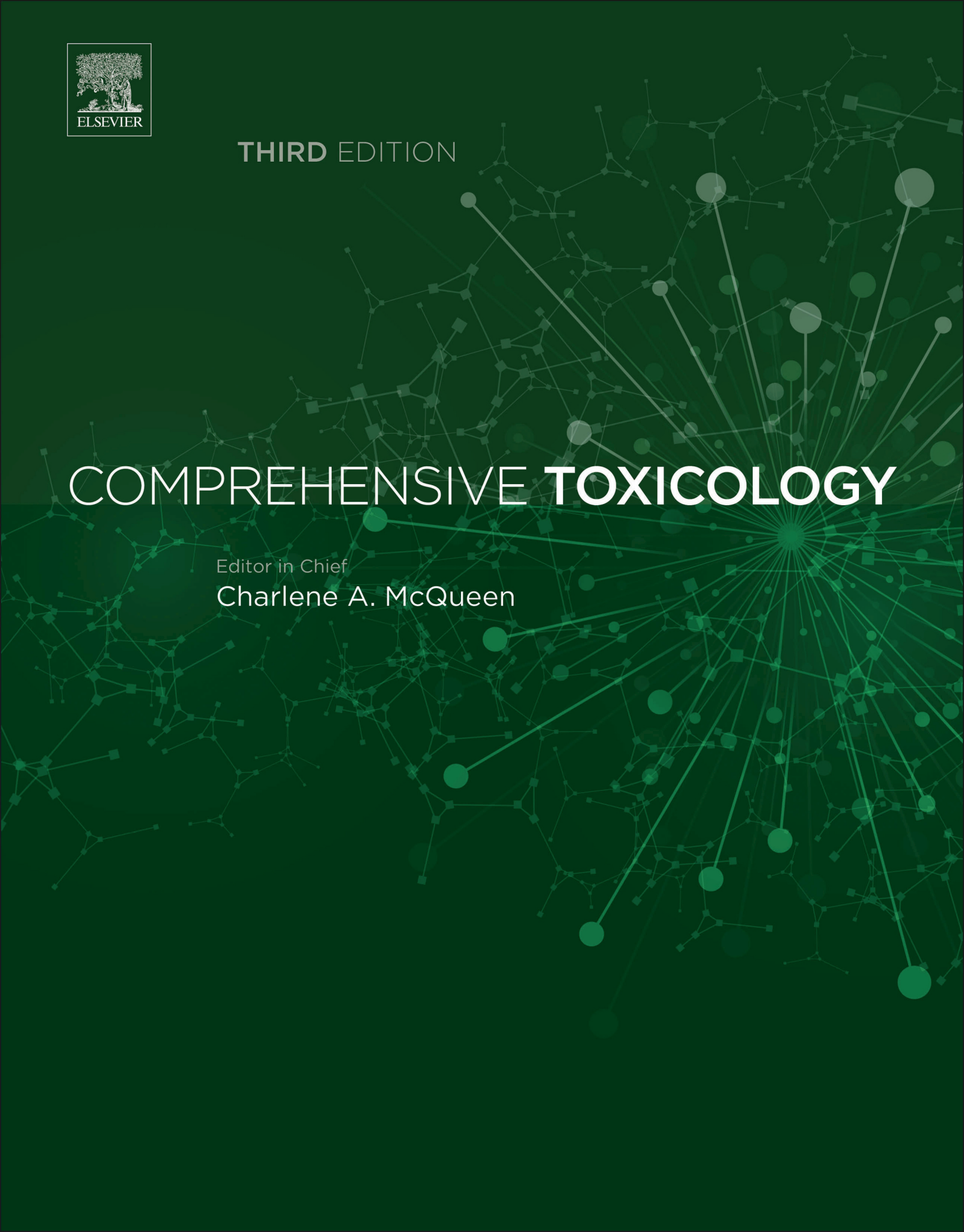
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COMPREHENSIVE TOXICOLOGY
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COMPREHENSIVE TOXICOLOGY

THIRD EDITION

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COMPREHENSIVE TOXICOLOGY

THIRD EDITION

EDITOR IN CHIEF

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VOLUME 1

GENERAL PRINCIPLES

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Charlene A. McQueen is a Professor in the Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona. Until January 2017, she was a Senior Scientist at the National Health Effects Research Laboratory of the USEPA. She served as the Director of the Integrated Systems Toxicology Division (2011–16). Prior to that, she held positions at the Harrison School of Pharmacy, Auburn University (2007–11) and the Department of Pharmacology and Toxicology at the University of Arizona (1990–2007). Dr. McQueen received a MS in Pharmacology from New York University and PhD in Human Genetics from the University of Michigan. Her work is in the areas of pharmacogenomics, toxicogenomics and chemical carcinogenesis. She has a particular interest in the genetic basis for response to xenobiotics. Her work with the arylamine N-acetyltransferase polymorphism has demonstrated that this genetic variation can affect drug efficacy as well as toxicity of aromatic amines and hydrazines. She was the Editor-in-Chief of the second edition of Comprehensive Toxicology and is continuing in that position for the third edition to be published in 2017. Dr. McQueen is an American Association for the Advancement of Science (AAAS) Fellow and a Fellow in the Academy of Toxicological Sciences (ATS). Dr. McQueen received the Society of Toxicology (SOT) Public Communications Award, the SOT AstraZeneca Traveling Lectureship Award and has served on numerous SOT committees. Dr. McQueen was on the Environmental Health Sciences Committee of the National Institute of Environmental Health Sciences, the Board of Scientific Councilors of the National Toxicology Program and the National Institutes of Health Cancer Etiology Study Section. She is a member of the Editorial Board of the Reference Modules in Biomedical Sciences (Elsevier). Dr. McQueen is a member of the Health and Environmental Sciences Institute (HESI) Board of Trustees, serving as the Board Secretary (2012–17) and is currently Vice-president elect (2017–18). Her current research interests include the discovery and development of Adverse Outcome Pathways (AOPs) for fatty liver disease and carcinogenesis as well as the role of the microbiome in xenobiotic biotransformation.

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VOLUME EDITOR: BIOGRAPHY

Dr. Eaton received his PhD in pharmacology from the University of Kansas Medical Center (KUMC) in 1978. He joined the faculty of the University of Washington in 1979. He is currently Dean and Vice Provost of the UW Graduate School, and serves as Professor of Environmental and Occupational Health Sciences, Professor of Public Health Genetics, and adjunct Professor of Medicinal Chemistry. Prior to becoming Dean and Vice Provost of the UW Graduate School in 2013, he served as Associate Vice Provost for Research for the UW from 2006–13, and as the Associate Dean for Research in the School of Public Health 1999–2005. Nationally, he has served as Secretary, and later as President, of the Society of Toxicology, and serves on a numerous scientific advisory boards for other centers and program grants. He served on the NAS/NRC Board of Environmental Studies and Toxicology and on numerous NAS/NRC/IOM Committees related to controversial public health issues in toxicology. He is currently a member of the National Advisory Environmental Health Sciences Council, and chairs the Research Committee for the Health Effects Institute. He served as Founding Director of the NIEHS Core Center, the Center for Ecogenetics & Environmental Health, for 18 years, and also directed the UW NIEHS Superfund Research Program. Dr. Eaton maintains his own active research and teaching program focused in the area of the molecular basis for environmental causes of cancer, with an emphasis on how chemical carcinogens are metabolized in the liver. He has published over 160 scientific articles and book chapters in the field of toxicology and risk assessment. He is an Elected Fellow of the American Association for the Advancement of Science, and of the Academy of Toxicological Sciences, and was elected to the National Academy of Medicine (formerly the Institute of Medicine) of the National Academies of Science and to the Washington State Academy of Sciences in 2011.

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CONTENTS OF VOLUME 1

VOLUME 1: GENERAL PRINCIPLES

CONTENTS OF ALL VOLUMES

VOLUME 1: GENERAL PRINCIPLES

VOLUME 2: HEPATIC TOXICOLOGY

VOLUME 3: GASTROINTESTINAL TOXICOLOGY

VOLUME 4: REPRODUCTIVE AND ENDOCRINE TOXICOLOGY

VOLUME 5: DEVELOPMENTAL TOXICOLOGY

VOLUME 6: NERVOUS SYSTEM AND BEHAVIORAL TOXICOLOGY

VOLUME 7: CARCINOGENESIS

VOLUME 8: CELLULAR AND MOLECULAR TOXICOLOGY

VOLUME 9: TOXICOLOGY TESTING AND EVALUATION

VOLUME 10: BIOTRANSFORMATION

VOLUME 11: IMMUNE SYSTEM TOXICOLOGY

VOLUME 12: HEMATOPOIETIC SYSTEM TOXICOLOGY

VOLUME 13: CARDIOVASCULAR TOXICOLOGY

VOLUME 14: RENAL TOXICOLOGY

VOLUME 15: RESPIRATORY TOXICOLOGY

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PREFACE

The field of toxicology has undergone a tremendous evolution since the publication of the first edition of Comprehensive Toxicology in 1997. In the intervening years, the science of toxicology has made significant strides that have resulted in a better understanding of how chemical and physical agents interact with biological systems. New technologies such as genomics, proteomics and metabolomics, novel *in vivo* and *in vitro* models, in silico and computational methods have enabled greater insight into mechanisms of toxicity. In turn that has improved the ability of risk assessors and regulators to protect human health and the environment.

The original goal of the series was to provide a strong foundation of the science utilizing a design that included basic principles and concepts coupled with in-depth volumes on major organ systems. This structure has been retained. The third edition of Comprehensive Toxicology consists of 15 volumes. Volumes have been expanded where needed and updated to include current references as well as new chapters, tables and figures. The Hematopoietic System Toxicology volume which was missing from the second edition has been reintroduced.

It is clearly a challenge to live up to the concept of a comprehensive work. There are always limitations that make it impossible to include every aspect of the discipline of toxicology. While certain areas have been omitted, Comprehensive Toxicology continues to be a resource for readers who are new to the field as well as experienced scientists exploring new areas.

I have been honored to be part of the 20 year journey of Comprehensive Toxicology as a co-editor-in-chief in the first edition and as editor-in-chief in the second and third editions. Each edition has required the efforts of many talented scientist and editorial staff. Without them, Comprehensive Toxicology would not exist.

The idea of a third edition of Comprehensive Toxicology began in discussions with Elsevier. From those initial conversations to publication has involved a tremendous effort by a large group of talented people. I have had the good fortune to work with an outstanding group of individuals. I would like to acknowledge the efforts and contributions of the volume editors, all the chapter authors and the Elsevier staff. All of you have my special thanks. Without you, this would not have been possible.

Charlene A. McQueen

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Figure 1A Toxicology Testing and Evaluation/Inhalation Toxicology Studies
Figure 2B Toxicology Testing and Evaluation/Inhalation Toxicology Studies
Table 2 General Principles/Biotransformation of Toxicants
Figure 1 Cardiovascular Toxicology/Halogenated Aromatic Hydrocarbons and Cardiovascular Disease
Figure 1 Reproductive and Endocrine Toxicology/Toxic Responses of the Adrenal Cortex
Figure 2 Reproductive and Endocrine Toxicology/Toxic Responses of the Adrenal Cortex
Figure 3 Reproductive and Endocrine Toxicology/Toxic Responses of the Adrenal Cortex
Figure 4 Reproductive and Endocrine Toxicology/Toxic Responses of the Adrenal Cortex
Figure 5 Reproductive and Endocrine Toxicology/Toxic Responses of the Adrenal Cortex
Figure 6 Reproductive and Endocrine Toxicology/Toxic Responses of the Adrenal Cortex
Figure 7 Reproductive and Endocrine Toxicology/Toxic Responses of the Adrenal Cortex
Table 6 Toxicology Testing and Evaluation/Toxicology Assessment of Endocrine Active Substances/New
Table 2 Renal Toxicology/Renal Xenobiotic Metabolism
Table 4 Gastrointestinal Toxicology/Pathologic Response of the Gastrointestinal Tract to Toxicants
Figure A E Hepatic Toxicology/Leading-Edge Approaches for In Vitro Hepatotoxicity Evaluation/New
Figure 3 Immune System Toxicology/T-Dependent Antibody Response/New
Figure 7 General Principles/Free Radicals and Reactive Oxygen Specie
Figure 8 General Principles/Free Radicals and Reactive Oxygen Specie
Table 2 General Principles/Free Radicals and Reactive Oxygen Specie
Figure 1 The Aryl Hydrocarbon Receptor and Immunity
Figure 2 The Aryl Hydrocarbon Receptor and Immunity
Figure 3 The Aryl Hydrocarbon Receptor and Immunity
Figure 4A The Aryl Hydrocarbon Receptor and Immunity
Figure 4B The Aryl Hydrocarbon Receptor and Immunity
Figure 12 General Principles/Genomics in Toxicology
Figure 1 Biotransformation/Enzymology of Amino Acid Conjugation Reactions
Figure 2 Biotransformation/Enzymology of Amino Acid Conjugation Reactions
Figure 3 Biotransformation/Enzymology of Amino Acid Conjugation Reactions
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Figure 8 Renal Toxicology/Renal Xenobiotic Metabolism
Figure 6B Hepatic Toxicology/Leading-Edge Approaches for In Vitro Hepatotoxicity Evaluation/New
Figure 3 Developmental Toxicology/Alternative Methods in Developmental Toxicology
Figure 5 Immune System Toxicology/T-Dependent Antibody Response/New
Figure 6 Immune System Toxicology/T-Dependent Antibody Response/New
Figure 7 Immune System Toxicology/T-Dependent Antibody Response/New
Figure 1 Developmental Toxicology/The Role of Biotransformation in Developmental Toxicity
Figure 3 Developmental Toxicology/The Role of Biotransformation in Developmental Toxicity

Figure 6 Developmental Toxicology/The Role of Biotransformation in Developmental Toxicity
Table B Cytokines: Role in Homeostasis and Disease States
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Figure 6 Efflux Transporters
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Figure 7 Renal Toxicology/Cell Adhesion Molecules in Renal Injury
Figure 8 Gastrointestinal Toxicology/Pathophysiological Mechanisms of Gastrointestinal Toxicity
Figure 9 Gastrointestinal Toxicology/Pathophysiological Mechanisms of Gastrointestinal Toxicity
Figure 11 Gastrointestinal Toxicology/Pathophysiological Mechanisms of Gastrointestinal Toxicity
Figure 12 Gastrointestinal Toxicology/Pathophysiological Mechanisms of Gastrointestinal Toxicity
Figure 2 Hepatic Toxicology/Toll-Like Receptors, PAMPs and DAMPs in Hepatotoxicity/New
Figure 2 Respiratory Toxicology/Cell Damage and Cell Renewal in the Lung
Figure 6 Carcinogenesis/Ionizing Radiation as a Carcinogen
Figure 6 General Principles/Metabolomics in Toxicology
Figure 7 General Principles/Metabolomics in Toxicology
Figure 3 Efflux Transporters
Figure 2 Developmental Toxicology/Epigenetics and the Developmental Origins of Health and Disease
Figure 3 Developmental Toxicology/Epigenetics and the Developmental Origins of Health and Disease
Figure 4 Developmental Toxicology/Epigenetics and the Developmental Origins of Health and Disease
Figure 4 Mitochondrial Genomics and Targeted Toxicities
http://www.nature.com

1.01 General Overview of Toxicology☆

DL Eaton, EP Gallagher, and TC Vandivort, University of Washington, Seattle, WA, United States

☆*Change History:* September 2016. TC Vandivort, DL Eaton and EP Gallagher have made extensive revisions to the chapter and added a revised **figure 9**. This is an update of D.L. Eaton and E.P. Gallagher, 1.01 - General Overview of Toxicology, In Comprehensive Toxicology (Second Edition), edited by Charlene A. McQueen, Elsevier, Oxford, 2010, Pages 1–46.

Abbreviations

2,4,5-T 2,4,5-Trichlorophenoxy acetic acid
2,4-D 2,4-Dichlorophenoxy acetic acid
A Adenine
AOP Adverse outcome pathway
ATP Adenosine triphosphate
BAL British anti-Lewisite
BoTox *Botulinum* toxin
BPA Bisphenol A
C Cytosine
CNS Central nervous system
CNV Copy number variant
CO Carbon monoxide
COHb Carboxyhemoglobin
CYP Cytochrome P450
DDT Dichlorodiphenyltrichloroethane
DEHP Di-2-ethylhexyl phthalate
DNA Deoxyribonucleic acid
EDC Endocrine disrupting compound
EPA Environmental Protection Agency
FDA Food and Drug Administration
G Guanine
GI Gastrointestinal
GLP Good laboratory practice
H_2S Hydrogen sulfide
HAB Harmful algal bloom
IARC International Agency for Research on Cancer
LOAEL Lowest-observed adverse effect level
MOA Mode of action
mRNA Messenger RNA
MTD Maximum tolerable dose
NIH National Institute of Health
NMDRC Nonmonotonic dose–response curve
NMR Nuclear Magnetic Resonance
NOAEL No observable adverse effect level
OECD Organization for Economic Cooperation and Development

PCB Polychlorinated biphenyl
PCP Pentachlorophenol
PNS Peripheral nervous system
PSP Paralytic Shellfish Poisoning
RfD Reference dose
RNA Ribonucleic acid
RNAi RNA interference
ROS Reactive oxygen species
siRNA Small-interfering RNA
SNP Single nucleotide polymorphism
T Thymine
VSD Virtually safe dose

1.01.1 Introduction

Toxicology is a multidisciplinary science that examines the adverse effects of chemicals on biological systems. Humans and other living creatures that inhabit the earth are increasingly exposed to a vast array of synthetic and naturally occurring chemicals. The science of toxicology has evolved over the past century from one that originally focused on the adverse effects of drugs and other therapeutic effects on patient populations, to one that now includes consideration of the adverse effects of chemicals found in industrial settings and in the environment, and the biochemical and molecular mechanisms that underlie toxic responses. These chemicals range from metals and inorganic chemicals to large complex organic molecules, and today include new materials, such as those associated with the burgeoning field of nanotechnology. Historically, the toxic metals, including lead, mercury, and arsenic, dominated the earliest outbreaks of poisoning episodes. With the onset of the industrial revolution and the emergence of the science of synthetic chemistry, a variety of new chemicals were synthesized. Today, the nature and the source of these chemicals will vary with the location. In agricultural settings, these chemicals may include herbicides and pesticides, while in the chemical industry these compounds include solvents, metals, intermediates of chemical manufacturing, or component manufacturing, such as nanoscale engineered materials.

Due to its broad scope, there are a variety of identifiable subdisciplines within the field of toxicology. This makes toxicology a challenging area of research and study. For example, *analytical* toxicologists use advanced chemical instrumentation to study the identification of toxicants, while *biomedical* toxicologists study the mechanisms underlying how toxicants cause human disease. *Forensic* toxicologists are often pathologists or medical examiners who specialize in evaluating the role of chemicals as a cause of death. *Occupational* toxicologists study the adverse effects of chemical exposure in the workplace, and this branch of toxicology is closely aligned with the field of industrial hygiene and occupational medicine. Despite the variety of identifiable subdisciplines within the field of toxicology, there is also extensive integration and overlap within these areas. For example, the subspecialty of *environmental toxicology* was originally developed to study the adverse effects of environmental chemicals on human health. However, the field of environmental toxicology has evolved to study the fate and effects of these compounds on fish and aquatic biota (*aquatic toxicology*), wildlife (*wildlife toxicology*), and upon ecological populations and communities (*ecotoxicology*).

A rapidly growing subdiscipline of toxicology, *molecular toxicology and toxicogenomics*, takes advantage of the technological advances developed in part from the Human Genome Project, as well as the remarkable increase in computing power that allows for analysis of enormous data sets. Fundamentally, most toxic responses generate a complex cellular response that is associated with changes in gene expression. Characterizing how a given cell type or tissue responds to a toxic insult through global analysis of changes in gene expression is now a common tool in the emerging field of toxicogenomics. Understanding how such molecular changes result in tissue or organismal responses serves as the basis for an integrative approach to toxicology, sometimes called "systems toxicology."

An important subdiscipline of toxicology is the field of *regulatory toxicology*. Regulatory toxicologists attempt to protect public health and the environment by establishing regulatory standards aimed at reducing the adverse public health and environmental impacts potentially associated with the manufacture, use, and disposal of a wide variety of potentially toxic materials. Regulatory toxicologists assess mechanistic information provided by research toxicologists to enhance the accuracy and relevance of toxicological evaluations conducted in experimental animals and provide a basis for their decisions. Although the background and training of the individuals involved in these various subdisciplines may vary greatly, they share a common body of knowledge related to toxicological principles. Toxicologists are employed in academia, government, private industry, or business (e.g., environmental consulting) settings.

1.01.1.1 Historical Aspects

Early humans were well aware of the poisonous effects of a number of animal- and plant-derived substances in their environment. Some of these poisons were used intentionally with the earliest weapons. Homicides using poison were quite common in ancient

Table 1 Historical developments and incidents in toxicology

Approximate year	*Development*
Early 1500s	Paracelsus provides a scientific basis for understanding poisons
1809	F. Magendie reports on the mechanisms of action of arrow poisons
1830–40	Orfila devises methods for detecting poisons, thereby proving that poisoning had taken place and establishing the field of forensic toxicology
1920s–30s	Delayed neurotoxicity in individuals who consume "bootleg liquor," in particular, "ginger jake," contaminated with tri-*o*-cresyl phosphate
1945	R.A. Peters, L.A. Stocken, and R.H.S. Thompson develop British anti-Lewisite (BAL) as an antidote for arsenic P. Muller introduces and studies DDT and related organochlorine compounds
1952	G. Schrader introduces and studies organophosphorus compounds
1950s	Over 200 cases of severe neurological disease reported in individuals consuming fish contaminated with methyl mercury in Minimata, Japan
1984	Approximately 2000 die in Bhopal, India, from acute lung disease associated with methylisocyanate release

Source: From Gallo, M. (2008). In: Curtis, P.D., Klaassen, D. (eds.) Casarett and Doull's toxicology: The basic science of poisons, pp. 3–10. New York: McGraw Hill.

Greece and later throughout Europe. A particularly noteworthy contribution to the original study of poisons was made by the Greek physician Dioscorides who classified poisons based upon animal, plant, or mineral origins, and also brought to light the value of emetics in the treatment of poisoning (**Table 1**). More significant contributions to the field of toxicology were made in the 16th century by Paracelsus (1493–1541), who saw the need for proper scientific experimentation, and thus gave toxicology a scientific basis (Gallo, 2013). Paracelsus recognized that chemicals often had both therapeutic and toxic properties and recognized that these may be indistinguishable except by dose. His observations laid the foundation for the concept of the dose–response relationship. Orfila (1787–1853) was a Spanish physician who devised methods for detecting poisons in the body, thereby proving that poisoning had taken place (Gallo, 2013). His work formed the foundation for the specialized area of forensic toxicology. More recently, Sir Rudolph Peters studied the mechanism of action of arsenical war gases and, in doing so, invented an antidote for war gas poisoning (anti-Lewisite) in 1945.

