THIRD EDITION

PRINCIPLES OF TOXICOLOGY

Environmental and Industrial Applications

Edited by

Stephen M. Roberts

Robert C. James

Phillip L. Williams

WILEY

PRINCIPLES OF TOXICOLOGY

Environmental and Industrial Applications

Third Edition

Edited By

STEPHEN M. ROBERTS
Professor and Program Director,
Center for Environmental & Human Toxicology,
University of Florida,
Gainesville, FL

ROBERT C. JAMES
Senior Principal Scientist,
ToxStrategies, Inc.,
Boise, ID

PHILLIP L. WILLIAMS
Dean, College of Public Health and Georgia Power Professor of Environmental Health Science,
University of Georgia,
Athens, GA

WILEY

Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada

Library of Congress Cataloging-in-Publication Data:

Principles of toxicology : environmental and industrial applications / [edited by] Stephen M Roberts, Robert C James, Phillip L Williams. – Third edition.
pages cm
Includes index.
ISBN 978-0-470-90791-7 (cloth)
1. Toxicology. 2. Industrial toxicology. 3. Environmental toxicology. I. Williams, Phillip L., 1952- II. James, Robert C.
III. Roberts, Stephen M., 1950-
RA1211.P746 2014
615.9′02–dc23

2014020557

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

PRINCIPLES OF TOXICOLOGY

CONTRIBUTORS

T. Renée Anthony, Ph.D., Associate Professor, Department of Occupational and Environmental Health, University of Iowa, University of Iowa, College of Public Health, Iowa City, IA

Lisa A. Bailey, Ph.D., Senior Health Scientist, Gradient, Cambridge, MA

Barbara D. Beck, Ph.D., DABT, FATS, Principal, Gradient, Cambridge, MA

Michael S. Bloom, Ph.D., Associate Professor, Departments of Environmental Health Sciences and Epidemiology and Biostatistics, University at Albany School of Public Health, State University of New York, Rensselaer, NY

Janice Britt, Ph.D., Senior Managing Scientist, ToxStrategies, Inc., Tallahassee, FL

Brenda S. Buikema, M.D., M.P.H, Assistant Professor (Clinical), Department of Occupational and Environmental Health, University of Iowa, College of Public Health; and Division of Pulmonary, Department of Internal Medicine, Critical Care, and Occupational Medicine, University of Iowa, Carver College of Medicine, Iowa City, IA

William M. Caudle, Ph.D., Assistant Professor, Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA

Harvey J. Clewell III, Ph.D., DABT, Director, Center for Human Health Assessment, Hamner Institute for Health Sciences, Research Triangle Park, NC

Rebecca A. Clewell, Ph.D., Hamner Institute for Health Sciences, Research Triangle Park, NC

Mary Jane Cunningham, Ph.D., Founder and President, Nanomics Biosciences, Inc., The Woodlands, TX

Steve Diamond, Ph.D., Vice President, Mid-West Division, Nanosafe, Duluth, MN

Michael R. Franklin, Ph.D., Professor, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT

Michele M. Garber, M.P.H., Senior Scientist, Hazardous Substance & Waste Management Research, Tallahassee, FL

Vincent F. Garry, M.D., MS., DABT, Professor (Emeritus), Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN

Fredric Gerr, M.D., Professor of Occupational and Environmental Health and Professor of Epidemiology, University of Iowa, College of Public Health; Iowa City, IA

Sherilyn A. Gross, Ph.D., Senior Health Scientist, Cardno ChemRisk Inc., Boulder, CO

Robert H. Heflich, Ph.D., U.S. Food and Drug Administration, National Center for Toxicological Research, Jefferson, AR

Georgia Hinkley, Ph.D., Research Associate, Center for Environmental & Human Toxicology, University of Florida, Gainesville, FL

