Foreword

WHAT'S THIS ABOUT "VETERINARY TOXICOLOGY"?

"Exciting!" "Challenging!" "Progressive!" "Rewarding!" Certainly not "Dull!" "Routine!" "Old stuff!" "Repetitious!" Just look at the titles of the chapters in this encyclopedic reference, and you'll get turned on to the relevant and cutting-edge science, diagnostics and real-world problem-solving going on in the discipline of "Veterinary Toxicology".

Where did it come from? How did it evolve to get that way? What is its current focus and where is it going? These are all stories worth telling. Let us start at the beginning . . .

In the beginning was folk medicine to deal with sufferings. That evolved into ritualistic "medicine", the use of herbs, plants, and incantations. Studies of how things work and categorizing illnesses organized into formal medicine, and then disciplined further to physiology. With the use and application of natural and synthetic chemicals to challenge body functions, pharmacology grew into its own specialty dealing with using these same compounds for therapeutic purposes. With even more sophistication, recognition of numerous dangerous effects from these products called for a clearer definition and understanding of what separated a "safe" medicinal from one that caused more pain and danger than the illness being treated. The thesis of "dose is everything" was born . . . and with it and its confounding parameters, toxicology was born!

And what a prodigious science that birth produced! Spurred on by the industrial revolution, urbanization, and "Better living through chemistry", the unfortunate ill effects of "too much" became obvious. Instances of chemical misuse, finding chemicals in unexpected locales, and the lack of the knowledge needed to deal with environmental, human, and animal toxic misadventures spurred public outcries. Government actions followed, but were also led by concerned and inquiring scientists. Their recognition that there is strength and opportunities in bonding together to encourage and share the development of this necessary and energetic science flowed into the growth of international organizations fostering and sponsoring the growth of toxicology in all its important ramifications.

As a specialty, veterinary toxicology was an early player in the toxicology arena. At first it was closely related to veterinary clinical medicine and pathology, particularly concerned with animal losses associated with the large scale use of open ranges for livestock grazing and then later the application of various insecticides to control insect-borne diseases in animals. But expanding agricultural needs triggered by the growing US population and scientific advances in chemistry, biochemistry and diagnostic techniques "pushed the envelope" to understand and resolve these losses and improve animal health. If animals were being challenged by these risks, could humans be far behind?

Increased sophistication of human pathology and forensic medicine stimulated similar developments in veterinary medicine. With the ever-increasing expansion of livestock populations on western ranges, concern developed for the death losses from consumption of poisonous plants. Poor grazing conditions and access to often unrecognized toxic range plants stimulated a wide range of investigations by federally employed as well as private veterinarians and resulted in large numbers of publications in the 1920s and the 1930s to inform livestock owners and to reduce losses. With this came the need for clearly identifying what toxic components were present and how they might be effectively treated. Such studies recognized the value of the veterinarian's training and utilized his multifaceted skills in toxicologic studies for clinical evaluations. This was rapidly recognized by industrial institutions and the veterinarian's talents were

sought for application to commercial drug trials in laboratory animals. These veterinary scientists wearing the hat of toxicology began expanding their responsibilities to biochemical separations, electron microscopy and studies of the cellular and molecular mechanisms of these chemical actions.

World War II and the extensive use of insecticides for pest control and speculative gas warfare resulted in veterinarians being employed by the armed forces in other experimental animal studies. After the war, these and newer chemicals were widely applied to problems in agriculture. Their use required skilled veterinary supervision and all too quickly veterinary treatment when the misuse of these potent chemicals occurred. Facilities at universities, such as Texas A&M, and at governmental research institutions, such as the Poisonous Plant Laboratory at Logan, Utah, focused efforts on the growing hazards and clinical problems resulting in domestic animals. The growth of the pesticide field, coupled with the intensive land use encroaching on plant and animal habitat, required increasing chemical and biological knowledge to understand and identify the disease processes involved.

Veterinary pathologists, such as C.C. Morrill, W.L. Sippel, K. McEntee, and P. Olafson, became increasingly interested in the toxic problems now being seen in expanding numbers. J.L. Shupe concerned himself with detail studies of fluorine intoxication associated with industrial pollution problems. Veterinary pharmacologists began to investigate specific toxicants and their effects on domestic animals; W.G. Huber studied toxic effects of chemotherapeutic agents and antiseptics; R.P. Link identified dicumarol as the anti-clotting factor in sweet clover poisoning and spoke out warning against insecticide poisonings; P.B. Hammond investigated heavy metal toxicities with particular interests in utilizing chelating agents to treat lead poisoning; O.H. Muth studied trace minerals and their interactions in animal intoxications; R.D. Radeleff worked extensively with insecticides and their harmful effects in domestic animals; J.S. Palmer worked closely with Radeleff performing similar investigations on herbicide and pesticide toxins; W. Binns and J.W. Dollahite studied the pathology and biochemistry of numerous poisonous plant intoxications in livestock. Information describing the specific pathology and biochemistry produced by the increasingly recognized number of xenobiotics and naturally occurring materials were coupled with the veterinary experiences discovered in diagnosing clinical cases and effectively providing treatment.

By the mid-1950s, toxicology was a highly active area of veterinary medicine. Biochemical and molecular interactions were discussed and the tools of other disciplines were brought to focus upon the problems of domestic animal poisonings. With it, a new breed of veterinarian emerged. The developing veterinary toxicologist had to understand physiology and pathology, but equally important he had to be a chemist with wide knowledge of separative and quantitative instrumental techniques. Professional judgment of clinical episodes and a working knowledge of metabolic and excretory processes were needed. He had to become intimately familiar with pharmacology and the molecular action of a wide variety of chemicals. Understanding treatments to be administered for specific intoxications was necessary, and finally he had to logically and scientifically put into perspective the often confusing and confounding assortment of signs, lesions and analytical results to reach rational interpretations and conclusions for the numerous problems being solved. Since increasing knowledge was being sought, this well-grounded veterinarian had also to be able to conduct significant independent research in well-equipped facilities. More and more veterinarians now were conducting toxicological research investigations as their primary mission.

In the presence of this increasing need and professional situation, a small group of veterinarians specializing in toxicology united to focus attention on the needs of veterinary toxicology and to assist the progress and growth of this discipline. The formation of the American College of Veterinary Toxicologists (ACVT) in 1958 was the beginning of formal development and recognition of veterinary toxicology. At a meeting in Salt Lake City, Utah, on January 15, 1958, the ACVT was formed by 11 veterinarians stimulated by, and engaged in, toxicology. The organizing committee consisted of Doctors Chapman, Christofferson, Furgeson, Harris, Hayden, Holmes, Jones, Phelps, Shupe, Spencer, and Vinsel. The group's objectives were: "To further the educational and scientific progress in veterinary toxicology and to encourage education, training and research in veterinary toxicology; To establish standards of training and experience for . . . specialists in veterinary toxicology; To further recognition of such qualified specialists . . .; To arrange meetings to promote discussion and interchange of ideas in the following fields of veterinary toxicology: teaching, research and development, diagnosis, nomenclature, public health . . .; To provide all possible aid and assistance to its members by the interchange of ideas and scientific information; To review manuscripts . . .; To review published material and keep a file on such reviews . . .; To accumulate and disseminate information in the field of veterinary toxicology . . .; To encourage adoption . . . of uniform clinical and laboratory reporting methods . . .; To suggest or direct basic research in those areas of deficient knowledge . . . " (Constitution, *American College of Veterinary Toxicologists*, Adopted 1958, Salt Lake City, Utah.)

By 1968 the ACVT grew to over 100 Fellows and Associate Fellows. It has worked efficiently and had stimulated national and international recognition of veterinary toxicology as a progressive and dynamic specialty.

This vitality was further stimulated in 1964 by the New York Academy of Sciences publishing a volume devoted to veterinary toxicology based on the proceedings of an international meeting held in New York City (Gabriel, K.L., editor. 1964. Veterinary toxicology. *Annals of the New*

York Academy of Science III, Art. **2**: 559–812). This symposium provided basic information on the energetic activities in veterinary toxicology at that time and had the effect of stimulating further growth and multidisciplinary efforts in the field. The increasing demand for specialized training in veterinary toxicology also encouraged academic training programs. Early efforts were established in universities at Cornell, Utah State, Iowa State, Florida, Kansas State and others. This proliferation has continued with training centers established in other universities and institutions, in veterinary diagnostic laboratories and including research training in molecular and genomic toxicology investigations throughout the United States and around the globe. These early centers and their offsprings have fostered the talents to understand and deal with numerous environmental and clinical problems in veterinary medicine.

Formal recognition of veterinary toxicology was initiated with the American Board of Veterinary Toxicology (ABVT) being formally recognized by the American Veterinary Medical Association (AVMA) in the mid-1960. Largely through the efforts of R.D. Radeleff during his term as president of the ACVT, an application for approval of the specialty was accepted by the AVMA Council on Specialty Organizations. A Certifying Board of W. Binns, J.W. Dollahite and R.D. Radeleff was designated to conduct the first examination leading to Diplomate status in the ABVT. Specific training and experience requirements were established for applicants and approval of each applicant's credentials was necessary before the candidate was admitted to the examination. Satisfactory completion of a comprehensive written examination was the final requirement for certification and the privilege of adding "DABVT" behind the successful candidate's name. The first ABVT certifying examination was held in July 1967 in Dallas, Texas. The five successful candidates joined the three original members of the Certifying Board to form the initial group of certified, i.e. "Boarded" Veterinary Toxicologists (Oehme, F.W. 1970. The development of toxicology as a veterinary discipline in the United States. *Clinical Toxicology* **3**: 211–20).

Since that time, annual certifying examinations of the ABVT have been given associated with the Annual Meeting of the AVMA. This certifying body has continued to set and maintain standards of qualification for veterinary toxicologists, and has complimented the continuing growth of veterinary toxicology experience and knowledge. By 2007 a total of 115 veterinarians have successfully completed the examination challenge and become Diplomates. Their special talents and skills continue to be professionally applied in academia, in industrial roles, as regulatory officials, at poison control centers and within diagnostic laboratories, and in consulting responsibilities throughout the world.