To meet the needs of growing populations in modern society, a great number and variety of chemicals and materials have been manufactured. The Toxic Substances Control Act Inventory (which is administered by the US Environmental Protection Agency (EPA)) currently lists approximately 85,000 chemicals in use in the United States, and an additional 200–1000 new chemicals are added each year. In the last decade, the emerging field of nanotechnology has resulted in the creation of new materials, called nanomaterials, with dimensions between 1 and 100 nm. Such nanomaterials have unique physical characteristics, come in a wide variety of shapes, sizes, and composition, and may possess unique biological properties. Although such materials have many remarkable uses and societal benefits, their unique biological properties suggest that they could also have unexpected toxicological properties. The wide array of chemicals and engineered materials used in commerce today may come into contact with various segments of the population, through manufacture, handling, consumption, or disposal. Thus, the enormous number of potentially toxic materials to which we may be exposed has created the need for organized study, as well as the promulgation of legislation that requires the testing of such materials for toxic effects. Unfortunately, industrial disasters have highlighted the need for knowledge of toxicity of chemicals used in industry, as well as of drugs or food additives. Also, during the last several decades, toxicology has moved from a phase of rapid development and has changed from an almost entirely descriptive science to one that is strongly based in the study of the biochemical and molecular mechanisms responsible for toxic effects.

1.01.1.1.1 Pesticides/herbicides/fungicides

Pesticides have been developed to control a wide variety of pests, primarily in agricultural and forest environments. Due to the very nature of their use in pest control, these compounds are common environmental contaminants. In fact, pesticides are the only class of chemicals that are designed specifically to kill higher biological organisms (e.g., animals and plants) and are intentionally released into the environment in large quantities. Prior to World War II, chemical control of insect and plant pests was accomplished by using a relatively small number of inorganic pesticides such as sulfur compounds and lead arsenate. However, with the development of dichlorodiphenyltrichloroethane (DDT) as an insecticide in the 1940s, there was a dramatic expansion in the development and use of a wide variety of synthetic organic pesticides. DDT was not only very effective in killing a wide range of insect pests and was relatively easy to manufacture, it also exhibited very persistent properties that allowed it to remain active for years. The success of DDT led to the development of other structurally similar organochlorine chemicals, such as aldrin, chlordane, heptachlor, and dieldrin. Unfortunately, the long residual life of these chemicals was also found to be a major contributor to their toxicity in nontarget organisms like fish and wildlife, and as a result, their use has largely been banned or restricted. Nevertheless, although today we tend to think of organochlorine compounds as "villains" to our well-being, they have also proven to be indispensible for controlling vector-borne diseases, like malaria. DDT in particular is now thought to have saved more human lives than any other single chemical, with the possible exception of penicillin.

Ultimately, the restrictions on use of DDT and other commonly used organochlorine pesticides led to increased use of other, more acutely toxic, synthetic compounds that would also be effective in pest control, but were much less persistent in the

environment. Chief among these groups were organic compounds known as organophosphates (OPs) and carbamates, which together were the foundation of insect control chemicals in the 1980s. Toxicity with both classes is due to inhibition of the acetylcholinesterase activity of nervous tissue (Costa, 2013), but carbamates are generally regarded as being less toxic because their inhibition is reversible (Gallo, 2013). While OPs and carbamate insecticides are still employed around the world, many have been heavily restricted or banned outright. In the United States, the mandates of the Food Quality Control Act of 1996 resulted in the EPA banning most residential uses of OPs, and the intervening years have seen further reductions in the remaining approved applications. For example, chlorpyrifos has been used since 1965 for a variety of both agricultural and nonagricultural applications, and remains one of the most broadly used OP pesticides. However, over the last 16 years, its use has been restricted in terms of application procedure and specific crops (e.g., tomatoes, apples, grapes, citrus, and tree nuts), due to the potential for human health impacts. In 2015, the EPA issued a proposal to revoke all chlorpyrifos tolerances, which would effectively cease all agricultural uses of the chemical if finalized.

Another widely used broad-spectrum insecticide with a different mechanism of action than the antiesterases, fipronil, has experienced increasing use since the 1990s. This compound acts by disrupting the insect's central nervous system (CNS) by blocking the passage of chloride ions through Gamma-aminobutyric acid (GABA) and glutamate receptors of the CNS (Tingle et al., 2003). Additional chemical groups of pesticides and herbicides, such as pyrethroids and plant growth regulators as well as biological controls, have been developed for agricultural and forest use. Since OP pesticides were largely banned from residential use by the EPA, pyrethroids have come to represent the majority of household insecticides. Though concerns still exist regarding the extreme sensitivity of aquatic insect species and fish to pyrethroids, these chemicals have ultimately proven to be more selective in their toxicity, and more compatible with the environment than their predecessors when properly applied.

Most herbicides are of relatively low acute and chronic toxicity, although controversy over reported chronic effects of phenoxy acid herbicides such as 2,4-dichlorophenoxy acetic acid (2,4-D) and 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) has led to changes in their manufacture and use. Due to the potential for contamination of 2,4,5-T with the highly toxic and unwanted by-product 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (commonly referred to as simply "dioxin"), this herbicide has been removed from commercial use in the United States. In contrast, 2,4-D continues to be widely applied. Its use has been repeatedly evaluated by regulatory bodies like the US EPA, and the EU's European Food Safety Authority and found to pose little risk to human health when use properly. Nevertheless, such evaluations are ongoing and subject to change. In June of 2015, the World Health Organization's International Agency for Research on Cancer (IARC) caused controversy when it reevaluated the safety of 2,4-D and proposed a designation of "possibly carcinogenic to humans (Group 2B), based on inadequate evidence in humans, and limited evidence in experimental animals." IARC indicated strong evidence in animal studies regarding the potential for 2,4-D to induce oxidative stress, and moderate evidence for immunosuppression, but stated that the epidemiological data regarding the risk of cancers such as non-Hodgkin's lymphoma (NHL) were not strong or consistent (IARC, 2015). The association between 2,4-D and NHL remains controversial (Garabrant and Philbert, 2002; Goodman et al., 2015). Similar concerns and controversy have arisen over an association between 2,4,5-T and/or dioxin exposure and a rare group of cancers collectively called soft tissue sarcomas (Bradberry et al., 2004).

The broad-spectrum herbicide paraquat is the most toxic of the commonly used herbicides and is considerably more toxic than many insecticides. Paraquat produces delayed-onset lung damage, similar to emphysema, which frequently is fatal (Dinis-Oliveira et al., 2008). Acute symptoms such as gastrointestinal (GI) distress, nausea, vomiting, and malaise may subside within a day after exposure, and prognosis may appear good, only to have the patient readmitted a week or two later with progressive failure of the lungs. This herbicide should be used carefully only by those who are trained in safe pesticide use. It is important to note that a closely related herbicide, diquat, does not share the potent lung toxicity as observed with paraquat (Jones and Vale, 2000) and thus does not share the similar restrictions on its application. In 2016 the US EPA proposed new regulations to curtain accidental poisonings with parquat (see: https://www.epa.gov/pesticides/epa-takes-strong-steps-prevent-poisonings-and-protect-workers-paraquat). In the proposed rule, EPA noted that since 2000, there have been 17 deaths, including 3 involving children, that were caused by accidental ingestion of paraquat, and 3 other deaths following dermal exposure. The deaths from ingestion resulted from the pesticide being illegally transferred to beverage containers and later mistaken for a drink and consumed. A single sip can be fatal. The new proposed ruling is focused largely on packaging and labeling, and additional training, including restricting the use to certified pesticide applicators.

1.01.1.1.2 Metals

Metals are a unique class of toxicants in that their chemical form may be changed as a result of environmental conditions, and these different physical forms may significantly affect toxicity. Many metals (essential metals) are needed (typically in very low concentrations) as cofactors for normal biochemical functions. Excessive amounts of trace metals may occur naturally as a result of normal geological conditions such as ore formation. Processes such as weathering or leaching may render these metals more biologically available in the environment. A significant source of metal contamination in the environment is through burning of fossil fuels, mining, smelting, and discharging domestic and industrial wastes. Due to their physicochemical properties, metals are typically persistent once released into the environment.

Some metals such as beryllium and mercury are directly hazardous such that even minimal exposure may adversely affect human health (**Table 2**). Other metals such as cadmium, copper, manganese, lead, nickel, and tin have also been demonstrated to cause a number of toxic effects. Although metals typically elicit a wide range of toxic effects, there are a number of toxicological features that are shared to some degree by many metals. These are (1) inhibition of enzymes which is a major mechanism of toxicity of metals, (2) many metals exist in chemical forms that allow them to enter into cells and disrupt normal cellular processes, and

Table 2 Examples of major toxic metals with multiple effects in humans

Metal	*Toxicity*
Arsenic	Neurotoxicity, liver injury, vascular disease, skin cancer
Beryllium	Lung disease, contact dermatitis, lung cancer
Cadmium	Lung disease, kidney disease, bone calcium loss, hypertension, lung cancer, prostate cancer
Nickel	Lung cancer, nasal cancer, contact dermatitis
Lead	Neurotoxicity, developmental effects, anemia, kidney toxicity, hypertension, sterility
Mercury	Neurotoxicity, gastrointestinal damage, kidney toxicity

(3) although such therapy can also deplete essential minerals, protection against metal toxicity is often possible by the administration of chelating agents that form stable bonds with metals and therefore limit their biological reactivity (Tokar et al., 2013). Exposure to metals can sometimes be quantitatively assessed by the presence and level of metal in the urine. As the metal becomes biologically stored or bound, however, urinary or blood metal levels will decline. Metals such as methyl mercury accumulate in the hair, and thus analytical analysis of hair methyl mercury concentrations has been used as a measure of exposure in areas with individuals subjected to methyl mercury poisoning.

From a public health perspective, one of the most important metals (actually, a "metalloid") is arsenic. Arsenic occurs naturally in groundwater in many regions of the world. A substantial body of epidemiological data has demonstrated that arsenic in drinking water is associated with an increased risk for several types of cancer, most notably lung, liver, and skin cancer. Remarkably, the carcinogenic effects of arsenic are not readily demonstrated in animal models, yet the human epidemiology data leaves little doubt that concentrations of arsenic in drinking water at concentrations in the range of 100–5000 ppb (micrograms of arsenic per liter of water) are associated with significant increases in cancer risk. Based on these studies, in 2007 the US EPA lowered the drinking water standard for arsenic from 50 to 10 ppb. Consumption of arsenic-contaminated drinking water has become a major public health disaster in parts of Bangladesh and India, where thousands of shallow groundwater wells were installed in an effort to reduce dysentery and vector-borne diseases that resulted from consumption of contaminated surface waters. Although well intentioned, the groundwater in many of the wells contained relatively high levels of arsenic, and thousands of people have developed arsenism (chronic arsenic poisoning) and arsenic-related cancers as a result. While local and international efforts to reduce exposure have been largely successful, there are small regions in Bangladesh that continue to suffer from arsenic toxicity from contaminated water supplies (Milton et al., 2012; Yunus et al., 2016).

1.01.1.1.3 Industrial chemicals

There are many thousands of chemicals that are used in industry. These range from inorganic compounds and metals to complex organic chemicals. As a group, organic solvents account for a large percentage of the chemicals used in industry. By definition, a solvent is any substance that has the ability to dissolve another substance (the solute). Typically, solvents are liquids and solutes are solids. Although water is often considered the "universal solvent," many substances are insoluble in water and therefore require alternative liquids for dissolution. Some typical examples of solvent used in industrial settings include those solvents used in the automotive industry with spray painting, metal trades, plastics, petrochemicals, wood working, and dry cleaning. Solvents of industrial use typically are very effective at dissolving fat-soluble (but water-insoluble) substances. Many industrial solvents are obtained from the distillation of crude oil (petroleum distillates), and therefore have the added hazard of flammability. Sometimes specific organic chemicals, such as toluene or xylene, are used as solvents in paints or other commercial products. Since petroleum-derived solvents often present a serious risk of explosion and/or fire, an alternative, nonflammable class of solvents has been developed synthetically for industrial use by chlorination of the simple one and two carbon hydrocarbons, methane and ethane. Thus, chlorinated solvents such as methylene chloride, trichloroethylene, 1,1,1-trichloroethane, and perchloroethylene are widely used in a variety of industries as degreasers or for other cleaning purposes.

In general, solvents may produce two types of toxic effect: defatting of the skin and depression of the CNS. The former is less serious but a common cause of dermatitis in industrial settings, whereas the latter is a major health concern in the occupational environment. CNS depression may occur following inhalation exposure to airborne solvents in the workplace, and at high concentrations can be lethal. However, a more common concern is secondary injuries that might occur when working around heavy equipment following inebriating exposure to organic solvents. The CNS depressant effect of industrial solvents is similar to that which occurs from consumption of alcohol. Ingestion of solvents used around the home is a frequent cause of childhood poisonings. Although most ingestion exposure to solvents will not result in serious CNS depressant effects, aspiration of the solvent into the lungs during vomiting is a serious and potentially fatal consequence of solvent ingestion. Thus, vomiting should never be induced in an individual who has ingested any organic solvent (including gasoline).

An interesting example of public health concerns related to solvents is the case of methyl-*tert*-butyl ether, or MTBE. MTBE is a widely used gasoline additive which was added to ostensibly improve air quality by reducing harmful automobile emissions. However, MTBE itself has some toxic properties (McGregor, 2007) and has been controversial, in part because of concerns about potential inhalation health effects and more recently because of concerns about groundwater contamination from leaking

underground gasoline storage tanks. Among the lessons that can be derived from the MTBE experience is the value of a thorough understanding of the risks, benefits, and trade-offs when substituting one solvent for another (Davis and Farland, 2001).

1.01.1.1.4 Vapors and gases

A number of toxicant responses are the result of absorption of chemicals that exist as either vapors or gases. Indeed, the most frequent cause of death from poisoning is due to the result of carbon monoxide (CO) exposure. CO is formed from incomplete combustion of organic matter, and as such may be produced in lethal quantities in automobile exhaust, faulty home heating systems, improperly used portable gas stoves and heaters, improperly vented wood stoves and fireplaces, and in many industrial situations. Cigarette smoke also contains relatively large quantities of CO, and it is common for heavy smokers to have two to three times more carboxyhemoglobin (COHb) than nonsmokers. CO has a high affinity for the iron molecule in hemoglobin (the oxygen-carrying pigment in red blood cells), and binding of CO to hemoglobin greatly decreases the ability of hemoglobin to deliver oxygen to tissues. Since the affinity of CO for hemoglobin is about 220 times greater than that for oxygen, breathing air containing CO at only 1/220th that of O_2 will result in the loss of 50% of the oxygen-carrying capacity of the blood. Thus, in an atmosphere of 21% O_2 (normal air), a CO concentration of 0.1% (1000 ppm) would result in 50% COHb, which is approaching a lethal level. Although the interference with the oxygen-carrying capacity of the red blood cells produced by CO is the major contributor to its toxicity, CO can also affect the ability of cells to utilize oxygen.

Individuals with normal red blood cell and hemoglobin amounts in the blood, and normal heart and lung function, do not generally have symptoms associated with COHb concentrations up to about 10%. However, COHb from 10% to 30% may result in tightness across the forehead, headache, and some dilation of blood vessels in the skin. As COHb increases to 30–50%, headaches may be quite severe, and accompanied by nausea, weakness, dizziness, increased pulse and respiration, and possibly fainting and collapse. COHb above 50–60% may be accompanied with all of the former and may readily lead to coma and death. Obviously, severely anemic individuals, and/or those who have preexisting lung or heart problems, may respond more severely to lower COHb concentrations. Individuals who survive the initial anoxic effects of CO poisoning may sometimes experience a delayed neuropsychiatric syndrome up to 240 days after the acute exposure, with symptoms ranging from subtle abnormalities such as personality changes and mild cognitive deficit, and in severe cases, dementia, psychosis, and Parkinsonism (Prockop and Chichkova, 2007).

Cyanide poisoning can result from inhalation exposure to hydrogen cyanide, or more commonly from inadvertent or intentional ingestion of cyanide salts. Cyanide anion acts by inhibiting the ability of cells to burn oxygen and sugars to produce energy (i.e., inhibition of cellular respiration). In contrast to the reduced form of iron (Fe^{2+}) in hemoglobin, the iron in cytochrome oxidase normally exists in the oxidized state (Fe^{3+}), for which cyanide anion binds tightly. Since oxygen utilization in the tissues is essentially blocked, venous blood may be as bright red as arterial blood, imparting a flushed appearance to skin and mucus membranes. The route of exposure is of consequence only because of the rate at which the chemical is absorbed. Since tissues which require high amounts of O_2 (e.g., brain and heart) are most readily affected, rapid absorption and distribution as occurs with inhalation exposure is usually rapidly fatal and frequently does not offer time for diagnosis and intervention. Ingestion of cyanide salts will result in considerably slower absorption relative to inhalation, such that diagnosis and effective treatment are sometimes possible (Nelson, 2006).

Hydrogen sulfide (H_2S) is a common component of "sewer gas," and is used and produced in many industrial processes, as well as by natural decay of organic matter high in sulfur (such as some seaweeds). H_2S has a strong, unpleasant, yet characteristic, odor of rotten eggs. However, loss of the ability to smell H_2S occurs rapidly such that the odor may seem to disappear quickly, even though dangerous amounts of gas may still be present, and thus the absence of odor does not indicate a safe environment. H_2S produces its toxic effects in essentially the same way as cyanide, although it is somewhat less toxic.

1.01.1.1.5 Naturally occurring toxins

In addition to the tens of thousands of anthropogenic (manufactured) chemicals, there exist a number of toxic substances produced by plants, animals, and fungi (toxins). Venomous animals occupy every continent and nearly every aquatic system on the earth, and numerous poisonous plants are present and adversely affect humans and animal populations. Animal toxins vary considerably with respect to their complexity and may include a diverse number of polypeptides and enzymes with different mechanisms of action (Gregus, 2013). While the majority of venoms exert their toxic effects directly upon the cells and tissues that they contact, many venoms damage a variety of tissues and organ systems, and produce a plethora of toxic effects. Venoms produced by elapid snakes (coral snakes and cobras), scorpions, and black widow spiders affect the nervous system and are termed neurotoxins. The most common clinical signs associated with rattlesnake bites are swelling and redness at the site of the bite. In addition, there is local pain and locomotion becomes stiff and painful. In some cases, the redness and swelling may extend over the entire limb or even over the entire body.

A variety of marine animals, including certain species of jellyfish, corals, sea anemones, mollusks, octopus, squid, sea urchins, and others, have either venomous glands or cells or spines. Those animals with toxic spines typically release a toxin directly into the skin when contacted, whereas in some animals the venom gland is part of the digestive system. In many areas shellfish consumption has resulted in poisoning. In such cases, these organisms themselves are not responsible for the production of the toxin. Instead, they typically harbor dinoflagellate unicellular algae that are responsible for toxin production. Thus, the shellfish itself is not affected by the toxin; however, animals or humans that ingest the contaminated shellfish become the victims. For example, saxitoxins are a class of compounds that induce a condition known as paralytic shellfish poisoning (PSP), which can cause muscular paralysis and respiratory failure with acute exposure. However, PSP is only one of at least five different types of poisoning

syndromes associated with shellfish consumption. Interestingly, several of these conditions, such as amnesic shellfish poisoning, caused by domoic acid, and azaspiracid shellfish poisoning have been identified in the last 30 years alone (Twiner et al., 2008).

The recent global increase in shellfish related poisonings is directly related to the growing occurrence of the harmful algal blooms (HABs) that produce them. While the exact contribution of human activities to these events is still unclear, there seems little doubt that we have aided this growth. Though efforts have long been underway to restrict their impact, costal eutrophication (excessive nutrient introduction) from agricultural processes, and the introduction of nonindigenous algal species through shipping ballast continue to help produce the ideal conditions necessary for HABs to occur. Furthermore, given the predicted increases in ocean temperatures, there also seems little doubt that such conditions will become even more common in the future. Many countries have begun developing specialized monitoring and warning systems to prevent shellfish consumption during such blooms, but the sporadic nature of HABs has thus far made adequate forecasting difficult (Berdalet et al., 2015).

A large array of the toxins produced by plants (phytotoxins) has evolved as defenses against herbivorous insects and animals. In some cases, these compounds may act more as repellents than physiologically crippling poisons. A group of alkaloids termed pyrrolizidine alkaloids are produced by plants of the *Senecio*, *Helotropium*, and *Crotalaria* species, many of which occur as weeds in many parts of the world (Albertson et al., 1994). In instances where these plants have contaminated cereal crops and consumption by humans has occurred, there have been cases of poisoning. Animals may also be exposed to pyrrolizidine compounds and suffer from toxic effects. Atropine, which is found in deadly nightshade berries (*Atropa belladonna*), and ricin, which is found in the seeds of the castor oil plants, are two of the most toxic plant products (Albertson et al., 1994). Other better known toxic substances derived from plants include cocaine, caffeine, nicotine, morphine, and cannabis (marijuana).

Mycotoxins are toxic, secondary fungal metabolites found in foods as a result of contamination by certain fungal molds. Thus, the mycotoxin is consumed in the diet. Mycotoxin poisonings typically appear in livestock, but can also be associated with human consumption of contaminated grains. For example, the aflatoxins represent a group of closely related toxic compounds produced by the common fungal molds, *Aspergillus flavus* and *Aspergillus parasiticus*. A number of adverse human health effects have been associated with dietary contamination with aflatoxins, including liver toxicity and liver cancer (Eaton and Gallagher, 1994). Worldwide, aflatoxins are considered a major public health problem, especially in developing countries where high heat and humidity favor the growth of the mold, and food storage is inadequate. The focus of this concern is almost universally on the carcinogenic effects of aflatoxins, as there is substantial research data to indicate a causative role of aflatoxins in the unusually high incidence of liver cancer in some areas of the world. Other mycotoxins may preferentially affect the nervous system, exert respiratory effects, or may cause reproductive disorders.