Mary L. Hixon, Ph.D., Senior Toxicologist, Gradient Corporation, Cambridge, MA

Keith A. Houck, Ph.D., Principal Investigator, National Center for Computational Toxicology, Office of Research & Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Robert C. James, Ph.D., Senior Principal Scientist, ToxStrategies, Inc., Boise, ID

Richard S. Judson, Ph.D., Principal Investigator, National Center for Computational Toxicology, Office of Research & Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

James E. Klaunig, Ph.D., Professor, Department of Environmental Health, Indiana University, Ellettsville, IN

Sailesh Konda, M.D., Procedural Dermatology/Mohs Surgery Fellow, Department of Dermatology, Loma Linda University Medical Center, Loma Linda, CA

J. Michael Kuperberg, Ph.D., Center for Biomedical & Toxicological Research, Florida State University, Tallahassee, FL

Lawrence H. Lash, Ph.D., Professor and Associate Chair, Department of Pharmacology, Wayne State University, School of Medicine, Detroit, MI

Ari S. Lewis, M.S., Principal Scientist, Gradient, Cambridge, MA

Cuiqing Liu, Ph.D., Postdoctoral Researcher, Davis Heart and Lung Research Institute, College of Medicine, The Ohio State University, Columbus, OH; and Associate Professor, Department of Physiology, Hangzhou Normal University, Hangzhou, People's Republic of China

Hongbo Ma, Ph.D., Assistant Professor, Zilber School of Public Health, University of Wisconsin – Milwaukee, Milwaukee, WI

Howard I. Maibach, M.D., ATS, Professor, Department of Dermatology, University of California San Francisco, San Francisco, CA

David B. Mayfield, M.S., DABT, Senior Toxicologist, Gradient, Seattle, WA

Gary W. Miller, Ph.D., Professor and Associate Dean for Research, Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA

Martha M. Moore, Ph.D., Senior Manager, ENVIRON International Corp., Little Rock, AR

Meagan B. Myers, Ph.D., Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR

David M. Reif, Ph.D., Associate Professor, Department of Biological Sciences, Bioinformatics Research Center, North Carolina State University, Raleigh, NC

Stephen M. Roberts, Ph.D., Professor and Program Director, Center for Environmental & Human Toxicology, University of Florida, Gainesville, FL

Eric S. Sobel, M.D., Ph.D., Associate Professor and Program Director, Rheumatology and Clinical Immunology, University of Florida College of Medicine, Gainesville, FL

Leah D. Stuchal, Ph.D., Research Assistant Professor, Center for Environmental & Human Toxicology, University of Florida, Gainesville, FL

Qinghua Sun, M.D., Ph.D, Divisions of Environmental Health Sciences & Cardiovascular Medicine, Davis Heart & Lung Research Institute, College of Public Health, The Ohio State University, Columbus, OH

Christopher M. Teaf, Ph.D., Associate Director, Center for Biomedical & Toxicological Research, Florida State University, Tallahassee, FL

Peter L. Truran, Ph.D., Resident Fellow, Center for Philosophy of Science, University of Minnesota, Minneapolis, MN

John E. Vena, Ph.D., Professor and Founding Chair, Department of Public Health Sciences, Medical University of South Carolina, Charleston SC

Phillip L. Williams, Ph.D., Dean, College of Public Health and Georgia Power Professor of Environmental Health Science, University of Georgia, Athens, GA

PREFACE

PURPOSE OF THIS BOOK

Principles of Toxicology: Environmental and Industrial Applications presents compactly and efficiently the scientific basis to toxicology as it applies to the workplace and the environment. The book covers the diverse chemical hazards encountered in the modern work place and natural environment and provides a practical understanding of these hazards for those concerned with protecting the health of humans and ecosystems.