In the years since the discipline's early embryonic period, veterinary toxicology has evolved into a multidisciplinary focus that embraces all of basic and clinical sciences. Its unique focus is not only the diversity of its embracing activities, but also the many talents and energies of its participants. It harbors a true global theme and is proud of its recognition and membership in "the only medical profession licenced for treating more than one animal species".

Veterinary Toxicology: Basic and Clinical Principles is an encyclopedic documentation of the developments in veterinary toxicology the past four decades and glimpses into the promises of exciting future growth. In a logical and well organized fashion, the contributors cover the vast and dynamic field of veterinary toxicology. Of special interest is the initial chapter on "General Principles of Veterinary Toxicology" by R.O. McClellan, one of the initial ABVT certified veterinarians from the 1967 examination in Dallas. The appropriateness of the first contributor to this volume being a 40-year boarded veterinary toxicologist should not be lost to the readers or the general toxicological community.

The initial part highlights the intensity and diversity of the veterinary contributions to toxicology. Pharmacokinetics, testing models, epidemiology and regulatory concerns, backed up by the timely heightened awareness of terrorism and the increasing necessity for legal compliance and actions are well documented.

Any toxicology text would be remiss if it did not focus on individual organ systems and their respective toxicological effects and clinical manifestations. Part 2 moves through each biological system and ends with immunotoxicology and the disastrous effect that various chemicals can have by upsetting this balance of nature.

Of more recent origin are the veterinary efforts of exploring nanoparticles, radiation, and the mechanisms and models of investigative carcinogenesis utilizing various animal species. The veterinary toxicologist is foremost in working with such models and evaluating study results. Of additional current importance are the chapters on over-the-counter drug toxicity and the prevalent potential of various drugs of abuse to affect animal health.

The traditional group of toxic elements are intelligently and dramatically discussed in Part 5, where metals and micronutrients ranging from aluminum through zinc are laid out in all their toxicity. No group of toxic elements is more historically relevant to toxicology than compounds such as arsenic, copper, fluoride, lead, mercury, selenium, and zinc, and when interspersed with some of the minor minerals a complete array of metal and mineral animal intoxications is provided in this part.

The original emphasis for development of veterinary toxicology comes to the forefront in the middle of this volume. The organochlorines and the organophosphates/carbamates are extensively reviewed. Rotenone sneaks in, but the more recent toxic developments with pyrethroids, fipronil, imidacloprid, amitraz, and ivermectin and selamectin are prominently presented. The part on

rodenticides and avicides, as well as the brief part on herbicides and fungicides, highlight the array of agricultural chemicals that have spurred not only the long-term developments in toxicology but also the environmental impact of widespread use of these groups of compounds.

The environmental areas of veterinary toxicology are discussed by reviewing industrial toxicants and the residual impacts of the biphenyls, dioxins and dibenzofurans. The environmental impact of these and other chemicals found in the environment are highlighted by extensive chapters dealing with their toxicity in birds, an introduction to ecotoxicology, and the distribution of chemicals in the global marine environment through aquatic toxicology, and the adverse effects of cyanobacterial toxins and others affecting marine animals.

Although reviewed in only two chapters, the extensive information on botulinum neurotoxin and the enterotoxins are not overlooked. Neither are the poisonous and venomous compounds generated by animals in the terrestrial environment. The chapter on "Caterpillars and mare reproductive loss syndrome" presents up-to-date information on this event's disastrous effect on equine breeding stables and the puzzling origin of these problems. An in-depth discussion on chemically induced estrogenicity brings readers current with this unique toxic hazard in all animal species including humans.

Part 14 is another expansive discussion of the still important poisonous plant concerns that contributed to and continue to stimulate the interests and skills of veterinary toxicologists. The groups of important United States' poisonous plants are reviewed, and then specific categories of plant toxins are presented: cyanide; nitrate/nitrite; oxalates; *Datura* and related plants; fescue; mushrooms; cottonseed toxicity; and the *Taxus* alkaloids. All these are common and highly concerning dietary risks for livestock and other animal species existing in the natural environment.

Fungal toxins are grouped under the "Mycotoxins" part where aflatoxins, trichothecenes, zearalenone, fumonisins, ochratoxins/citrinin, slaframine, ergot, and the interestingly and dynamic tremorgenic mycotoxins are nicely presented. These compounds present not only animal hazards, but are also important public health concerns for the dietary contamination of grains and other human food sources. Other dietary contaminants are reviewed in the part dealing with "Feed and water contaminants". Ionophores and nonprotein nitrogen dietary supplements are highlighted. Not to be overlooked, water quality and contaminants of water sources alert diagnosticians to the hazards and often animal-threatening risks involved with these aqueous contaminants.

The concluding parts in this book of facts and knowledge address how current methodology allows confirmation of specific poisonings and the appropriate means by which poisoned animals may be treated and managed. After reviewing the basic concepts of analyses, appropriate sample submission requirements for such procedures, the use of proteomics for diagnostic application, the application of microscopic analyses of feeds and animal ingesta for toxic components, and the complementing role of pathology in the diagnostic process are presented. To wrap it all up, a concluding part on therapeutic measures offers recommendations on how to prevent poisonings and, if necessary, what treatments may be applied to treat individual intoxications.

In a full circle, the basic principles of veterinary toxicology have been utilized to understand the mechanisms of toxicology, to relate to the numerous and challenging individual chemical constituents that offer risk and produce injury to animals and indirectly to humans, and to offer current information and recommendations for identifying such problems and specifically managing their animal and public health effects.

It should be apparent that Veterinary Toxicology is about everything – from initial concerns of animal illness to specific molecular and genomic impacts in all of society. The veterinary toxicologist is well equipped and active in identifying the opportunities and challenges presented. The discipline stands increasingly ready to contribute to medical science by utilizing its broad talents to have significant impacts for the health of all animals on this globe.

What's Veterinary Toxicology all about? Those answers are what this encyclopedic volume offers! Enjoy them and use the information to the benefit of society and science!

Frederick W. Oehme

Preface

Veterinary toxicology is a very complex, yet fascinating, subject as it deals with a wide variety of poisons of chemical, mineral, plant, fungal, and animal origins. Presently, synthetic compounds constitute the largest class of chemicals that are most frequently encountered in animal poisonings. Veterinary toxicology is greatly complicated by the wide variations in responses of domestic, aquatic, wild, and exotic animal species to toxicants. In the last few decades, veterinary toxicologists have faced the enormous task of dealing with a flood of new farm chemicals and household products. Understanding the complete profile, especially the mechanism of toxicity, of each toxicant is the biggest challenge for today's veterinary toxicologists. At the present time, toxicologists are facing many new problems. For example, during the incident of September 11, 2001, a large number of pets died in the collapse of the World Trade Center in New York City, while the survivors continue to suffer from respiratory illnesses (Ground Zero Illnesses) caused by dust, debris, and toxic chemicals. In 2005, Hurricanes Katrina and Rita, devastated the lives of many animals in the Gulf coast states (Louisiana and Mississippi). Thousands of animals died, while a large number of others suffered from intoxication with high levels of metals, pesticides, mold, and other toxic substances. Recently, a fatal food from Diamond Pet Foods Company has sparked concern as more than 125 dogs died in more than 25 states in the United States. Aflatoxin was proven to be the culprit. From time to time, unusual toxicological problems are encountered on a large scale, and this trend is likely to continue in the future. Around the world, animals and humans are living in a more polluted environment today than ever before. Many of the toxicological problems are global, while others are regional. Unfortunately, antidotes for common poisons are not readily available, resulting in either delayed or no treatment. Thus, veterinary toxicologists have the tremendous task ahead of facing new challenges of the 21st century.

The primary objective of this book, *Veterinary Toxicology: Basic and Clinical Principles*, is to offer a comprehensive text/reference source to research veterinary toxicologists, students, teachers, clinicians, and environmentalists. The volume is organized into 18 parts, with a total of 91 chapters, in order to offer a stand alone chapter on as many topics as possible. Although the book is heavily focused on target organ toxicity (Part 2), it has many novel chapters on timely topics, such as veterinary toxicology and the law, physiologically based pharmacokinetic modeling, *in vivo/in vitro* toxicity testing models, neurotoxic oxidative stress, nanoparticles, radiation, immunotoxicity, reproductive/endocrine/placental toxicity, chemical terrorism, and carcinogenesis. Poisonous plants, mycotoxins, feed, and water contaminants are covered extensively. Several chapters provide the latest information on problems related to industrial, environmental, aquatic, marine, avian, and zoo toxins. A significant part of the book (Part 16) is devoted to diagnostic toxicology, which includes basic principles, method validation and QA/QC, sample submission, current diagnostic criteria, toxicoproteomics, pathology, and microscopic analysis of feed. Finally, the last part of the book emphasizes prevention and therapeutic measures of common poisonings.

In the past few years, veterinary toxicologists from many parts of the world have realized the need for a standard book that can provide a detailed coverage of the basic and clinical principles of veterinary toxicology. This book addresses global as well as regional toxicological problems, and offers practical solutions. A stand alone chapter is provided on every major topic, with major references for further reading. This book represents the collective wisdom of more than 75 authors, and offers a unique text/reference source for those involved in

veterinary medicine in general and toxicology in particular. Contributing authors for chapters of this book are the most qualified and well-experienced authorities in their respective areas of veterinary toxicology.

The editor is deeply indebted to all the authors for their sincere and dedicated contributions. Technical assistance of Joan Jenkins, Debra Britton and Robin Doss is immensely appreciated. The editor and the authors would like to thank Renske Van Dijk, Keri Witman, Mara Conner, and Tari Broderick, the editors at Academic Press/Elsevier, for their vital input in preparation of this book.