1.01.1.1.6 Drugs

Since drugs are compounds that are designed to have biological activity, it is not surprising that under certain conditions they may elicit toxic reactions. The danger to the individual depends upon several factors, including the nature of the toxic response, the dose necessary to produce the toxic response, and the margin between the therapeutic dose and the toxicity threshold. Thus, the use of a very dangerous drug with only a narrow margin between the therapeutic and toxic dosage may not be justified if a safer drug for that particular disease is available. Furthermore, drug toxicity is affected by factors that influence the toxicities of other chemicals, including genetic variation, age, sex, diet, and coexposure to other chemicals. For the most part, the adverse reactions associated with drug exposure are associated with wrongful use. There are several different types of toxicities associated with drugs including adverse or side effects associated with proper therapeutic usage, immediate (acute) toxicity associated with overdose, interactions with other drugs that lead to toxic side effects, and habitual use of drugs leading to toxicity. However, it must be emphasized that the toxic side effects of drugs are generally uncommon and may occur more frequently in susceptible individuals or populations.

Drug overdoses are the leading cause of injury deaths in the United States, at nearly 44,000 per year. Deaths from drug overdose have more than doubled in the past 14 years, with the majority associated with prescription drugs. Annual drug overdose mortality exceeds motor vehicle-related deaths in 36 states and Washington, D.C. (http://healthyamericans.org/reports/injuryprevention15/). These sobering facts have led to a multitude of initiatives aimed at both creating awareness among prescribers, and probing for potential signs of abuse. The development or strengthening of Prescription Drug Monitoring Programs in many states has been particularly instrumental in accomplishing the latter. These programs provide pharmacists and prescribers with information regarding a patient's history with controlled substances, as well as monitoring for any indication that such drugs are being directed into illegal channels (see: https://www.cdc.gov/drugoverdose/pdmp/index.html). While these collective efforts seem to have had a positive outcome over the last few years, the abuse continues to be a major threat to public health around the world.

1.01.1.2 Dose–Response

Four centuries ago, Paracelsus stated that "All substances are poisons; there is none which is not. Only the dose differentiates a poison from a remedy." The relationship between the dose of a compound and the response elicited is a fundamental concept in toxicology. Regardless of the source-toxic animal venoms, pesticides, industrial chemicals, or therapeutic drugs—the responses of living organisms largely show a dose–response relationship (see "**Hormetic and Nonmonotonic Dose Responses**" section, for discussions of nonmonotonic response curves). Inherent in this relationship is the tenant that the magnitude of the effect increases with dose. This concept is depicted graphically in **Fig. 1**. For any living organism and chemical, there exists a dose below which no adverse or toxic effect will be observed. However, the body has a certain finite ability to handle the chemical before toxicity is observed. This ability may vary among individuals and across species. This threshold level is also influenced by a number of intrinsic

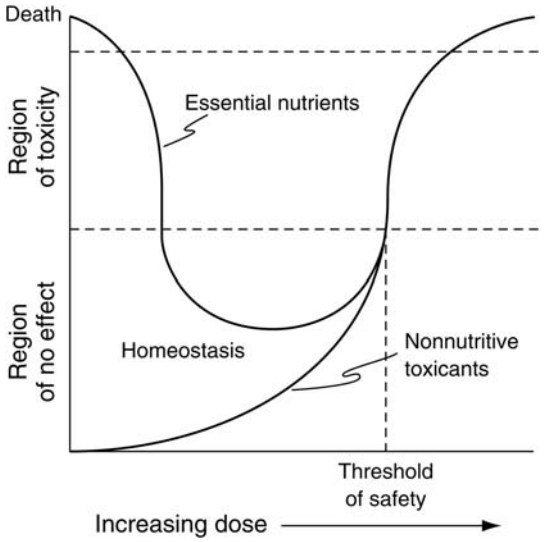

Fig. 1 Dose–response relationship for an individual exposed to either an essential substance or a nonnutritive substance. As observed, a threshold exists for most types of toxic response such that below the threshold, no toxicity is evident. For essential substances such as certain vitamins and trace metals, doses well above or below the safety threshold may elicit toxicity. (Reproduced from Rosenstock, L., Cillen, M.R., Redlich, C.A., Brodkin, C.A. (eds.) (2005). In: Textbook of clinical occupational and environmental medicine (2nd edn.), p. 84. Philadelphia, PA: Elsevier Saunders (Chapter 5). With permission of Elsevier Saunders.)

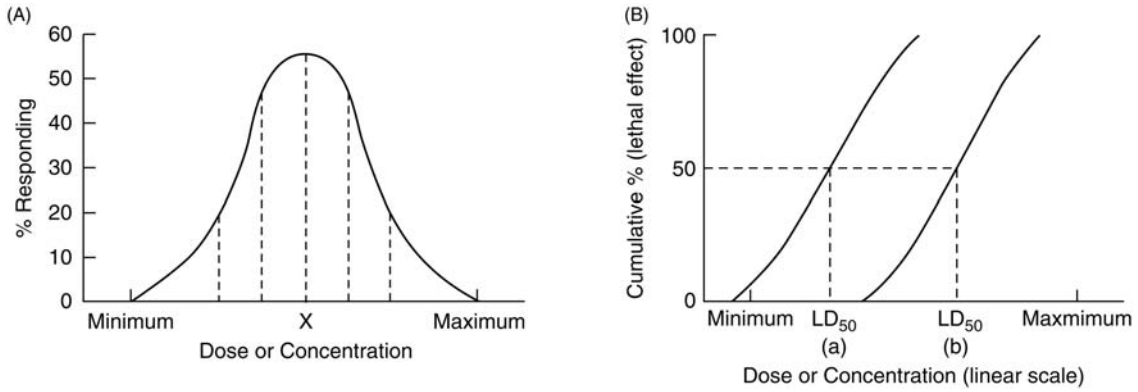

Fig. 2 Frequency distribution and quantal dose–response relationship. (A) Represents a frequency response distribution curve for the response of a given population on exposure to a toxic substance. (B) Plots the data for two different chemicals (a) and (b) as cumulative quantal dose–response curves, with the midpoint of the curves representing the LD_{50} if the quantal response is death. (Reproduced from Rosenstock, L., Cullen, M.R., Redlich, C.A., Brodkin, C.A. (eds.) (2005). In: Textbook of clinical occupational and environmental medicine (2nd edn.), p. 85. Philadelphia, PA: Elsevier Saunders (Chapter 5). With permission of Saunders.)

factors including age, gender, weight, and genetics, and by extrinsic factors such as smoking and exposure to other chemicals. Since such variations exist, there will always be individuals within a population who are relatively sensitive and are therefore at increased risk of exposure to some chemicals. Conversely, there are others who are resistant and who require relatively greater exposure to elicit similar toxic responses.

The variation in population response to toxic chemicals often follows a classical "bell-shaped curve" also called a Gaussian distribution (Fig. 2A), which is frequently depicted as a cumulative, quantal dose–response curve (Fig. 2B). These curves identify the response of a population to varying doses of a toxic chemical, and the midpoint of the curve represents the effective dose 50, or ED_{50}, that is, the dose at which 50% of the population responds. If the effect that is measured is death, then the ED_{50} is expressed as the lethal dose 50, or LD_{50}. The LD_{50} measures only the acute, or single dose, response to chemicals, and the only response it refers to is death. It says nothing about other types of responses such as neurological effects, carcinogenic potential, teratogenic potential, reproductive effects, or other serious adverse effects that may well occur at doses far below the LD_{50}. Furthermore, the LD_{50} values are always based on laboratory animal data, and thus poorly reflect the diversity of human conditions and experiences that may drastically alter response to toxic chemicals. It is therefore never safe to assume that exposures far below the LD_{50} are always harmless without a much greater understanding of the types of effects a chemical produces.

1.01.1.3 Hormetic and Nonmonotonic Dose Responses

In addition to the dose–response relationships described earlier, there is increasing evidence that dose–response curves associated with some nonnutritional toxic substances may exhibit nontraditional shapes in their dose–response. In particular, these agents may impart beneficial or stimulatory effects at low doses, but at higher doses, the agents produce the expected adverse effects—a concept referred to as "hormesis." Hormesis was first described for radiation effects (Wang et al., 2008) but also pertains to many other chemical responses (Calabrese, 2013). In these circumstances, a plot of response over a wide range of doses results in a "U-shaped" dose–response curve (**Fig. 1**). Hormetic responses are often viewed as adaptive in character and the result of natural selection. Hormetic responses, by definition, have unique quantitative features that describe the magnitude and width of the low-dose stimulatory response (Calabrese, 2008). They are expected to occur in all types of biological systems and in all types of physiological systems and cells. The application of the concept of hormesis to whole-animal toxicological dose–response relationships may also be relevant, but requires that the "response" on the ordinate be variant with dose, such that different types of responses are recognized to occur at different doses. An example is the substantial clinical and epidemiological evidence to indicate that low to moderate consumption of alcohol may reduce the incidence of coronary heart disease, whereas chronic high-dose alcohol consumption can increase the risk of liver cirrhosis and liver cancer, as well as cancer of the esophagus.

Another example of hormesis relevant to toxicology is the adaptive response to "oxidative stress." It is now widely recognized that the generation of oxygen free radicals ("reactive oxygen species," or ROS), at relatively low-dose levels, from a host of potentially toxic substances stimulates a change of events that lead to transcriptional activation of a set of genes that code for proteins that facilitate the elimination of ROS and enhance repair of deoxyribonucleic acid (DNA) damage. This type of adaptive response hormesis may be particularly relevant to both low-dose toxicological response to toxic chemicals, and normal aging (Gems and Partridge, 2008). Even the induction of expression of xenobiotic biotransformation enzymes that occurs with many chemicals at doses below those that cause evident toxicity may be viewed as a type of hormetic response.

It has also been reported that certain toxicants, including natural hormones and their synthetic mimics (see "**Toxic responses of other organ systems**" section for a discussion of endocrine disrupting chemicals), can show bi- or even multiphasic adverse responses. In contrast to classical dose–response curves, which show a linear relationship between dose and effect above certain dose levels (the so-called "threshold" levels), these so-called nonmonotonic dose response curves (NMDRCs) are characterized by one or more changes in the sign of the slope across a wide range of the doses examined (Vandenberg et al., 2012). In practice, this distinction means that the high dose exposures often utilized in traditional toxicity testing to identify target organs may substantially underestimate the hazard posed by environmental exposure. Such a response has been described for the plasticizer Bisphenol A, which has been reported to influence reproductive outcomes including spermatogenesis and seminal vesicle weight, and overall body weight in males (Richter et al., 2007). While the findings regarding some NMDRC agents have been hindered by poor reproducibility between studies, they have also led multiple organizations, including the EPA and Food and Drug Administration (FDA), to reevaluate the essential assumptions underlying chemical testing procedures, especially for chemicals that may act as modulators (agonists or antagonists) of endocrine pathways.

1.01.2 Concepts of Absorption, Distribution, Metabolism, and Excretion

1.01.2.1 Absorption

With the exception of local effects at the site of contact, a toxicant can only cause injury if it is absorbed by the organism, for example, if it crosses from the external environment of the lung, skin, or GI tract into the bloodstream. The rate and site of absorption are critical factors in the eventual toxicity elicited by a compound. Most toxic chemicals are absorbed via the process of simple diffusion across cellular membranes. A cell membrane generally consists of a bimolecular layer of lipid molecules with proteins scattered throughout the membrane (**Fig. 3**). The structure of biological membranes is a key determinant of their functional

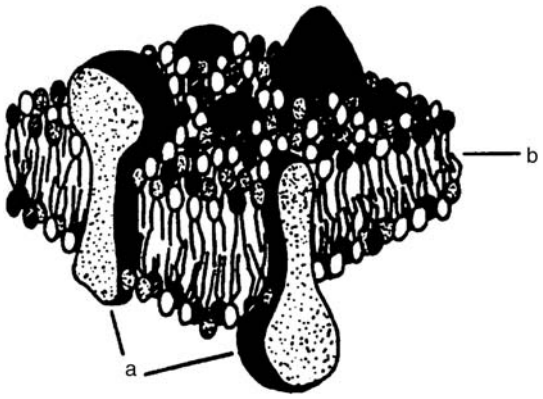

Fig. 3 Three-dimensional structure of an animal cell membrane showing the interspersion of proteins (A) among the phospholipid bilayer (B). (Reproduced from Timbrell, J.A. (2001). Introduction to toxicology, p. 20. With permission of Taylor & Francis.)

characteristics. From a toxicological perspective, these structural characteristics render biological membranes selectively permeable. In essence, this means that only certain substances are able to pass through them, depending upon the physicochemical characteristics of the chemical.

There are four basic mechanisms by which a toxicant may cross cellular membranes. The most common is passive diffusion through the membrane. For passive diffusion to occur, the compound must be neutral (uncharged) and must be lipid soluble. Furthermore, there must exist a concentration gradient across the membrane. The rate of diffusion is described by Fick's law:

$$\upsilon_0 = \frac{dX}{dt} = \frac{P \times A/(C_2 - C_1)}{d}$$

where υ_0 is the rate of flux of a chemical across a membrane, P is the permeability coefficient, A is the surface area, d is the diffusion distance, C_2 is the concentration gradient outside the membrane, and C_1 is the concentration gradient inside the membrane. The extent of absorption is directly proportional to the surface area exposed, the concentration gradient, and the length of time over which exposure occurs, and is inversely proportional to the thickness of the particular diffusion barrier. The permeability coefficient, P, is an arbitrary constant that is determined by both the physicochemical characteristics of the chemical and the particular diffusion barrier. Other transport processes include filtration through membrane pores, active transport, and pinocytosis/phagocytosis (engulfing by the cell). Small molecules such as urea typically cross membranes by filtration, whereas phagocytosis and pinocytosis are mechanisms by which particles of insoluble substances such as asbestos are absorbed. Active transport of chemicals across membranes requires metabolic energy to operate and is normally for endogenous compounds such as nutrients. Often, however, analogs and physically similar molecules may undergo active transport across cellular membranes.

There are three major sites for absorption of foreign compounds: the skin (dermal or percutaneous absorption), the lungs (pulmonary or inhalation absorption), and the GI tract (oral absorption). Since the diffusion barriers of skin, GI tract, and lungs (respiratory tract) are made mostly from lipids (fats), the rate of absorption is dependent upon the solubility of the chemical in lipids. Thus, lipid solubility, as determined by "octanol–water partition coefficient," is frequently used as a crude predictor of the ability of chemicals to be absorbed. Chemicals which are highly fat soluble are in general quite well absorbed, whereas highly water-soluble substances are generally poorly absorbed.

1.01.2.1.1 Absorption of chemicals via the GI tract

Since the GI tract functions physiologically as an organ of absorption, it is not surprising that a variety of chemicals are well absorbed when ingested. Ingestion of contaminated food and water is also a common route of exposure to environmental pollutants for this reason. While some chemicals are caustic and irritating to the mucosa, most toxic chemicals do not cause any adverse effects until they are absorbed. The absorption of weak acids and bases is greatly influenced by the pH at the site of absorption. The extent of ionization of a weak acid or base is a function of both the pH and the pK_a of the chemical, as described by the Henderson–Hasselbalch equation:

$$\text{For weak acids, } pK_a - \text{PH} = \log\frac{[\text{nonionized}]}{[\text{ionized}]}$$

Thus, weak organic acids, which generally have a pK_a of 3–4, exist predominantly in the nonionized (lipid soluble) form at pH values >3. In contrast, weak organic bases, which have pK_a values of 6–8, exist in the ionized, water-soluble form at low pH. Thus, the stomach, which has a pH of 2–3, is a significant site of absorption for weak acids such as benzoic acid. In contrast, weak acids exist in the ionized form in the intestine (pH 6–7), and are thus less readily diffusible there. The intestine favors absorption of weak bases such as aniline. However, while the rate of absorption for some compounds may be faster in the stomach, the extent of absorption by those compounds is often greater in the intestine because of longer residence time and higher surface area in the intestine. The specialized transport systems that exist in the intestine for the absorption of nutrients may also carry toxicants such as lead and thallium across intestinal membranes.

1.01.2.1.2 Absorption of chemicals across the skin

In contrast to the GI tract, the physiological function of the skin is to act primarily as a barrier to absorption of exogenous substances from the environment, and to prevent excessive loss of water and electrolytes from the body. Despite its protective function, some chemicals can be absorbed through the skin in sufficient quantities to cause systemic effects. The protective function of the skin lies exclusively in the epidermis, the outermost layer of cells (Fig. 4). The outermost layer of the epidermis, called the *stratum corneum*, consists of multiple layers of flattened, dead cells. Absorption of chemicals across the skin (percutaneous or dermal absorption) occurs exclusively by simple diffusion. There is little question that the primary barrier to absorption of chemicals across the skin is the stratum corneum. Upon penetration of the stratum corneum, diffusion through the inner layers of the skin is extremely rapid for most substances.

There are many factors which can influence both the rate and the extent of absorption of chemicals across the skin. The single most important one is the integrity of the stratum corneum. Damage to this barrier will result in greatly enhanced penetration, as will irritation, inflammation, and other forms of injury. The age of the skin may be important, as children and the elderly tend to have higher rates of skin absorption than young adults. The second phase of percutaneous absorption occurs when the toxicant

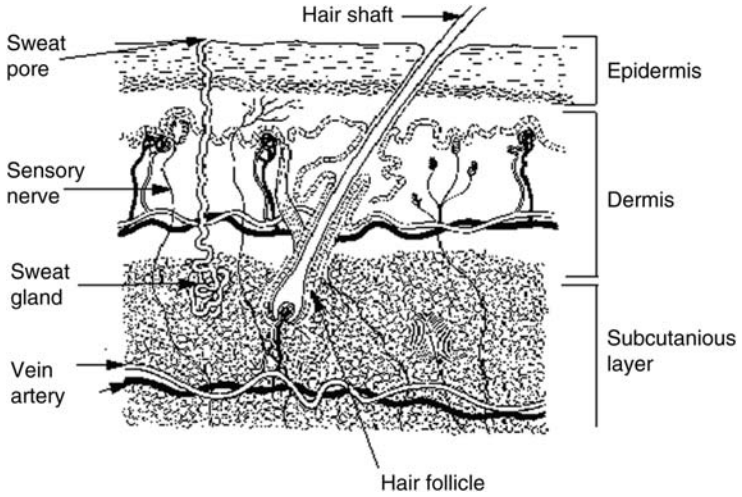

Fig. 4 Diagram of a cross section of human skin. (http://www.cancerindex.org.)

diffuses through the lower layers of the epidermis. These layers include the *stratum granulosum*, *stratum spinosum*, and *stratum germinativum* (Fig. 4). These cell layers are not as efficient as the stratum corneum as barriers to toxicant diffusion. Toxicants enter the general circulation after passing through these areas and enter the systemic circulation through the venous and lymphatic capillaries in the dermis. Skin appendages include hair follicles, sebaceous glands, and sweat glands. However, their total contribution to percutaneous absorption is considered negligible.

Among the various physicochemical factors that can influence skin absorption, the extent of hydration (amount of moisture in the skin) is one of the most important. The permeability of skin has been shown to increase as much as four- to fivefold following hydration. Dehydration may also enhance absorption by causing damage to the integrity of the outermost layer of skin. At water concentrations below 10% the skin becomes brittle and looses its functional integrity. Tightly covering the skin—for example, with rubber gloves—is an especially effective means of enhancing skin absorption; in one study occlusion with an impermeable barrier resulted in a 50-fold increase in penetration when compared to the same chemical in an identical formulation without occlusion. The use of gloves which serve as an incomplete barrier to chemicals may actually enhance skin absorption. Thus, it is imperative that gloves worn to protect against skin contact from chemicals be truly impermeable to the chemical of concern.

1.01.2.1.3 Absorption of chemicals via the respiratory tract

The main site of absorption in the respiratory tract is the lungs. Like the GI tract, the lung is designed for optimal absorption. It has a large total surface area (50–100 m^2), high blood flow (4–5 L min^{-1}), and a very thin diffusion distance. The lungs serve as an important site of contact with chemicals in the external environment. This is especially true for gases such as CO and for vapors of volatile liquids such as benzene and carbon tetrachloride. However, absorption of chemicals from inspired air can occur regardless of the physical form. For gases and fine vapors, absorption occurs via direct diffusion across the cells in the deep reaches of the lung (alveolar air spaces), whereas for aerosols and other types of particle, deposition occurs along various aspects of the tracheobronchial tree, with the specific location depending on size and density of the particle. Such contact can result in direct damage to the lungs, or may lead to toxicity following absorption into the bloodstream.

Particles (aerosols) deposit on the lung surface primarily via physical forces. Inhaled aerosols are variable in size. The mass median diameter is a common means of describing aerosol size, as it considers not only the physical diameter of particles, but their density as well. The rate of absorption of gases and vapors by the lung is largely a function of the blood–gas partition coefficient. Chemicals with high blood–gas partition coefficients will have a high rate of uptake into the bloodstream, relative to those chemicals with low blood–gas partition coefficients. For chemicals with a low solubility in blood, only a small fraction of the gas present in the lung will be removed, and an increase in breathing rate will not enhance uptake. Conversely, an increase in breathing rate can significantly enhance the extent of absorption of gases which are readily soluble in blood, as the delivery of gas to the blood, and not dissolution into blood, may be rate limiting in uptake (ventilation limited). Solubility of a gas in blood should not be equated to its solubility in water, because components of blood other than water can greatly affect the solubility of some gases. For example, CO, because of its high affinity for hemoglobin, has an extremely high apparent blood–gas partition coefficient even though it is only sparingly soluble in water.

Particles may be deposited on the respiratory tract epithelium by three fundamental processes: inertia, sedimentation, and diffusion. In the process of inertia, particles with sufficient mass will collide with the surface of the lung at points of branching and curvature. As the direction of air velocity changes, the inertial force of particles will prevent them from changing direction at the same rate as the airflow. The greater the mass, the less the ability of particles to change direction with airflow. Thus, deposition of particles occurs via impaction. In the process of sedimentation, particles that are of sufficiently small size to escape deposition via inertia, such as nanoscale materials, may deposit on the surface of the lung via sedimentation once the velocity of airflow becomes low.

Diffusion is a deposition process that is important for extremely small particles. Brownian motion, in which small suspended particles are bombarded by surrounding gaseous molecules, is the principal means of deposition. Although the particle size is the principal determinant of deposition, other factors, including breathing pattern, airway diameter, and the anatomy of the nasal, oral, and pharyngeal areas, are also important.

1.01.2.2 Distribution of Toxic Chemicals

After a chemical has been absorbed, it passes into the bloodstream and is distributed throughout the body. The part of the vascular system into which the compound is absorbed will depend upon the site of absorption. Absorption through the skin leads to the peripheral blood supply, whereas absorption through the lung will cause the compound to be distributed through the pulmonary circulation. Oral absorption will be followed by entry of the compound into the portal vein supplying the liver with blood from the GI tract.

