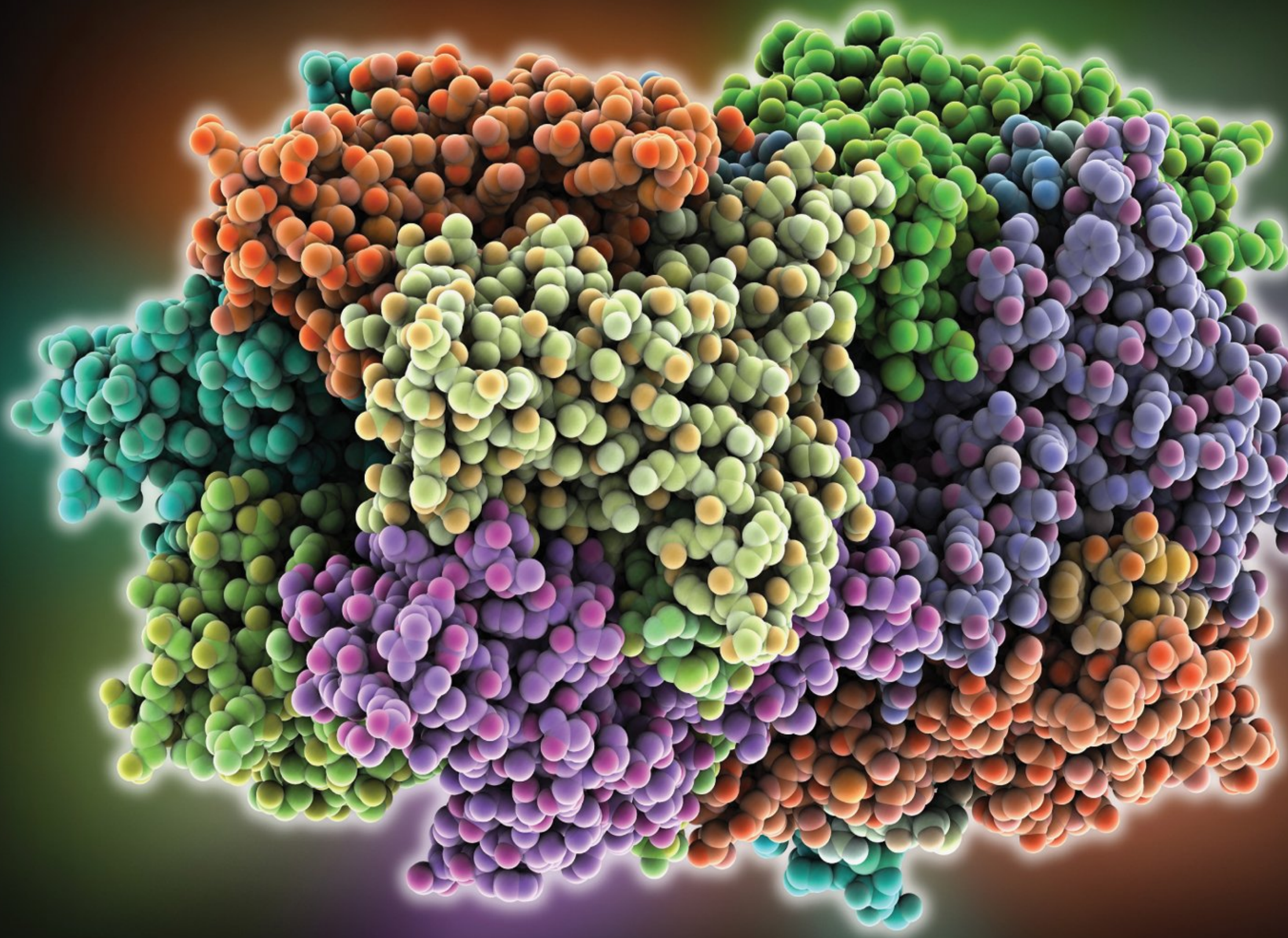
Curtis D. Klaassen
John B. Watkins III



CASARETT & DOULL'S

ESSENTIALS of TOXICOLOGY

Third Edition

Mc Graw Hill Education
LANGE®
INTERNATIONAL EDITION

Casarett & Doull's Essentials of Toxicology

NOTICE

Medicine is an ever-changing science. As new research and clinical experience broaden our knowledge, changes in treatment and drug therapy are required. T e authors and the publisher of this work have checked with sources believed to be reliable in their efforts to provide information that is complete and generally in accord with the standards accepted at the time of publication. However, in view of the possibility of human error or changes in medical sciences, neither the authors nor the publisher nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they disclaim all responsibility for any errors or omissions or for the results obtained from use of the information contained in this work. Readers are encouraged to confirm the information contained herein with other sources. For example and in particular, readers are advised to check the product information sheet included in the package of each drug they plan to administer to be certain that the information contained in this work is accurate and that changes have not been made in the recommended dose or in the contraindications for administration. T is recommendation is of particular importance in connection with new or infrequently used drugs.

Casarett & Doull's Essentials of Toxicology

Third Edition

Editors

Curtis D. Klaassen, PhD
University Distinguished Professor
Division of Gastroenterology
Department of Internal Medicine
University of Kansas Medical Center
Kansas City, Kansas

John B. Watkins III, PhD
Associate Dean and Director
Professor of Pharmacology and *T*oxicology
Medical Sciences Program
Indiana University School of Medicine
Bloomington, Indiana

Mc Graw Hill Medical

New York Chicago San Francisco Lisbon London Madrid Mexico City
Milan New Delhi San Juan Seoul Singapore Sydney Toronto

ISBN: 978-0-07-184709-4

MHID: 0-07-184709-X

The material in this eBook also appears in the print version of this title: ISBN: 978-0-07-184708-7,
MHID: 0-07-184708-1.

eBook conversion by codeMantra
Version 1.0

Contents

Contributors

S. Satheesh Anand, PhD, DABT
Senior Research Toxicologist
Haskell Global Centers for Health and Environmental Sciences
Newark, Delaware
Chapter 24

Michael Aschner, PhD
Professor
Department of Pediatrics
Vanderbilt University Medical Center
Nashville, Tennessee
Chapter 16

T omas M. Badger, PhD
Distinguished Faculty Scholar
Professor
Departments of Pediatrics and Physiology/Biophysics
University of Arkansas for Medical Sciences
Director
Arkansas Children's Nutrition Center
Little Rock, Arkansas
Chapter 27

John C. Bloom, VMD, PhD
President
Bloom Consulting Services, LLC
Special Government Employee
FDA
Adjunct Professor of Pathology
Schools of Veterinary Medicine
University of Pennsylvania and Purdue University
Indianapolis, Indiana
Chapter 11

Windy A. Boyd, PhD
Biologist
Biomolecular Screening Branch
National Toxicology Program Division
National Institute of Environmental Health Sciences, NIH
Research Triangle Park, North Carolina
Chapter 23

William K. Boyes, PhD
Neurotoxicology Branch
Toxicity Assessment Division
National Health and Environmental Effects Research Laboratory
Office of Research and Development
US Environmental Protection Agency
Research Triangle Park, North Carolina
Chapter 17

John T. Brandt, MD
Eli Lilly & Co. (retired)
Indianapolis, Indiana
Chapter 11

James V. Bruckner, PhD
Professor of Pharmacology & Toxicology
Department of Pharmaceutical & Biomedical Sciences
College of Pharmacy
University of Georgia
Athens, Georgia
Chapter 24

David B. Buckley, PhD
Chief Scientific Officer
XenoTech, LLC
Lenexa, Kansas
Chapter 6

George A. Burdock, PhD, DABT, FACN
President
Burdock Group Consultants
Orlando, Florida
Chapter 31

Louis R. Cantilena Jr., MD, PhD
Professor, Medicine and Pharmacology
Department of Medicine
Uniformed Services University
Bethesda, Maryland
Chapter 33