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CHAPTER

1

Concepts in veterinary toxicology

Roger O. McClellan

INTRODUCTION

Toxicology, from the Greek words toxicon for poison and logos for scientific study, is the study of poisons. Veterinary medicine is that branch of medical science concerned with the diagnosis, treatment and prevention of diseases of animals. The adjective veterinary is derived from Latin – veterinae, beasts of burden. Obviously, the modern field of veterinary medicine extends beyond the "beasts of burden" to include all the domesticated animal species, both livestock and companion animals, as well as non-domesticated species. Indeed, it has expanded to include non-mammalian species. While the focus of toxicology remains on chemicals, it is generally acknowledged that the study of effects of ionizing radiation is a part of the field or at least a closely related specialty. Pharmacology, from the Greek words pharma for drugs and logos for scientific study, is a closely related field concerned with the science of drugs: their preparation, properties, effects and uses in the diagnosis, treatment and prevention of disease.

The field of toxicology is very broad including the identification and characterization of poisons, their physical and chemical properties, their fate in the body and their biological effects. In addition, toxicology is concerned with the treatment of disease conditions caused by poisons. The terms toxicant and poison are used interchangeably. A toxicant is a material that when it contacts or enters the body via ingestion, inhalation, dermal contact or injection, interferes with the normal biological processes and causes adverse health effects. The term toxin is used to describe poisons originating from biological processes. The term toxic is used to describe the effects of a poison on biological systems. Toxicosis is the term used to describe the syndrome of adverse health effects that result from exposure to a toxicant. During the last several decades, increased concern has developed for the effects of long-term low-level exposures to toxicants. With these exposures, adverse health effects, if they occur, may be manifest in a non-specific manner as an increase in the incidence of common diseases in a population.

A wide range of materials produces toxic effects when exposure occurs at sufficiently high levels. Indeed, with extreme levels of exposure most agents can produce adverse effects. For example, while both water and oxygen are required to sustain life, they are toxic when the level of intake is excessive. The nature of the toxic responses depends not only on the toxicant, but also the route of exposure, the duration and intensity of the exposure and the characteristics of the exposed individual, i.e. species, gender, age, pre-existing disease states, nutritional status and prior exposure to the agent or related compounds. The exposure may be brief or prolonged. The response may occur acute or chronic and occur soon after exposure or much later and only after prolonged exposure. The response may be relatively unique to the toxicant, i.e. a specific toxicosis, or distinguishable from common diseases caused by natural processes or exposure to other agents. In many cases, sophisticated statistical methods are required to associate some excess health risk, over and above that caused by other factors, with a particular toxicant exposure. This is especially true today after much progress has been made in controlling exposure to toxic materials.

In this chapter, I first provide a brief historical perspective on the development of veterinary toxicology as a subspecialty of the veterinary medical profession and as a specialized area within the general field of toxicology. This

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is followed by a section on the evolution of veterinary toxicology from an observation-based profession and science to one that places increasing reliance on science developed through experimentation. This includes a discussion of the risk paradigm which has become an integral part of toxicology in recent decades. In the next section, I offer several related paradigms for acquiring, organizing and using knowledge in veterinary toxicology so as to maximize its potential impact. Next, there is a section on the sources of knowledge that may be obtained either through observation or experimentation. These sources may include studies on the species of interest, i.e. people or some other specific animal species, controlled exposure studies in the species of interest, studies in other species, investigations using tissues and cells and structure–activity analyses. This is followed by a section discussing the design of experimental studies to optimize the interpretation and use of the results. This chapter concludes with a discussion of key toxicological descriptors and a brief conclusion section.

HISTORICAL PERSPECTIVE

Historical events

The father of modern toxicology is generally acknowledged to be Auroleus Phillipus Theostratus Bombastus Von Hohenheim (1493–1541), who referred to himself as a Paracelsus, from his belief that his work was beyond the work of Celsus, a first century Roman physician (Pagel, 1958). Paracelsus is credited with the well-known statement: "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy." Paracelsus advanced many views that were revolutionary for his time that are now accepted as fundamental concepts for the field of toxicology. In contrast to earlier emphasis on mixtures, he focused on the toxicon as a specific primary chemical entity that was toxic. Paracelsus advanced four fundamental concepts:

1 Experimentation is required for examining responses to chemicals.
2 A distinction should be made between the therapeutic and toxic properties of chemicals.
3 The therapeutic and toxic properties are something closely related and distinguishable by dose.
4 It is possible to ascertain a degree of specificity for chemicals and their therapeutic or toxic effects.

It is obvious from the foregoing that toxicology and pharmacology are closely related fields of scientific endeavor. Pharmacology is focused on drugs, including both their effectiveness and safety. Toxicology is concerned with all kinds of chemicals and other agents that may, at some level of exposure, cause adverse health effects. As will be noted at several places in this chapter, toxicology is increasingly concerned with low-level exposures for which the effects, if any are observed, may not be specific to a particular chemical.

Toxicology, in a sense, dates back to the earliest activities of humans. By observation, people came to learn that which could be ingested without harm and, by contrast, the foodstuffs to be avoided because of their harmful properties. They also came to know which animal venoms, plant extracts and other materials could be used for hunting, warfare and assassination. No doubt as animals were domesticated, it became apparent that the human observations and practices could be extended to domestic animals. Unfortunately, domestic animals are not always as astute as people in learning to avoid poisonous plants and other harmful situations. Thus, veterinary practitioners still encounter toxicoses involving animals ingesting poisonous plants.

The history of toxicology has been well documented by several contemporary authors (Milles, 1999; Borzelleca, 2001; Gallo, 2001). The history of veterinary toxicology has not been as well documented, although it is apparent that veterinary toxicology has been an integral part of veterinary medicine since the origins. Veterinary medicine is a specialized branch of medical science with formal programs of study leading to a professional degree. The history of veterinary medicine has been reviewed by several authors (Smithcors, 1957; Stahlheim, 1994; Swabe, 1999; Wilkinson, 2005). The role of veterinary toxicology in the veterinary curriculum is well documented for one of the earliest veterinary medical colleges, that at the Free University of Berlin. Wilsdorf and Graf (1998) provide an account of the development of veterinary toxicology at that University from 1790 to 1945. Oehme (1970) has briefly reviewed the development of veterinary toxicology as a discipline in the United States.

Textbooks

In the English language, the earliest veterinary toxicology publication I could find was a synopsis of *Veterinary Materia Medica, Therapeutics and Toxicology* (Quitman, 1905) apparently used at Washington State University College of Veterinary Medicine in the early part of the 20th century. I am uncertain of the extent to which this synopsis is based on a French text by Kaufmann (1901). The earliest English language veterinary toxicology textbook I was able to locate was that authored by an Englishman, Lander (1912). This book was also prepared in a second edition (1926) and a third edition was prepared by an Irishman, Nicholson (1945). I am uncertain how widely it was used in the United States. The text included four sections: a brief introduction to toxicology followed by sections on

classes of toxicants; mineral or inorganic poisons; organic poisons and drugs; and poisonous plants. This last section represented half of the book.

Many early students in veterinary medicine in the United States used textbooks prepared for physicians such as Kobert (1897), *Practical Toxicology for Physicians and Students*. It was also common to use either textbooks in pharmacology or veterinary pharmacology that contained a brief coverage of toxicology. Indeed, few veterinary medical colleges prior to the 1950s had full-time veterinary toxicologists on their faculty. Lectures on toxicology were usually included in courses in pharmacology, pathology and clinical medicine.

The first veterinary toxicology text I personally used was authored by Garner (1957) who was then a Senior Lecturer in Chemical Pathology (Veterinary) at the University of Bristol in the United Kingdom and later Head of the Radiobiology Department at the Agricultural Research Council Field Station, Compton, Berks, UK. The text by Garner (1957) was intended as a successor to the third edition of Lander's *Veterinary Toxicology*. A second edition was prepared by Garner (1961) after he became Head of the Public Health Section, Radiological Protection Division, UK Atomic Energy Authority, Harwell, Berks, UK. Later, Garner came to the United States where he was initially associated with Colorado State University directing studies of the long-term effects of radiation on beagle dogs. I recall asking Garner in the early 1970s about the possibility of preparation of a third edition of his *Veterinary Toxicology* text. He indicated that the field of veterinary toxicology had become so broad that it was not readily feasible for a single individual to author a text in veterinary toxicology and he was not interested in "shepherding" a herd of individual chapter authors with specialized knowledge of various aspects of veterinary toxicology.

Radeleff (1964) authored one of the first veterinary toxicology texts published in the United States. A second edition appeared in 1970. This was followed by a text prepared by Osweiler *et al.* (1985). Several books published in the 1960s became classics on the effects of poisonous plants (Kingsbury, 1954, 1964; Hulbert and Oehme, 1968). Recent books on poisonous plants have been authored by Garland and Barr (1998), Burrows and Tyrl (2001, 2006) and Knight and Walter (2001). Murphy (1996) has authored a field guide to common animal poison. It is organized by the organ system affected and then by toxicant.

Osweiler (1996) has authored a text focused on toxicology as part of the National Veterinary Medical Series for Independent Study. It has been widely used by individuals preparing for the National Board Examinations for Veterinary Medical Licensing. Roder (2001) has prepared a text, *Veterinary Toxicology*, as part of a series *The Practical Veterinarian*. Plumlee (2004) has edited *Clinical Veterinary Toxicology* and Peterson and Talcott (2001, 2006) have edited two editions of *Small Animal Toxicology*. The present multi-authored text promises to be the most comprehensive text on veterinary toxicology published to date. A *Veterinary Toxicology* text edited by Chapman (2007) is in preparation.

There are a number of comprehensive general toxicology texts available today. I will note four that the serious student of toxicology will find useful to have in their reference library. *Casarett and Doull's Toxicology: The Basic Science of Poisons* edited most recently by Klaassen (2001) was first published in 1975 and is now in its sixth edition. Hayes (2001), *Principles and Methods in Toxicology*, is now in its fourth edition. *Toxicology*, edited by Marquadt *et al.* (1999), built on an earlier German text by Marquadt and Schafer. *Biological Concepts and Techniques in Toxicology: An Integrated Approach* edited by Riviere (2006) was just released. Serious students will also want to be aware of a 13 volume comprehensive set of toxicology text edited by Sipes *et al.* (1997). Moreover, there are numerous text and reference books available now covering various sub-specialty areas such as *Inhalation Toxicology*, *Reproductive and Developmental Toxicology* and *Dermal Toxicology*.

