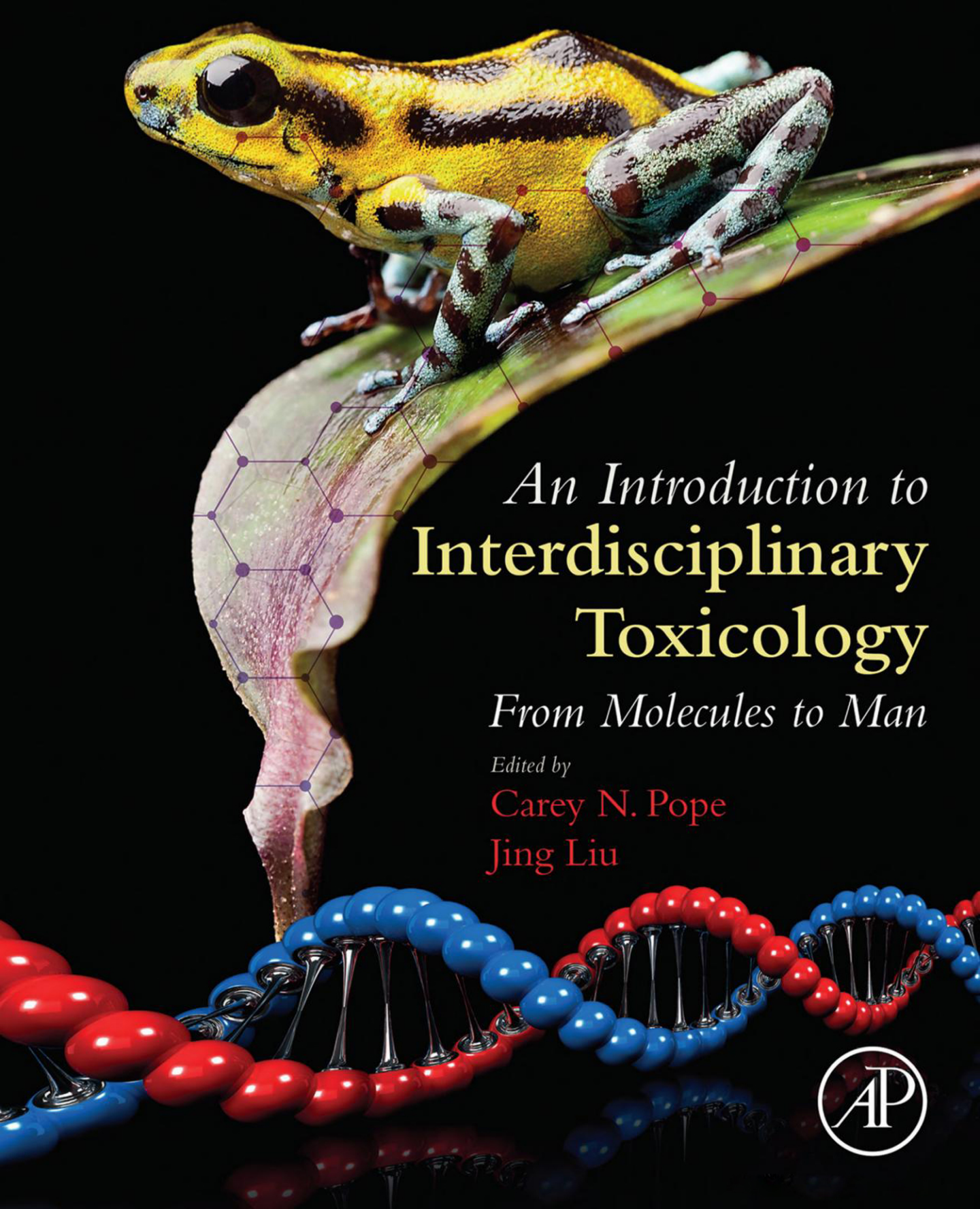
An Introduction to
Interdisciplinary
Toxicology
From Molecules to Man
Edited by
Carey N. Pope
Jing Liu