Daniel L. Costa, PhD
Office of Research and Development
National Program Director for Air, Climate, and Energy
Research Program
US Environmental Protection Agency
Research Triangle Park, North Carolina
Chapter 29

Lucio G. Costa, PhD
Professor
Department of Environmental and Occupational
Health Sciences
School of Public Health
University of Washington
Seattle, Washington
Chapter 22

Maciej Czerwinski, PhD
Principal Scientist
XenoTech, LLC
Lenexa, Kansas
Chapter 6

Richard T. Di Giulio, PhD
Professor
Nicholas School of the Environmental
Duke University
Durham, North Carolina
Chapter 30

David L. Eaton, PhD
Professor
Department of Environmental and Occupational
Health Sciences
Associate Vice Provost for Research
University of Washington
Seattle, Washington
Chapter 2

Elaine M. Faustman, PhD
Professor
Institute for Risk Analysis and Risk Communication
Department of Environmental and Occupational
Health Sciences
School of Public Health
University of Washington
Seattle, Washington
Chapter 4

Jodi A. Flaws, PhD
Professor
Department of Comparative Biosciences
University of Illinois
Urbana, Illinois
Chapter 21

Paul M.D. Foster, PhD
Chief
Toxicology Branch
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina
Chapter 20

Donald A. Fox, PhD
Professor of Vision Sciences
Biology and Biochemistry, Pharmacology, and Health and
Human Performance
University of Houston
Houston, Texas
Chapter 17

Jonathan H. Freedman, PhD
Laboratory of Toxicology and Pharmacology
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina
Chapter 23

Michael A. Gallo, PhD
Environmental and Occupational Health Sciences
Institute
Rutgers-T e State University of New Jersey
UMDNJ-Robert Wood Johnson Medical School
Piscataway, New Jersey
Chapter 1

Steven G. Gilbert, PhD
Director
Institute of Neurotoxicology & Neurological Disorders
Seattle, Washington
Chapter 2

Bruce A. Goldberger, PhD
Professor and Director of Toxicology
Departments of Pathology and Psychiatry
University of Florida College of Medicine
Gainesville, Florida
Chapter 32

Terry Gordon, PhD
Professor
Department of Environmental Medicine
NYU School of Medicine
Tuxedo, New York
Chapter 29

L. Earl Gray Jr., PhD
Reproductive Toxicology Branch
United States Environmental Protection Agency
Adjunct Professor
North Carolina State University
Raleigh, North Carolina
Chapter 20

Zoltán Gregus, MD, PhD, DSc, DABT
Professor
Department of Pharmacology and T erapeutics
Toxicology Section
University of Pecs
Medical School
Pecs, Hungary
Chapter 3

David G. Hoel, PhD
Principal Scientist
Exponent, Inc
Alexandria, Virginia
Distinguished University Professor
Department of Medicine
Medical University of South Carolina
Charleston, South Carolina
Chapter 25

George R. Hof mann, PhD
Professor
Department of Biology
College of the Holy Cross
Worcester, Massachusetts
Chapter 9

Michael P. Holsapple, PhD, ATS
Senior Research Leader
Systems Toxicology
Health and Life Sciences Global Business
Battelle Memorial Institute
Columbus, Ohio
Chapter 12

Patricia B. Hoyer, PhD
Professor
Department of Physiology
College of Medicine
T e University of Arizona
Tucson, Arizona
Chapter 21

Robert H. Hurt, PhD
Professor
School of Engineering
Director
Institute for Molecular and Nanoscale Innovation
Brown University
Providence, Rhode Island
Chapter 28

Hartmut Jaeschke, PhD, ATS
Professor and Chair
Department of Pharmacology, Toxicology & T erapeutics
University of Kansas Medical Center
Kansas City, Kansas
Chapter 13

Norbert E. Kaminski, PhD
Professor
Department of Pharmacotherapy and Toxicology
Director
Center for Integrative Toxicology
Michigan State University
East Lansing, Michigan
Chapter 12

Agnes B. Kane, MD, PhD
Professor
Department of Pathology and Laboratory Medicine
Brown University
Providence, Rhode Island
Chapter 28

Y. James Kang, DVM, PhD, FATS
Professor and Distinguished University Scholar
Department of Pharmacology and Toxicology
University of Louisville School of Medicine
Louisville, Kentucky
Chapter 18

Barbara L.F. Kaplan, PhD
Assistant Professor
Center for Integrative Toxicology
Department of Pharmacology and Toxicology and
Neuroscience
Program
Michigan State University
East Lansing, Michigan
Chapter 12

Faraz Kazmi, BS
Senior Scientist
XenoTech, LLC
Lenexa, Kansas
Chapter 6

Rebecca D. Kapler, PhD
School of Freshwater Sciences
University of Wisconsin-Milwaukee
Milwaukee, Wisconsin
Chapter 28

James E. Klaunig, PhD, ATS, IATP
Professor
Environmental Health
Indiana University
Bloomington, Indiana
Chapter 8

Frank N. Kotsonis, PhD
Retired Corporate Vice President
Worldwide Regulatory Sciences
Monsanto Corporation
Skokie, Illinois
Chapter 31

Lois D. Lehman-McKeeman, PhD
Distinguished Research Fellow
Discovery Toxicology
Bristol-Myers Squibb Company
Princeton, New Jersey
Chapter 5

George D. Leikauf, PhD
Professor
Department of Environmental and Occupational Health
Graduate School of Public Health
University of Pittsburgh
Pittsburgh, Pennsylvania
Chapter 15

T eodora M. Mauro, MD
Professor and Vice-Chair
Dermatology Department
University of California, San Francisco
Service Chief
Dermatology
San Francisco Veterans Medical Center
San Francisco, California
Chapter 19

Virginia C. Moser, PhD, DABT, FATS
Toxicologist
Toxicity Assessment Division
National Health and Environmental Effects Research Laboratory
US Environmental Protection Agency
Research Triangle Park, North Carolina
Chapter 16

Michael C. Newman, MS, PhD
A. Marshall Acuff Jr. Professor
Virginia Institute of Marine Science
College of William & Mary
Gloucester Point, Virginia
Chapter 30

Gunter Oberdörster, DVM, PhD
Professor
Department of Environmental Medicine
University of Rochester
School of Medicine & Dentistry
Rochester, New York
Chapter 28

Brian W. Ogilvie, BA
Principal Scientist
XenoTech, LLC
Lenexa, Kansas
Chapter 6

Gilbert S. Omenn, MD, PhD
Professor of Internal Medicine, Human Genetics and Public Health
Director
Center for Computational Medicine and Bioinformatics
University of Michigan Department of Computational Medicine and Bioinformatics
Ann Arbor, Michigan
Chapter 4

Oliver Parkinson, PhD
XPD Consulting, LLC
Shawnee, Kansas
Chapter 6

Andrew Parkinson, PhD
CEO
XPD Consulting, LLC
Shawnee, Kansas
Chapter 6

Martin A. Philbert, PhD
Professor of Toxicology and Dean
School of Public Health
University of Michigan
Ann Arbor, Michigan
Chapter 16

R. Julian Preston, MA, PhD
Associate Director for Health
National Health and Environmental Effects Research Laboratory
US Environmental Protection Agency
Research Triangle Park, North Carolina
Chapter 9

Robert H. Rice, PhD
Professor
Department of Environmental Toxicology
University of California
Davis, California
Chapter 19

Rudy J. Richardson, ScD, DABT
Toxicology Program
University of Michigan School of Public Health
Neurology Department
University of Michigan School of Medicine
Ann Arbor, Michigan
Chapter 16

John M. Rogers, PhD
Toxicity Assessment Division
National Health and Environmental Effects Research Laboratory
Office of Research and Development
United States Environmental Protection Agency
Research Triangle Park, North Carolina
Chapter 10