In addition to text and reference books, there are numerous journals published in the field of toxicology that regularly contain articles that relate recent findings in veterinary toxicology. Many clinically oriented veterinary medical journals contain articles on veterinary toxicology. The on-line search capabilities serving the medical sciences including toxicology and veterinary toxicology are expanding at an exponential rate. Of special note are those maintained under the auspices of the National Library of Medicine, MEDLINE and TOXLINE.

Organizations

A number of professional scientific organizations have been created as the field of toxicology, including veterinary toxicology, has matured. The most noteworthy include the American College of Veterinary Toxicology (ACVT), American Board of Veterinary Toxicology (ABVT), Society of Toxicology (SOT), American Board of Toxicology (ABT) and Academy of Toxicological Sciences (ATS). The ACVT was one of the earliest scientific societies in the field being founded in 1958. It now exists as the American Academy of Veterinary and Comparative Toxicology (AAVCT). The ACVT was instrumental in fostering the creation of the ABVT and its recognition by the American Veterinary Medical Association (AVMA) as the approved certifying specialty organization for veterinary toxicology. Three well-known veterinary toxicologists, W. Binns, J.W. Dollahite and R. Radeleff, were accepted by the AVMA as Charter Members of the ABVT. They prepared the first certifying ABVT examination which was given in 1967 (see www.abvt. org). I was pleased to be one of seven

individuals in the first class certified, based on examination, as Diplomates of the ABVT.

The SOT, with the world's largest membership of toxicologists, was organized in 1961 (see www.sot.org). Many of the organizers of the SOT were members of the American Society for Pharmacology and Experimental Therapeutics (ASPET) who felt toxicologists needed a "home" of their own. I recall attending an organizational meeting of the SOT held in conjunction with an ASPET meeting at the University of Rochester and the excitement and enthusiasm of the attendees for creating the SOT. As an aside, it would be a few years before I felt my credentials were sufficient that I could apply for membership in the SOT. The SOT fostered the creation of the ABT which held its first certifying examination in 1980 (see www.abtox.org). I was pleased to be one of the first class of individuals certified, based on examination, as Diplomates of the ABT. The SOT includes a number of specialty sections including the Comparative and Veterinary Specialty Section.

A third certifying entity, the ATS, which accepts individuals as Fellows based on a review of credentials, was created in 1981 (see www.acadtoxsci.org). Many veterinary toxicologists belong to all of the organizations noted above and some have been certified by one or more of the certifying organizations: the ABVT, ABT and ATS. Veterinary toxicology has evolved greatly over the past several decades.

EVOLUTION OF VETERINARY TOXICOLOGY

Roots in veterinary medicine and toxicology

The evolution of veterinary toxicology occurred concurrently with evolution of its two roots: the profession of veterinary medicine and the science of toxicology. The veterinary medicine profession was initially focused on domestic animals, particularly those used for food, fiber, transportation and to provide power for agricultural endeavors and transportation. With the growth of more specialized agriculture and production practices, the profession with its linkage to domestic livestock stimulated growth of the profession. Veterinary toxicology focused on poisonous plants and then on antidotes for various toxins. The early part of the 20th century presented a special challenge for veterinary medicine as the use of horses and mules in agriculture decreased in favor of the use of equipment powered by internal combustion engines. During this period of time, there must have been considerable uncertainty as to the future of the profession.

By the mid-20th century three movements transformed veterinary medicine. The first related to the traditional roots of the profession in animal agriculture and related to the increasing emphasis given to large-scale highly specialized livestock endeavors. The second related to the increased attention given to providing veterinary medical services to a growing population of companion animals. In both areas the science of veterinary medicine was strengthened as observation-based medical practice was complemented and, ultimately, supplemented by science-based medicine. During this period, veterinary toxicologists began to play an important role in veterinary medical diagnostic laboratories, both in veterinary medical colleges and in state and federal agencies. With the strengthening of the science base of veterinary medicine, including the quality of the science in the veterinary medical curriculum, the third movement, the emergency of the comparative medicine character of veterinary medicine, became more apparent and was enhanced (Wilkinson, 2005). These changes in the profession were accompanied by increased involvement of veterinarians in research on the species of traditional concern to the profession, domestic and companion animals (Stahlheim, 1994), and to participation in a broader range of biomedical research activities, involving use of the traditional laboratory animal species, driven largely by concern for human health (Wilkinson, 2005).

Emergence of science-based toxicology

Toxicology, like veterinary medicine, was also rapidly changing and evolving in the mid-20th century. The previous strong emphasis on field observations was first complemented and then supplemented by experimentation. This led to the current strong mechanistic orientation of toxicology. With this shift in toxicology came an increased awareness of the utility of a comparative medicine orientation in research directed primarily toward improving human health (Wilkinson, 2005). With this comparative medicine orientation came increased opportunities for individuals educated in veterinary medicine, including veterinary toxicology, to contribute to general toxicology and biomedical science.

These changes in the veterinary medical profession and the emergence of toxicology as a science came during a period when the public was giving increased attention to the health risks, and its counterpoint safety, of new technologies and products. A landmark of the era was publication of Rachel Carson's book, *Silent Spring* (Carson, 1962). She focused on both human health impacts and impacts on the total ecosystem of which people were just a part. Her book was certainly one of the key stimuli to a tidal wave of legislative actions in the United States that focused broadly on the environment with concern for clean air and water; safe food, pharmaceuticals, pesticides, fungicides, rodenticides and consumer products; and a safe working environment.

The legislative actions and related administrative actions in the 1970s created the US Environmental Protection

Agency (USEPA), the Consumer Product Safety Commission, the National Institute of Occupational Safety and Health (NIOSH), the National Center for Toxicological Research, the National Institute of Environmental Health Sciences and the Cancer Bioassay Program within the National Cancer Institute, which evolved into the National Toxicology Program (NTP) now administered by the National Institute for Environmental Health Sciences. This was also a period of rapid expansion of research activities in the pharmaceutical food, chemical and petroleum industries. The chemical industry in 1976 started the not-for-profit Chemical Industry Institute of Toxicology, which now exists as the CIIT Center for Health Sciences, to test commodity chemicals, investigate the mechanisms of chemical toxicity and train additional toxicologists. The Food and Drug Administration (FDA) continued its traditional dual emphasis of ensuring both the efficacy and the safety of drugs and medical devices continued. Increased emphasis was given by the FDA to veterinary drugs and to the potential for veterinary drugs to contaminate meat and milk.

Increasing public concern for safety/risk and the resulting legislation led to the development of increasingly formalized approaches to both safety and risk analysis. This included more clearly defined roles for using the results of toxicological studies, including studies with laboratory animals, to assess the safety, or conversely risk, to humans of the use of pharmaceuticals, other products in commerce, and technologies.

Toxicology joined to the risk paradigm

As noted earlier, federal legislation passed in the 1970s focused on the health impacts of environmental and occupational exposures and led to more formalized approaches to evaluating the risks and safety of various exposures. The risk paradigm built on the long-standing paradigm linking sources to exposure to dose to adverse health outcomes that had guided toxicology from its earliest days (Figure 1.1). I have reviewed elsewhere the development of the risk analysis paradigm (McClellan, 1999). The risk analysis paradigm originally proposed by the National Research Council (NRC, 1983) and used by the USEPA is shown in Figure 1.2. A later report *Science and Judgment in Risk Assessment* (McClellan, 1994; NRC, 1994) and reports from the Risk Commission (1997) re-affirmed use of the risk paradigm which continues to be a cornerstone of activities not just at EPA but in other national and international agencies and in the private sector.

The original key elements of the risk paradigm were (1) hazard identification, (2) exposure–response assessment, (3) exposure assessment and (4) risk assessment. The NRC (1994) report emphasized the importance of a fifth element – using the results of the risk analysis to guide future research and, thus, reduce uncertainty in future risk estimates. In addition, I have added a sixth over-arching element - risk communication. The hazard identification element has been a source of contention and confusion both with the public and in the scientific community, especially with regard to cancer as I will discuss later.

Hazard is defined as the potential for an agent under some conditions of exposure to cause an adverse effect (NRC, 1983, 1994; McClellan, 1999). With this definition the level of exposure or dose required to produce an adverse health effect is not considered. An agent may be classified as a hazard irrespective of whether or not the exposure conditions required to elicit adverse effects are relevant to human situations. The exposure–response assessment involves characterization of this relationship as it may pertain to likely levels of human exposure. The exposure assessment quantifies, either retrospectively or prospectively, the likely duration and intensity of human

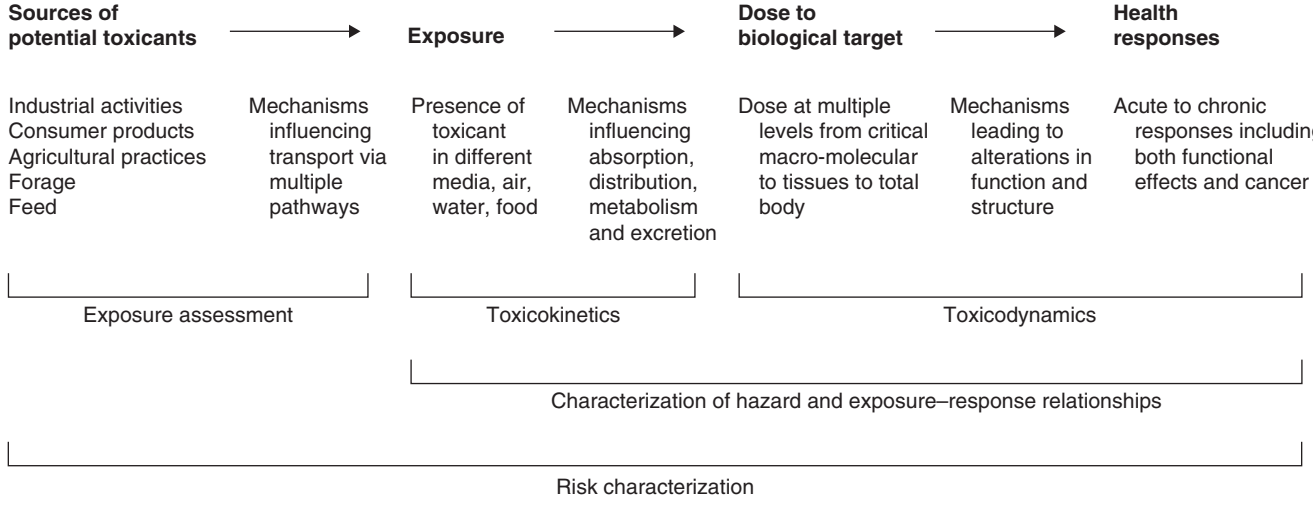


FIGURE 1.1 Critical linkages for integrating information from sources of toxicants to the development of adverse health effects.