AN INTRODUCTION TO INTERDISCIPLINARY TOXICOLOGY

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FROM MOLECULES TO MAN

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Foreword

Toxicological risk can be defined by the simple risk equation: RISK = INTRINSIC TOXICITY × EXPOSURE. As will be seen in this volume, this equation encapsulates all aspects of toxicology, from fundamental definitions of toxicology to its many subdisciplines. Through its comprehensive coverage of this broad field, this work provides a useful and logical description of toxicology in a meaningful and impactful manner. Spanning molecular toxicology, organ systems and organismal toxicology, ecotoxicology, and ultimately population impact, *An Introduction to Interdisciplinary Toxicology* covers the waterfront of the discipline of toxicology.

Chemical exposure is widely explored in this text because of its central role in defining toxicity. From absorption, distribution, metabolism, and elimination of a chemical in an organism to environmental and occupational exposures, the general principles of chemical exposure are systematically examined. The roles of competing pathways of metabolism, including the opportunity for induction of metabolic enzymes with overall effects to magnify or lessen the toxicity, are described.

Pathways to toxicity, including receptor interaction, intracellular signaling pathways, and covalent binding, are thoroughly discussed in pharmacological and molecular terms. In many cases, the mechanistic basis for a chemical's toxicity is the disruption of an endogenous biological pathway. Outcomes of such disruption may be cancer or reproductive toxicity, yet other mechanisms such as DNA covalent binding or nongenomic alterations, including epigenetic mechanisms, may play a pivotal role.

At the organ system level, the impacts of toxicants on the hepatic, renal, respiratory, and cardiovascular systems are extensively examined. The sensitivity of these systems, including the immune and reproductive systems, is appraised. Distribution of receptor systems, metabolic capability, enzymatic pathways, and signaling pathways are examined as modulators of potential toxicity.

Potentially toxic chemicals can be found almost anywhere, including homes, workplaces, and communities. Exposure to potential toxicants may vary widely in these different environments, but knowledge of exposure scenarios and routes of exposure may provide protective strategies for adults and children.

The principles of ecotoxicology are examined along with environmental impact of exposures to chemicals. The concept of environmental justice is thoroughly examined and forces that control it are discussed. Because wildlife and plant life can be affected, the entire ecosystem must be considered. Even the smallest of physico-chemical entities (i.e., nanoparticles) are evaluated for their relative toxicity profiles compared with more traditional forms of those same chemicals.

The toxicological world has several branches that are firmly attached to the major trunk of the toxicology world. Among those examined are clinical, veterinary, forensic, and regulatory toxicology, each with its own focus of interest

but all firmly related to general toxicological principles.

Finally, model systems and various risk assessment approaches and tools are presented to strengthen and reinforce the principles of toxicology. These approaches allow prediction and a quantitative definition of the risk associated with toxicant exposure. This comprehensive and all-encompassing treatise on toxicology provides the basis for understanding the importance of the principles of toxicology.

William Slikker
National Center for Toxicological Research, U.S. Food and Drug Administration

Preface

The Interdisciplinary Toxicology Program (ITP) was established at Oklahoma State University (OSU) in 2012, with the recognition that complex environmental issues of our time surrounding chemical contamination will require the efforts of investigators across disciplines and the cross-training of their students to be effective investigators. Faculty and students in our program come from 12 different departments, 6 colleges, and 2 campuses. Our earlier experience with an undergraduate toxicology program at the University of Louisiana at Monroe emphasized the value of starting simple in developing and transferring knowledge in toxicology through coursework and laboratory experiences, highlighting important concepts and skills in easy-to-understand approaches. This same concept of education and training applies to graduate students in an interdisciplinary program, with students coming from diverse multiple disciplines and sometimes very different experiences.