Martin J. Ronis, BA, MA, PhD
Professor
Department of Pharmacology & Toxicology
College of Medicine
University of Arkansas for Medical Sciences
Associate Director for Basic Research
Arkansas Children's Nutrition Center
Arkansas Children's Hospital Research Institute
Little Rock, Arkansas
Chapter 27

Andrew E. Schade, MD, PhD
Senior Director
Clinical Diagnostics Laboratory
Diagnostics Research and Development
Eli Lilly and Co.
Indianapolis, Indiana
Chapter 11

Rick G. Schnellmann, PhD
Professor and Chair
Department of Pharmaceutical and Biomedical Sciences
Medical University of South Carolina
Charleston, South Carolina
Chapter 14

Kartik Shankar, PhD, DABT
Arkansas Children's Nutrition Center
Department of Pediatrics
University of Arkansas for Medical Sciences
Little Rock, Arkansas
Chapter 27

Danny D. Shen, PhD
Professor
Departments of Pharmaceuticals and Pharmacy
School of Pharmacy
University of Washington
Seattle, Washington
Chapter 7

Courtney E.W. Sulentic, PhD
Associate Professor
Department of Pharmacology & Toxicology
Boonshof School of Medicine
Wright State University
Dayton, Ohio
Chapter 12

Peter S. T orne, MS, PhD
Professor and Head
Department of Occupational and Environmental Health
College of Public Health
T e University of Iowa
Iowa City, Iowa
Chapter 34

Erik J. Tokar, PhD
Biologist
Inorganic Toxicology Group
Division of the National Toxicology Program
National Toxicology Program
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina
Chapter 23

Michael P. Waalkes, PhD
Chief
National Toxicology Group
Division of the National Toxicology Program
National Toxicology Program
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina
Chapter 23

D. Alan Warren, MPh, PhD
Program Director
Environmental Health Science
University of South Carolina Beaufort
Beaufort, South Carolina
Chapter 24

John B. Watkins, III, PhD
Associate Dean and Director
Professor of Pharmacology and Toxicology
Medical Sciences Program
Indiana University School of Medicine
Bloomington, Indiana
Chapter 26

Diana G. Wilkins, MS, PhD
Director
Center for Human Toxicology
Research Associate Professor
Department of Pharmacology and Toxicology
University of Utah
Salt Lake City, Utah
Chapter 32

This page intentionally left blank

Preface

This updated full-color edition of Essentials of *T*oxicology distills the major principles and concepts of toxicology that were described in detail in the eighth edition of Casarett & Doull's *T*oxicology: The Basic Science of Poisons. We are grateful to the authors who contributed to the eighth edition of Casarett & Doull's *T*oxicology: The Basic Science of Poisons; their chapters in the parent text provided the foundation for the chapters in this edition of Essentials of *T*oxicology.

Essentials of *T*oxicology concisely describes the expansive science of toxicology, and includes important concepts from anatomy, physiology, and biochemistry to facilitate the understanding of the principles and mechanisms of toxicant action on specific organ systems. We trust that this book will assist students in undergraduate and graduate courses in toxicology, as well as students from other disciplines, to develop a strong foundation in the concepts and principles of toxicology.

The book is organized into seven units: (1) General Principles of Toxicology; (2) Disposition of Toxicants; (3) Nonorgan-directed Toxicity; (4) Target Organ Toxicity; (5) Toxic Agents; (6) Environmental Toxicology; and (7) Applications of Toxicology. A summary of key points is included at the beginning of each chapter, and a set of review questions is provided at the end of each chapter. We invite readers to send us suggestions of ways to improve this text and we appreciate the thoughtful recommendations that we received on the last edition.

We would like to acknowledge all individuals who were involved in this project. We particularly give a heartfelt and sincere thanks to our families for their love, patience, and support during the preparation of this book. We especially appreciate Richard J. Batka and Alyssa Shapiro who provided invaluable assistance on this project. The capable advice, guidance, and assistance of the McGraw-Hill staff is gratefully acknowledged. Finally, we thank our students for their enthusiasm for learning and what they have taught us during their time with us.

Curtis D. Klaassen
John B. Watkins III

This page intentionally left blank

UNIT 1 GENERAL PRINCIPLES OF TOXICOLOGY

CHAPTER

History and Scope of Toxicology

1

Michael A. Gallo

KEY POINTS

- Toxicology is the study of the adverse effects of xenobiotics on living systems.
- Toxicology assimilates knowledge and techniques from biochemistry, biology, chemistry, genetics, mathematics, medicine, pharmacology, physiology, and physics.
- Toxicology applies safety evaluation and risk assessment to the discipline.

HISTORY OF TOXICOLOGY

Modern toxicology goes beyond the study of the adverse effects of exogenous agents by assimilating knowledge and techniques from most branches of biochemistry, biology, chemistry, genetics, mathematics, medicine, pharmacology, physiology, and physics and applies safety evaluation and risk assessment to the discipline. In all branches of toxicology, scientists explore the mechanisms by which chemicals produce adverse effects in biological systems. Activities in these broad subjects complement toxicologic research.

Antiquity

Knowledge of animal venoms and plant extracts for hunting, warfare, and assassination presumably predate recorded history. One of the oldest known writings, the Ebers Papyrus (circa 1500 b.c.), contains information pertaining to many recognized poisons, including hemlock, aconite, opium, and metals such as lead, copper, and antimony. The Book of Job (circa 1400 b.c.) speaks of poison arrows (Job 6:4) and Hippocrates (circa 400 b.c.) added a number of poisons and clinical toxicology principles pertaining to bioavailability in therapy and

overdosage. T eophrastus (370–286 b.c.), a student of Aristotle, included numerous references to poisonous plants in De Historia Plantarum. Dioscorides, a Greek physician in the court of the Roman emperor Nero, made the first attempt at classifying poisons as plant, animal, and mineral in his book De Materia Medica, which contains reference to some 600 plants.

One legend tells of Roman King Mithridates VI of Pontus, who was so fearful of poisons that he regularly ingested a mixture of 36 ingredients as protection against assassination. On the occasion of his imminent capture by enemies, his attempts to kill himself with poison failed because of his successful antidote concoction. T is tale leads to use of the word mithridatic as an antidote or protective mixture. Because poisonings in politics became so extensive, Sulla issued the Lex Cornelia (circa 82 b.c.), which appears to be the first law against poisoning and later became a regulatory statute directed at careless dispensers of drugs.

Middle Ages

T e writings of Maimonides (Moses ben Maimon, a.d. 1135–1204) included a treatise on the treatment of poisonings from insects, snakes, and mad dogs (*T*reatise on Poisons and T eir Antidotes, 1198). Maimonides described the subject of bioavailability, noting that milk, butter, and cream could delay intestinal absorption. In the early Renaissance and under the guise of delivering provender to the sick and the poor, Catherine de Medici tested toxic concoctions, carefully noting the rapidity of the toxic response (onset of action), the effectiveness of the compound (potency), the degree of response of the parts of the body (specificity and site of action), and the complaints of the victim (clinical signs and symptoms).

Renaissance

All substances are poisons; there is none that is not a poison. T e right dose differentiates a poison from a remedy.

Paracelsus

Philippus Aureolus T eophrastus Bombastus von Hohenheim-Paracelsus (1493–1541) was pivotal, standing between the philosophy and magic of classic antiquity and the philosophy and science willed to us by figures of the seventeenth and eighteenth centuries. Paracelsus, a physician-alchemist, formulated many revolutionary views that remain integral to the structure of toxicology, pharmacology, and therapeutics today. He focused on the primary toxic agent as a chemical entity, and held that (1) experimentation is essential in the examination of responses to chemicals, (2) one should make a distinction between the therapeutic and toxic properties of chemicals, (3) these properties are sometimes but not always indistinguishable except by dose, and (4) one can ascertain a degree of specificity of chemicals and their therapeutic or toxic effects. T ese principles led Paracelsus to articulate the dose–response relation as a bulwark of toxicology.