INTENDED AUDIENCE

This book is a third edition and represents an update and expansion on the previous, very successful texts. The first edition of this book was entitled *Industrial Toxicology: Safety and Health Applications in the Workplace.* The current edition retains the emphasis on applied aspects of toxicology, while extending its scope to cover new areas of toxicology such as toxicokinetics, omics technology, nanotoxicology, and computational toxicology. The book was written for those health professionals who need toxicological information and assistance beyond that of an introductory text in general toxicology, yet more practical than that in advanced scientific works on toxicology. In particular, we have in mind industrial hygienists, occupational physicians, safety engineers, environmental health practitioners, occupational health nurses, safety directors, and environmental scientists.

ORGANIZATION OF THE BOOK

This volume consists of 23 chapters. The early chapters establish the scientific basis to toxicology, which is then applied through the rest of the book. It discusses concepts such as absorption, distribution, and elimination of toxic agents from the body. Chapters 5–11 discuss the effects of toxic agents on specific physiological organs or systems, including the blood, liver, kidneys, nerves, skin, lungs, and the immune system.

The next part of the book addresses specific areas of concern in the occupational and environmental settings—both toxic agents and their manifestations. Chapters 12–15 examine the areas of great research interest—reproductive toxicology, developmental toxicology, mutagenesis, and carcinogenesis. Chapters 16–18 examine the toxic effects of metals, pesticides, and organic solvents.

The final part of the book is devoted to specific areas and applications of the toxicological principles from both the environmental and occupational settings. Chapters 19 and 20 cover the emerging areas of nanotoxicology and computational toxicology. Chapters 21 and 22 discuss epidemiologic issues and occupational/environmental health. Chapter 23 covers risk assessment.

FEATURES

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

- The book is compact and practical, and the information is structured for easy use by the health professionals in both industry and government.
- The approach is scientific, but applied, rather than theoretical. In this it differs from more general works in toxicology, which fail to emphasize the information pertinent to the industrial environment.
- The book consistently stresses evaluation and control of toxic hazards.

- Numerous illustrations and figures clarify and summarize key points.
- Case histories and examples demonstrate the application of toxicological principles.
- Chapters include suggested reading bibliographies to provide the reader with additional useful information.
- A comprehensive glossary of toxicological terms is included.

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

CONTENTS

1

GENERAL PRINCIPLES OF TOXICOLOGY

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

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

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

1.1 BASIC DEFINITIONS AND TERMINOLOGY

The literal meaning of the term *toxicology* is "the study of poisons." The root word toxic entered the English language around 1655 from the Late Latin word *toxicus* (which meant poisonous), itself derived from *toxikón*, an ancient Greek term for poisons into which arrows were dipped. The early history of toxicology focused on the understanding and uses of different poisons, and perhaps even today most people tend to think of a chemical or products labeled as a "toxic" substance" as that group of chemicals for which minimal exposure inevitably leads to death or some serious long-term adverse effect like cancer. As toxicology has evolved into a modern science it has expanded to encompass all forms of adverse health effects that any substance might produce. The following definitions are provided to help the reader understand several basic terms that may be used in this and other chapters:

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

Toxicity—any toxic (adverse) effect that a chemical or physical agent might produce within a living organism.

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

As these definitions indicate, the toxic responses that form the study of toxicology span a broad biological and physiological spectrum. Effects of interest may range from something relatively minor such as irritation or tearing to a more serious response like acute and reversible liver or kidney damage, to an even more serious and permanent disability such as cirrhosis of the liver or liver cancer. Given this broad range of potentially adverse effects to consider, it

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

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

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

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

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

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

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

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

Subacute exposure—resembles acute exposure except that the exposure duration is greater, for example, from several days to 1 month in animal studies.

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

Chronic exposure—exposures (either repeated or continuous) over a long period of time. In animal testing this exposure ranges between 90 days to a lifetime. It is generally any exposure that occurs for the majority of that species' lifetime. In occupational settings it is generally considered to be for a number of years or more and may include either a working lifetime or an entire lifetime of an individual.