This book is modeled after one of the courses in the OSU ITP, *Toxicology: from molecules to ecosystems*. The course begins with principles and goes on to cover from toxicant-target interactions to proteotoxicity, cellular responses, toxicokinetics, organ systems, ecotoxicology, forensics, population effects, the sociology of chemical contamination episodes, and other topics, matching the strengths of the participating faculty and the interests of their students. While covering the subject matter can be a challenge for both the students and the instructors, most agree that synergy can develop when bringing different emphasis areas, concepts, and approaches together. Active participation between the students and instructors is an important part of the course and facilitates an understanding among all for their specific interests and experiences.

One advantage for putting this book together was a necessary emphasis on what we were teaching and how it could be made more succinct and clear, in addition to having the opportunity to recruit other OSU faculty for coverage of new areas of emphasis. Expert authors from other institutions contributed chapters as well, and a number of those have already visited or will visit OSU as part of our annual ITP symposium. We are indebted to the efforts of all of the chapter contributors without which completion of the book could not have happened. We hope that our book provides an easy-to-understand survey of timely topics in toxicology suitable for graduate students across disciplines entering into this exciting area of investigation.

Carey N. Pope and Jing Liu
September 2019

PART I

General concepts

CHAPTER

1

History and basic concepts of toxicology

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1.1 A brief history of toxicology

There is substantial evidence indicating that humans have been aware of, and in some cases utilized, the toxicity of various substances since antiquity. While there is little evidence of poisonings in the Paleolithic and Neolithic periods in Europe, around 18,000 years ago Maasai hunters in Kenya used arrow and dart poisons (likely cardiac glycosides of *Strophanthus* species) to increase the effectiveness of their weapons. Indeed the term *toxicology* is derived from the Greek terms *toxikos* (bow) and *toxicon* (poison into which arrowheads are dipped).[1]

In the bronze (3000−1000 years BCE) and iron ages (800−100 years BCE), people started to communicate with writing, providing lasting documentation of accidental and intentional intoxications and the use of toxic substances in executions. During the Bronze Age, metal alloys were first developed using tin, aluminum, lead, manganese, and other trace elements. During the Iron Age, the development of iron and steel industries was instrumental in the maintenance of power and order by European monarchies and feudal overlords. One can assume that human exposure to heavy metals was a constant threat due to the smelting, iron casting, and other activities such as painting and tanning.

In the past, *medical toxicology* concerned natural substances including metals, plants, fungi such as mushrooms and mycotoxins (ergotism), bacterial exotoxin (botulism), and venomous animals as well as carbon oxides produced by combustion of carbonaceous materials. The Eber's papyrus, an ancient Egyptian text written around 1500 BCE, is among the earliest of medical texts, describing a variety of ancient poisons including aconite, antimony, arsenic, cyanogenic glycosides, hemlock, lead, mandrake, opium, and wormwood.

The basis of pharmacology was clearly stated in *Phaedo* by Plato (428−348 BCE), and

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further developed by Aristotle (384–322 BCE). At this time, the toxicity of plants and venomous animals was well known as illustrated by the modus operandi for Socrates' sanctioned execution by self-ingestion of hemlock (470–399 BCE), while much later the Egyptian queen Cleopatra died from a self-inflicted fatal snake bite (51–30 BCE). The Roman empire followed by the Middle Age and Renaissance inaugurated a long period during which murder using poisonous substances was a common practice, using knowledge held by "wizards" and alchemists. The Greek physician Galen (c. CE 129–200) described Mithridates' experiences in a series of books on *Antidotes*. Chemical warfare and infectious agents were commonly used during sieges. A number of historians suggested a relationship between the large use of lead for the numerous pipelines supplying Rome's drinking water and chronic lead poisoning of the Roman population leading to the twilight and eventual fall of the Roman Empire in the mid-5th century CE.