Come bitter pilot, now at once run on
T e dashing rocks thy seasick weary bark!
Here's to my love! O true apothecary!
T y drugs are quick. T us with a kiss I die.

Romeo and Juliet, act 5, scene 3

Although Ellenbog (circa 1480) warned of the toxicity of mercury and lead from goldsmithing and Agricola published a short treatise on mining diseases in 1556, the major work on the subject, On the Miners' Sickness and Other Diseases of Miners (1567), was published by Paracelsus. T is treatise addressed the etiology of miners' disease, along with treatment and prevention strategies. Occupational toxicology was further advanced by the work of Bernardino Ramazzini when he published in 1700 his Discourse on the Diseases of Workers, which discussed occupations ranging from miners to midwives and including printers, weavers, and potters. Percival Pott's (1775) recognition of the role of soot in scrotal cancer among chimney sweeps was the first report of polyaromatic hydrocarbon carcinogenicity. T ese findings led to improved medical practices, particularly in prevention.

Age of Enlightenment

Experimental toxicology accompanied the growth of organic chemistry and developed rapidly during the nineteenth century. Magendie (1783–1885), Orfila (1787–1853), and Bernard (1813–1878) laid the groundwork for pharmacology, experimental therapeutics, and occupational toxicology.

Orfila, a Spanish physician in the French court, used autopsy material and chemical analysis systematically as legal proof of poisoning. His introduction of this detailed type of analysis survives as the underpinning of forensic toxicology. Orfila published a major work devoted expressly to the toxicity of natural agents in 1815. Magendie, a physician and experimental physiologist, studied the mechanisms of action of emetine and strychnine. His research determined the absorption and distribution of these compounds in the body. One of Magendie's more famous students, Claude Bernard, contributed the classic treatise, An Introduction to the Study of Experimental Medicine.

German scientists Oswald Schmiedeberg (1838–1921) and Louis Lewin (1850–1929) made many contributions to the science of toxicology. Schmeideberg trained approximately 120 students who later populated the most important laboratories of pharmacology and toxicology throughout the world. Lewin published much of the early work on the toxicity of narcotics, methanol, glycerol, acrolein, and chloroform.

20TH CENTURY TOXICOLOGY: THE AWAKENING OF UNDERSTANDING

Toxicology has drawn its strength and diversity from its proclivity to borrowing from almost all the basic sciences to test its hypotheses. T is fact, coupled with the health and occupational

regulations that have driven toxicology research since 1900, has made this discipline exceptional in the history of science.

With the advent of anesthetics and disinfectants in the late 1850s, toxicology as it is currently understood began. T e prevalent use of "patent" medicines led to several incidents of poisonings from these medicaments, which, when coupled with the response to Upton Sinclair's exposé of the meatpacking industry in T e Jungle, culminated in the passage of the Wiley Bill in 1906, the first of many U.S. pure food and drug laws.

During the 1890s and early 1900s, the discovery of radioactivity and the vitamins, or "vital amines," led to the use of the first large-scale bioassays (multiple animal studies) to determine whether these "new" chemicals were beneficial or harmful to laboratory animals.

One of the first journals expressly dedicated to experimental toxicology, Archiv für *T*oxikologie, began publication in Europe in 1930. T at same year the National Institutes of Health (NIH) was established in the United States. As a response to the tragic consequences of acute kidney failure after taking sulfanilamide in glycol solutions, the Copeland bill was passed in 1938. T is was the second major bill involving the formation of the U.S. Food and Drug Administration (FDA). T e first major U.S. pesticide act was signed into law in 1947. T e significance of the initial Federal Insecticide, Fungicide, and Rodenticide Act was that for the first time in U.S. history a substance that was neither a drug nor a food had to be shown to be safe and efficacious for approval.

AFTER WORLD WAR II

You too can be a toxicologist in two easy lessons, each of ten years.
Arnold Lehman (circa 1955)

T e mid-1950s witnessed the strengthening of the U.S. FDA's commitment to toxicology. T e U.S. Congress passed and the president of the United States signed the additives amendments to the Food, Drug, and Cosmetic Act. T e Delaney clause (1958) of these amendments stated broadly that any chemical found to be carcinogenic in laboratory animals or humans could not be added to the U.S. food supply. Delaney became a battle cry for many groups and resulted in the inclusion at a new level of biostatisticians and mathematical modelers in the field of toxicology. Shortly after the Delaney amendment, the first American journal dedicated to toxicology, *T*oxicology and Applied Pharmacology, was launched. T e founding of the Society of Toxicology followed shortly afterward.

T e 1960s started with the tragic thalidomide incident, in which several thousand children were born with serious birth defects, and the publication of Rachel Carson's Silent Spring (1962). Attempts to understand the effects of chemicals on the embryo and fetus and on the environment as a whole gained momentum. New legislation was passed, and new journals were founded. Cellular and molecular toxicology developed as a subdiscipline, and risk assessment became a major product of toxicologic investigations.

Currently, many dozens of professional, governmental, and other scientific organizations with thousands of members and over 120 journals are dedicated to toxicology and related disciplines. In addition, the International Congress of Toxicology is composed of toxicology societies from Europe, South America, Asia, Africa, and Australia, which brings together the broadest representation of toxicologists.

21 ST CENTURY TOXICOLOGY

T e sequencing of the human genome and that of several other organisms has markedly affected all biological sciences, including toxicology. Genetically modifying organisms is now commonplace and those possessing orthologs of human genes (e.g., zebrafish [Danio rerio], roundworms [Caenorhabditis elegans], and fruit flys [Drosophila melanogaster]) are widely used in toxicology. Deeper understanding of epigenetics has provided novel approaches to studying the fetal origin of adult diseases including cancers, diabetes, and neurodegenerative diseases and disorders.

Toxicology has an interesting and varied history. Perhaps as a science that has grown and prospered by borrowing from many disciplines, it has suffered from the absence of a single goal, but its diversification has allowed for the interspersion of ideas and concepts from higher education, industry, and government. T is has resulted in an exciting, innovative, and diversified field that is serving science and the community at large. Few disciplines can point to both basic sciences and direct applications at the same time. Toxicology—the study of the adverse effects of xenobiotics—may be unique in this regard.

BIBLIOGRAPHY

Bryan CP: T e Papyrus Ebers. London: Geoffrey Bales, 1930.

Carson R: Silent Spring. Boston, MA: Houghton Mif in, 1962.

Gunther RT: T e Greek Herbal of Dioscorides. New York: Oxford University Press, 1934.

Guthrie DA: A History of Medicine. Philadelphia, PA: Lippincott, 1946.

Hays HW: Society of *T*oxicology History, 1961–1986. Washington, DC: Society of Toxicology, 1986.

Munter S (ed.): *T*reatise on Poisons and T eir Antidotes. Vol. II of the Medical Writings of Moses Maimonides. Philadelphia, PA: Lippincott, 1966.

Pagel W: Paracelsus: An Introduction to Philosophical Medicine in the Era of the Renaissance. New York: Karger, 1958.

T ompson CJS: Poisons and Poisoners: With Historical Accounts of Some Famous Mysteries in Ancient and Modern *T*imes. London: Shaylor, 1931.

http://www.toxipedia.org/display/toxipedia/History+ of+ Toxicology

QUESTIONS

1. Which one of the following statements regarding toxicology is true?
   - **a.** Modern toxicology is concerned with the study of the adverse effects of chemicals on ancient forms of life.
   - **b.** Modern toxicology studies embrace principles from such disciplines as biochemistry, botany, chemistry, physiology, and physics.
   - **c.** Modern toxicology has its roots in the knowledge of plant and animal poisons, which predates recorded history and has been used to promote peace.
   - **d.** Modern toxicology studies the mechanisms by which inorganic chemicals produce advantageous as well as deleterious effects.
   - **e.** Modern toxicology is concerned with the study of chemicals in mammalian species.