Acute toxicity—an adverse or undesirable effect that is manifested within a relatively short time interval ranging from almost immediately to within several days following exposure (or dosing). An example would be chemical asphyxiation from exposure to a high concentration of carbon monoxide (CO).

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

Local toxicity—an adverse or undesirable effect that is manifested at the toxicant's site of contact with the organism. Examples include an acid's ability to cause burning of the eyes, upper respiratory tract irritation, and skin burns.

Systemic toxicity—an adverse or undesirable effect that can be seen anywhere within the organism. It typically involves an organ in the body with selective tissue vulnerability to the toxic effect of the chemical distant from the point of entry of the toxicant (i.e., toxicant requires absorption and distribution within the organism to produce a systemic effect). Examples would include the adverse effects on the kidney or central nervous system (CNS) resulting from the acute or chronic ingestion of mercury.

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

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

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

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

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

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

Toxin—any toxicant produced by an organism (floral or faunal, including bacteria), that is, naturally produced toxicants. An example would be the pyrethrins, which are natural pesticides produced by pyrethrum flowers (i.e., certain chrysanthemums) that serve as the model for the man-made insecticide class pyrethroids.

Potency—a measure of the ability of a chemical to express its toxicity per unit of dose or dosage. The more potent a chemical, the less dosage needed to induce the toxicity it produces. In general terms, the less potent a chemical is, the safer it is because the probability of achieving a dose sufficient to induce toxicity via a particular route of exposure is lessened. Similarly, more potent chemicals tend to be more dangerous because it takes a smaller dose from an exposure to be able to induced toxicity.

Hazard—the qualitative nature of the adverse or undesirable effect (i.e., the type of adverse effect or toxicity the chemical produces) resulting from exposure to a particular toxicant or physical agent. For example, asphyxiation is the hazard from acute exposures to CO. Cancer, liver toxicity, and immunotoxicity are other hazards (types of toxicities) a chemical exposure might potentially represent. A hazard typically refers to the kind(s) of toxic effect(s) the chemical can produce if the exposure/dose is sufficient.

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

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

Risk assessment—the process by which the potential (or probability of) adverse health effects of exposure are characterized. In risk assessment, a safe exposure concentration is extrapolated from the dose–response curve for an adverse effect produced by the chemical that is used to derive a safe exposure concentration. Alternatively, a risk assessment might determine the probability and/or acceptability of a toxicity occurring at a known or measured exposure level.

1.2 TOXICOLOGY: A DIVERSE SCIENCE WITH TWO BASIC GOALS

Toxicology has become a science that builds on and uses knowledge developed in many related medical sciences, such as physiology, biochemistry, pathology, pharmacology, medicine, and epidemiology, to name only a few. Toxicology has evolved from the study of poisons to the study of all adverse effects induced by all chemicals or substances. Although toxicology is a science where a number of areas of specialization have evolved, all toxicologists fall into three principal areas of endeavor: descriptive toxicology, research/mechanistic toxicology, and applied toxicology.

Descriptive toxicologists are scientists whose work focuses on the toxicity testing of chemicals. This work is done primarily at commercial and governmental toxicity testing laboratories, and the studies performed at these facilities are designed to generate basic toxicity information that identifies the various organ toxicities (hazards) the test agent is capable of inducing over those exposure conditions necessary to induce each effect. A thorough description of a chemical's toxicology would identify all possible acute and chronic toxicities, including the genotoxic, reproductive, teratogenic (developmental), and carcinogenic potential of the test agent. It would identify important metabolites of the chemical that are generated as the body attempts to break down and eliminate the chemical, as well as understand how the chemical is absorbed into the body and distributed to tissues throughout the body, identify tissue accumulation or elimination, and ultimately determine how it is excreted from the body. Hopefully, appropriate dose–response test data are generated for those toxicities of greatest concern and that toxicity produced at the lowest dose during the completion of the descriptive studies so that the relative safety of any given exposure or dose level that humans might typically encounter can be predicted.