The bean of the Calabar plant (*Physostigma venonosum*) and seeds of a variety of other plants were used in Africa and Madagascar for likely hundreds of years as "ordeal poisons" to determine guilt of someone accused of a crime. While the substance and methods for using an ordeal poison varied, the suspect was typically forced to eat or drink the substance and the reaction was observed. If the material was expelled by vomiting, he or she was assumed to be innocent. If the individual did not eliminate the poison, toxicity would follow shortly and the accused would be considered guilty by the negative outcome.[1,2]

The term "poison" appeared first in the English literature around CE 1225 to describe a potion that was prepared with deadly ingredients. Since the Middle Age, members of aristocracy used "tasters" to shield themselves from potential poisoners by having them first sample their beverages and meals before consuming themselves. Interestingly, the concept of making a "toast" arose from a common fear of poisoning. It was believed that if all present would drink from the same container at the same time, it would likely be devoid of any deadly poison. Obviously, a martyr (person who will die for a cause) could make this strategy less protective.

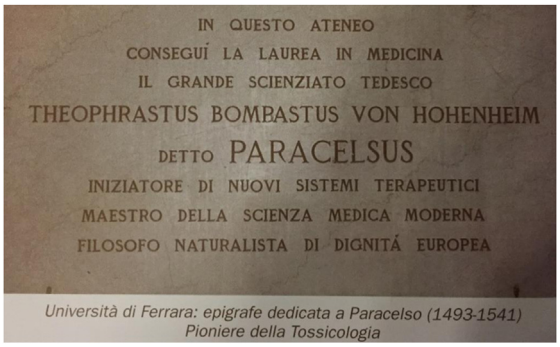

FIGURE 1.1 Commemorative to Paracelsus, University of Ferrara, Italy. In this University, the great scientist Theophrastus Bombastus von Hohenheim Paracelsus obtained a degree in Medicine. Initiator of a new system in therapeutics. Master of the modern medical sciences. Naturalist philosopher of Europe. Pioneer of Toxicology.

During the Italian Renaissance, Paracelsus (1493–1541) at the University of Ferrara in Italy described a number of principles of human toxicology (see Fig. 1.1). The most well known is the prominent role of the dose of the substance in toxicity, reported as *No substances are safe, all substances are poisonous. The major parameter of toxicity is the dose*. However, Paracelsus' ideology should not be restricted to this major principle. His work led to the description of some types of toxicants as xenobiotics (toxic substances originating from outside of the human body) and to the field of *organ toxicology*.

In the mid-17th century, Bernardino Ramazzini (1633–1714) first developed the area of *occupational medicine*. In 1700 he wrote *De Morbis Artificum Diatriba* (diseases of workers), the first comprehensive text discussing the relationship between disease and workplace hazards. Ramazzini described diseases associated with 54 occupations, including solvent poisoning in painters, mercury poisoning

in mirror makers, and pulmonary diseases in miners. Around 1775, Sir Percivall Pott uncovered the association between *workplace exposures and cancer, when he reported* a high incidence of scrotal cancer in English chimney sweeps, whose occupation was associated with direct and chronic exposure to incomplete combustion products such as complex polycyclic aromatic hydrocarbons.

About one century later, the French physician Bonaventure Orfila (1787–1853) highlighted the role of toxicology as a distinct discipline separated from clinical medicine and pharmacology. His treatise *Traité des Poisons* (1814) is regarded as the foundation of *experimental and forensic toxicology*, promoting the use of chemical analysis and autopsy for medicolegal purposes. The French physician *Claude Bernard* (1813–78) was instrumental in discovering the mechanism of toxicity of carbon monoxide through its binding to hemoglobin. He also provided the first compelling evidence for a *synapse* between a motor neuron and the muscle cell with which it communicates. Interestingly, much of Bernard's work in this context relied on the effects of one of the arrow poisons, curare. He promoted experimental studies in physiology to assess the accuracy of hypotheses regarding *mechanism of toxicity* and advised the use of poisons to study organ function, summarized in his aphorism: "The poison is for the physiologist like the scalpel is for the surgeon."