Once in the bloodstream the rate of distribution to each organ is dependent upon the blood flow through the organ, the ease of penetration of the compound across local capillaries and cell membranes, and the affinity of components of the organ for the chemical. Only nonionized compounds will pass out of the bloodstream into tissues by passive diffusion. The concentration of the compound in the plasma reflects the distribution of the compound. Compounds that are lipid soluble (e.g., carbon tetrachloride) will be distributed to all tissues and will tend to have low plasma concentrations. In contrast, compounds that are ionized at the pH of the plasma, and/or tightly bind to plasma proteins, will not readily distribute into tissues, and thus tend to have higher plasma levels. The relationship between distribution and plasma concentration of a compound can be quantified as the apparent volume of distribution (VD).

The VD may sometimes indicate that a chemical is localized within a particular tissue or is confined mainly to the plasma. Thus, if a chemical distributes mainly into fat, it can be seen from the VD that the plasma concentrations will be low and thus the VD will be rather high.

$$\mathrm{VD} = \frac{\text{dose}}{\text{plasma concentration}}$$

1.01.2.2.1 First-pass effect

As indicated, after a chemical has entered the bloodstream, it is potentially available to all tissues in the body. However, biologic activity at or near the site of absorption may greatly reduce the availability of the chemical to distant sites. This phenomenon is termed the first-pass effect and is most often described in the context of absorption of chemicals following ingestion. In general, the first-pass effect after oral absorption is the result of efficient uptake and metabolism of compounds by the liver. Since the blood filtering through the GI tract is collected in the portal vein, all substances absorbed with blood must first enter the liver prior to distribution to other organs. The liver has a high capacity for extraction and biotransformation of compounds, and thus may efficiently limit the availability of chemicals from reaching other sites in the body. Although hepatic extraction is generally considered the most important site of removal of chemicals demonstrating a first-pass effect following oral administration, extraction and biotransformation by epithelial cells of the GI tract may also occur.

1.01.2.2.2 Binding and storage

Binding of chemicals to proteins can also have a dramatic effect on the toxicological effect of an absorbed dose. There are two general types of binding: covalent and noncovalent. In the covalent type, binding is irreversible and may be associated with a relatively high degree of toxicity. Noncovalent binding is reversible and is usually associated with the major portion of the absorbed dose. Many chemicals bind to plasma proteins, especially albumin and globulins. Since only the unbound chemicals are free to react with targets in tissues, extensive protein binding can often limit the toxicity of a chemical. However, because the binding is often reversible, it permits the bound chemical to dissociate from the protein, thereby returning the chemical to the circulation. For chemicals that are highly bound to plasma proteins, even a relatively small shift in protein binding can have a substantial effect on the distribution. For example, if the equilibrium binding of an absorbed chemical is 98%, then a shift to 96% binding would result in a doubling of the concentration of free chemical in the bloodstream, and thus a greater propensity for toxicity. This is especially a problem for drug–drug interactions. For example, many antidiabetic drugs are bound to proteins but may be displaced by sulfonamide drugs, which have a higher affinity for the plasma proteins. Once released, the antidiabetic drugs may then trigger a hypoglycemic coma.

If a chemical undergoes binding to intracellular proteins in a tissue, that tissue may serve as a "sink" for accumulation of that chemical. For example, cadmium is a highly toxic trace metal that undergoes binding to an intracellular storage protein called "metallothionein." This low molecular weight binding protein is present in high concentrations in the liver and kidney. Following oral ingestion, cadmium binding in the liver by metallothionein occurs, causing a dramatic increase in its concentration at this site. While the preferential binding of cadmium by metallothionein serves as a protective mechanism against cadmium injury to more critical proteins, the cadmium–metallothionein complex may be slowly transported out of the liver. Once released into the bloodstream, the metal–protein complex is extracted by specific membrane transport processes in the proximal tubules of the kidney. As the metallothionein protein is degraded by normal physiological processes, the released cadmium is free to react with kidney metallothionein or other similar proteins. Over time, cadmium may become bioconcentrated in the kidney and remain

there for many years. After the concentrations of cadmium exceed the available binding sites on metallothionein proteins, irreversible and potentially fatal kidney damage may result.

The adipose tissue is an important storage site for lipid-soluble chemicals such as DDT and polychlorinated biphenyls (PCBs). However, the potential exists for release of these chemicals into the bloodstream on breakdown of this fatty tissue during starvation. For example, rapid weight loss from either gastric by-pass surgery or to disease state is associated with an increase in the blood concentration of PCBs, due the reduction in lipid mass without effective elimination of the mobilized PCBs. One study found that a 15% decrease in body mass index (from 40 to 34) over 6 months resulted in a 45% increase in blood PCB concentrations (Dirinck et al., 2016). Bone is a major storage site for certain elements such as lead and fluoride. Since they share certain physicochemical characteristics, calcium in bone may be readily replaced by lead or strontium.

1.01.2.2.3 Barriers to distribution

The blood–brain barrier is an effective anatomic barrier to the penetration of water-soluble chemicals into the brain. The barrier is the result of a number of unique anatomic features, including a system of tightly joined capillary endothelial cells, a number of glial foot processes that effectively surround the capillary endothelium, and a contiguous basement membrane. In addition, the relatively low protein concentration of the interstitial fluid of the brain limits the amount of protein binding of toxicants.

While being very effective in limiting the amount of water-soluble substances reaching the brain, this barrier presents little impediment to the diffusion of lipid-soluble substances into the brain. As a result, compounds such as methyl mercury and almost all organic solvents can enter the brain and produce effects on the CNS.

Other barriers are present in the eyes, testicles, and placenta. The *placental* barrier is comprised of multiple layers of cells between fetal and maternal blood. The number of layers of cells in the placental barrier varies among species. The *blood–testis* barrier also limits the availability of waterborne toxicants to germinal cells. However, like the other barriers, it provides little protection against lipid-soluble chemicals.

1.01.2.3 Toxicokinetics

On entering the body, a chemical immediately begins undergoing changes in concentration, physical form, and location. As previously discussed, it may be transported by several routes in the circulatory system, absorbed by various tissues, stored, or cause toxic effects. As these factors are in a constant state of flux, each of these and related processes may be described mathematically by rate constants (k). The use of these biochemical constants to model the time course of a potentially hazardous substance's deposition in the body is called "toxicokinetics," and constitutes a highly specialized branch of toxicology.

Simple first-order kinetics is typically used to describe individual rate processes for modeling chemical distribution after entry into the body. These sites, or compartments, represent locations in the body that have similar characteristics with respect to the behavior of the compound. A one-compartment open model typically assumes that a chemical is instantaneously distributed equally throughout the body (**Fig. 5**). Therefore, the one-compartment model treats the concentration of a chemical at any point in the circulatory system as representative of the concentration throughout the compartment. The two-compartment model is used to describe the distribution of a chemical that slowly moves from systemic circulation into the tissues (**Fig. 5**). The central compartment in a two-compartment model conceptually represents the vascular space and rapidly perfused tissues, whereas the rest of the body represents the peripheral compartment. Rate constants describe the rate of exchange between the two compartments, and also the rate of influx into the central compartment and the rate of elimination from the central compartment. For chemicals that slowly distribute to "deep" compartments such as fat or bone, a three-compartment model may be employed. In this model, different rate constants between the central and the two peripheral compartments may be used to explain the very long biologic half-life of some chemicals.

1.01.2.4 Metabolism of Toxicants

After a chemical has been absorbed into the body, it may undergo metabolism (also termed biotransformation). For many chemicals, the toxic effects are highly dependent upon the metabolic fate of the chemical in the body. The metabolic fate of a chemical can also have an important effect on its disposition in the body and its excretion. There are a number of pathways for the body to biotransform a toxic chemical. These pathways are all mediated by specialized proteins (enzymes) in the cells, which are designed for the purpose of converting unwanted, foreign chemicals to nontoxic metabolites. The products of metabolism are typically more water soluble than the original compound, and are thus more easily excreted. When the excretion of a chemical is facilitated, it typically has a shorter residence time in the body and hence its potential to elicit toxic effects is reduced. The enzymes that catalyze these reactions are typically found in highest concentrations in the liver. This is because most foreign chemicals enter the body through the GI tract and the portal blood supply that goes directly to the liver.

While biotransformation ultimately aims to limit the toxic potential of chemicals (i.e., detoxification reactions), these processes can also exacerbate toxicity (i.e., activation reactions). Some, but not all, of the chemicals that undergo bioactivation are presented in **Table 3**. A well-known example of an activation reaction is the metabolism of ethylene glycol, a component of antifreeze, to oxalic acid, which partially contributes to its toxicity. There are a multitude of enzymatic pathways that are capable of these reactions, and the qualitative and quantitative differences in the ability of different organs to conduct such reactions often underlie the organ-specific effects of many chemicals. For example, the metabolism of sulfonamides in urine by acetylation results in the formation of crystals

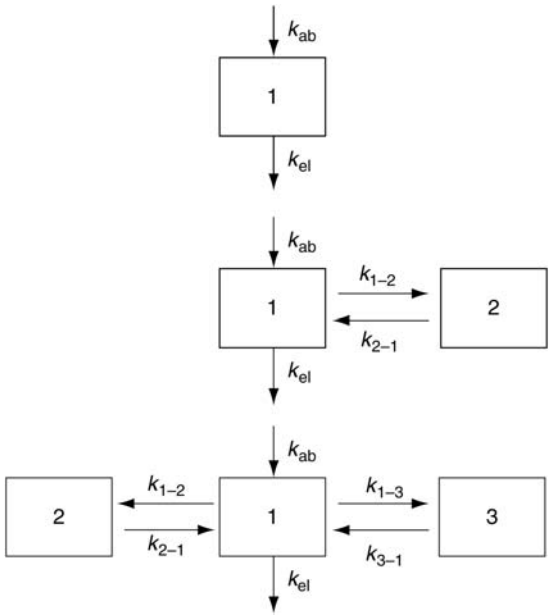

Fig. 5 Schematic representation of one-compartment open models. K_{ab} represents the absorption rate constant and K_{el} the elimination rate constant. The numbering of the rate constants (k) indicates the originating compartment (first numeral) and the receiving compartment (second numeral). The one-compartment model (*top*) represents the simplest approach to understanding the distribution of chemicals in the body and assumes that the chemical is instantaneously distributed throughout the compartment (body). Two- and three-compartment models (*middle* and *bottom*) take into account a slower distribution phase between the central compartment (blood) and the peripheral compartments (tissues).

Table 3 Some chemicals that undergo bioactivation

Acetaminophen
Aflatoxin B_1
Benzo[*a*]pyrene
Benzene
Chlorpyrifos
Diethylhexylphthalate
Ethylene glycol
Halothane
Parathion
Pyrrolizidine alkaloids
Vinyl chloride

and tissue damage in the kidney. In contrast, the liver is the most common site of toxicity for chloroform and carbon tetrachloride, largely because of the ability of this organ to rapidly biotransform these compounds into reactive intermediates.

Biotransformation reactions are commonly divided into three broad categories: phase I, phase II, and phase III reactions. Phase I reactions are so named because they are generally the first biotransformation step in what is often a multistep process leading to the eventual excretion of the biotransformation products. Phase I reactions can occur as (1) oxidation reactions, where an atom of oxygen is added to the chemical, (2) reduction reactions, where hydrogen atoms are added, or (3) hydrolysis reactions, where a molecule of water is incorporated into the chemical, often resulting in the "breaking in two" of the chemical. Phase II reactions are those enzymatic processes which utilize the products of phase I reactions to impart further structural changes, usually greatly increasing water solubility. Phase II reactions include the so-called "conjugation" reactions, where an endogenous (present naturally in the body) molecule, such as sugar or amino acid, is added on to the foreign chemical. There are many types of conjugation reaction, and the products of this reaction are usually highly water soluble and thus can be rapidly eliminated in the urine and/or bile. Virtually all biotransformation reactions of biological significance can be identified as proceeding by one of the four described pathways (oxidation, reduction, hydrolysis, and conjugation). Finally, phase III reactions refer to the processes by which transporter proteins facilitate the transport and elimination of conjugated xenobiotics across biological membranes. Because most xenobiotics that undergo phase I and phase II reactions within a cell (e.g., hepatocyte) lack sufficient lipid solubility to diffuse across cell membranes, it is necessary for these water-soluble metabolites to be actively transported across the cell membrane. In recent years, many genes have been discovered to code for such membrane transport proteins.

1.01.2.4.1 Factors that affect metabolism

Idiosyncratic reactions to drugs and toxic chemicals can often be explained by individual differences in the ability to carry out certain biotransformation reactions. In some instances there are profound genetic differences, such as the complete lack of a particular enzyme, that determine such adverse responses. Unusual differences in bodily functions that result from genetic differences between individuals are called genetic polymorphisms. Genetic variation is particularly important in the human population, which is genetically mixed.

The mechanisms underlying these interindividual differences in the toxicological and pharmacological effects of drugs and environmental chemicals have been an area of intense investigation during recent decades. Interindividual variability in drug metabolism has been shown to influence drug efficacy and toxicity directly. Furthermore, a large number of the potentially genotoxic environmental chemicals and natural products to which humans are exposed require metabolic activation to exhibit their mutagenic and carcinogenic effects. The initial events in chemical carcinogenesis are often the metabolism or activation of the carcinogen to reactive intermediates followed by binding to DNA. We now know that differences in the level of expression of several drug-metabolizing enzymes can greatly contribute to the variation in cellular response to these compounds. The majority of these activation reactions involve oxidation reactions and are primarily mediated by cytochrome P450 (CYP) enzymes. There are at least 57 different human genes that code for distinct CYP enzymes. As a result, the relative expression of individual P450 enzymes may be a major factor in determining individual susceptibility to both adverse drug reactions and chemical carcinogenesis. A dominant high-affinity enzyme can be identified for the primary metabolism of many compounds, thus allowing for the development of noninvasive biomarker assays for potential risk factors to be used in human molecular epidemiology studies. Literally thousands of different genetic polymorphisms in drug-metabolizing enzymes have been identified in humans. It has been proposed that detailed genetic analysis of polymorphisms in drug-metabolizing enzymes may ultimately lead to more effective "precision medicine," where specific drugs and dosing regimens would be tailored to an individual's own genetic makeup (Duffy, 2015). While the development of such approaches has long been limited by numerous ethical, financial, and technological challenges, continued innovations in data storage and high-throughput "omics" platforms (see "**Toxicogenomics and Systems Toxicology**" section) have engendered much promise for "precision medicine." In 2015, United States President Barack Obama announced the "Precision Medicine Initiative," which among other objectives allocates funds for a "PMI Cohort Program." This program will utilize one million American volunteers to generate a national database of patient information (e.g., medical records, genomic and metabolic information, environmental and lifestyle data) in order to "empower patients and clinicians and advance individual, community, and population health" (White House Office of the Press Secretary, 2015).

In addition to genetic differences, there are other factors which can affect the metabolism, and therefore the toxicity of many chemicals. For example, species often vary widely in their responses to toxic compounds. This is of prime importance since drugs are tested in animals for their eventual use in humans. Similarly, veterinary products may be used on a variety of species, but not all animals or strains may exhibit similar dose–response relationships with respect to toxicity. Males and females can also differ in their responses due to metabolic and hormonal differences. In some species, males metabolize certain compounds more rapidly than females. The difference in susceptibility to chloroform-induced kidney damage is such an example. Male mice are more susceptible to chloroform toxicity, but this difference can be negated by castration. Another factor which can affect metabolism is co-exposure to other chemicals in the environment. Furthermore, unlike experimental animals, humans may also take several different drugs at one time, which may influence response to chemical exposure. There are many chemicals in the diet that can inhibit or induce enzymes that are responsible for the metabolism of other chemicals. For example, charcoal broiling of beef results in the production of compounds, called polyaromatic hydrocarbons (PAHs), that when ingested at adequate doses induce the level of certain phase I enzymes responsible for the activation of certain environmental chemicals. Dietary exposure to dioxins and PCBs also has the potential to induce hepatic biotransformation enzymes, such as cytochrome P4501A1 (CYP1A1). However, at the concentrations of PCBs found in the general population, significant enzyme induction is not likely to occur, as the tissue concentrations measured in the National Health and Nutrition Survey (http://www.cdc.gov/nchs/nhanes.htm) are several orders of magnitude lower than the concentrations needed to produce significant enzyme induction in human liver cells in vitro (Silkworth et al., 2005). A study of placental CYP1A1 activity in Inuit women with relatively high levels of PCBs, compared to age-matched controls from Quebec women with lower levels of PCBs, failed to find any difference in placental Ethoxyresorufin-O-deethylase (EROD) activity (a measure of CYP1A1 activity) when controlled for smoking history. However, there was a large, dose-related increase in placental EROD activity in moderate and heavy smokers from both populations, demonstrating that environmental exposures to CYP1A1-activating compounds such as PAHs can and does occur in humans (Pereg et al., 2002).

1.01.2.5 Excretion of Toxic Chemicals From the Body

The elimination of chemicals from the body is a critical determinant of their biological effect. In the case of toxicity, rapid elimination of a chemical will reduce the likelihood that a toxic effect will occur and reduce the duration of the effect. The ability of the body to rid itself of toxic chemicals is largely dependent upon the physicochemical characteristics of the chemical. Chemicals which have very low blood–gas partition coefficients (e.g., are poorly soluble in blood and have a high vapor pressure) may be effectively eliminated via exhalation, whereas chemicals which are highly water soluble will generally be eliminated by excretion into urine or bile. As many chemicals of occupational and environmental concern lack either of the above characteristics, accumulation in the body is likely to occur unless biotransformation processes alter the chemical to a more readily excreted form. Thus, elimination of a chemical from the body occurs via two processes: direct excretion of the unchanged substance or biotransformation to a different chemical form which may then be excreted as a metabolite.

The most important routes of excretion are through the kidneys into the urine and excretion into the bile.

1.01.2.5.1 Urinary excretion

The kidney is highly efficient at removing many foreign substances from the bloodstream. The kidney receives about 25% of the cardiac output and about 20% of this is filtered at the glomeruli of the kidney. Toxic agents may also be excreted from the blood into the urine by passive diffusion through the kidney tubules and also by active secretion. The extent and rate of urinary excretion is highly dependent upon the water solubility of the substance. Excretion from the bloodstream into the urine predominantly occurs for relatively small water-soluble chemicals. Large molecules such as proteins are too large to pass through the filtering apparatus of the kidney. In addition, fat-soluble chemicals are not effectively eliminated in the urine. Since many functions of the kidney are not completely developed at birth, newborns may eliminate some chemicals more slowly than do adults. Therefore, these chemicals may be more toxic to newborns than to adults.

1.01.2.5.2 Biliary excretion

In contrast to urine, bile is not an ultrafiltrate of plasma, and the biliary system has very little direct contact with the bloodstream. Therefore, all substances which enter bile from the plasma must do so by passing through liver cells. Once a chemical enters the liver cell, it is generally biotransformed to a more water-soluble form, which may either reenter the circulation for elimination in urine or be transported into bile. The bile passes directly into the GI tract, where the chemical can then be eliminated in the feces. However, if it remains fat soluble, it will be reabsorbed in the intestine. Therefore, many organic chemicals are conjugated before excretion into the bile. Such conjugates are too water soluble to be reabsorbed. However, intestinal microflora may hydrolyze off the polar conjugate groups of the chemical, rendering it sufficiently lipid soluble for reabsorption. Reabsorption of a chemical completes an enterohepatic recycle, which can dramatically increase the biologic residence time of the chemical.

The bile is an important route of elimination for some metals. For example, methyl mercury is secreted in the bile, although it too is subject to reabsorption in the intestinal tract.

1.01.2.5.3 Other routes of excretion

The lungs are an important route of excretion for gaseous metabolites and volatile compounds such as benzene. No specialized transport systems have been described for the excretion of toxic chemicals by the lungs. Instead, they seem to be excreted by simple diffusion. Chemicals may also be excreted into body fluids such as breast milk, sweat, or semen. Excretion into breast milk can be an important route of excretion for lipid-soluble chemicals such as DDT or PCBs. Thus, newborn animals are at particular risk from toxic compounds excreted into the milk. Secretion of toxic chemicals into milk is also important because toxic compounds may be passed from cows to people by dairy products.

1.01.3 Types of Toxic Effect

1.01.3.1 General Considerations

Toxic effects vary greatly with respect to nature, scope, target tissue, and mechanism of action. As observed in Fig. 6, toxic effects are a result of biochemical interactions that occur between toxicants and certain target structures of the exposed organism. Typically, these target structures may be a specific cell type or subcellular organelle within a tissue. However, the target structure may also be nonspecific, such as any tissue or organ that comes into direct contact with the toxicant. The nature of the toxic effect can vary from organ to organ. The variety of toxic effects observed can be classified according to the duration, target organ, and mechanism of action. In addition, reversible effects of toxicant exposure are those that disappear following cessation of exposure. Irreversible effects, in contrast, will persist or worsen after exposure is discontinued. Examples of irreversible effects of toxicant exposure include cirrhosis of the liver and cancer.

1.01.3.1.1 Duration of exposure

There are two basic exposure conditions for toxic compounds: acute and chronic. Acute exposure applies to a single episode where a particular amount of a compound enters the organism. While acute exposure usually refers to a single dose of a chemical, repeated exposures may be given within a brief period of time (typically >24 h) for less toxic chemicals. Repeated exposures for >24 h are considered chronic, which may then cause a cumulative toxic effect. However, the frequency of repeated exposure in laboratory animal studies is often subdivided into three categories: subacute, subchronic, and chronic. Subacute exposure refers to repeated exposure to a chemical for 1 month or less, subchronic for 1–3 months, and chronic for >3 months.

For many chemicals, the toxic effects resulting from acute exposure are far different from those resulting from chronic exposure. For example, acute exposure to benzene typically results in central nervous depression, while chronic exposure may cause leukemia. If a chemical is rapidly absorbed into the body after acute exposure, it is likely that some type of immediate toxic effect will result. However, acute exposure can also produce some delayed toxic effects that are similar to those occurring with chronic exposure. Carcinogenic effects of chemicals occur after a long latency period, often 20–30 years, before tumors are observed in humans. Also, delayed neurotoxicity is observed after exposure to certain OP agents that have anticholinesterase action. The most notable of the compounds that produce these effects is triorthocresylphosphate, which produces delayed neurotoxic effects several days to weeks after exposure (Costa, 2013).