2. Knowledge of the toxicology of poisonous agents was published earliest in the:
   - **a.** Ebers papyrus.
   - **b.** De Historia Plantarum.
   - **c.** De Materia Medica.
   - **d.** Lex Cornelia.
   - **e.** *T*reatise on Poisons and T eir Antidotes.

3. Paracelsus, a physician-alchemist, formulated many revolutionary views that remain integral to the structure of toxicology, pharmacology, and therapeutics today. He focused on the primary toxic agent as a chemical entity and articulated the dose–response relation. Which one of the following statements is not attributable to Paracelsus?
   - **a.** Natural poisons are quick in their onset of actions.
   - **b.** Experimentation is essential in the examination of responses to chemicals.
   - **c.** One should make a distinction between the therapeutic and toxic properties of chemicals.
   - **d.** T ese properties are sometimes but not always indistinguishable except by dose.
   - **e.** One can ascertain a degree of specificity of chemicals and their therapeutic or toxic effects.

4. T e art of toxicology requires years of experience to acquire, even though the knowledge base of facts may be learned more quickly. Which modern toxicologist is credited with saying that "you can be a toxicologist in two easy lesions, each of 10 years?"
   - **a.** Claude Bernard.
   - **b.** Rachel Carson.
   - **c.** Upton Sinclair.
   - **d.** Arnold Lehman.
   - **e.** Oswald Schmiedeberg.

5. Which of the following statements is correct?
   - **a.** Claude Bernard was a prolific scientist who trained over 120 students and published numerous contributions to the scientific literature.
   - **b.** Louis Lewin trained under Oswald Schmiedeberg and published much of the early work on the toxicity of narcotics, methanol, and chloroform.
   - **c.** An Introduction to the Study of Experimental Medicine was written by the Spanish physician Orfila.
   - **d.** Magendie used autopsy material and chemical analysis systematically as legal proof of poisoning.
   - **e.** Percival Potts was instrumental in demonstrating the chemical complexity of snake venoms.

CHAPTER

2

Principles of Toxicology

David L. Eaton and Steven G. Gilbert

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.
- T e 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. T e 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. T e expanding inf uence 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.

T ere 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. T ird, 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_{50}). Chemicals producing death in microgram doses are often considered extremely poisonous. Note that measures of acute lethality such as LD_{50} may not accurately ref ect 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_{50} of some representative chemical agents.

Agent	LD_{50}, 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_{50} 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. T ese agents may be discussed in terms of their target organs, use, source, and effects. T e term toxin generally refers to toxic substances that are produced by biological systems such as plants, animals, fungi, or bacteria. T e 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 UNDESIRED EFFECTS

T e spectrum of undesired 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 ef ects. 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. T ese 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

T e 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. T e "vehicle" (the material in which the chemical is dissolved) and other formulation factors can markedly alter absorption. In addition, the route of administration can inf uence 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. T us, 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. T e other time-related factor that is important in the temporal characterization of repeated exposures is the frequency of exposure. T e 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 2 U 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. T e important consideration, then, is whether the interval between

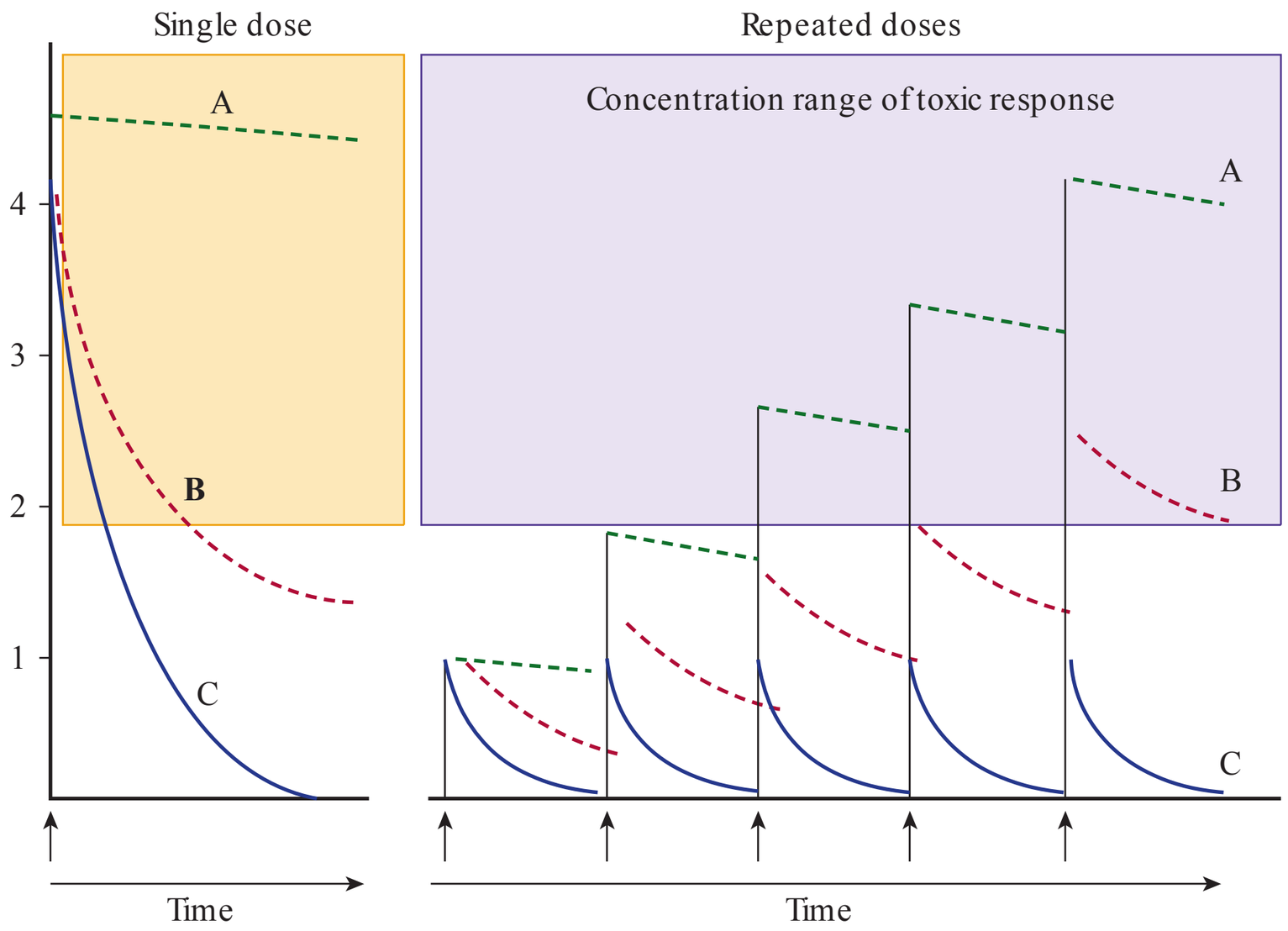


FIGURE 2–1 **Diagrammatic view of the relationship between dose and concentration at the target site under different conditions of dose frequency and elimination rate.** Line A. A chemical with very slow elimination (e.g., half-life of 1 year). Line B. A chemical with a rate of elimination equal to frequency 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

T e 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. T e 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