While one can identify through literature when chemicals were first being used for poisonings, it is more difficult to determine a time when people first started using substances for recreational purposes. It is known however that marijuana (*Cannabis* sp.) has been used for millennia. Many natural plants, herbs, and seeds contain psychoactive substances which have been used in traditional medicines. Written communication did not start in China until the 1700s, but it is suggested that the Chinese have been using herbal medicines for likely thousands of years. In Europe in the 16th century, Paracelsus was promoting the medical use of opium. In the 17th century, the English physician Thomas Sydenham proposed a formulation of opium tincture for various purposes.

Alice Hamilton (1869–1970) was first to highlight *occupational toxicology*. By living and working in a working class neighborhood in Chicago, she identified "dangerous trades" including those working with rubber, dyes, lead, enamelware, copper, mercury, and explosives, documenting the different types of disorders. Her work on lead intoxication was one of the first that focused on gender differences in response to toxicants.

The awareness of toxicological hazards to which the general population may be exposed is a relatively recent phenomenon. The establishment of *regulatory authorities* appeared only very recently. Interestingly, in France, a progressive and continuing decrease in attempted murders using poisonous substances was associated with increasing legal freedom to divorce starting in the late 18th century. The US Pure Food and Drug Act of 1906 was the first federal legislative antipoisoning regulatory initiative.[1] The Federal Caustic Poison Act of 1927 was the first federal legislation to specifically address household poisonings. In fact, the US Food and Drug Administration was born out of a major drug-related poisoning disaster. In the early–mid 1930s, sulfamides were developed as potent antimicrobial agents. Unfortunately, the antimicrobials were given intravenously in a diethylene glycol solvent, leading to the deaths of hundreds of patients from acute renal failure. After this tragedy, the policies that required safety testing of new drugs before marketing were developed and implemented. Nowadays, in addition to therapeutics and drugs of abuse, environmental contaminants, and ecotoxicology are major concerns, and governmental agencies are addressing to change large-scale activities. The development of *Poison Control Centers* in the mid-20th century was also a major step worldwide for vigilant tracking of human

responses to xenobiotics, determining toxic relationships between exposure to newly released or currently marketed drugs and environmental contaminants.

1.2 Important concepts in toxicology

Chemical contamination episodes occur relatively often and can be found in reports by various news outlets. The public's *perception* of these events plays a major role in how communities deal with such episodes and how those communities, interest groups, and local, state, federal, and international governments may respond. A basic understanding of the principles of toxicology is important for communicating the *relative* nature of chemical hazards and informing public perception.

1.2.1 The dose–response relationship

A key factor for placing in context any intoxication or chemical contamination event, and a hallmark of toxicology as a scientific field, is the concept of the *dose–response relationship*, that is, the relationship between the incidence or magnitude of a toxic response and the extent of the chemical exposure. As noted in Section 1.1, the Swiss physician Theophrastus von Hohenheim (1493–1541), who took the name Paracelsus later in life, was an early proponent of the application of chemistry in medicine and medical education.[3] In the 16th century, Paracelsus was the first to propose that a predictable relationship exists between the extent of exposure to a substance and its relative therapeutic or toxic effect. His quote *dosis sola facit venenum* (dose alone makes the poison) is widely paraphrased. Because of the paramount importance of the dose–response relationship in chemical toxicity, Paracelsus is commonly recognized as the father of toxicology.[4]

Toxicity can be defined as the inherent capacity of a chemical to do harm to a living organism. *Hazard* is defined as the probability or practical certainty that an adverse effect (harm) *will occur* when a chemical is used under stated conditions (amount, dose, concentration, exposure, duration of exposure, use of personal protective equipment, etc.). In contrast, *safety* is the practical certainty that toxicity *will not occur* when a chemical is used under defined conditions. The hazard/safety associated with the use of any chemical therefore depends not only on its inherent chemical properties, but also on the likelihood (and if so the extent) of exposure when the chemical is used under defined conditions. An important corollary of Paracelsus' centuries-old concept is that while all chemicals can elicit toxicity, any chemical can be used safely if its toxic potential is recognized and the exposures are effectively controlled.