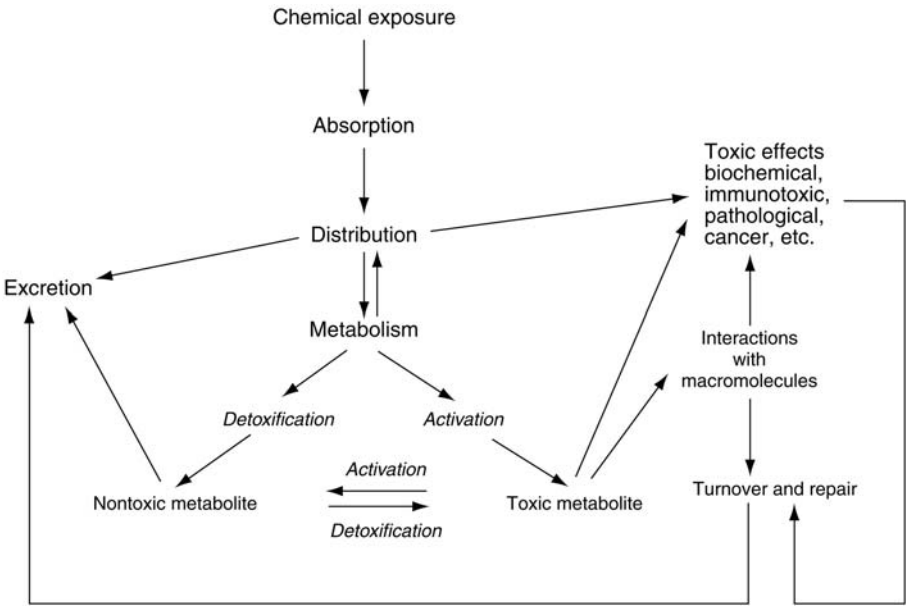

Fig. 6 Schematic representation of the sequence of events following exposure to toxic chemicals.

1.01.3.2 Idiosyncratic and Allergic Reactions

Although humans will generally respond to toxic chemicals in a manner similar to laboratory animals, and usually in doses that are relatively similar on a body weight basis, there are often individuals within a population that have some genetic variation that causes them to respond at a dose far below the anticipated dose. This type of hypersensitivity is often referred to as an idiosyncratic response and, if it occurs at all, is usually seen only in a very small percentage of the population. Most of the identified chemical idiosyncratic responses are associated with administration of therapeutic drugs and likely have a genetic component to their etiology. For example, 3–5% of people are genetically deficient in an enzyme in the bloodstream known as butyrylcholinesterase (also called pseudocholinesterase) (Daly et al., 1993). This enzyme apparently plays little or no role in normal human functions. However, when such individuals are given a muscle-paralyzing drug (called succinylcholine) for surgical procedures, they respond by remaining paralyzed for much greater periods of time than the average person with adequate pseudocholinesterase enzyme. The mechanism underlying this adverse response is that the pseudocholinesterase enzyme is primarily responsible for breaking down (metabolizing) succinylcholine. Similarly, individuals with a deficiency in Nicotinamide adenine dinucleotide (NADH) methemoglobin reductase exhibit a marked sensitivity to nitrites and other chemicals that produce methemoglobinemia (Scott and Griffith, 1959). These individuals typically have 10–50% of their circulating blood hemoglobin in the form of methemoglobin. Numerous other examples of genetically determined hypersusceptibility to the adverse effects of drugs and nondrug chemicals have been described and may be important in determining susceptibility to nonacute responses such as cancer and birth defects.

In addition to the normal (expected) responses and the idiosyncratic types of responses, some individuals may develop allergic reactions to chemicals. These reactions result from previous sensitization to a toxicant or a chemically similar compound. While the occurrence of this appears to be rather small, for some substances it may be an important consideration. Unlike normal toxicological responses, allergic reactions do not follow the classical population dose–response curve, that is, allergic individuals will respond at doses far below nonallergic individuals, and even within an allergic population, the magnitude of response is not always clearly dose related. However, within the allergic individual, the magnitude of the response to an allergen is usually related to the magnitude of exposure. In contrast to normal toxicological responses, an individual who subsequently becomes allergic to a chemical will show no response upon the first exposure. This is because, unlike normal toxic responses, the allergic response is dependent upon the presence of specific "antibodies" in the body which are directed against an "antigen." Most chemicals are not large enough to stimulate the immune system directly, but must first combine with a normal body protein. The chemical is then referred to as a hapten and the chemical–protein complex becomes the antigen. Once an antigen is formed in the body, the production of antibodies requires several weeks following this first, or sensitizing, exposure. However, once antibodies have been formed, a subsequent exposure may result in a rapid and severe allergic response which in turn may result in any of a number of different physiological effects. The most common allergic responses are associated with skin rashes, while others may present as asthmatic responses (difficulty in breathing), or less commonly as disorders of the blood such as hemolytic anemia (rupturing of red blood cells). Rarely, an individual may respond by a rapid, life-threatening anaphylactic reaction, where blood pressure falls to dangerously low levels (e.g., as may occur with some individuals allergic to bee stings or peanuts).

Although allergic and idiosyncratic reactions normally occur in a relatively small percentage of the total population, it is quite possible that they may be associated with a high percentage of adverse responses to chemicals in the workplace. This is because workplace controls of hazardous substances are not always be adequate to protect highly sensitive individuals.

1.01.3.3 Systemic Toxicology

1.01.3.3.1 Toxic responses of the liver

Liver injury induced by chemicals has been recognized as a toxicological problem for over 100 years. Hepatic lesions produced by carbon tetrachloride and chloroform were among the first to be studied by scientists. The liver is the largest organ in the body and is often the target for chemically induced injuries. This is because most chemicals enter the body through the GI tract and, after absorption, are transported to the liver through the hepatic portal vein. A second factor that plays a significant role in toxicant-induced liver injury is the presence of high concentrations of CYP-dependent monooxygenase enzymes that can bioactivate chemicals via oxidation reactions to toxic metabolites (**Table 3**). Often the area of the liver subjected to the highest damage is the centrilobular area, which contains very high concentrations of CYP enzymes. The occurrence of liver injury is typically dependent upon the nature of the chemical agent and the duration of exposure. After acute exposure, one usually observes the appearance of hepatic lipids in cells (fatty liver) that have been injured, followed by cell death (hepatocellular necrosis) and, in extreme cases, liver dysfunction. The most common types of liver injury include fatty liver, cell necrosis, cholestasis, cirrhosis, hepatitis, and liver cancer. Examples of different hepatotoxicants and their associated types of liver injury are presented in **Table 4**. No single biochemical mechanism seems to underlie the appearance of degenerative hepatocellular changes or loss of liver function. Furthermore, some forms of liver injury are permanent, while others are reversible.

Cell necrosis (a type of cell death) is usually an acute injury that is preceded by a number of biological and morphological changes. Hepatic necrosis is often a result of bioactivation reactions described earlier. For example, both carbon tetrachloride and chloroform are bioactivated by hepatic enzymes to produce reactive intermediates that damage critical cellular macromolecules and cause hepatic necrosis (**Table 4**; Bruckner et al., 2013). Acetaminophen-induced liver injury is also caused by a chemically reactive metabolite. The formation of this metabolite occurs at a very low level after subtoxic doses, but increases as the dose approaches the toxic range. Endogenous compounds such as glutathione, a low molecular weight tripeptide found in cells, play an essential role in protecting liver cells from injury from chemically reactive intermediates.

Overdoses of drugs such as amitriptyline, estradiol, and diazepam can cause a diminution or cessation of bile flow. Inflammation or blockage of the bile ducts can result in the retention of bile salts, or *cholestasis*. This condition can also cause the accumulation of bilirubin, leading to jaundice. *Cirrhosis* is a progressive disease caused by the accumulation of collagen in the liver, typically due to chronic consumption of ethanol. *Hepatitis* is an inflammation of the liver typically caused by a virus. However, a type of chemical-

Table 4 Some hepatotoxicants and their associated types of liver injury

Type of injury	*Compound*
Necrosis	Acetaminophen
	Bromobenzene
	Chloroform
	Carbon tetrachloride
	Thioacetamide
Fatty liver	Chloroform
	Carbon tetrachloride
	Ethanol
	Puromycin
	Tetracycline
Cholestasis	Amitriptyline
	Imipramine
	Sulfanilamide
Hepatitis	Colchicine
	Halothane
	Phenylbutazone
	Zoxazolamine
Cancer	Aflatoxin B_1
	Pyrrolizidine alkaloids
	Urethane
	Vinyl chloride

Source: From Plaa, G.B. (1991). In: Amdur, M.O., Doull, J., Klaassen, C.D. (eds.) Casarett and Doull's toxicology: The basic science of poisons, pp. 334–353. New York: Pergamon Press; Lu, F.C. (1991). Basic toxicology: Fundamentals, target organs, and risk assessment (2nd edn.). New York: Hemisphere.

induced hepatitis, which closely resembles that produced by viral infections, can occur with exposure to certain drugs (Table 4). Although a wide variety of chemicals have been shown to cause liver cancer in experimental animals, only a few are known to be human carcinogens. Two known primary human liver carcinogens are vinyl chloride and the mycotoxin aflatoxin B_1.

1.01.3.3.2 Toxic responses of the kidney

In addition to the excretion of wastes, the kidney plays a significant role in the regulation of overall body homeostasis through the regulation of fluids and electrolytes. Furthermore, the kidney produces a number of critical hormones that influence metabolic functions. Accordingly, a toxicological insult to the kidney can have an impact on any of these functions. The kidney is particularly sensitive to the toxic effects of a variety of chemicals, primarily because of its unique anatomy and physiological features. For example, the extensive filtering and reabsorptive capabilities of the kidney cause remaining materials to be concentrated. Thus, a nontoxic concentration of a chemical in the plasma could become toxic in the kidney as the urinary filtrate is concentrated to form urine. Although the two kidneys comprise less than 1% of the total body mass, they receive approximately 25% of the cardiac output. Due to the high blood flow to the kidneys, any toxicant that is present in the systemic circulation will be delivered to the kidney in significant amounts.

A number of chemicals found commonly in the environment may be toxic to the kidney (nephrotoxicity). For example, many metals, such as mercury and cadmium, are potent nephrotoxicants. At low doses, a variety of metals may cause alterations in ion transport capacity (aminoaciduria or glucosuria), whereas higher exposure can cause kidney cell necrosis and death. Extensive data has accumulated on the nephrotoxicity of mercury; the potential for nephrotoxicity of this compound is highly dependent upon its chemical form. The kidney is a primary target of toxicity following accidental or suicidal ingestion of mercuric salts. Cadmium is another metal that can cause kidney injury. Cadmium has an extremely long half-life in the body (20–30 years) and accumulates primarily in the kidney. Thus, low levels of chronic exposure will eventually result in accumulation to toxic levels. Kidney damage has also been observed following administration of chromium, arsenic, gold, lead, and thallium.

Many chlorinated hydrocarbons such as chloroform and hexachlorobutadiene also cause renal toxicity. In the case of chloroform, nephrotoxicity is somewhat dependent upon bioactivation to a toxic intermediate. Interestingly, the nephrotoxicity of several of the halogenated hydrocarbons may be related to the activation in the kidney of a conjugation product between the toxicant and an endogenous compound that is formed in the liver. Certain antibiotics are nephrotoxicants in humans when present in high doses or over prolonged periods of time. In particular, the aminoglycoside antibiotics, including streptomycin, neomycin, and gentamicin can cause kidney damage after prolonged use. The immunosuppressant drug, tacrolimus, and similar "calcinurin inhibitor" drugs used to reduce organ rejection in transplant patients are nephrotoxic in a significant percentage of transplant patients and may cause complete renal failure. The toxicity appears to be associated with renal metabolism of the compound, and genetic differences in a specific CYP (CYP3A5) might contribute to individual differences in susceptibility (Dai et al., 2006). Finally, some plant toxins are specifically nephrotoxic. For example, aristolochic acid, produced in the seeds of *Aristolochia* spp. of plant, has been implicated in a chronic disease known as Balkan endemic nephropathy, and also resulted in a cluster of deaths following ingestion of an herbal preparation that contained aristolochic acid (Grollman, 2013).

1.01.3.3.3 Pulmonary toxicology

As previously indicated, inhalation is a very important route of toxicant exposure, especially in workplace environments. The lung efficiently absorbs many types of inhaled substances. Since the lung receives all of the cardiac blood supply, the distribution of inhaled toxicants from the lung to other organs can be rapid. Thus, it is important to distinguish between inhalation toxicology, which defines the route of exposure, and pulmonary toxicology, which specifically assesses the response of the lung to toxic agents. The lung is in direct contact with the external environment and is exposed to infectious agents as well as toxic particles and gases. Since the primary purpose of the respiratory system involves the exchange of gases, impairment of this process may affect the functions of the entire body, depending upon the degree of severity of damage.

Over 40 different cell types are required to perform the diverse functions of the respiratory tract. In response to toxicant exposure, many of these lung cells are known to release a variety of chemical mediators that are designed to neutralize or remove the inhaled toxic material. The type of response mounted by the lung ultimately depends upon the physical and chemical properties of the agent. Some toxicants may elicit nonspecific responses involving clearance of the toxicant. Unlike most organs, the lung can respond to a toxic insult or agent by initially trying to remove or neutralize it and then repair the damage. These nonspecific responses provide a considerable degree of protection against injury from a wide variety of inhaled agents. In contrast, specific defense mechanisms are immunological in nature and are stimulated by the constant exposure to inhaled toxic antigens. The mammalian lung has a well-developed immune system. Once sensitized to a particular antigen, the immune system can mount an amplified response to extremely small concentrations of that toxic antigen.

Despite the specific and nonspecific defenses of the lung, chronic injury to the lung as a result of toxicant exposure occurs all too often. Chronic lung injury occurs when the defenses and repair processes of the lung simply cannot cope with the damage resulting from either high levels of acute toxicant exposure or repeated exposure to low levels of the material. The result of the struggle between repair and injury can produce a wide range of pulmonary responses including fibrotic diseases, obstructive pulmonary diseases, and cancer. A number of workplace toxicants induce inflammatory processes at concentrations sufficient to cause fibrosis after chronic exposure. In particular, silicosis is a common fibrotic disease that occurs after chronic occupational exposure to crystalline silica. One major obstructive disease that can be caused by pollutant exposure is *emphysema*. Emphysema is characterized by the destruction of certain airspaces of the lung, resulting in a steady progression of functional disability. Emphysema is clearly

associated with heavy cigarette smoking and occurs late in life. In general, the contribution of occupational and environmental agents toward lung disease is overshadowed by damage attributed to cigarette smoke.

1.01.3.3.4 Neurotoxicology

The nervous system consists of two major parts: the CNS and the peripheral nervous system (PNS). The CNS is made up of the brain and spinal cord, whereas the PNS includes the motor and sensory nerves of the cranium and of the spine. The PNS also includes the nerves arising from the thoracic and lumbar regions of the spine (sympathetic nervous system) and also nerve fibers leaving the CNS through the cranial nerves and the sacral region of the spine (parasympathetic nervous system). The brain, spinal cord, and peripheral nerves are covered with a lining of specialized cells that restrict entry of molecules from adjacent tissue. In addition, the endothelium of the brain is protected from some blood-borne toxicants through an anatomically defined barrier termed the blood–brain barrier. The principal basis for this barrier is the tight junction that exists between endothelial cells of the nervous system. To cross the "blood–brain barrier" and gain access to the nervous system, molecules must be able to pass through the plasma membranes of the cells, rather than between cells. Despite this barrier, certain toxicants, including methyl mercury, trimethyltin, organophosphorous insecticides, and carbon disulfide, are specific for cells of the nervous system and result in serious nervous system impairment, often leading to death, when exposure is severe enough.

The effects of neurotoxicants are typically classified based upon their site of action (Costa, 2013). Certain toxicants are specific for neurons, the principal cells of the nervous system. The loss of a neuron is irreversible. Examples of compounds that are associated with neuronal injury include methyl mercury, trimethyltin, and carbon disulfide.

Myelin provides the electrical insulation of nerve cells, and its loss leads to a slowing of electrical impulses along nerve cells, or *myelinopathy*. Compounds that are associated with injury to myelin include ethidium bromide, tellurium, and triethyltin. The neurotoxic disorders termed *axonopathies* are those in which the primary sites of action are the long elements of the neurons, or *axons*. Toxicity may occur in the proximal or distal regions of the axons. Since long axons have more targets for toxic damage than shorter axons, the longer axons are overrepresented among axonopathies. Compounds associated with axonal injury include carbon disulfide, hexane, lead, and certain OP insecticides. Toxicants such as tetrodotoxin, the toxic principle of puffer fish, and saxitoxin, the toxic component of certain dinoflagellates associated with "Red Tides," act on nerve cell membranes and interfere with impulse conduction. *Botulinum* toxin, now widely used in cosmetic procedures, causes muscle paralysis by impairing release of the neurotransmitter acetylcholine from motor nerve endings. Conversely, black widow spider venom interferes with synaptic transmission by causing a massive release of acetylcholine.

Alterations in behavior or psychological health after chemical exposure are frequently an initial clue that a given chemical is neurotoxic. Neurological examinations often provide an indication as to the site of neurotoxicity. Motor examinations, which include inspection of muscles for weakness or atrophy, may indicate dysfunction of lower motor neurons. Neurological signs usually develop rapidly with neuropathies, but slowly with axonopathies. The former generally affect both the sensory and motor fibers, while the latter predominantly affect the sensory fibers.

Recently there has been a rapid growth in interest in the potential role for environmental neurotoxicants as contributors to the etiology of chronic neurological diseases such as Parkinson's disease (Wirdefeldt et al., 2011). While a causal connection between agricultural chemicals and Parkinson's disease has yet to be established, human epidemiological data has largely supported the association between cumulative exposure and the development of disease. In addition, studies in laboratory animals have demonstrated that certain pesticides, such as paraquat and rotenone, are capable of causing selective loss of dopaminergic neurons, which is the hallmark characteristic of Parkinson's disease. There is, however, strong evidence that occupational exposure to high levels of the divalent metal, manganese, is associated with the development of a syndrome known as "Manganism," which is quite similar to Parkinson's disease (Kwakye et al., 2015).

1.01.3.3.5 Toxic responses of other organ systems

In addition to the organ systems listed earlier, chemicals can also selectively affect the heart or vasculature. Generally, after a functional change in the heart, the risk of lethality is greater than the risk associated with other internal organs. In contrast to other tissues, the skin displays a fairly limited variety of toxic responses. Since the surface of the skin is so visible, toxic reactions to the skin are typically described on the basis of morphological, as opposed to functional, changes. The impact of new chemicals or drugs on embryonic and fetal development has been accentuated by the tragic thalidomide incidence in the 1960s. There are several sites of interference of chemicals that can affect the human reproductive system. A number of cancer chemotherapeutic agents cause severe damage to the germ cells of the gonads. Chemicals such as benzene, carbon disulfide, formaldehyde, cigarette smoke, and vinyl chloride have been associated with reproductive dysfunction in women. The relatively new awareness of reproductive hazards in the workplace has lead to a number of corporate policies and legal considerations. The pesticide ethylene dibromide, used to kill soil pests that damage pineapples, strawberries, and other crops, caused reduced sperm count and complete loss of fertility in a large number of workers occupationally exposed to this volatile compound. The interaction of environmental chemicals with the cells and tissues of the immune system was discussed previously. Examples of agents that alter the immune system include certain metals, resins and plasticizers, and pesticides. Systemic exposure to these agents can adversely affect the immune response and alter resistance to infectious agents and cancer.

In the field of ecotoxicology, there is mounting evidence to indicate that exposure to natural and synthetic chemicals in the environment can act as "endocrine disrupting agents" which can affect the reproductive health of wildlife populations. In this regard, the endocrine system controls the development and regulation of such tissues as pituitary, pancreatic, and adrenal glands, as well as

hormonal homeostasis. In particular, the hormones secreted by these glands act as natural messengers which bind to receptors and control a variety of developmental functions. An example of this is the secretion of estrogen from the ovaries which controls fertility and is also essential for normal development. The modes of action of endocrine disrupting chemicals are fairly diverse and include those compounds that act as *agonists* and *antagonists* to hormonal receptors, including androgen, estrogen, and thyroid receptors. The chemicals that have been shown in laboratory or field studies to have the potential to disrupt endocrine homeostasis have been termed "endocrine disrupting compounds" (EDCs) and include components of municipal sewage discharges and certain industrial effluents, as well as certain pesticides, herbicides, and metals. For example, despite being banned from use decades ago, certain metabolites of DDT such as *p,p′*-dichlorodiphenyldichloroethylene still persist in the environment and may affect hormonal homeostasis (Quinn et al., 2006; Steinhardt, 2004). The scenario of pollution-induced disruption of normal endocrine function in wildlife species has recently given rise to national and international research efforts directed toward developing and testing strategies for EDCs (Fuhrman et al., 2015; Vogel, 2005).

1.01.3.4 Mutagenesis

One of the most important types of toxic response a chemical can produce is the production of mutations. Mutations arise when the DNA in a cell is damaged in such a way that the information contained in the genetic code is altered.

1.01.3.4.1 Structure and function of DNA

The genetic information contained within DNA is stored in the form of four different molecules, called nucleotide bases, which include two purines, adenine (A) and guanine (G), and two pyrimidines, thymine (T) and cytosine (C) (**Fig. 7A**). These bases are joined via a sugar (deoxyribose) phosphate backbone to form specific sequences, and assembled into discrete packages called chromosomes. Most DNA exists as a double-stranded helix, with two complementary strands of nucleotide bases joined by hydrogen bonding between fixed complementary base pairs. G on one strand always binds with C on the other, and A always binds with T (**Fig. 7B**). Thus, a segment of DNA with the sequence 5′-CGT TCA ACA-3′ would have complementary strand with the sequence 3′-GCA AGT TGT-5′. When cells divide, the double-stranded DNA within chromosomes separates and the two copies are duplicated by enzymes called *DNA polymerases*. These enzymes read the sequence of bases on one strand and then assemble the duplicate strand using complementary bases. As a result, every nucleated cell in our body contains the complete genetic blueprint for human life, with individual cells only differing only in how they utilize this information.