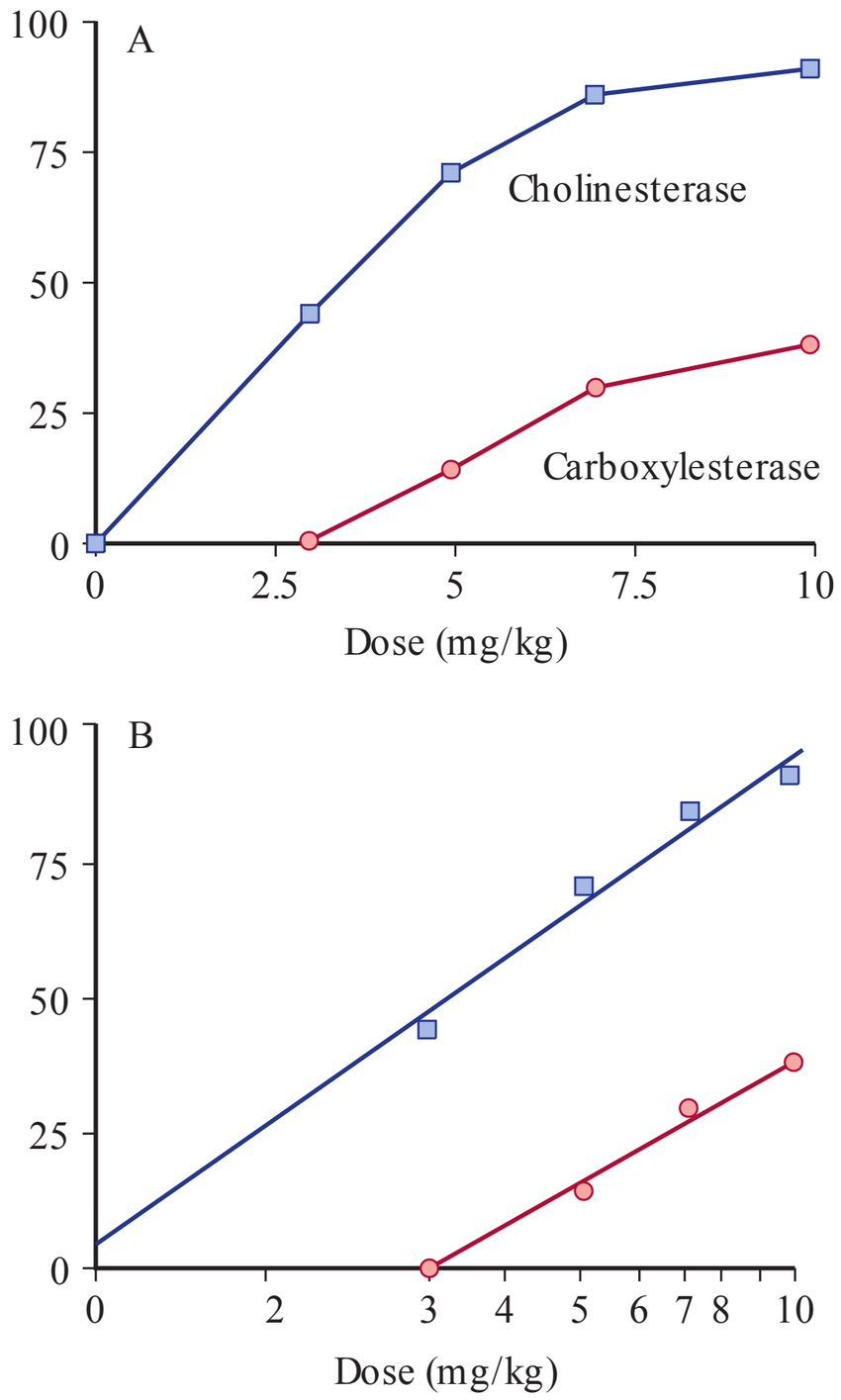


FIGURE 2–2 **Dose–response relationship between different doses of the organophosphate insecticide chlorpyrifos and esterase enzyme inhibition in the brain.** Open circles and blue lines represent acetylcholinesterase activity and closed circles represent carboxylesterase activity in the brains of pregnant 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.)

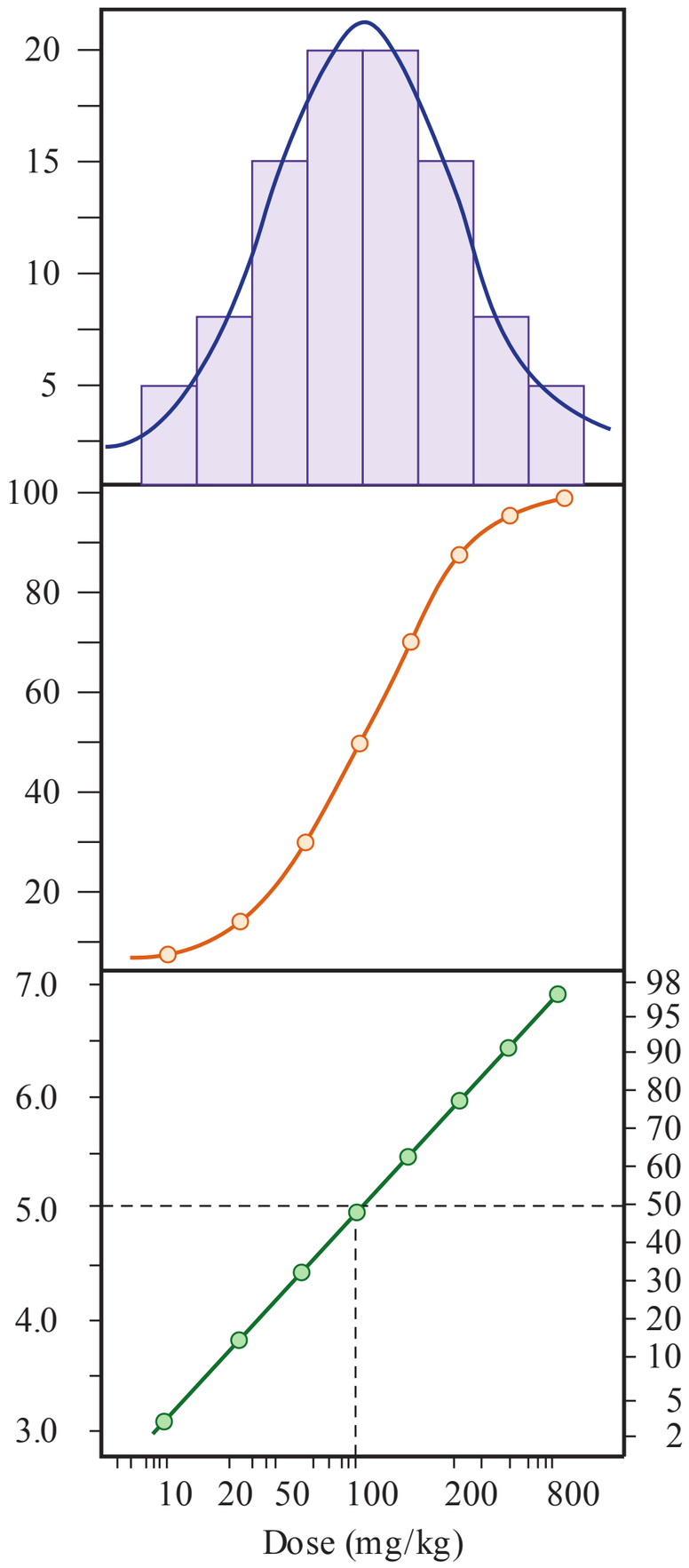


FIGURE 2–3 **Diagram of a quantal dose–response relationship.** The abscissa is a log dosage of the chemical. In the top panel the ordinate is response frequency, in the middle panel the ordinate is percent response, and in the bottom panel the response is in probit units (see text).

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. T e 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.

T e 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.

T e top panel of Figure 2–3 shows that quantal dose–responses exhibit a normal or Gaussian distribution. T e frequency histogram in this panel also shows the relationship between dose and effect. T e bars represent the percentage of 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. T us, we have a bell-shaped curve known as a normal *f*requency distribution. T e 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.

T e sigmoid curve has a relatively linear portion between 16% and 84%. T ese values represent the limits of 1 standard deviation (SD) of the mean (and the median) in a population with truly normal distribution. T us, the mean ± 1 SD represents 68.3% of the population, the mean ± 2 SD represents 95.5% of the population, and the mean ± 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). T us, 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.

T e 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. T e 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 "f at" dose–response curve, showing that a large change in dosage is required before 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. T e 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.