Basic research or mechanistic toxicologists are scientists who study the chemical or agent in depth for the purpose of gaining an understanding of how the chemical or agent initiates those biochemical or physiological changes within the cell or tissue that result in the toxicity (adverse effect). The goal of mechanistic studies is to understand the specific biological reactions (i.e., the adverse chain of events) within the affected organism that ultimately result in the toxic effect being studied. Mechanistic experiments are performed at the molecular, biochemical, cellular, and tissue level of the affected organism. So, mechanistic assessments may incorporate and apply the knowledge of a number of many other related scientific disciplines within the biological and medical sciences (e.g., physiology, biochemistry, genetics, molecular biology, pathology). Because animal species are generally used to identify chemical-induced hazards, and because there may be significant species-specific responses to a chemical, mechanistic studies help provide the information on those key changes required to induce toxicity, and help reduce the uncertainty of the animal-to-human extrapolation we need to make to develop a safe exposure guideline.

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

safe exposure guidelines for each exposure pathway (e.g., air, skin, ingestion exposure to the chemical) in that environment. Toxicologists who work in this area of toxicology use descriptive and mechanistic toxicity studies to limit the dose received by each or all exposure pathways to a total dose of the chemical that is believed to be safe. The process whereby this safe dose or level of exposure is derived is generally referred to as the area of *risk assessment.* Within applied toxicology a number of subspecialties occur. *Forensic toxicology* is that unique combination of analytical chemistry, pharmacology, and toxicology concerned with the medical and legal aspects of drugs and poisons; it is concerned with the determination of which chemicals are present and responsible in exposure situations of abuse, overdose, poisoning, and death that become of interest to the police, medical examiners, and coroners. *Clinical toxicology* specializes in ways to treat poisoned individuals and focuses on determining and understanding the toxic effects of medicines, simple over-the-counter (nonprescription) drugs, and other household products. *Environmental toxicology* is the subdiscipline concerned with those chemical exposure situations found in our general living environment. These exposures may stem from the agricultural application of chemicals, the release of chemicals during modern-day living (e.g., chemicals released by household products), regulated and unintentional industrial discharges into air or waterways, and various nonpoint emission sources (e.g., the combustion by-products of cars). Within this area there may be even further subspecialization (e.g., ecotoxicology, aquatic toxicology, mammalian toxicology, avian toxicology). *Occupational toxicology* is the subdiscipline concerned with the chemical exposures and diseases found in the workplace, the identification of the hazards or injuries that overexposure to an occupationally used chemical might represent, and the prevention of these exposures or the treatment of the injuries they might produce.

Regardless of the specialization within toxicology, or the types of toxicities of major interest to the toxicologist, essentially every toxicologist performs one or both of the two basic functions of toxicology, which are to (1) examine the nature of the adverse effects produced by a chemical or physical agent (*hazard/toxicity identification* function) and (2) assess the probability of these toxicities occurring under specific conditions of exposure (*dose–response and risk assessment* function). Ultimately, the goal and basic purpose of toxicology is to understand the toxic properties of a chemical so that these adverse effects can be prevented by the development of appropriate handling or exposure guidelines.