In addition to replication during cell division, DNA also functions by providing a blueprint for the synthesis of specific functional units in the cell, known as proteins. These macromolecules are responsible for carrying out the majority of the functions of the cell, from the generation of cellular energy, to the synthesis of major structural components such as lipids and complex carbohydrates. The roles that individual proteins serve are dictated by their three-dimensional shape once assembled, which in turn is

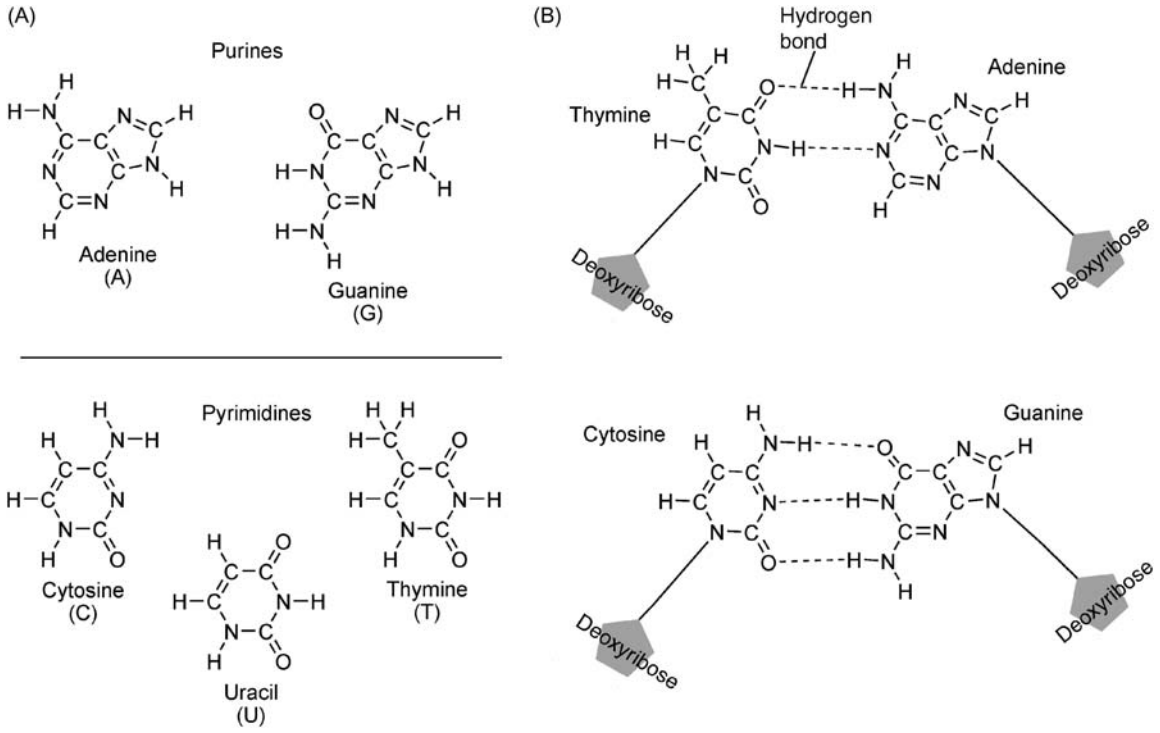

Fig. 7 Purine and pyrimidine bases of nucleic acids (A) and hydrogen bonding between the A–T and G–C base pairs (B).

determined by their underlying amino acid composition. Specific protein "blueprints" are contained within DNA in the form of discrete units of code called genes, but before the information in DNA can be used to assemble amino acids into proteins, an intermediate step, called transcription, is required.

Transcription is similar to DNA replication and involves the conversion of specific sequences of DNA, known as genes, into complementary, single-stranded nucleotide sequences known as ribonucleic acid (RNA). RNA is similar to DNA, except that the pyrimidine uracil is used in place of T, and the sugar ribose is used in the strand backbone in place of deoxyribose. Thus, when transcribed, the DNA sequence 5′-CGT TCA CAA-3′ will produce a strand of RNA with the sequence 3′-GCA AGU GUU-5′. Once in this form, triplets of RNA nucleotides can be read by the translational machinery of the cell and transformed into specific amino acids. For example, translation of the above sequence of RNA will result in the synthesis of a three-amino acid "peptide," with the sequence alanine–serine–cysteine, because the RNA sequence GCA codes for alanine, AGU codes for serine, and UGU codes for cysteine. Note that if the fourth base in the DNA sequence shown here (CGT**T**CA) were mutated from a T to an A, this would result in a change in the RNA sequence to GCA**U**GU in the translated peptide to become alanine–cysteine–cysteine. However, it is also worth noting that RNA does not necessarily need to be translated to be functional. Recently, the discovery of small pieces of double-stranded RNA, called microRNAs or "small-interfering RNAs" that help regulate transcription, has led to the realization that there is much more to the sequence of DNA than simply coding for proteins. Thus, changes in the sequence of DNA can have profound implications to the organism, regardless of whether the change in sequence at a specific base, or point mutation, causes a change in a codon for a specific amino acid.

Genes contain two major parts: the regulatory region and the coding region. The regulatory region provides important information that determines when, and to what extent, a gene is transcribed; in other words, it functions like a switching mechanism, turning the gene on or off in response to other signals from the cell or its environment. As the name suggests, the coding region contains the specific DNA information that will produce a protein, called exons. However, the vast majority of the genome (around 97% of the 3 billion base pairs) does not produce a functional protein. These sections can be found both within and between gene sequences, but when they are contained within the gene they are known as introns. These sections are ultimately "spliced out" of the mature RNA such that the RNA contains only the exon sequences attached together. Although in the early days of molecular genetics it was often thought that introns had no function, it is now apparent that some intronic sequence provide important information that determines, in part, the level of expression of particular genes and the stability of the resulting RNA. Recently, the discovery of small pieces of double-stranded RNA, called microRNAs or "small-interfering RNAs" that help regulate transcription, has led to the realization that there is much more to the sequence of DNA than simply coding for proteins. In addition, there are also vast areas of noncoding sequence that fall between individual genes, long known as "junk" DNA, which have increasing been shown to be rich in other regulatory elements.

Changes in the primary sequence of DNA are referred to as *mutations*. Mutation can occur in two general sources of DNA: DNA in germinal cells (eggs and sperm) and DNA in somatic cells. Somatic cells represent all other cells in the body other than germinal cells.

1.01.3.4.2 Germinal mutations

Mutations that occur in the DNA of germinal cells are of critical importance because they can be passed on to future generations. Thus, all hereditary diseases are a result of an acquired mutation in a sperm or egg cell that occurred in a preceding generation. Of course, if the mutation occurs in a gene that is required for the survival of the germ cell itself, then it cannot become a heritable mutation because the cell will die and thus be unable to pass on the mutated DNA during fertilization. It is also possible that a germinal mutation will result in loss of a vital gene necessary for the survival of the fertilized egg (zygote). This also will not necessarily result in a heritable mutation, but rather could result in a miscarriage (failure of the fertilized egg to develop into a viable offspring). However, every offspring is given two copies of genetic information, one from each parent. If the function of a gene is *dominant*, then a mutation in either parental copy could result in a malfunction of the gene function. If the function of a gene is *recessive*, then both parental copies (alleles) must be altered in order to produce an abnormal gene function, because one good copy of the gene may be all that is necessary for the offspring to function properly. However, most biological functions are "multigenic," and thus most inherited traits are not easily identified as coming from one or the other parent.

Although mutational events are extremely common in DNA, most of these mutations are either inconsequential or lethal to the cell. It is only when a mutation in a germinal cell occurs in a gene that performs some important, but noncritical (in terms of survival and reproductive function), function that a mutation becomes potentially heritable. Obviously, for a mutation to be passed on to future generations, the mutated offspring must be able to survive to reproductive age and be capable of successful reproduction. Germinal mutations may occur in the sex-linked chromosomes (X or rarely Y), or in any of the 22 other autosomal chromosomes. Thus, heritable mutations are usually classified as autosomal dominant, autosomal recessive, or sex-linked (which may also be recessive or dominant). Although not considered strictly a mutation, a serious alteration in DNA can occur during cell division if the chromosomes are broken or the proper number does not segregate normally. For example, Down's syndrome results when an extra copy of chromosome 21 is present in the fertilized ovum. The condition where an abnormal number of chromosomes are present is called *aneuploidy*. Aneuploidy in offspring arises from uneven chromosomal segregation that occurs during germ cell meiosis. Such large changes in the structure or number of chromosomes in a cell are referred to as *cytogenetic* changes, or more specifically, *chromosomal aberrations* and will not be dealt with further here.

1.01.3.4.3 Somatic cell mutations

A far more common consequence of exposure to chemical mutagens is alteration of DNA in somatic cells. Mutations can occur through alterations of single bases in the DNA, or by loss or rearrangement of large sections of DNA. Single base changes are called *point mutations* and can occur when one base is substituted for another. If a purine substitutes for another purine (e.g., A is changed to G) or a pyrimidine is changed to another pyrimidine (e.g., T changed to C) the mutation is called a *transition*, whereas substitution of a purine for a pyrimidine, or vice versa, is called a *transversion*. Both types of base substitution mutation can occur. Base pair substitution mutations in the coding region (exons) of genes have a reasonably high chance of being silent (having no effect) because of the redundancy of the genetic code; about one-quarter of all possible base substitutions in codons will not result in amino acid changes and will therefore likely be silent. Most base pair substitution mutations in exons will at worst result in the change of only one amino acid in the protein sequence. Depending on the position of this amino acid in the protein structure, this may or may not have any functional consequence. However, a base pair substitution could result in a triplet codon changing from an amino acid codon to a stop codon, resulting in premature termination of the protein assembly. Point mutations in non-coding regions of the gene may also be critically important if the coding sequence containing the point mutation happens to be involved in the regulation of transcription (e.g., change in nucleotide in the regulatory region of a gene that alters the ability of a transcription factor to bind to the gene). Mutation of an intronic base that is at an intron–exon boundary may also be important, as it may cause the entire exon to be skipped in the process of transcription. Single nucleotide changes in other parts of the gene may alter the ability of small-interfering RNA (siRNA) molecules to bind to DNA, thereby altering transcription.

Another type of point mutation occurs when a single base is deleted or added to an exon in a gene. Since the genetic code is strictly based on the triplet codon arrangement that is read in one direction (from 5′ to 3′), deleting or adding one base in a sequence will cause a shift in the reading frame, and thus such mutations are called "frameshift" mutations. For example, in our previous example of a DNA sequence, 5′-CGT TCA ACA-3′, the addition of one G base between the two Ts would result in the sequence 5′-CGT GTC AAC A-3′. Now the triplet code for all amino acids prior to the base addition (or deletion) will remain the same, but the sequence following will be out of phase: the second codon is now GTC, rather than TCA; the third is AAC, rather than ACA; and so on. Thus, all of the genetic code beyond the insertion or deletion will be incorrect. Obviously, this will have a profound effect on the characteristics of the gene product.

It should be noted that single nucleotide differences between individuals are very common. A difference in one nucleotide at a specific site in DNA, when compared to the "common sequence" found in a population, is referred to as a "single nucleotide polymorphism," or SNP. The analysis of SNPs in specific genes has become a common feature in the rapidly growing field of molecular epidemiology. SNPs are not really "mutations," because by definition they are relatively common in a population (most definitions of a polymorphism indicate that the variant allele should be present at a frequency of 1% or more in a population). However, common SNPs did arise through a germinal mutation in DNA, but usually thousands of years ago in a "founder" population.

There are several ways in which chemicals can induce point mutations. One of the most common is by forming adducts with a particular base in the DNA. Many chemicals that interact with DNA do so by forming covalent bonds between an electrophilic part of the molecule and a nucleophilic part of DNA. For example, the potent mold toxin and liver carcinogen, aflatoxin B_1, is biotransformed in the body to a highly chemically reactive epoxide intermediate. This epoxide is highly electrophilic and will react quickly with nucleophilic sites in the cell. One such site is the nitrogen in position 7 of the DNA base, G. Thus, one consequence of aflatoxin exposure will be the formation of aflatoxin-N^7-guanine adducts in DNA (Eaton and Groopman, 1994; **Fig. 8**). Since this adduct is bulky, it will change the shape of the double-stranded DNA molecule, which may lead to mispairing of bases on the strand or to errors in DNA replication, typically base pair substitutions. Other molecules may interact with DNA by intercalating between the two strands of DNA. There are grooves in the double helix configuration of DNA and certain planar molecules fit within these grooves. The presence of intercalated molecules may cause errors in DNA replication or DNA repair, thereby introducing mutations in the DNA. Finally, chemically reactive forms of oxygen that are generated in many different ways in the body may interact with and damage DNA. One form, hydroxyl free radicals may react with G at carbon 8 to form 8-hydroxy-guanine. This results in an unstable base pairing and may ultimately lead to the introduction of mutations into DNA. It now appears that oxidative damage to DNA is a very common event and may be important in the process of aging, as well as the development of cancer. There is currently much interest in developing ways to combat the so-called "oxidative stress" in the body that may lead to oxidative damage to DNA. Both natural dietary antioxidants and synthetic antioxidants have been proposed to help reduce cancer risks and slow the aging process

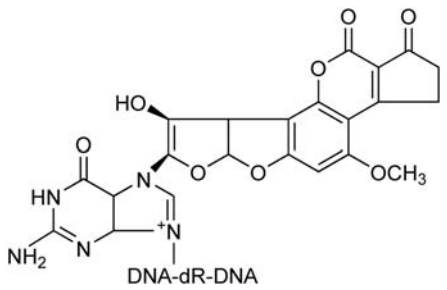

Fig. 8 Aflatoxin B_1-N^7 guanine adduct.

by reducing the effects of reactive oxygen molecules (Wattenberg, 1985). Much remains to be understood about the actions of antioxidants in the body and their effectiveness in reducing the long-term adverse effects of oxidative stress.

The consequences of somatic mutations are twofold: (1) excessive cell death and (2) cancer. Excessive mutations, and/or mutations in critical genes, may result in the death of a cell. If too many cells are killed, then the functions of the organ comprised of those cells will be altered. Since DNA is most susceptible to mutations immediately prior to and during DNA replication for cell division, it is not surprising that the cells that are most susceptible to mutations are those cells that are rapidly dividing. In fact, the cytotoxic effects from extensive DNA damage are the basis of most chemotherapeutic treatments for cancer. Cancer cells by definition are rapidly growing and thus are quite susceptible to being killed by DNA-reactive chemicals. Unfortunately, so are certain normal cells that typically undergo relatively rapid cell division; cells lining the GI tract, cells in the bone marrow that produce red and white blood cells, and cells in the skin and hair follicles. Thus, major side effects of many chemotherapeutic agents are related to the GI system (nausea, vomiting, diarrhea), the blood (anemia, low white blood cells, and associated immune suppression resulting in sensitivity to infection), and the skin (loss of hair, dermatitis).

Mutations in genes that are involved in normal cellular growth control and differentiation may ultimately result in the development of cancer. There is strong evidence that somatic mutations are a requisite step in the development of all cancers. Since somatic mutations may accumulate over a lifetime and are a relatively common event (resulting from the production of reactive oxygen, exposure to chemical carcinogens in our diet and environment, and random errors that occur during DNA replication and repair), it is unfortunate but not surprising that cancer is a relatively common disease that occurs much more frequently as we get older.

1.01.3.5 Toxicogenomics and Systems Toxicology

1.01.3.5.1 Toxicogenomics

In the past decade, numerous new genome-based technologies have become available that allow for the large-scale analysis of biological responses to external stimuli. Traditional scientific approaches to elucidate the biochemical and molecular effects of toxic substances focused largely on examining biochemical pathways that were logically connected to observed responses identified through gross pathology, histology, blood chemistry, or behavioral observations. Such "hypothesis-driven" research into understanding mechanism of action remains a mainstay of current scientific investigations in toxicology; however, technologies now available allow one to examine the entire "universe" of biological responses to a toxic substance. These new "hypothesis-generating" technologies include genomics (characterization of much or all of the genome of an organism), transcriptomics (characterization of most or all of the messenger RNAs (mRNAs), or transcriptome, expressed in a given cell/tissue), proteomics (characterization of most or all of the proteins expressed in a given cell/tissue), and metabonomics (also called "metabolomics"; characterization of most or all of the small molecules in a cell or tissue, including substrates, products, and cofactors of enzyme reactions). The integration of multiple levels of molecular function (genomics, transcriptomics, proteomics, metabonomics, etc.) are essential to understand how a living organism functions at the cellular level. This integrative approach is called "Systems Biology" (Weston and Hood, 2004). However, because each level of analysis generates a very large quantity of data, the collection, organization, evaluation, and statistical analysis is in itself an enormous undertaking. The field of "Bioinformatics" has been developed to address the many computational and statistical challenges of "omics" data. In the field of toxicology, the term "toxicogenomics" is used to define the area of research that "combines transcript, protein, and metabolite profiling with conventional toxicology to investigate the interaction between genes and environmental stress in disease causation" (Waters and Fostel, 2004).

Genomics: The genome of an organism represents the full complement of genes that are determined at fertilization by the combination of the parental DNA. Thus, each cell of an organism has the same genome, characterized by the nucleotide sequences inherited from its parents. The human genome consists of approximately 3 billion base pairs of deoxyribonucleotides organized into discrete units referred to as genes (see "**Structure and function of DNA**" section for a review on the structure and function of DNA). There are approximately 24,000 genes that code for proteins in the human genome. Within the human genome, there is, on average, about 0.1% variability in DNA sequence between any two individuals, and it is these differences that contribute to the uniqueness of each person. Most of this variability exists as "SNP," although larger segments of DNA may be variable between individuals, including the duplication or loss of entire genes (referred to as "Copy Number Variants," or CNV). The identification of particular genetic variants, such as the glutathione *S*-transferase (GSTM1) polymorphism that might contribute to interindividual differences in susceptibility to chemicals or other environmental factors, represents a relatively new and growing area of study that aims to understand the complex interactions between the human genome and the environment (Costa and Eaton, 2006).

In addition to sequence-based differences, it is now recognized that the so-called epigenetic modifications made to DNA can influence an individual's susceptibility to toxicants by modifying "how" genes are expressed. Common examples of such modifications include the covalent addition of various chemical groups (i.e., methylation, acetylation, phosphorylation). Importantly, although such epigenetic changes do not result in the alteration of the actual genomic sequence, they can result in heritable phenotypic changes. Thus, genomic analyses in toxicology may also include techniques to identify toxicant-induced changes in DNA modification patterns as well (Watson and Goodman, 2002).

Transcriptomics: In order for a gene to produce a functional protein, it must first be transcribed into an intermediate known as an mRNA. The sum of mature mRNA species present at a given time is referred to as the transcriptome. Because the transcriptome represents the steady state between the rate of synthesis (transcription) and degradation of mRNAs in a cell, it is often the first battery to be perturbed in response to toxicants. In addition to coding for RNAs that provide the blueprint for protein synthesis, genomic DNA

also generates siRNAs (microRNAs), which are biologically active and can participate in the regulation of gene expression. Microarray technologies are the most common method for looking at changes in the transcriptome. By anchoring tens of thousands of unique oligonucleotides (or cDNAs) on solid matrix, toxicologists can quantitatively measure the expression of thousands of unique mRNAs in a single sample. However, this technology is being rapidly replaced by a newer, and much more comprehensive method known as RNA-seq (RNA-sequencing), which uses RNA-sequencing (i.e., next-generation sequencing) in lieu of DNA hybridization.

Transcriptomics approaches are useful for a variety of toxicological applications. For example, in the subdiscipline of *ecotoxicology*, transcriptomics can be used to identify molecular mechanisms of action of environmental chemicals, generate biomarkers of exposure, and determine detrimental effects in nontarget organisms (Ankley et al., 2006). This approach is based on the assumption that measurable gene expression effects in target tissues of wild animals are temporally, spatially, and mechanistically related to either chemical exposures or adverse effects. With regards to the latter, this is accomplished by linking the *-omic* profile to a response that has been linked to fish condition, such as tissue damage, histopathological abnormalities, endocrine alterations, and cancer (Iguchi et al., 2006; Neumann and Galvez, 2002). These types of phenotypic anchoring studies (because they anchor genome markers with a particular phenotype such as tissue pathology, see below) are more prevalent in the biomedical literature than in aquatic studies.

Proteomics: Although changes in gene expression often contribute to, or are reflective of, phenotypic changes that occur in response to a toxic substance, the transcriptome is still somewhat removed from the ultimate cellular response that it elicits. This is because transcripts generally need to be translated into a protein before they can serve a function. As a result there is also great interest in the "proteome"—the entire complement of *proteins* that are present in a cell or tissue at a given point in time. Analysis of the proteome of a cell or tissue is much more difficult than analysis of the transcriptome, primarily because it is not yet possible to "amplify" the number of copies of proteins in a cell. Furthermore, unambiguous identification of specific proteins is much more difficult than for individual mRNAs. Identification of specific proteins can be performed using a microarray chip containing previously prepared antigens (as is often done in transcriptomics), but is most commonly carried out using an approach known as bottom-up proteomics (also shotgun proteomics). This method involves the proteolytic digestion of a sample to generate a peptide mixture, which is then separated (e.g., 2D-gel electrophoresis, high-performance liquid chromatography) and analyzed via tandem mass spectrometry (Zhang et al., 2014). The large and complex set of peptide mass fragments is then analyzed and compared with a large database of mass fragments of known peptides/proteins for identification. In addition, top-down proteomic approaches (i.e., without a digestion step) have also been developed. Such methods are valuable as they derive analysis from intact protein, and therefore provide information that is lost with digestion (i.e., protein mass, posttranslational modifications, degradation artifacts), but have suffered from low throughput until recently.

As with transcriptomics, it is anticipated that changes in protein expression can be used as specific biomarkers for particular types of toxic responses, or early markers of disease onset such as Alzheimer's or Parkinson's disease. There is precedence for these applications as similar conceptual approaches have been used in medicine for years. For example, use of serum transaminase proteins as indicators of liver damage, or the presence of prostate-specific antigen in serum as a potential biomarker of early stage prostate hyperplasia or cancer. Relative to other approaches, the potential power of proteomics lies in the ability to identify unique patterns of protein expression, or the identification of unique proteins or peptides, that are predictive of early toxic response or later development of disease.