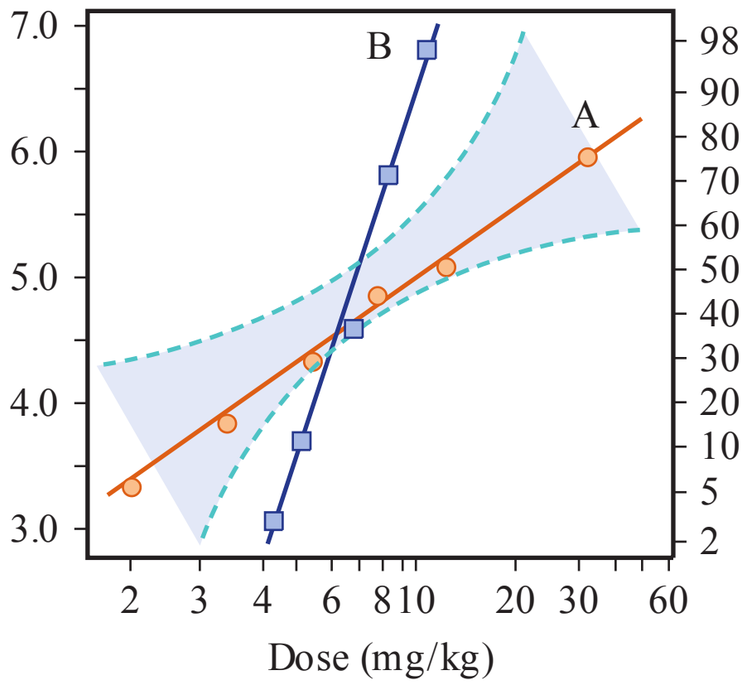


FIGURE 2–4 **Comparison of dose–response relationship for two different chemicals, plotted on a log dose–probit scale.** Note that the slope of the dose–response relationship is steeper for chemical B than for chemical A. Dotted lines represent the confidence limits for chemical A.

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 × (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^2)*	Fold Difference, Relative to Humans, Normalized by Body Weight		
			mg/kg	$(BW)^{2/3}$	$(BW)^{3/4}$
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	1 410	1	3.5	2.6
Cat	2	1 710	1	3.2	2.4
Monkey	4	2 720	1	2.6	2.0
Dog	12	5 680	1	1.8	1.5
Human	70	18 500	1	1.0	1.0

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

by the two alternatives. If a scaling factor of (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 (mg/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 is 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 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

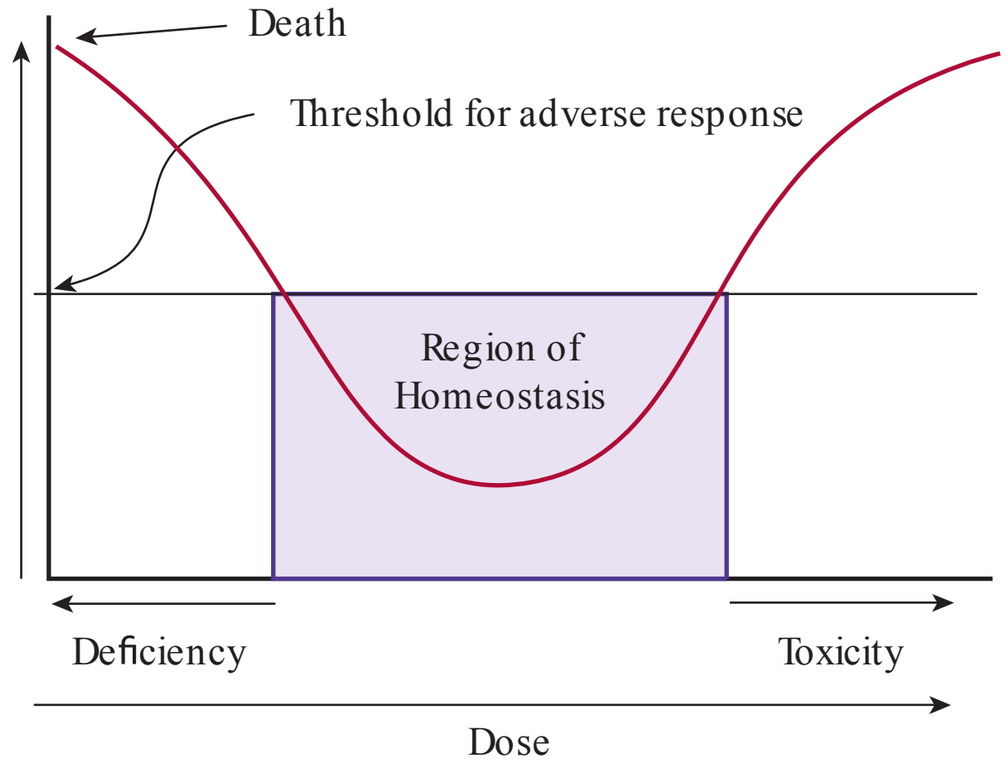


FIGURE 2–5 **Individual dose–response relationship for an essential substance such as a vitamin or trace element.** It is generally recognized that, for most types of toxic responses, a threshold exists such that at doses below the threshold, no toxicity is evident. For essential substances, doses below 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.