1.3 HAZARD IDENTIFICATION FUNCTION

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

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

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

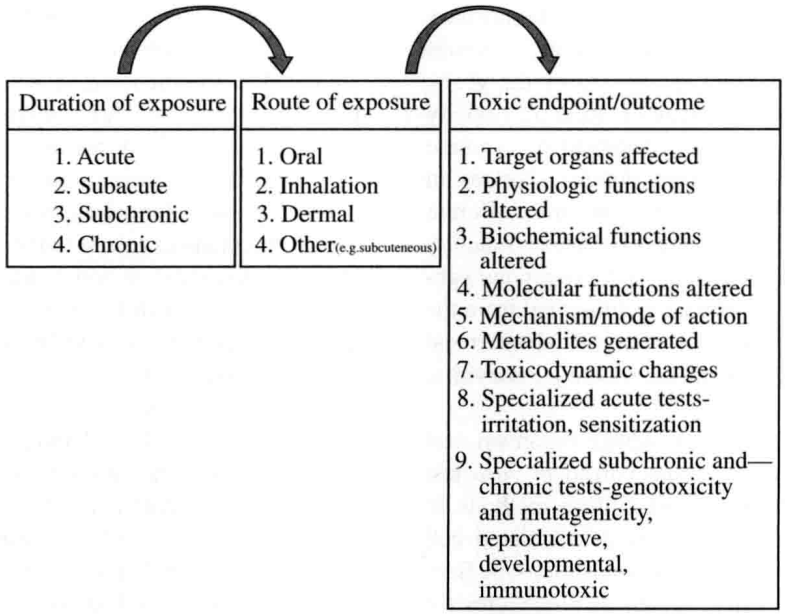

FIGURE 1.1 A generic toxicity testing scheme that shows the ways in which a toxicity test might differ because of the different choices to be made regarding the duration of exposure, the route of exposure, or the endpoint to be measured in the study.

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

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

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

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

For example, besides animal or whole organism test results, a toxicologist might use a specialized *in vitro* test system that involves test tube or cell culture methods to examine effects on cellular macromolecules, isolated cell fractions, cellular organelles (e.g., mitochondria), tissue fractions, and isolated perfused whole organs as procedures for examining specific molecular, physiological, or biological functions. A toxicologist might also perform *in vivo* tests in a variety of nonmammalian organisms ranging from simple, single cell organisms (e.g., bacteria, algae) to larger and more complex nonmammalian organisms like nematodes, fruit flies, *Daphnia magna*, or fish, particularly when attempting to identify the ecological hazards or an environmental pollutant.

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

In addition to animal methods, hazard information associated with human exposure to the chemical may also be available. As discussed in more detail elsewhere, there can be significant species differences in the both the beneficial and adverse responses induced by a chemical. So, in the final hazard assessment for a chemical, a toxicologist would like to review as much human data as are available. There are four basic categories of epidemiological information that can assist the hazard evaluation. These categories are occupational epidemiology (mortality and morbidity studies), clinical exposure studies, accidental acute poisonings, and chronic environmental epidemiology studies. The advantages and disadvantages of the hazard information typically provided by these four categories of human toxicological information and that of traditional *in vitro* and animal toxicity tests are summarized and compared in Table 1.2.

1.4 DOSE–RESPONSE/RISK ASSESSMENT FUNCTION

It is probably safe to say that among lay individuals there exists considerable confusion about the term toxic. If asked, most lay individuals would probably define a toxic substance using either a definition that one would apply to highly poisonous or very potently toxic chemicals or something that implies that only some chemicals produce adverse effects in humans and so can be described as toxic chemicals or those substances that we should all avoid. To help illustrate this point, and to begin to emphasize the fact that the toxicity is a function of dose, the reader is invited to take the following pop quiz. First, cross-match the doses shown in column A that produce lethality in 50% of the animals (lethal dose [LD_{50}]) with the chemicals listed in column B. These chemicals are a collection of food additives, medicines, drugs of abuse, poisons, pesticides, and hazardous substances for which the correct LD_{50} is listed somewhere in column A. To perform this cross-matching, first photocopy Table 1.3 and simply mark the ranking of the dose (i.e., the number corresponding next to the dose in column A) you believe correctly corresponds to the chemical it has been measured for in column B. (*Note:* The doses are listed in descending order, and the chemicals have been listed alphabetically. So, the three chemicals you believe to be the safest should have the three largest doses [you should rank them as 1, 2, and 3], and the more unsafe or dangerous you perceive the chemical to be, the higher the numerical ranking you should give it. After testing yourself with the chemicals