Metabonomics/metabolomics: The terms *metabonomics and metabolomics* are often used interchangeably to describe the analysis of the "universe" of small molecules that serve as substrates, products, and cofactors of the milieu of enzymatic reactions and other metabolic processes that define living cells, and thus the organism. However, there is growing consensus that "metabolomics" refers to this "universe" as it pertains to normal physiologically function, whereas "metabonomics" refers to analysis of the abnormal molecular changes that arise from environmental stimulation (i.e., toxin/toxicant, diet, lifestyle, etc.). Regardless of the specific term used (metabonomics will be used here), the ability to quantitatively analyze toxicant-induced changes in the "metabolic profile" (the "metabonome," or "metabolome") of a cell, tissue, or body fluid is truly exciting from a functional perspective. Ultimately, changes in the metabonome should reflect the biologically relevant changes in gene transcription, translation, protein function, and other cellular processes, while also ignoring biologically irrelevant changes in these factors. Although conceptually superior to either transcriptomics or proteomics for predictive toxicology, metabonomics lags significantly in technological development of readily accessible tools for thorough analysis of the metabonome. While these technologies are under continuing development, two approaches for identifying and measuring hundreds, or even thousands, of small molecules in biological samples have emerged—nuclear magnetic resonance, and mass spectrometry (Emwas, 2015). Both have their advantages and limitations, and it is likely that the most successful approaches to applying metabonomics to toxicological problems will utilize both techniques.

Bioinformatics: One feature in common among all of the various "omics" technologies is the ability to generate very large volumes of data (literally millions of data points from a single experiment). Both the data management and the statistical evaluation of toxicogenomics studies represent an enormous challenge. The emerging field of bioinformatics has developed to address these challenges. Numerous commercial platforms for conducting microarray analysis of the transcriptome are available, and sophisticated software is available for both data management and analysis. One of the major challenges in statistical analysis of large data sets is the large number of "false positives" that will result from multiple comparisons. In a typical gene array experiment, it is not uncommon for an investigator to make $>20{,}000$ different comparisons. At the typical "95%" statistical confidence limit, one would expect >1000 of the noted differences to occur just by chance alone. Thus, more rigorous statistical methods have been developed to reduce the so-called "false discovery rate" in such experiments (Storey et al., 2005).

Challenges in using "omics" technologies for predictive toxicology and risk assessment: Toxicogenomics' tools are becoming indispensable for research aimed at identifying the mechanisms and mode of action (MOA) of toxic substances. However, the incorporation of such approaches into routine toxicity assessment presents numerous challenges (Wilson, et al., 2013; Maggioli et al., 2006). One of the primary challenges to incorporating toxicogenomic data into the risk assessment paradigm is related to dynamic nature of toxic responses. While traditional measure of toxicity, such as histopathological changes in a tissue, tend to be stable or even irreversible, the myriad of molecular, biochemical, and cellular changes that give rise to the toxic response(s) are often much more transient. Thus, the snapshot profiles of mRNAs, proteins, and/or metabolites captured at a single point in time may be dramatically different depending on the specific point in time the sample was collected.

"Systems toxicology" refers to a conceptual framework by which classical toxicology is assimilated with the data derived from the above technologies to inform predictive toxicology assessments. This approach has several key components including: (1) large databases of treatment-specific information, such as results of transcriptomic, proteomic, and metabonomic analyses from target tissues and/or body fluids derived from toxicant-treated animals, (2) genomic databases that describe the DNA sequence information from the species of interest, (3) computational tools that extract information from these and other databases and the published literature to identify critical pathways and networks that are altered by the toxicant treatment, and (4) comparison with traditional toxicological endpoints to ensure that the observed "omics" responses are closely aligned with the toxicant-related pathophysiology in the animal (histopathology, clinical chemistry, etc.)—a process called "phenotypic anchoring" (Waters and Fostel, 2004).

The toxicology in the 21st century (Tox21) program is just one example of the value that systems toxicology holds for real world problems. Begun in 2008 as a joint National Institute of Health (NIH)/EPA/FDA initiative, the goal of Tox21 is to "develop more efficient and less time-consuming approaches to predict how chemicals may affect human health." By generating high-throughput, in vitro screening systems, Tox21 has thus far been able to screen more than 10,000 chemicals in the Tox2110K library to determine which require further study. This information has been made available, free of charge, to both scientific investigators and the public, reducing the need for animal testing at all phases of the assessment (NCATS, 2015). As such, the Tox21 program also exemplifies the potential for animal reduction, refinement, and replacement (known as the "3Rs") inherent in systemic approaches to toxicity testing (Kroeger, 2006).

1.01.3.6 Carcinogenesis

It has been stated, and many people believe, that we are in an epidemic of cancer and that this epidemic is due in large part to our unprecedented exposure to environmental pollutants associated with increased industrialization and environmental pollution. However, there are many important considerations that one must take into account when assessing the impact of chemical pollution on cancer rates.

1.01.3.6.1 Trends in cancer incidence and mortality in the United States

There is little question that more people are dying of cancer today than ever before. However, before jumping to conclusions about cancer trends over time, three points must be considered when evaluating cancer statistics.

First, cancer is a disease that occurs much more frequently in the elderly. Thus, as the population grows increasingly older (because we are prematurely dying less frequently from infectious diseases, heart disease, and other common causes of death), the fraction of the population dying from cancer is bound to increase. For this reason, trend comparisons in cancer statistics utilize age-adjusted rates to account for demographic shifts in the age of the population that occurs over time. The second important point to consider when examining cancer trends over time is that cancer is not a single disease, but rather a conglomeration of different diseases with different causes that share many common characteristics. Thus, it is most useful to consider trends and statistics for specific forms of cancer, rather than lumping them all together. Finally, it is important to define whether the statistics are for mortality or incidence. For some types of cancer (those which are uniformly lethal and are not amenable to effective treatment), the differences between incidence and mortality statistics are not large. However, for other types, such as skin cancer, such differences may be very large. Furthermore, incidence statistics may be more subject to apparent variation over time because of differences in efficiency of collecting, classifying, and reporting the cases, rather than a true change in disease incidence. Mortality data tend to be less subject to such reporting differences, although such data can still be the subject of some error in trend analysis. With these caveats in mind, some remarkable changes in cancer mortality have occurred in this century.

Fig. 9 shows age-adjusted incidence of cancer mortality in the United States for males (Fig. 9A and B) and females over the period 1930–2012 (American Cancer Society, 2016). The first and most dramatic feature of these plots is the large increase in lung cancer deaths in males. Prior to the early 1930s, lung cancer was relatively rare, with the death rates >10 per 100,000. Since the late 1930s, there has been a steady and dramatic increase in lung cancer mortality in American males continuing until the mid-1980s, when the increase peaked at an annual death rate of 87 per 100,000 and has slowly declined to about 58 per 100,000 in 2011. Lung cancer has also increased dramatically in women, but less so. This increase did not begin until the early 1960s and essentially reached a plateau in the early 2000s, with a slight reduction over the last 10 years. In 1985, lung cancer deaths in women surpassed breast cancer as the leading cause of cancer-related deaths in women. Not surprisingly, the increase in lung cancer mortality in both men and women is paralleled by an increase in *per capita* consumption of cigarettes in the United States, with an approximately 20-year "lag," that is, trends in smoking increased about 20 years before the increase in lung cancer became evident.

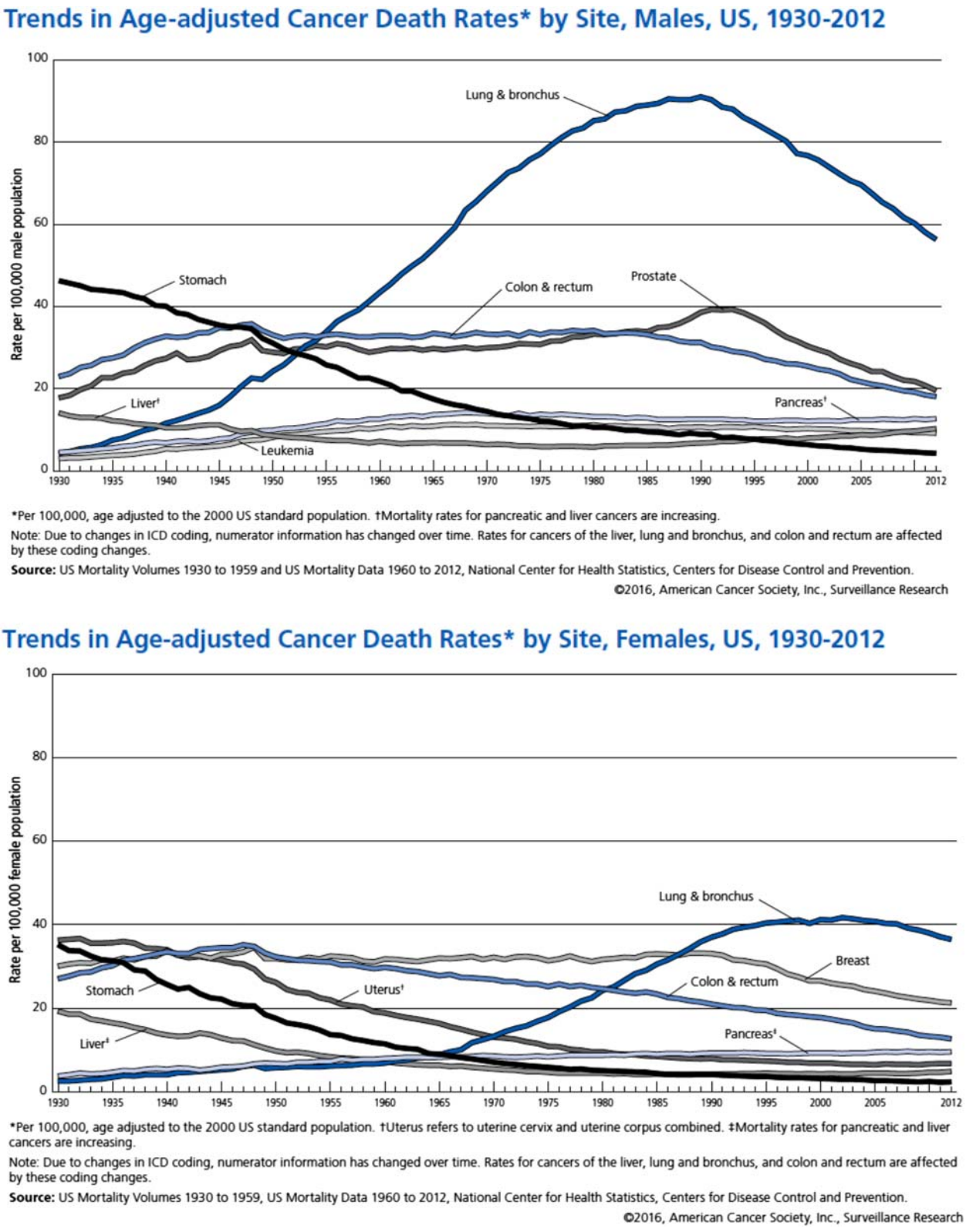

Fig. 9 Age-adjusted incidence of cancer mortality in the United States for (A) males and (B) females over the period 1930–2004 (http://www.cancer.org/docroot/STT/st_0_2008.asp?sitearea=STT&level=1). (Reproduced from American Cancer Society. (2016). Cancer facts & figures 2016. Atlanta, GA: American Cancer Society, with permission of the American Cancer Society.)

In contrast to lung cancer, stomach cancer death rates have declined dramatically over the same 60-year period in both US men and women. However, in some parts of the world, most notably Japan, stomach cancer remains at a relatively high level. Studies of cancer incidence and mortality data among ethnic groups residing in different parts of the world suggest that most cancers have a strong environmental component. For example, Japanese-Americans who have adopted the diet, lifestyle, and environment of America have a relatively low stomach cancer incidence reflective of the US population, rather than the high incidence reflective of Japanese populations. Of some interest was the recognition in the 1980s that a large proportion of stomach cancers are actually due in part to a bacteria, *Helicobacter pylori*. With this recognition, preventive measures using antibiotics have further contributed to the decline in stomach cancer mortality. Many other epidemiology studies have led many cancer researchers to conclude that approximately 80–90% of all cancers are environmentally related and are thus potentially preventable. However, it must be recognized that the use of the phrase "environmentally related" should be construed in its broadest meaning, that is, everything that is not genetic. Thus, environmental causes by this definition include such things as diet, smoking and drinking habits, and other lifestyle and social habits that may influence cancer risk. Certainly included in the broad category of "environmental causes" is industrial chemical pollution of our air, water, and food, but this is not generally thought to contribute to a large proportion of all cancers, although such cancers should largely be preventable with appropriate research to identify chemicals that pose a potential cancer risk and regulatory control to limit exposures. Undoubtedly, there are strong interactions between environment and genetics that ultimately determine which individual actually develops the disease. Current research into genetic risk factors has revealed extensive genetic heterogeneity in the human population that may be reflective of large genetically determined differences in susceptibility to environmental carcinogens.

1.01.3.6.2 The causes of cancer

In the United States and many other parts of the world, smoking is considered the leading single cause of human cancer. In the United States, it has been estimated that approximately 32% of all cancers are related to smoking (American Cancer Society, 2016). Although the majority of these cases are lung cancer, smoking also increases the risk for other types of cancer, including cancers of the bladder, mouth, pharynx, larynx, esophagus, pancreas, uterine cervix, and kidney. Smoking is responsible for 83% of lung cancer deaths in US men and 76% in women (American Cancer Society, 2016). Individuals who smoke two or more packs of cigarettes per day for 20+ years will have more than a 20-fold increased risk of developing lung cancer compared to nonsmokers.

Poor diet, excess alcohol consumption, and physical inactivity are also considered to be major factors in the development of many cancers, and an estimated 20% of US cancers are attributed their combined effects (American Cancer Society, 2016). However, the exact components of the diet that influence cancer risk are not well understood. For many years it was believed that the amount of fat in the diet was an important risk factor for breast cancer in women, with a higher fat diet associated with greater risk. While there is some support for a relationship between diet and breast cancer, there is controversy over whether the important factor is simply total fat or particular types of fat (e.g., saturated, unsaturated), or whether the association is due to other nonfat dietary factors that are correlated with fat intake.

It is now recognized that there are many naturally occurring chemicals in our diet that may positively and negatively influence cancer risk. For example, the mycotoxin aflatoxin B_1, which occurs in corn, peanuts, and occasionally other grains contaminated with the mold *A. flavus*, appears to be an important cause of liver cancer in certain areas of the world (some parts of China, Southeast Asia, and Central Africa). While aflatoxin itself is a potent liver carcinogen in laboratory rats, it appears to act most effectively in humans in the presence of hepatitis B virus infection. In some parts of the world where hepatitis B (or C) viruses are endemic, and aflatoxin is present at relatively high levels in the diet, liver cancer is the leading cause of cancer-related deaths (Eaton and Groopman, 1994). In the United States, where dietary aflatoxin contamination of corn and peanuts is kept relatively low through proper harvest and storage and frequent monitoring, and where hepatitis viral infections are relatively rare, aflatoxins are not thought to be a significant cause of liver cancer.

Naturally occurring chemicals in plants, particularly certain vegetables and fruits, seem to afford resistance to the effects of other chemical carcinogens present in the diet and may also be important in protecting against oxidative damage to DNA. Thus, diets high in fruits and vegetables have been associated with lower incidence for several types of cancer. A specific example of a dietary phytochemical that may influence health is the isothiocyanate, sulforaphane. Sulforaphane and other isothiocyanates are present as a glucosinolate conjugates in a variety of cruciferous vegetables, especially broccoli and broccoli sprouts. Numerous studies have demonstrated that sulforaphane is able to alter the transcriptional activation of a number of genes that may help protect against cellular injury from oxidative stress.

The presence of environmental pollutants in our diet, such as pesticide residues on fruits and vegetables, chlorinated organic chemicals such as PCBs and polybrominated diphenyl ethers in animal fat, and chemical contaminants in our drinking water have been proposed to contribute to our total burden of carcinogen exposure. However, most cancer researchers believe that the contribution of these pollutants to overall cancer incidence is small (but probably not 0) because the concentration of contaminants is usually quite low, and the duration and/or frequency of exposure is often short. However, particular instances of excessively high contamination of a food commodity or water supply could represent a significant risk to exposed individuals if the exposure were to occur frequently over a prolonged period of time. For example, contamination of groundwater with arsenic has undoubtedly contributed to a large number of cancers, most notably lung, bladder, and skin cancer, worldwide. Thus, such potential sources of exposure should not be dismissed as unimportant.

1.01.3.6.3 Basic mechanisms of chemical carcinogenesis

Although there is a general consensus that chemical carcinogenesis is a multistep process, the specific biological processes involved in the development of cancer are not completely understood. However, most, but not all, chemicals which increase tumor development do so by interacting with DNA (Fig. 10). Such chemicals are called *genotoxic* carcinogens because they damage DNA. However, for a chemical to bind covalently to DNA it must be chemically reactive, and most chemicals encountered in our environment and diet are not sufficiently reactive to bind to DNA. Thus, the first step for many chemical carcinogens is the activation in the body of a chemically reactive form capable of binding to DNA. Once a procarcinogen has been biotransformed to a DNA-reactive "ultimate" carcinogen, it may bind to DNA, but other biotransformation pathways in the body may effectively protect DNA by detoxifying the reactive chemical. Depending on the relative rates of activation to detoxification, some of the activated chemical may bind to and damage DNA. Most DNA damage is either inconsequential, lethal to the cell, or produces damage which is efficiently repaired by special DNA repair enzymes (Fig. 10). However, some DNA damage may escape repair or, worse, be repaired incorrectly, and thus cause a mutation in the cell. If the mutated gene is important in the regulation of cell division, it may result in a change rate in cell growth a change rate and/or cell differentiation.

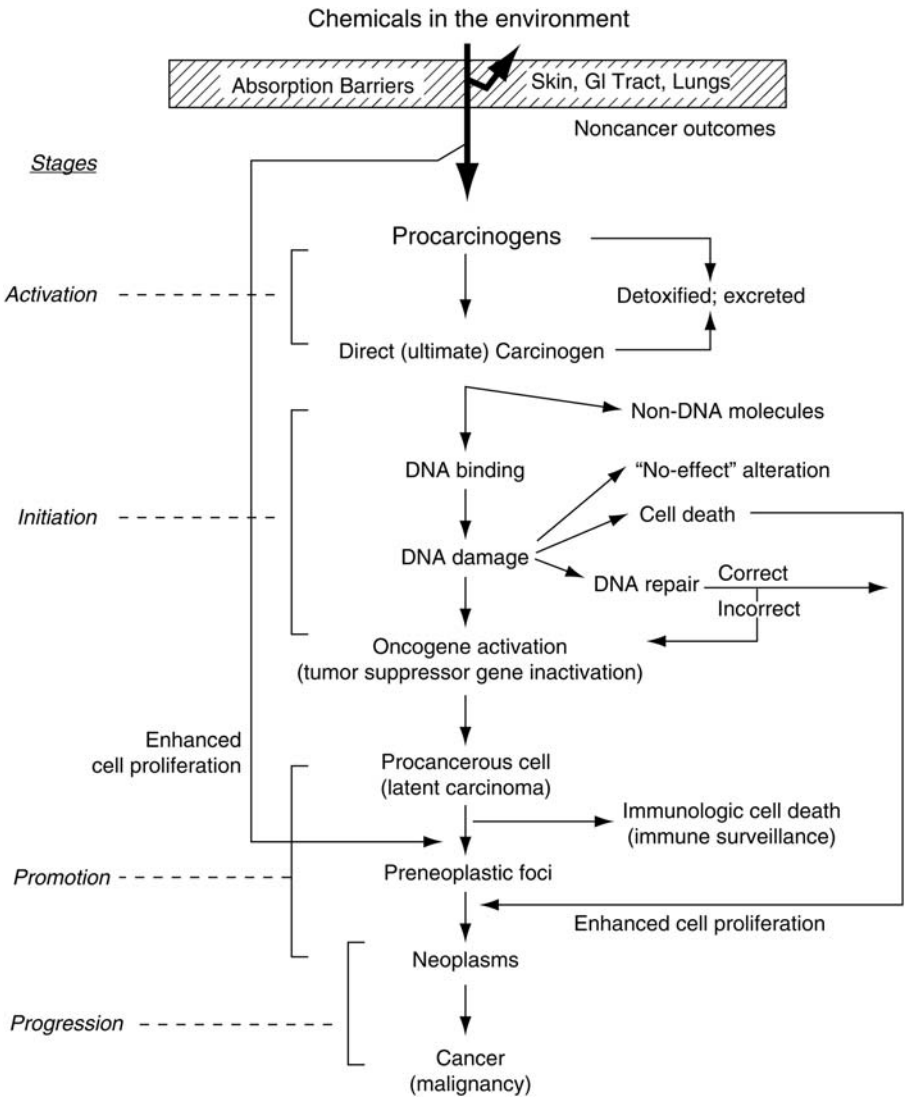

Fig. 10 Process of chemical carcinogenesis. As illustrated, carcinogenic chemicals must undergo a sequence of events prior to tumor formation. Many chemicals require enzyme-mediated bioactivation to produce reactive intermediates before producing somatic mutations. Initiation occurs when genes involved in cellular growth and differentiation (oncogenes) undergo mutation, whereas the stimulation of clonal expansion of the mutated cell to a colony of cells containing the mutated oncogene is referred to as promotion. Cancers develop when cells within this population acquire additional mutations which favor growth of the new cell population. This later stage is often referred to as tumor progression, and it may involve successive changes in phenotype leading to increasing degrees of malignancy. (Reproduced from Rosenstock, L., Cullen, M.R., Redlich, C.A., Brodkin, C.A. (eds.) (2005). In: Textbook of clinical occupational and environmental medicine (2nd edn.), p. 88. Philadelphia, PA: Elsevier Saunders (Chapter 5). With permission of Saunders.)

There are certain genes present in all cells, called *proto-oncogenes*, which when altered by mutation are changed from a normal cell into a precancerous cell. There are genes that normally function to inhibit cell division, called tumor suppressor genes. Mutation of tumor suppressor genes is a critical factor in most, if not all, cancers. For example, one particular tumor suppressor gene, called "p53," is mutated to an abnormal form in a high percentage of human cancers (Hollstein et al., 1991). The product of this gene appears to function as a "checkpoint" in normal cell division, ensuring that the cell has adequate time for DNA damage to be repaired before it divides. In the absence of this regulation (e.g., mutated p53), cells are much more likely to replicate their DNA before they are fully repaired, resulting in mutations in daughter cells.

The process by which chemicals damage DNA to produce a cell with altered growth potential is called *initiation*. Initiation is a critical early step in the development of chemically related cancers. However, initiation of DNA damage by itself may be insufficient to cause a full-blown cancer and additional events are necessary (**Fig. 10**). Initiated, precancerous cells may be present in tissues for many years, but without additional stimulation to undergo division will not become cancerous. Furthermore, frequently such transformed cells express surface proteins which mark the cell as abnormal or foreign to the body, such that the immune system will destroy it. However, if this immune surveillance is ineffective or incomplete, the precancerous cell may be stimulated by other factors to proliferate into tumors and eventually spread (metastasize) to distant sites. Stimulation of clonal expansion of precancerous cells to small populations of identical precancerous cells is referred to as *promotion* (**Fig. 10**). The multiple steps from initiation to early neoplastic foci and tumor development (promotion) to metastatic carcinomas (*progression*) are not fully understood. Promotion and progression are often associated with factors that increase the rate of cell division. Thus, an increase in the rate of cell division and attendant DNA replication in a normally nondividing cell population will greatly increase the chances of permanent incorporation into the DNA of mutations that arise continuously from endogenous processes and exposure to environmental mutagens. Therefore, drugs or other chemicals which increase the rate of cell division may enhance the expression of mutations accumulated over time, increasing the chances for mutated cells to expand in number and acquire additional mutations.