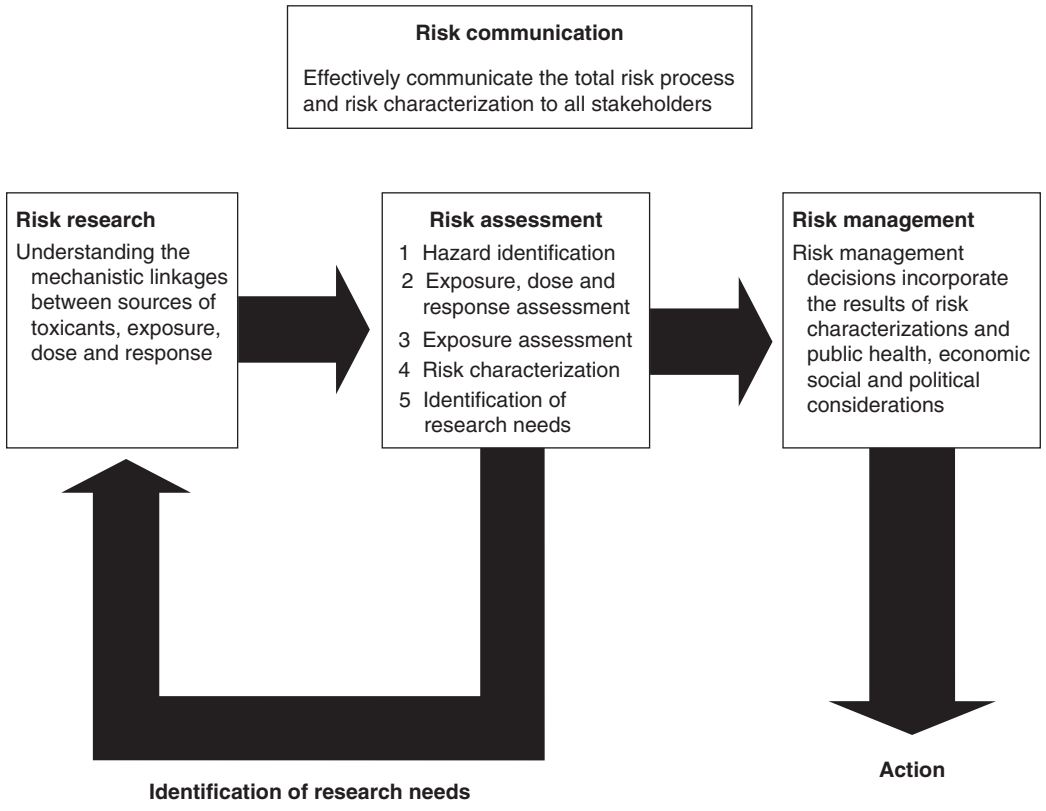


FIGURE 1.2 Risk paradigm for evaluating potential health impacts of a toxicant.

exposure to the hazardous agent. The risk assessment element brings together information from the other three elements to characterize risk as illustrated in Figure 1.1. Risk is defined as the probability of occurrence of an adverse health effect from exposure to a hazardous agent at a specified duration and intensity of exposure. As an aside, especially in Europe, the word hazard is used as risk has been defined in the United States. Safety is defined as being a condition with a high probability of freedom from any increase in adverse health outcome when the agent is used in a specified manner. Obviously, both safety and risk are relative recognizing that it is not possible to ensure absolute freedom from some small level of risk.

The more formalized risk analysis approaches developed starting in the 1970s built on approaches developed earlier for providing guidance for controlling occupational exposures, the intake of contaminants in food and the safety of pharmaceutical agents. Pre-World War II, the primary focus was on adverse health outcomes that caused functional impairment such as decreased respiratory function. As will be discussed later, the issue of carcinogenic responses received limited attention before World War II. The approach to developing guidance for the control of toxicants was based on the assumption that a threshold exists in the exposure (dose)–response relationship – just as discussed by Paracelsus. The threshold exposure–response

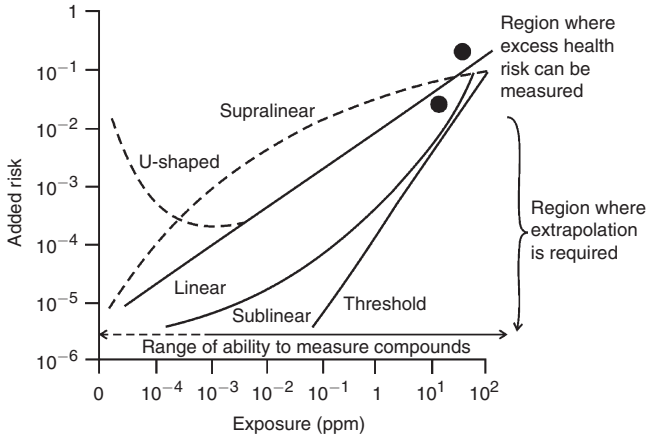


FIGURE 1.3 Schematic rendering of exposure–response relationships for various toxicants.

relationship is shown in Figure 1.3 along with four other relationships: sub-linear, linear, supralinear and a U-shaped or hormetic function. Note that both scales in this schematic rendering are logarithmic.

Technically, in hormesis there is a beneficial effect at some low level of exposure which decreases with increasing exposure/dose and at yet higher levels adverse effects become apparent. During the last decade, there has been

increased discussion of the concept of hormesis in which very low-level exposures have positive effects with negative effects observed only at higher exposure levels (Calabrese and Baldwin, 2003; Calabrese and Blain, 2005). The concept of hormesis is well known to veterinarians who are aware that certain agents, such as vitamins and minerals, are essential for life at low concentrations and can produce toxicity with excess intake.

As an aside, there has been on-going debate for decades as to whether linear exposure–response relationships, especially for cancer, are realistic, i.e. an added level of exposure, regardless of how small, results in a calculable monotonic increase in cancer risk. It has been argued by some that the linear exposure–response model is appropriate for regulatory purposes for assessing cancer risks because every dose of a new agent is added to a background of genetic damage in somatic cells arising from multiple agents and endogenous factors.

The early development of threshold limit values (TLVs) for control of occupational exposures by the American Conference of Governmental Industrial Hygienists (ACGIH), organized in 1938, assumed the existence of thresholds in exposure–response relationships. The initial data were provided primarily by opportunistic studies of exposed human populations. In the absence of human data, data from controlled exposure studies in laboratory animals were used. This necessitated the use of safety factors to account for inter-individual variability, inter-species extrapolation and duration of the study as will be discussed later. The original safety factors were formally proposed by Lehman and Fitzhugh (1954) of the FDA. Later, the USEPA was organized and began using the same factors. However, the EPA identifies them as uncertainty factors apparently out of a desire to avoid use of the potentially contentious word – safety.

Post-World War II increased public concern developed for the occurrence of cancer. This was stimulated by multiple factors. Extensive research conducted during and after the war on the effects of both external ionizing radiation and internally deposited radionuclides emphasized the importance of cancer as a radiation-induced disease. Concern for radiation-induced cancer was further heightened when the intensive follow-up of Japanese A-bomb survivors revealed an increase, first in hematopoietic neoplasms, and, later in solid cancers. These findings soon led to abandoning a threshold approach to evaluating radiation risks in favor of using a probabilistic approach to assess the health risks of using radiation devices in space and nuclear power. The probabilistic approach using the linear exposure–response model discussed earlier was convenient to use because it could be readily applied to assessing the risks to individuals or populations. My first experience with probabilistic risk assessment came in the mid-1960s when I was on a temporary assignment with what was then the US Atomic Energy Commission (AEC). I worked with a joint AEC–National Aeronautical and Space Administration assessing potential human cancer risks of accidents involved with the launch of spacecraft containing plutonium-238 fueled thermal electric power systems.

Another factor influencing public concern was the increasing incidence of total cancers being observed in all of the economically developed countries including the US driven largely by lung cancer. It is now well known that the increase in lung cancer, first observed in men and then in women, was largely related to cigarette smoking. Rachel Carson's book also helped to create concern for exposure to man-made chemicals contributing to the increasing incidence of cancer. It is now known that this is not the case (Gold *et al.*, 2003).

The experience with radiation soon resulted in its use as a proto-typical carcinogen in developing approaches to risk analysis and risk regulation. Albert (1994) documented the development of the USEPA's approach to assessing cancer risks. Key assumptions in the approach were (a) cancer-causing chemical agents acted like radiation in causing cancer; (b) there was a linear relationship between exposure (dose) and increased risk of cancer extending to the lowest levels of exposure; (c) agents causing cancer in laboratory animals could be viewed as also causing cancer in people and (d) exposure–response relationships could be extrapolated between species by considering differences, body weight and surface area, i.e. metabolic activity. These assumptions were viewed as default options to be used in the absence of specific scientific data to the contrary (McClellan, 1994, 1999, 2003; NRC, 1994).