Exposures can be considered in a number of ways. They can be based on the amount of chemical in the ambient environment, on the amount of chemical absorbed into the organism, or most importantly on the amount of chemical that reaches receptors within an organism that initiate a toxic response. While it is appreciated that the magnitude of a toxic response is related to the concentration or dose of the toxicant, what is critical is the concentration of the chemical at the receptor site, with the toxicant–receptor interaction constituting a *molecular initiating event* that progresses through key events to an ultimate toxic response. In essence, a toxicant must interact with a receptor on/in a cell or tissue to initiate toxicity. Theoretical and practical implications of the toxicant–receptor interactions continue to impact how chemicals are evaluated and regulated for protecting public health and the environment.[4] The frequency and duration, when repeated exposures occur, are also vital in the expression of dose-related toxicity.

All chemicals have the capacity to elicit toxic responses. It is therefore important to consider a chemical's toxicity in context with other substances. The most recognized endpoint in toxicology for comparing substances is historically

the lethal dose 50 (LD_{50}), that is, a statistically determined *dose* of a chemical that leads to death in 50% of a group/population of exposed organisms. The standard LD_{50} approach has been progressively replaced in many areas by assessment with other methods such as estimating maximum tolerated dose (MTD) approaches generally requiring less animals to derive an estimate of acute lethality.

In ecological studies, the environmental medium is typically used for exposure, with those exposures being quantified by the substance concentration within the medium. Thus toxicity is often expressed as the *concentration* in the medium that kills 50% of the exposed population, that is, the LC_{50}. It is important to differentiate between concentration and dose, since the former does not measure internal (target/receptor site) content of the chemical but only measures the chemical's concentration in the medium. Concentration is also generally used to characterize in vitro and other exposures, for example, in inhalation toxicity studies.

Knowledge of doses or concentrations of a chemical that either do or do not elicit toxicity is essential in characterizing that chemical's *relative potency*. There are two major types of dose–response or concentration–response relationships, that is, those which exhibit a *threshold* and those which do not. Fig. 1.2 provides examples of both (data in these figures are not from any real study but are merely for example purposes). In Fig. 1.2A, both *chemical X* and *chemical Y* elicit a dose-related increase in toxicity. With lower exposures (0.03 mg/kg/day for chemical X and 0.03 – 1 mg/kg/day for chemical Y), no incidence of the response is noted. As the dose increases, however, the percent of individuals showing toxicity also increases. Note that the dose or concentration in dose–response relationships is typically shown on a semilog scale and dose–response relationships often show an "S-shaped" curve similar to chemical X in Fig. 1.2A. The data portrayed in Fig. 1.2A provide an example of a threshold dose–response relationship. In essence, while lower doses do not elicit toxicity, at some "threshold" level of exposure, a toxic response is noted (in this case in a proportion of individuals) which then increases in incidence with higher doses (or increases in magnitude when the degree or extent of a response is measured). The concept that a threshold exists in exposures below which no toxic response occurs has been the foundation for chemical risk assessments and regulatory decision-making for decades. It is assumed that if levels of exposure below the threshold do not elicit toxicity, then regulating/managing chemicals such that exposures fall below the threshold will maintain public safety and environmental health.