Chemicals that by themselves are incapable of inducing cancer, but when given before or during exposure to initiators increase the potency or effectiveness of the carcinogen are called *cocarcinogens*. Often such chemicals alter the effectiveness of protective pathways in the multistep process leading from initiation to tumor progression. For example, some chemicals can decrease the relative fraction of procarcinogen which is activated to the ultimate carcinogenic form by increasing the level of detoxification enzymes. Conversely, chemicals which decrease immune function or alter DNA repair processes will enhance the carcinogenic potency of other carcinogens and will increase the chances of a tumor arising from nonspecific, background mutations.

1.01.3.7 Teratogenesis

Concern over the potential for occupational and/or environmental chemical exposure to produce birth defects has heightened in the last few decades. As the developing embryo is often highly sensitive to toxic chemicals, it is necessary to consider women of childbearing age to be at particularly high risk from toxic chemical exposures.

1.01.3.7.1 Causes of birth defects

Many causes of birth defects, such as maternal malnutrition, drugs, alcohol, genetic factors, and certain viruses and bacteria, have been identified, but the cause of most human birth defects remains unknown.

Literally hundreds of chemicals have been identified as having the ability to cause birth defects (called teratogens), usually at high doses in laboratory animals, but the list of documented human teratogens remains surprisingly small, however, and many of these are subject to debate. The large species differences that exist in sensitivity to some teratogens are of critical importance; moreover, the timing of exposure and stage of embryonic development have made reliable, quantitative extrapolation of animal data to humans difficult at best. For example, the drug thalidomide, used in Europe to ameliorate nausea and vomiting during the early stages of pregnancy, was found to produce an extraordinarily high incidence of a rare birth defect, phocomelia (failure of limbs to develop), among offspring of women who took this drug during a specific period early in their pregnancies. Only later was it found that humans are more than 1000-fold more sensitive to the teratogenic effects of thalidomide than most rat or mouse strains (Levi et al., 1987).

Even though species differences complicate greatly the interpretation of laboratory animal studies to predict human risks, there are some basic concepts about the teratogenic response that appear to be true in all animals, including humans. First, the age of development of the embryo at the time of exposure to the teratogen is very important. The period of greatest sensitivity to chemical teratogens is during the first trimester of pregnancy, a time of maximal rate of cell differentiation.

In humans, this highly sensitive period for teratogenic effects extends from about 2 weeks through 10 weeks of development. Exposures to many toxic substances during the first 2 weeks of embryonic development are likely to result in death of the embryo, and it is probable that the woman would not even be aware that she was pregnant and had an early miscarriage. Exposures to teratogens occurring beyond the 10th–12th week of pregnancy are far less likely to result in physical or morphological abnormalities because all of the major physical characteristics (extremities, palate, ribs, organs, etc.) have been formed. However, other developmental problems, particularly relating to the nervous system, may result from exposure during the later stages of fetal development, because the brain is not fully differentiated and developed until birth or slightly thereafter. Methyl mercury, lead, and alcohol are all examples of chemicals which can affect the developing nervous system in utero during later stages of pregnancy.

The second important factor relating chemical exposure to teratogenic responses is the dose of teratogen. Most, if not all, toxic substances can adversely affect the developing embryo and/or fetus, either directly or indirectly if they produce a toxic response in

the mother. What generally defines whether a chemical is truly teratogenic depends on whether it affects embryonic and/or fetal development at a dose below that which produces maternal toxicity. As with other types of response, teratogenicity follows the dose–response relationship for a population, although often the slope of this curve is exceedingly steep (the dose range is very small between no effect in the population and a very high percentage of the population responding). While a steep dose–response curve argues for the concept of a *threshold dose* in experimental animals, it is very difficult to extrapolate such information to the human situation. Furthermore, because of the very wide genetic variability among individuals within the human population relative to the highly inbred characteristics of laboratory animals, it is likely that the threshold for teratogenic response will vary more substantially among individuals within the human population, making it more difficult to establish a safe level of exposure for everyone. Due to our relative ignorance about the causes of most birth defects and the likely high sensitivity of the developing organism to some toxic chemicals, a high degree of prudence in establishing the so-called "safe" levels of exposure for pregnant women would seem appropriate.

1.01.4 Toxicity Testing in Experimental Animals

1.01.4.1 Basic Approaches and Principles of Toxicity Testing

Two main principles underlie all descriptive animal toxicity testing. The first is that the effects of chemicals in laboratory animals, when properly qualified, are applicable to humans. In general, doses are determined on a body weight basis, and doses in both humans and laboratory animals are usually presented in units of milligram of chemical per kilogram of body weight. However, extrapolation of dosage across species often correlates better when dose is expressed on the basis of dose per unit of body surface area. Thus, scaling factors are often used to convert animal dose to human dose. When extrapolating dose from small animals such as mice, this equates to approximately a factor of 10.

The second main principle is that exposure of experimental animals to toxic substances in large doses is a necessary and valid method of identifying possible hazards to humans. This principle is based on the classical quantal dose–response relationship. Practical considerations require that the number of animals used in toxicology experiments will be small compared with the size of human populations at risk. To obtain statistically valid results from a relatively small sample size (number of animals) requires the use of relatively large doses so that the effect will occur frequently enough to be detected. For example, an incidence of a serious toxic effect (such as cancer), as low as 0.01%, would represent 20,000 people in a population of 200 million and would be considered unacceptably high. To detect such a low incidence in experimental animals would directly require a minimum of about 30,000 animals. For this reason, there is no choice but to give large doses to relatively small groups and then use toxicological principles in extrapolating the results to estimate the risk at low doses.

Toxicity tests are not designed to demonstrate that a chemical is safe, but rather to characterize what toxic effects a chemical can produce. There are no set toxicology tests that have to be performed on every chemical intended for commerce. Depending on the eventual use of the chemical, the toxic effects produced by structural analogs of the chemical, as well as the toxic effects produced by the chemical itself, all contribute to determine what toxicology tests should be performed. However, the FDA, EPA, and Organization for Economic Cooperation and Development have written good laboratory practice standards. These guidelines are expected to be followed when toxicity tests are conducted in support of the introduction of a chemical to the market.

1.01.4.2 Acute Lethality

Usually the first toxicity test performed on a new chemical is acute toxicity. The LD_{50} and other acute toxic effects are determined after one or more routes of administration (one route being oral or the intended route of exposure), in one or more species. The species most often used are the mouse and rat, but sometime other species are employed. In mice and rats, the LD_{50} is usually determined as described earlier in this article, but in the larger species only an approximation of the LD_{50} is obtained by increasing the dose in the same animal until serious toxic effects of the chemical are demonstrated. Studies are performed in both adult male and female animals. Food is often withheld the night prior to dosing. The number of animals that die during a 14-day period after a single dosage is tabulated. In addition to mortality and weight, daily examination of test animals should be conducted for signs of intoxication, lethargy, behavioral modifications, morbidity, food consumption, and so on. The acute toxicity tests provide the following: (1) a quantitative estimate of acute toxicity (LD_{50}) for comparison with other substances, (2) identification of target organs and other clinical manifestations of acute toxicity, (3) establishment of the reversibility of the toxic response, and (4) dose-ranging guidance for subsequent studies.

If there is a reasonable likelihood of substantial exposure to the material by dermal or inhalation exposure, then acute dermal and acute inhalation studies are performed. The acute dermal toxicity test is usually performed in rabbits. The site of application is shaved. The test substance is kept in contact with the skin for 24 h by wrapping with an impervious plastic material. At the end of the exposure period, the wrapping is removed and the skin is wiped to remove any test substance still remaining. Animals are observed at various intervals for 14 days and the LD_{50} is calculated. If no toxicity is evident at 2 g $(kg)^{-1}$, further acute dermal toxicity testing is usually not performed. Acute inhalation studies are performed similar to the other acute toxicity studies except the route of exposure is via the respiratory tract. Most often, the length of exposure is 4 h.

1.01.4.3 Subacute Studies

Subacute toxicity tests are intended to evaluate the toxicity of the chemical after repeated administration and also to help in establishing doses for the longer-term subchronic studies. Most subacute studies utilize three to four different dosages of the chemicals, administered by mixing it in the feed. For rodent studies, 10 animals of each sex are usually used at each dose, whereas for dogs 3 dosages and 3 to 4 animals per sex are used. The chemical is typically administered for 14 days, after which the animals are killed and complete clinical chemistry and histopathology analyses are performed.

1.01.4.4 Subchronic Studies

Most toxicity assessment protocols call for a more prolonged administration of chemical than is done with the 14-day subacute study. Typically, such subchronic exposure studies last for 90 days. The main goals of the subchronic study are to establish a no observable adverse effect level (NOAEL) and to evaluate further the effects of repeated administration of the test compound on specific organ(s). These studies also provide the lowest-observed adverse effect level (LOAEL) for the species tested. The values obtained for the NOAEL and LOAEL will depend on the number of dose groups used, the distance between doses, and the number of animals in each group. NOAELs and LOAELs are utilized frequently for regulatory purposes. For example, the EPA utilizes the NOAEL to calculate the reference dose, which may be used to establish regulatory values for acceptable pollutant levels (Eaton and Gilbert, 2013). An alternative to the NOAEL approach, referred to as the *benchmark dose*, attempts to use all of the experimental data to fit one or more dose–response curves. These curves are then used to calculate a benchmark dose, which is the statistical lower bound on a dose corresponding to a specified level of risk. Although subchronic studies are frequently the primary source of experimental data to determine both the NOAEL and the benchmark dose, these approaches are frequently applied to other types of toxicity testing protocols, such as that for developmental toxicity. If chronic studies have been completed, these data would generally be used for the NOAEL and LOAEL estimates in preference to data from subchronic studies.

Subchronic studies are usually conducted in two species (rat and dog), with the test substance administered in the diet or by gavage. Occasionally, for volatile compounds inhalation exposure may be used, but these are difficult and costly studies to complete. Most protocols require at least three doses; the highest dose should produce evident toxicity but should not produce more than 10% fatalities, whereas the lowest dose should produce no apparent toxic effects. Usually 10–20 rats and 4–6 dogs of each sex per dose are used. Any animals that die during the course of the study should be kept for gross and histopathological evaluation. At the end of the 90-day study, all remaining animals are killed, and blood and tissues are collected for further analysis. Gross and microscopic condition of the organs and tissues (about 15–20) and the weight of the major organs (about 12) are recorded and evaluated. Hematology and blood chemistry measurements are generally done prior to, in the middle of, and at the end of exposure.

1.01.4.5 Chronic Studies

Chronic exposure studies are similar to subchronic studies except the period of exposure is longer than 90 days. In rodents, chronic studies are usually for the lifespan of the animal, which is approximately 2 years. Chronic studies in other species are usually for 1 year but may be longer.

Chronic toxicity tests are performed to assess the cumulative toxicity of chemicals, but often include consideration of the carcinogenic potential of chemicals so that additional lifetime feeding studies do not have to be completed. To ensure that 30 rats per dose survive the 2-year study, many study protocols call for 60 rats per group per sex. Both gross and microscopic pathologic examinations are made on all animals, including those that die prematurely.

Dose selection is the single most important consideration in these studies. Most regulatory guidelines require that the highest dose administered be the estimated maximum tolerable dose (MTD). This is generally derived from subchronic studies, but additional, intermediate time studies (e.g., 6 months) may be necessary if delayed effects or extensive cumulative toxicity are identified in the 90-day subchronic study. One widely used definition of the MTD is the dose that suppresses body weight gain slightly (i.e., 10%) in a 90-day subchronic study, although some regulatory agencies are critically evaluating the use of parameters other than weight gain, such as physiologic and pharmacokinetic considerations and urinary metabolite profiles, as indicators of a more mechanistically relevant MTD. Generally, one or two additional doses, usually some fraction of the MTD (e.g., 1/2 and 1/4 MTD), and a control group are tested.

Although such protocols have been widely used in chronic bioassays for decades, the predictive value of such studies has come under fire from multiple perspectives. Criticisms include concerns that biological responses seen at high doses may overwhelm protective pathways that are functional at much lower doses relevant to human exposures, and thus high dose results in rodents may overestimate risk to humans at much lower doses. Conversely, some argue that toxic effects that occur at high doses may mask important biochemical perturbations in disease pathways that occur at low doses, thereby underestimating potential risk. This is especially a concern for chemicals that act via modulation of endocrine pathways. Yet a third argument has been made that high dose studies in animals may fail to identify stimulatory, potentially beneficial, effects that might occur at low doses (Hormesis).

1.01.4.6 Developmental and Reproductive Toxicity

Four types of animal tests are utilized to examine the potential of a chemical to affect development and/or reproduction. General fertility and reproductive performance (segment I or phase I) tests are usually done in rats, with two or three doses (20 rats per sex per dose) of the test chemical. If three doses are used, the highest dose may show some evidence of maternal toxicity, but neither of the lower two doses should produce any evidence of maternal toxicity. Males are given the chemical 60 days and females 14 days prior to mating, and for the females this is continued throughout gestation and lactation. Typical observations made are the percentage of the females that become pregnant, the number of stillborn and live offspring, and the weight, growth, survival, and general condition of the offspring during the first 3 weeks following birth.

Tests to evaluate the ability of chemicals to cause malformations (birth defects, teratogenic effects) in the offspring are also frequently done in laboratory animals (referred to as segment II studies). Teratogens are most effective when administered during the period when the vital organs are developing (period of organogenesis), which occurs between 6 and 12 days of development in rodents. Rabbits, which have a somewhat longer gestation period, are also used frequently in teratology studies. The animals (12 rabbits and 20 rats or mice per group) are usually exposed to one of three dosages during organogenesis (day 6–15 in rats and 6–18 in rabbits) and the fetuses removed by Cesarean section 1 day before the estimated end of gestation (rabbit—day 31, rat—day 21). The uterus is excised and weighed, and then examined for the number of live, dead, and resorbed fetuses. Live fetuses are weighed, and one-half of each litter is preserved and the skeletons are stained to reveal skeletal abnormalities; the remaining half of the fetuses are examined grossly and under a dissecting microscope for soft tissue anomalies.

Perinatal and postnatal toxicity of a chemical may also be evaluated (the so-called segment III studies). This test is performed by administering the chemical to rats from the 15th day of gestation throughout delivery and lactation. The birth weight, survival, and growth of the offspring during the first 3 weeks of life are then evaluated to reveal any perinatal and/or postnatal toxicity from the chemical, distinct from effects that may have occurred during the earlier stages of development.

To identify possible effects of chemicals on the development of the reproductive system, a multigeneration study is often conducted. Shortly after weaning (30–40 days of age), groups of 25 female and 25 male rats are administered the test chemical. Usually three dose groups and a control are used. These rats are referred to as the F0 generation. Dosing continues throughout breeding, gestation, and lactation. The offspring (F1 generation) thus have been exposed to the chemical in utero, via lactation, and thereafter in the feed. When the F1 generation is about 140 days old, 25 females and 25 males are bred to produce the F2 generation, and the administration of the test chemical is continued. The F2 generation is thus also exposed to the chemical in utero and via lactation. The F1 and F2 litters are examined immediately after delivery. The percentage of F0 and F1 females that become pregnant, the number of pregnancies that go to full term, the litter size, the number of stillborn, and the number of live births are recorded. Viability counts and pup weights are recorded at birth: 4, 7, 14, and 21 days of age. The fertility index (percentage of mating resulting in pregnancy), gestation index (percentage of pregnancies resulting in live litters), viability index (percentage of animals that survive 4 days or longer), and lactation index (percentage of animals alive at 4 days that survived the 21-day lactation period) are then determined. Gross necropsy and histopathology are performed on all weanlings and some of the parents (F0 and F1), with a focus on the reproductive organs in the parents.

1.01.4.7 Mutagenicity Assays

Several in vivo and in vitro procedures have been devised for testing chemicals for their ability to cause mutations. The most frequently used test for mutagens is the Ames Salmonella/microsome test. This test uses several mutant strains of *Salmonella typhimurium* that have been genetically engineered such that they are unable to grow in a histidine-deficient medium unless a reverse or back mutation to the wild-type has occurred. Additional mutations have been incorporated into these bacteria to enhance penetration of substances into the bacteria and decrease the ability of the bacteria to repair DNA damage. These changes make the bacteria very sensitive to mutagenic chemicals. Since many chemicals are not mutagenic or carcinogenic unless they are biotransformed to a toxic product, rat liver microsomes that contain the necessary enzymes for biotransformation are usually added to the medium containing the mutant strain and the test chemical. The number of reverse mutations is then determined by the number of bacterial colonies that grow in a histidine-deficient medium. This type of test is very useful for identifying chemicals that cause point mutations. There are many other short-term mutagenicity tests in use today. Since each test may produce false positives or false negatives, there is a general desire to conduct a battery of different tests and then make an evaluation of the relative mutagenic potential of a chemical based on a composite evaluation of all of the tests.

1.01.4.8 Skin and Eye Irritation Tests

The ability of a chemical to irritate the skin and eye after an acute exposure in the past was usually determined in rabbits. However, for eye irritation and the potential to cause skin allergies, new in vitro tests have been developed that have dramatically decreased the use of animals in such tests.

1.01.4.9 Sensitization Reaction (Allergic) Assays

Information about the potential of a chemical to sensitize skin is needed in addition to irritation testing for all materials that may repeatedly come into contact with the skin. In the past, Guinea pigs were most frequently used to determine the potential of

substances to induce a sensitization reaction in humans (delayed hypersensitivity reaction). Although in vivo testing for skin sensitization is sometimes required, alternative tests, such as the "local lymph node assay," have greatly reduced the number of animals used in testing for dermal sensitization.

1.01.4.10 Other Toxicity Tests

Most of the tests described earlier are included in standard toxicity testing protocols and are often required by the various regulatory agencies. Additional tests may also be included in the chemical evaluation to provide information relating to a special route of exposure (i.e., inhalation) or to a special effect (i.e., behavior). Toxicity tests in animals exposed via inhalation are usually carried out in flow-through chambers rather than in static chambers, to avoid particulate settling and complications from exhaled gases. Special dispersing and analytic methodologies are necessary, depending on whether the agent to be tested is a gas, vapor, or aerosol. Other special types of animal toxicity tests include immunotoxicology and toxicokinetics (absorption, distribution, biotransformation, and excretion), which are increasingly becoming a part of routine toxicological evaluation.

1.01.5 Risk Assessment and Regulatory Toxicology

1.01.5.1 Introduction

Much of what we know about the toxic effects of chemicals in humans comes from workplace exposures, attempted suicides, and/or industrial accidents. However, for a great many chemicals, there is relatively little information obtained directly in humans. Under these circumstances, we are forced to rely upon studies in experimental animals. While responses to toxic chemicals observed in laboratory rats and mice are often similar to that in humans, extrapolating these data to humans is often fraught with uncertainty and unsubstantiated assumptions. This issue is particularly problematic for genotoxic carcinogenic chemicals, which in contrast to the majority of compounds are generally assumed not to require a minimum threshold dose for adverse effects to occur. For these chemicals, a well-described process known as *quantitative risk assessment* is used to set virtually safe dose (VSD) based on levels of risk that are considered low enough to be acceptable. Such assessments have benefited in recent years from a growing emphasis on both the MOA of toxic effects, and technological advances in biologically based modeling.

1.01.5.2 Quantitative Risk Assessment for Chemical Carcinogens

1.01.5.2.1 General considerations

Although virtually all toxicological responses follow a dose–response relationship (i.e., the magnitude of the effect is proportional to dose), it is recognized that for nearly all types of toxic response, there is a threshold dose, below which the probability of someone responding adversely is essentially 0. However, with the discovery in the 1950s that somatic mutations could result in cancer, the single-hit theory of chemical carcinogenesis was developed. This theory holds that if a single mutation in one cell is sufficient to initiate the cancer process, then a single molecule of a chemical capable of causing mutation will have some small but finite probability of causing cancer. In other words, the probability of a mutagenic chemical causing cancer is directly proportional to dose at all doses. There is no threshold dose and thus there is no absolutely safe dose. Although this simplistic logic has since been shown to be false (the process of chemical carcinogenesis is now recognized as a multievent process, requiring several hits at various stages of cancer progression), it still remains ingrained in our regulatory process.

The area of risk estimation that is subject to the most uncertainty, and thus the most controversy, is that of carcinogenic risk assessment. The procedures currently used for estimating the magnitude of human cancer risk from a potential exposure to a potentially carcinogenic chemical are referred to as quantitative risk assessment. Risk from chemical exposure is defined simply as the product of hazard (the innate ability of the chemical to induce a specific toxic effect) times the magnitude of exposure:

$$P = \text{intrinsic toxicity}\left(\text{mg kg}^{-1}\,\text{d}^{-1}\right) \times \text{dose}\left(\text{mg kg}^{-1}\,\text{d}^{-1}\right)$$

where P is the lifetime probability of developing the toxic effect in question; hazard is the potency of a chemical to produce a given adverse effect, usually described in reciprocal dose units of $(\text{mg kg}^{-1}\,\text{d}^{-1})^{-1}$; dose is estimated from the routes of exposure to the chemical (ingestion, inhalation, dermal absorption, etc.) and is expressed in units of milligram of chemical per kilogram of body weight per day.

Hazard is estimated from one of two sources, human epidemiological data, and/or experimental animal tests, and generally represents the slope of the linear dose–response relationship, estimated at low doses. The scientific validity of the risk estimate is, of course, related to the validity of the data, as well as the assumptions that are inherent in the process. Uncertainty is a central issue to the controversy over the value of quantitative risk assessment as a tool for decision making. Utilization of laboratory animal data requires extrapolation from the very high doses administered in animals to the much lower human doses. Extrapolations frequently extend over four orders of magnitude.

1.01.5.2.2 Extrapolation of animal data to humans

Low-dose risk assessments from animal data are further extended to humans. Complicating these extrapolations are differences in lifespan, genetics, body size, routes of metabolism, and rates of exposure. Animal studies nearly always utilize high doses because of