In response to public concern for chemicals causing cancer, the International Agency for Research on Cancer (IARC) became the first organization to propose a scheme for classifying agents as to their carcinogenic potential (IARC, 1972). The view was that if cancer-causing chemicals or other agents, such as radiation, or workplace conditions involving exposure to chemicals or other agents could be identified, then these could be controlled and the occurrence of cancer in people reduced. The IARC carcinogen classification scheme considers human, laboratory animal and supporting data to classify agents or workplace conditions as (1) carcinogenic to humans, (2) probably carcinogenic to humans, (3) possibly carcinogenic to humans, (4) not likely to be carcinogenic to humans or (5) not classified as to carcinogenicity. The IARC classification is strictly hazard oriented, it does not formally evaluate the potency of these agents for causing cancer at a specific level of exposure. The USEPA, the NTP and other organizations have developed similar carcinogen classification schemes (EPA, 1986, 2005a, b; NTP, 2005). In recent years, IARC (1991) has made provision for increased use of mechanistic data in classifying chemicals as human carcinogens. Both the EPA and NTP now also give increased emphasis to the use of mechanistic data in classifying chemicals as carcinogens

(EPA, 2005a, b) unlike IARC and the NTP, the EPA does develop estimates of cancer-causing potency for some agents classified as having cancer-causing potential. This in turn, using measurements or estimates of exposure, provides the basis for calculating lifetime cancer risks for individuals or populations.

It should be apparent that the cancer classification of a given agent is insufficient for characterizing cancer risk since the hazard-based classification does not include an estimate of the agent's potency. The USEPA has estimated the carcinogenic potency for a number of chemicals. The results are usually related as the concentration of a chemical in water or air that will result in a calculated one in a million probability of cancer occurring above the background incidence (EPA/IRIS, 2006). To estimate the cancer risk for any agent and exposure situation, it is also necessary to estimate the exposure to the agent, both as to intensity and duration. In short, risk is a product of exposure and the potency of the agent for causing the effect.

There has been a tendency for regulatory agencies, such as the USEPA, to use their experience with classifying chemicals as to their carcinogenic potential as a template for also classifying chemicals as to their potential for producing other non-cancer hazards. Thus, there has been a trend toward classifying chemicals as to their potential hazard for causing different health outcomes and labeling them as such, i.e. neurotoxins, reproductive toxins, hepatic toxins, etc. Indeed, some even broader classifications have emerged, i.e. endocrine disrupting chemicals. In my view, this short-hand approach to identifying and classifying hazardous agents as to their potential to cause cancer or other effects is confusing to the public. In my view, the labeling approach has contributed to both radiation reactions and chemical phobia and sometimes irrational actions. It certainly flies in the face of the fact that for many chemicals the admonishment of Paracelsus that "the dose makes the poison" remains true for many chemicals. For many chemicals, even when toxic effects are apparent at high doses, these same adverse effects are no longer manifest at sufficiently low doses. Gold *et al.* (2003) have discussed the challenge of using high exposure (dose) animal studies to identify either man-made or natural chemicals as human carcinogens.

A FRAMEWORK FOR ACQUIRING INFORMATION

Linkages from sources to health impacts

The purpose of this section is to provide a conceptual framework for using information to evaluate specific cases of actual or alleged toxicosis and to facilitate the acquisition of new knowledge that will have impact in understanding potential toxic effects. Earlier, in Figure 1.1, a conceptual framework was provided for evaluating the linkages extending from a source of a toxic material to manifestation of an adverse health outcome in an individual or a population. The conceptual framework is equally applicable to humans or other animal species.

The source to exposure linkage has been expanded in Figure 1.4 (Paustenbach, 2001). In this example, an industrial plant is illustrated as the source. The figure serves to illustrate the complex nature of the exposure pathways that may be encountered including the role of livestock. The focus in the figure is on the multiple pathways by which a potential toxicant may reach a human population: inhalation, drinking water, dermal absorption, ingestion of soil, and ingestion of a variety of foodstuffs including milk and meat from domestic animals. All of these pathways might also serve to expose the cow in the figure to the toxicant. The quantities of the toxicant taken in by the cow could cause toxicity in a herd of cows. Equally as important is the role of the cow as a pathway for the toxicant to reach people. For example, the figure illustrates that a toxicant could be present in cow's milk and the milk could be consumed by people. The cow could also be slaughtered and the meat

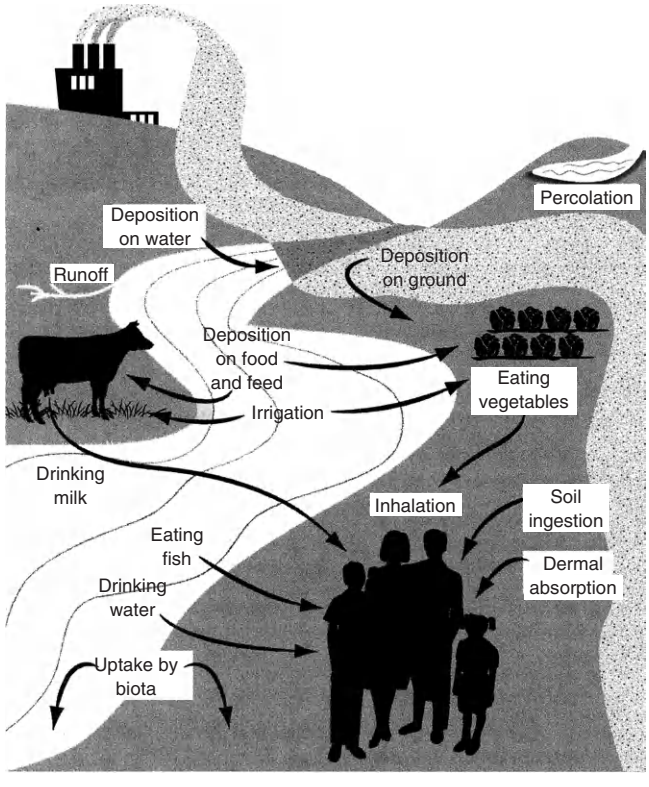


FIGURE 1.4 Schematic rendering illustrating exposure pathways extending from a source of toxicants to exposure of livestock and people (from Paustenbach, 2001).

ultimately consumed by people. Thus, it is important to recognize that the cow, or any other food animal species, can both manifest toxic effects and serve as a pathway for toxicants to reach people via the food supply.

It is readily apparent that the schematic rendering shown in Figure 1.4 can be expanded or contracted. In natural ecosystems, multiple species might be involved as a toxicant moves from a source or multiple sources via various pathways. In some cases, various species in the ecosystem may be impacted as individuals. Moreover, natural populations may be impacted. In addition, these pathways may ultimately result in the toxicant reaching people. An example is mercury in fish. In practice, veterinarians may encounter situations where poisonous plants in the pasture or in harvested forage may be the source of the toxicant. Feed may be contaminated at a mill and serve as the pathway by which a toxicant reaches the livestock. In other cases, the potential human toxicant may be a pharmaceutical purposefully given to the cow.

Toxicokinetics

The simple schematic rendering shown in Figure 1.1 can be used to illustrate several important concepts. First, it is important to recognize that contrary to common usage, exposure and dose are not the same. The exposure environment is characterized by the concentration of the toxicant in the media, be it water, air or feed, the quantities taken in and the time course of the intake. Dose is the concentration, over time, of the toxicant in the various tissues of the subject, whether it be a cow, a human or a laboratory rat. The characterization of the kinetics linking exposure with dose is referred to as toxicokinetics (for a toxic agent) or pharmacokinetics (for a pharmaceutical). In actual practice, the term pharmacokinetics is frequently used when it would be more appropriate to use the term – toxicokinetics. Several chapters in this book deal specifically with kinetics of toxicants and pharmaceuticals.

Toxicokinetics (see Figure 1.1) are used to describe the movement and disposition of the toxicant in the organism. This includes consideration of the route of entry: ingestion, inhalation, dermal or purposeful administration by injection. A complete description of the toxicokinetics of a toxicant will take into account (a) the intensity and duration of the exposure, (b) the rate and amount of absorption of the toxicant from the site of entry, (c) the distribution of the toxicant within the body, (d) potential biotransformation to less, equal or more toxic form and (e) the rate of excretion by route (urine, feces or exhalation). All of these aspects of toxicokinetics may be influenced by species differences in physiological and biochemical characteristics. Modern approaches to modeling toxicokinetics attempt to take account of both species differences and similarities in influencing the fate in the body of toxicants. It is also important to recognize that the exposure or dose level may influence the kinetics of a toxicant and its metabolite(s). This is an especially important consideration in extrapolating from laboratory studies that may be conducted at high doses to lower more environmentally relevant exposures/doses.

Toxicodynamics

The linkage between dose and adverse health outcome shown in Figure 1.1 involves multiple mechanisms as various toxicants may potentially impact all the cells and organ systems of the body. Increasingly, scientists have attempted to model these relationships which, in parallel to the nomenclature for the kinetic phase, are called toxicodynamic or pharmacodynamic models. It is obvious that multiple pathways may be involved in a toxicant producing disease and that knowledge of the individual steps will increase as knowledge of basic biological mechanisms increases. For example, the explosion of knowledge of basic biology at the level of the genome (genomics), proteins (proteomics) and metabolism (metabolomics) has provided a basis for exploring the mechanistic basis of toxicant-induced disease with a degree of refinement that could not even be envisioned even a short time ago.

A later chapter reviews the basic mechanisms of toxicity. In addition, many of the chapters on organ toxicity and specific toxicants contain detailed information on mechanisms of toxicity. As the reader reviews this material, and especially the detailed discussion of biochemical mechanisms of action, it will be important to place those in the context of processes at the cellular and tissue level; i.e. inflammation, cell death, cell proliferation, hypertrophy, hyperplasia, metaplasia and neoplasia. A strength of the veterinary medical curriculum, as with the human medical curriculum, is the emphasis given to understanding both normal and disease processes extending from the molecular level to cells to tissues to organs and, ultimately, to the integrated mammalian organism. A special opportunity exists for medically trained personnel, both veterinarians and physicians, to put the expanding knowledge of molecular and cell processes into the context of overt disease. After years of emphasis on a reductionist approach to basic biomedical science, it has become recognized that this approach needs to be complemented by an integrative approach. This has recently been termed systems biology. In my view, this is not really a new concept. It is more a rediscovery and refinement of the concepts of integrated biology and pathobiology used in veterinary medicine for decades.

There has been great enthusiasm for the use of mechanistic information in safety/risk evaluations as will be discussed later. Recognition of the difficulty of characterizing of all the individual mechanistic steps has given rise to a new term – mode of action. The mode of action has

been defined as the dominant step(s) involved in producing a given toxic endpoint. An example is the role of cell killing as the mode of action for large intakes of chloroform (Butterworth *et al.*, 1995) or formaldehyde (Conolly *et al.*, 2004), over extended periods of time causing tumors in rodents. The exposure–response relationship for cell killing may likely have a threshold which must be considered in extrapolating the findings from high exposure level studies in rodents to humans exposed to low concentrations of these chemicals.