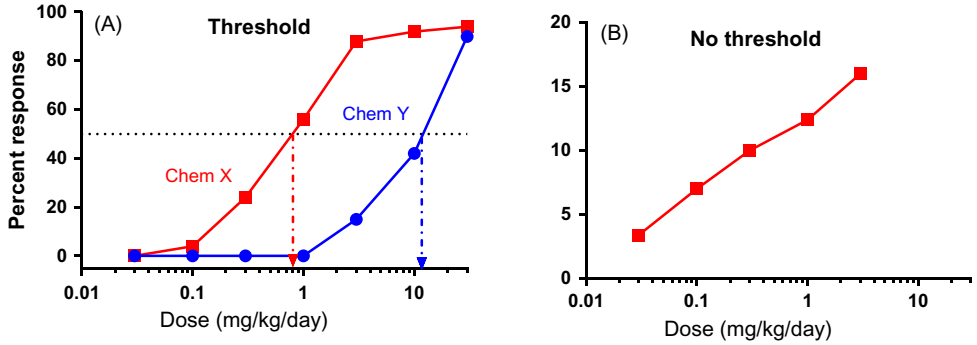

FIGURE 1.2 **Basic types of dose–response relationships.** A threshold (A) and no threshold (B) dose–response relationship is shown. The threshold dose–response relationship has been the cornerstone for regulating noncarcinogens while the no threshold dose–response relationship is generally considered in estimating risk for genotoxic carcinogens.

Several conclusions can be extracted from threshold dose–response data. First, when comparing chemicals X and Y (Fig. 1.2A), one can see that chemical X is more *potent*, that is, it elicits toxicity at lower levels of exposure. If you draw a line at the 50% response level, you can graphically estimate the dose of chemical X that would elicit toxicity in 50% of the individuals (around 1 mg/kg/day). Similarly, the dose of chemical Y that elicits toxicity in 50% of the individuals can be estimated at about 10 mg/kg/day. Thus you can consider based on the toxic response being measured that chemical X is roughly 10 times more potent than Chemical Y. Second, both chemicals can elicit the toxic response in essentially all of the individuals exposed, as long as the dose is high enough. Third, these types of data allow you to operationally define a "no effect" or *no observed adverse effect level* (NOAEL). For a given dataset (in the case of Fig. 1.2A, doses of 0.01, 0.03, 0.1, 0.3, 1, and 3 mg/kg/day), the highest dose in the study associated with no toxicity is defined as the NOAEL. For chemical X, the NOAEL would thus be defined as 0.03 mg/kg/day, while the NOAEL for chemical Y would be 1 mg/kg/day. Chemical-specific NOAEL values derived primarily from experimental studies on chemicals that exhibit threshold dose–response relationships, along with considerations of uncertainty based on extrapolating results from animal studies to humans, and variability among different people, have historically been essential in estimating safe levels of exposures and protecting public health.

In contrast, Fig. 1.2B shows the second major type of dose–response relationship, that is, one in which no apparent threshold is exhibited. In this case, as before, increasing dose leads to an increased proportion of individuals exhibiting toxicity, but there is no clear-cut "break" between exposures that do or do not elicit toxicity. Genotoxic carcinogens often exhibit non-threshold dose–response relationships. Even very low exposures may elicit some incidence of toxicity. The process for evaluating risk of chemicals that do not show a threshold is conducted by a different paradigm compared to those that show thresholds, based at least partly on the uncertainty of responses at very low levels of exposure, which are very difficult to study in experimental models for a variety of reasons.

Two substances with exceedingly different toxic potencies can be used to illustrate how both the chemical's inherent properties and the type of exposure interact to influence whether or not toxicity occurs. Let us first consider botulinum toxins. These toxins exist as a family of eight distinct polypeptides (referred to as types A–H) that are produced by the bacterium, *Clostridium botulinum* and/or related microorganisms. Severe muscle paralysis is a potentially lethal response to botulinum toxin exposure. Nerve cells in complex organisms communicate with other neurons (and other cell types, e.g., muscle cells) by releasing specific neurotransmitters which interact directly with the target cell (see Chapter 6: Disruption of extracellular signaling and Chapter 20: Nervous system). All subtypes of botulinum toxin act by binding to specific proteins within the nerve terminal to block neurotransmitter release and thereby disrupt cellular communication.[5] Neurons that supply or *innervate* skeletal muscles release the neurotransmitter acetylcholine to cause that muscle cell to contract. A botulinum toxin acting on those neurons will therefore block acetylcholine release, leading to reduced muscle contractions and potentially paralysis of the affected muscles.