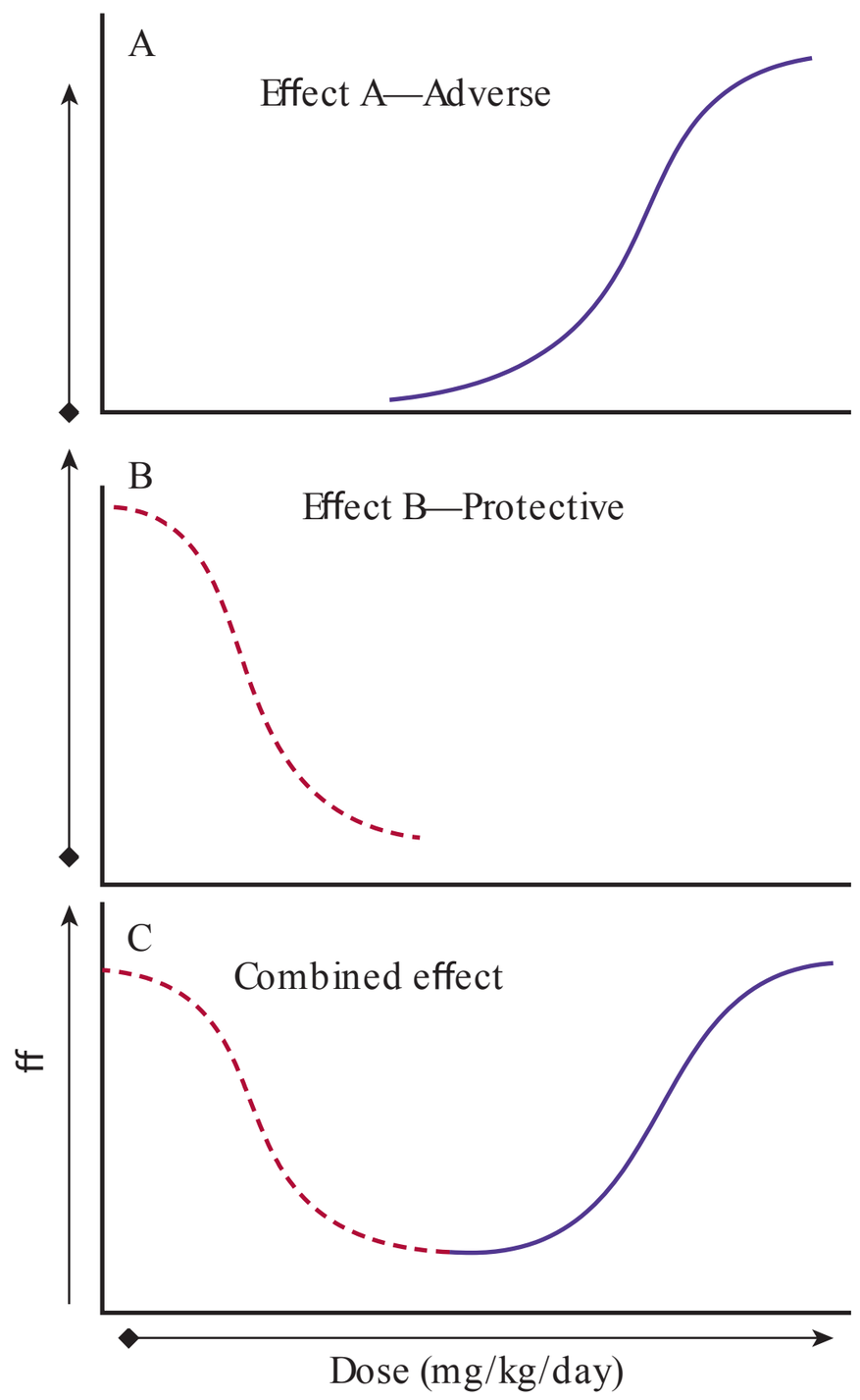


FIGURE 2–6 **Hypothetical dose–response relationship depicting characteristics of hormesis.** Hormetic effects of a substance are hypothesized to occur when relatively low doses result in the stimulation of a beneficial or protective response (B), such as induction of enzymatic pathways that protect 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 inf ections in the shape of the dose–response curve rather than absolute thresholds. T at 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 inf ections 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. T ese agents produce the so-called nonmonotonic dose–response curves. T ese curves may result from upregulation of some receptors at low doses with downregulation of those receptors at higher doses. T e 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. T e first is that the response is due to the chemical administered, a cause-and-effect relationship.

T e second assumption is that the magnitude of the response is in fact related to the dose. T is 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.

T e 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. T e same admonition applies to any pair of parallel "effect" curves or any other pair of toxicity or lethality curves.

Therapeutic Index—T e 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. T e 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.

T e 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

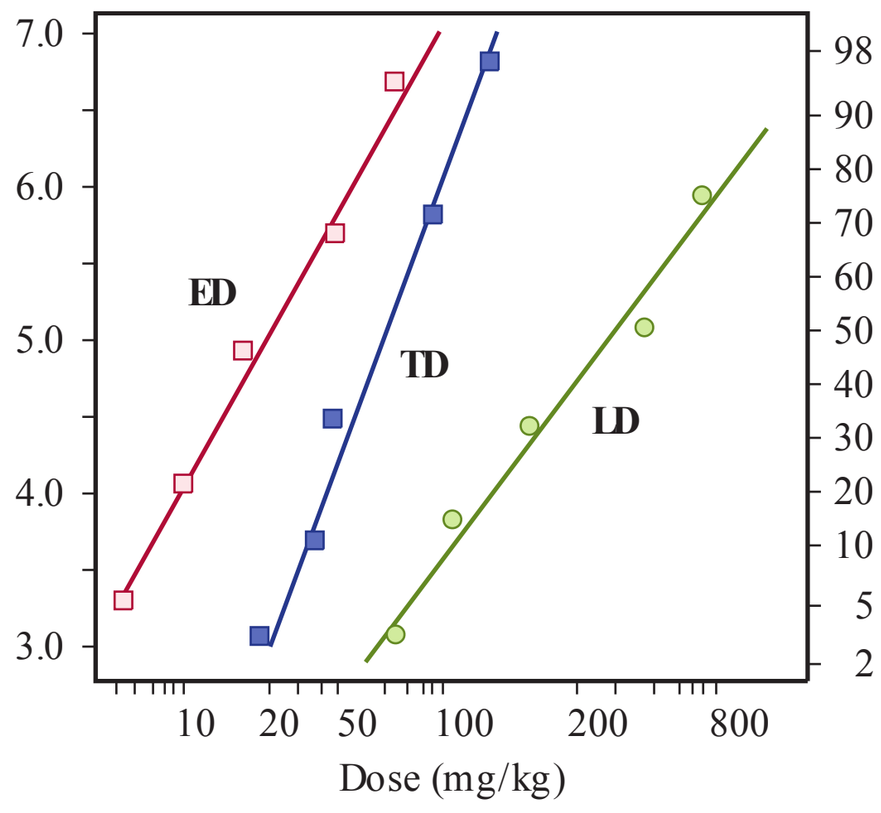


FIGURE 2–7 **Comparison of effective dose (ED), toxic dose (TD), and lethal dose (LD).** The plot is of log dosage versus percentage of population responding in probit units.

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.

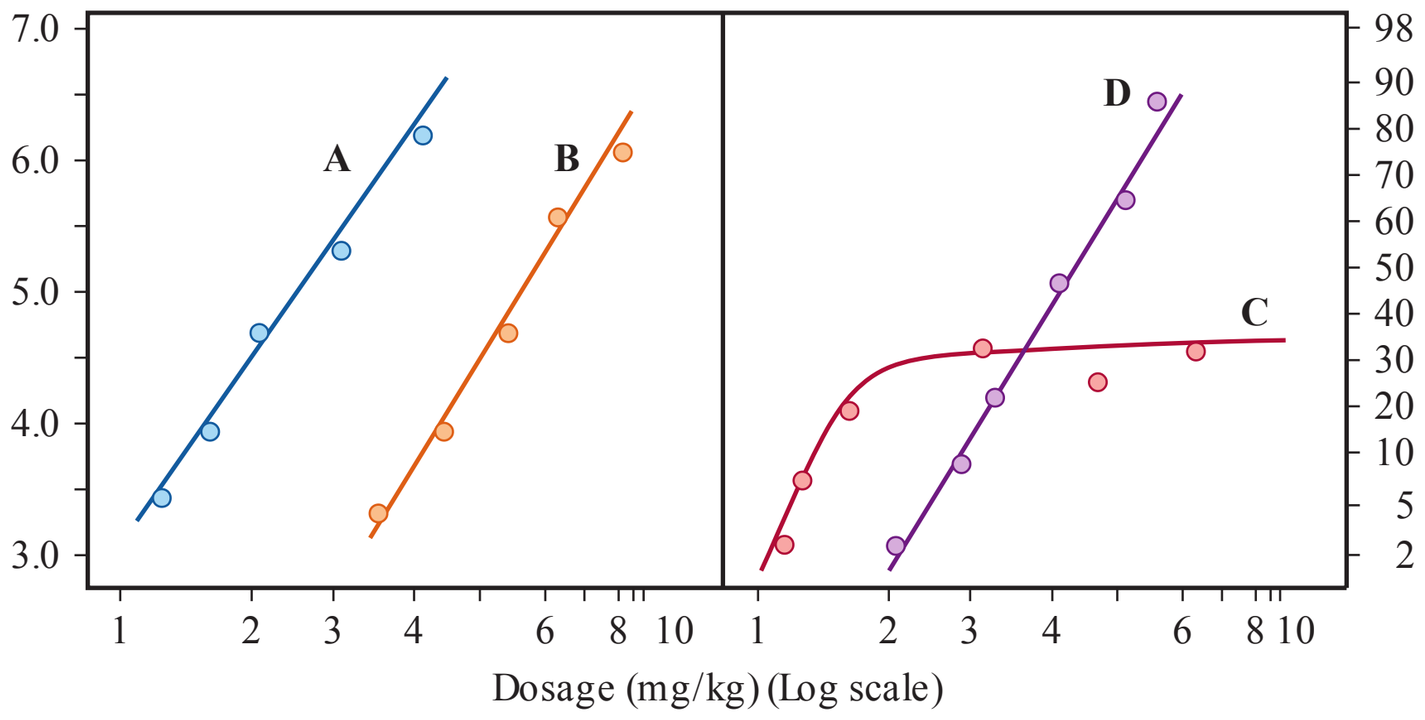


FIGURE 2–8 **Schematic representation of the difference in the dose–response curves for four chemicals (A–D), illustrating the difference between potency and efficacy (see text).**