It is my contention that understanding the basic concepts conveyed in Figures 1.1, 1.2 and 1.4 can be very useful in investigating a range of situations where the objective is to establish or refute a causal association between a given source and toxic agent and an increased incidence of an adverse health outcome. I use the term, increased incidence, advisably recognizing in most situations involving domestic animals, either as commercial herds or as companion animals, the situation is one of presence or absence of a given disease and the "ruling out" of other differential diagnoses. However, in situations involving human populations the issue frequently encountered is whether a given toxicant exposure has caused an increase in a disease recognizing that most diseases may have multiple etiologies, e.g. hypertension and diabetes. This is especially the case in evaluating diseases that typically occur late in life, such as cancer and chronic diseases, and with exposure to toxicants that may occur at low levels over long periods of time. In some cases, such as lung cancer and cardiorespiratory disease in humans, a risk factor such as cigarette smoking is so substantial, it is a challenge to determine if low-level exposure to other toxicants such as air pollutants has chronic effects at low exposure concentrations.

Veterinary toxicology is multi-faceted

It will be apparent to the reader of this book that veterinary toxicology is multi-faceted. Thus, there are many ways to organize and synthesize the knowledge base that we call veterinary toxicology. One dimension is the various classes of toxicants. Another dimension of the field relates to the media that contains the toxicant: air, water, soil and feed. Another dimension considers the various routes of exposure of toxicants: inhalation, ingestion, dermal or purposeful injection. It is also convenient to consider the various organ systems and processes that may be affected by toxicants. This is the basis for organization of a major section in this book. It is also important to consider the individual toxicants or classes of toxicants. This approach is used in organizing another major section of this book. Finally, veterinary toxicologists recognize the necessity of considering the various species of concern. Increasingly veterinary medical practitioners have become more specialized with many focusing their clinical skills on a single species. This book does not include a section addressing the toxicology of individual species. To have done so would have substantially increased the size of this text. However, chapter authors have endeavored to discuss species variations in responses to toxic agents. It is noteworthy that at least one textbook, that of Peterson and Talcott (2006), focuses on small animals. Some of the major comprehensive veterinary medicine texts that focus on other species include chapters on toxicology related to that species such as the *Current Veterinary Therapy* series.

SOURCES OF INFORMATION

Case observations in the species of interest

There are multiple sources of scientific information for characterizing the relationship between exposure to a toxicant and toxicant-induced response. Figure 1.5 is a schematic rendering of the multiple sources of information that may be used to understand the toxicity of a given agent.

As discussed earlier, the origins of veterinary toxicology and toxicology, in general, are both rooted in observations. An adverse health effect, either a pattern of morbidity or death in an individual or population, is observed and the disease linked to exposure to a toxicant. Typically, the time interval between exposure and the adverse health outcome was brief which aided in deducing an association. Because the causal association was identified in the species of interest, whether it be a person, a horse or a cow, it was

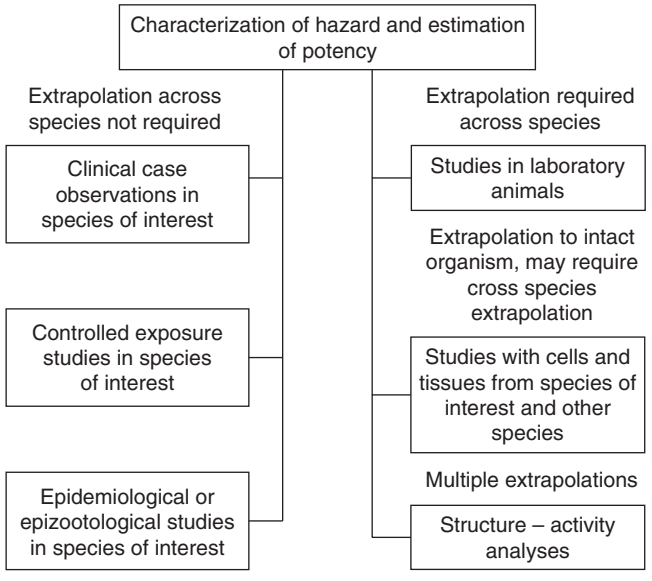


FIGURE 1.5 Sources of information for evaluating potential toxicants.

not necessary to extrapolate between species. Nor was it necessary to explore in depth the mechanistic basis for the causal association to either diagnose a particular case or prevent future cases. Action to prevent exposures and, thus, prevent disease, could be based on empirical observations.

As you read many of the chapters in this book, you will note that details of the mechanism by which a particular toxicant causes disease have been elucidated to a variable extent. When the toxicant is exclusively of concern in veterinary medicine and has no implications for human health, there has been limited impetus for developing a mechanistic understanding of how a toxicant causes disease. Concern for human health has been a major driver of the biomedical research agenda. An obvious exception is when the toxicoses observed in veterinary medicine have large economic impact or toxicants can reach people via animal products.

There are many circumstances where observational knowledge is not adequate and it is necessary to conduct experiments to characterize the toxicology of an agent. It is obvious that if concern for the potential toxic response is in a non-human species, controlled experiments can be conducted using the species of interest. This is obviously the case for domestic livestock as well as companion animals.

A much more common situation is when concern focuses on potential toxicity of a newly developed agent for use in people or animals. For example, it is necessary to establish the safety of a potential new pharmaceutical or consumer product before it is introduced into commerce. In these instances experimental animals are used as a "first approximation" of the safety of the new compounds to humans. In the case of products intended for use in animals, studies on both efficacy and safety can be conducted in the species of interest. This remains an imperative step in the safety evaluation of new products. There are also circumstances in which it is desirable to extend limited observations from opportunistic studies on people or animals that have been exposed. When a new product is developed and marketed, either a pharmaceutical or a consumer product, various post-marketing surveillance systems should be put in place to attempt to detect any unexpected adverse outcomes.

Epidemiological/epizootological studies

If a particular chemical has been used for an extended period of time and human exposure has occurred either in the workplace or from the environment, it may be feasible to conduct epidemiological studies. Epidemiology is the study of how disease is distributed in the population and the factors that influence or determine this distribution. The design of a particular epidemiological study will be guided by the hypothesis being tested and the nature of the population(s) available for study. As an aside, the term epidemiology (epi for across, dem for people and ology for scientific study) is applicable to people while the more appropriate related term for studies on animals would be epizootology (epa for across, zoo for animal and ology for scientific study). The details of conducting epidemiological or epizootological studies are beyond the scope of this chapter. A relevant reference for basic concepts in epidemiology is the text by Gordis (2005).

Retrospective epidemiological studies may be feasible for previously introduced agents for which prior exposure has occurred or prospectively for a newly introduced agent. If the agent is new it is obvious that it is not feasible to conduct epidemiological studies to retrospectively evaluate the potential safety/hazard of the agent. If the ultimate interest is in the effects on people, it may be feasible to conduct controlled exposure studies with human volunteers. It is advisable for the planning of such studies to be based on a solid database on the potential toxicity of the agent acquired from studies in laboratory animals. The design and conduct of such human studies must be guided first and foremost by ethical considerations (NRC, 2004). If a non-human species is the target species of concern, then it is obvious that the most relevant information is that acquired from studies conducted in that species.

Experimentation

An additional option for acquiring information is to conduct toxicological studies in the typical laboratory animal species. Such studies are the cornerstone of research conducted to evaluate the safety/risk of newly synthesized agents whether they be a potential new pharmaceutical, pesticide or herbicide, a significant consumer product or a new chemical or intermediate to be used in commerce. It is well recognized, certainly by veterinarians, that no single laboratory animal species is a miniature version of the human species, i.e. 15 cm in height, weighing 180 g and sharing all of the common biological traits of humans. Fortunately, humans and laboratory animals do share many common biological traits. Knowledge of the extent to which there are similarities and differences between humans and a given laboratory animal species can be used to guide the selection of a species to serve as a surrogate for humans in developing data for safety/risk evaluations for humans. It is encouraging that some veterinary medical schools are recognizing the importance of extending the range of species studied in the core curriculum from the usual companion animal and domestic livestock species to include the common laboratory animal species.

At this juncture, it is appropriate to note the importance of animal welfare issues. The Animal Welfare Act (AWA), initially enacted in 1966 and amended in 1970, 1976, 1985, 1990 and 2002, is the principal federal statute in the United

States governing the sale, handling, transport and use of animals. The AWA applies to all species of warm-blooded vertebrate animals used for research, testing or teaching excluding animals used for agricultural research. The US Department of Agriculture, Animal and Plant Health Inspection Service has responsibility for implementing the AWA. The 1985 Amendments to the AWA clarified the importance of humane care, minimization of pain and distress, consideration of alternatives, the role of institutional animal care and use committees, the psychological well-being of primates and exercise for dogs. The primary reference on animal care and use is the *Guide for the Care and Use of Laboratory Animals* prepared under the auspices of the Institute of Laboratory Animal Resources of the National Academy of Sciences/National Research Council (ILAR, 1996). All toxicologists involved with laboratory investigations should be familiar with the contents of the guide irrespective of the species they use for their research.

An additional matter the experimentalist should be aware of is the need for use of good laboratory practices (GLPs) in the conduct of research intended to be used for regulatory decisions. Both the FDA (FDA, 2001) and the EPA (TSCA, 1985; FIFRA, 1991) have requirements for the use of GLPs. The FDA GLP requirements do not extend to exploratory, mechanism of action or efficacy studies. The basic elements of GLPs are (1) the appointment by the institution of a study director, (2) the use of an independent quality assurance unit, (3) the use of documented standard operating procedures, (4) a written protocol for each study and (5) preparation of a final report containing a GLP compliance statement for each study. The use of GLPs is not required by FDA for studies with domestic livestock. However, investigators conducting studies using domestic livestock would be well advised to attempt to adhere to the basic principles that under-gird GLPs to help ensure the quality and reproducibility of the data being generated.