Botulinum toxin A is considered the most toxic substance known to man, with reported LD_{50} values in the low ng/kg range (i.e., an amount approximately 100 trillion-fold lower than the weight of a human).[6] It would therefore make inherent sense to avoid *any* exposure to these exceptionally toxic substances. As is well known however, botulinum toxins have

been developed as therapeutic agents to reduce muscle contractions in disorders that are associated with excessive muscle contractions. Moreover, therapeutic applications for botulinum toxins to treat other medical conditions continue to be pursued.[7] Thus the most potent toxic substances in the world can be used effectively and safely, but only by understanding their inherent toxic potential and by strictly controlling exposure.

On the other end of the spectrum from botulinum toxins is water, an absolutely essential substance for all living organisms on Earth. One would assume that any hazard associated with systemic water exposure would be minimal, and that is in fact, generally the case. Water is not without an inherent capacity to do harm, however. A reduction in blood sodium levels (hyponatremia) by excess water consumption can increase fluid uptake due to disruption of the sodium concentration gradient between blood and the organs/tissues. If excess fluid accumulates in the brain, swelling of the tissue will lead to increased pressure (due to the rigid, bony skull) and damaged/dead cells within the brain, potentially leading to severe effects including seizures, unconsciousness, respiratory arrest, and death.

Excessive water consumption has been reported in attempts to dilute a person's urine before a drug test, leading to serious complications.[8] Although infrequent, cases of child abuse have been reported involving forced water consumption and subsequent water intoxication.[9] Some case studies report excessive water intake and water intoxication in marathon runners after a race. What is clear from these examples is that although water is absolutely essential for all living organisms, excessive intake (as with any substance) can lead to toxicity. Botulinum toxins and water therefore provide evidence that on the one hand all chemicals are toxic, and on the other even the most toxic substances can be used safely.

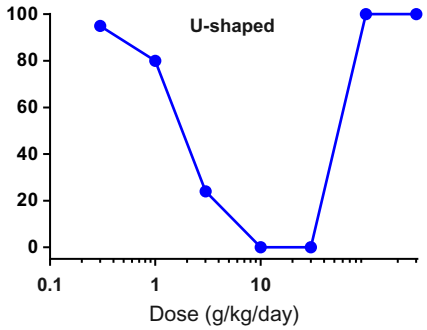

FIGURE 1.3 A U-shaped dose–response relationship. This type of relationship is exhibited by essential substances.

The extreme case of water intoxication provides the opportunity to consider a third type of dose–response relationship, one that is exhibited by substances which are essential for the organism. Fig. 1.3 shows a hypothetical dose–response relationship for water intoxication. Very low water is associated with dehydration, with fluid levels insufficient to maintain homeostasis, tissue hydration, ionic balances, and sufficient blood volume, leading to some form(s) of toxicity. Within a certain range of higher exposures, fluid homeostasis is maintained and no adverse effects are noted. With excessive (much higher) exposures however, adverse effects occur which can be life-threatening.

Other types of dose–response relationships can be observed. For example, some endocrine disrupting chemicals (see Chapter 17: Organ system effects: endocrine toxicology) have been reported to elicit toxicity at low levels of exposure, but not at higher levels. Some chemicals can elicit *beneficial* effects at low levels of exposure, but adverse effects with higher exposures. These other *nonmonotonic dose*–response relationships may be based on adaptive changes (e.g., receptor upregulation or downregulation) or feedback loops that occur at one end of the dosing spectrum, but not at the other.