Another option for acquiring useful toxicity data is to conduct investigations in *in vitro* using tissues or cells from mammalian species, both humans and laboratory animals, and using bacteria and yeasts. An additional option is to conduct structure–activity analyses on the new agent using the large data bank of structure–activity information already available on other related chemicals.

All of the options outlined, to some extent, create extrapolation issues. Even if studies are conducted in the species of interest, it is typically necessary to extrapolate from the high levels of exposure or administered doses studied experimentally to lower exposures or doses anticipated to be representative of intended use. It may also be necessary to extrapolate from a relatively short period of study, say days or a few weeks, to the intended period of use, over months or years. If the studies are not conducted in the species of ultimate interest, there is need to extrapolate between species. It may also be necessary to extrapolate observations made in a population of healthy individuals to a population that includes individuals with pre-existing disease. Some aspects of the extrapolation between species and across exposure/dose levels may be facilitated by physiologically based toxicokinetic and toxicodynamic modeling. However, toxicodynamic modeling is still in its relative infancy.

It is important to recognize that even with today's level of knowledge of these extrapolation issues, it is not possible to estimate, with absolute certainty, the precise numerical level of human exposure to a given agent that may be without any risk of potential harm or will produce a specific level of harm. This is generally recognized in contemporary safety/risk evaluation methodology such that conservative approaches are used in estimating safe levels of human intake of chemicals. By taking a conservative approach to setting standards or providing guidance to limit exposures, there can be a high degree of confidence that an agent can be used safely if used as intended. Ultimately, all processes that develop guidance or standards to limit exposures and thus limit disease require judgments to be exercised. In short, science can inform the standard or guidance development process; however, it cannot prescribe specific standards.

SCHEMATIC EXPERIMENTAL DESIGNS

The experimental design for testing of any specific hypothesis must be matched to the hypothesis, the desired statistical power and the resources available. Inevitably, decisions on an experimental design involve making difficult choices among options because of resource constraints. In this section, two schematic experimental designs will be discussed to illustrate some of the key issues that must be addressed in planning toxicological studies. The discussion in both cases will assume that the species to be used in the study has already been selected.

Acquiring toxicokinetic data

The first design, Figure 1.6, illustrates an approach to acquiring data for understanding the link between exposure and internal dose, the kind of data that can be used for toxicokinetic modeling. Recall the toxicokinetic linkage in Figure 1.1. The design shown is based on a single brief intake of the test agent. However, the design can be modified for studying chronic intake of an agent. A critical decision is the choice of the route of administration or intake of the test material. Obviously, such studies are most readily carried out with parenteral administration of the agent. This may be the most appropriate route for a pharmaceutical

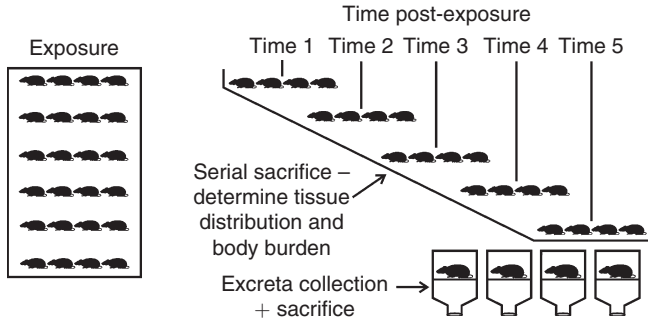


FIGURE 1.6 Schematic rendering of an experimental design for evaluating the kinetics of an administered toxicant.

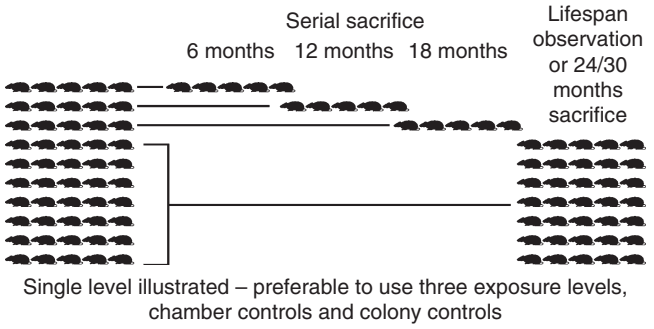


FIGURE 1.7 Schematic rendering of an experimental design for evaluating exposure (dose)–response relationships for a toxicant.

agent that is to be parenterally administered. However, the resulting data may be of limited relevance to other routes of intake. For example, it may not appropriately mimic oral intake since only a small fraction of some toxicants may be absorbed from the gastrointestinal tract. In short, the route of administration should be matched to the route of concern for real-world exposure to the agent.

With inhalation, the particle size distribution of the airborne toxicant will influence what portion of the inhaled material will be deposited and where it is deposited in the various regions of the respiratory tract. The pattern of retention and subsequent translocation of the deposited material will depend on the size, chemical composition and dissolution properties of the deposited material.

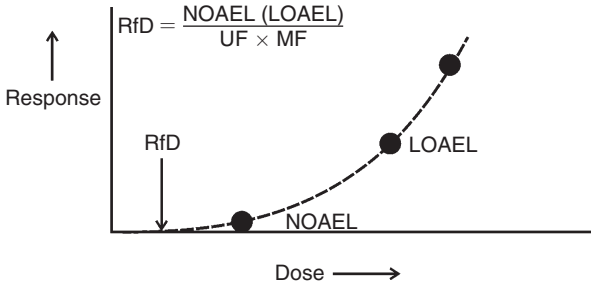
Another key decision is whether conduct of the toxicokinetic studies may be facilitated by using a test agent labeled with radioactive or stable element tracers. Analytical considerations for the initial toxicant as well as any metabolite are of major importance in the conduct of toxicokinetic studies.

The schematic design (Figure 1.6) shows a group of animals maintained for collection of excreta and, perhaps, even sampling of expired air. Data from these analyses can be used along with tissue analyses to obtain a mass balance between the quantity administered and the quantity recovered. The schematic design shows multiple times at which animals will be euthanized and tissues collected for analysis. This allows the development of a dynamic profile of how the body handles the administered material. For organic compounds, provision needs to be made for analyzing for both the parent compound and potential metabolites.

The selection of the sacrifice times will be guided by the anticipated kinetic profile of the agent and its metabolites. It may be useful to obtain preliminary information on retention kinetics from pilot studies. Some organic compounds may be rapidly metabolized leading to the need to schedule all of the sacrifices over a few hours. On the other hand, certain inhaled relatively inherent materials may have long-term retention in the lungs extending over hundreds of days. It is important to recognize that the quantity of material administered may influence the kinetics of the material. Hence, it is desirable to use multiple administered exposure/dose levels as an experimental variable. Without question, the design of any particular toxicokinetic study requires the exercise of considerable professional judgment. Toxicological research is not a "cookie cutter" or "check the box" science.

Acquiring exposure (dose)–response data

A schematic experimental design for a study to evaluate exposure (dose)–response relationships for toxicants is shown in Figure 1.7. Recall the exposure–response linkage shown in Figure 1.1. The design shown is typical of that which might be used in the conduct of a 2-year bioassay, typically to evaluate carcinogenicity, in rats and mice. The same design, and indeed the same experiment, can be used to evaluate other endpoints and to conduct shorter-term studies. The study should involve administration of the material by a route matched to likely exposure conditions to be encountered with the agent. Administration of an agent by gavage may be acceptable as a surrogate for ingestion, especially when it is desirable to administer specific quantities of material. However, I am not enthusiastic about the repeated use of gavage as a substitute for ingestion of an agent in feed. The use of intratracheal instillation as a surrogate for conducting inhalation exposures to an agent remains controversial. It is my professional opinion that intratracheal administration is a non-physiological mode for delivery of materials to the respiratory tract. It may result in exaggerated quantities of material being deposited in some regions of the respiratory tract while other regions are spared any exposure. This unusual pattern of distribution of the agent is very likely to influence the toxic response of the airways and alveoli. Thus, I am hesitant to even recommend intratracheal instillation for mechanistic studies; the mechanistic information acquired may be irrelevant to the inhalation exposure situations that are of concern for people.



$UF = UF_H \bullet UF_A \bullet UF_S \bullet UF_L$
UF_H = Human variability (10×)
UF_A = Animal to human (10×)
UF_S = Subchronic to chronic (10×)
$UF_{L=}$ = LOAEL to NOAEL (10×)
MF = Modifying factor for completeness of data (1–10×)
RfD = Reference dose

FIGURE 1.8 Schematic rendering of a threshold exposure–response relationship.

It is critical that exposure–response studies utilize multiple exposure levels, perhaps three or four exposure levels. The choice of the specific exposure levels is one of the most important decisions to be made in planning such studies. One consideration relates to the potential level(s) of exposure to be encountered with intended use. Higher additional levels can be selected above this base level. Selection of exposure/dose levels can also be informed by the results of the kinetic studies. For example, it would not be desirable to use only exposure levels above a level at which metabolic processes are saturated. Another consideration emphasized by the EPA and NTP, especially when cancer is an endpoint, is to select a maximum tolerated dose (MTD) level as the highest exposure/dose level and establish lower levels by some fraction of the MTD level, perhaps 1/2 and 1/4 or 1/3 and 1/9. The use of an MTD has been justified on the grounds that it is necessary to maximize exposure to potentially observe carcinogenic responses recognizing the blunt experimental approach (NRC, 1993).

The extent to which animal bioassays are a blunt approach to detecting the carcinogenic potential of agents is illustrated in Figure 1.8. It can be noted that for a species and train of animals with a background incidence of 1%, a study of 50 animals will require a 20% response to detect a statistically significant effect. As an aside, a population of non-smoking people will experience about a 1% lifetime incidence of lung cancer. A population of two pack a day cigarette smokers will experience about a 20% lifetime incidence of lung cancer compared to the 1% expected in non-smokers. Consideration of statistical information such as these emphasizes the importance of using care in interpreting the results of cancer bioassays using the typical 100 animals per exposure level. The interpretation of the relevance of the results of animal studies for estimating human hazards will be greatly enhanced by knowledge of the mechanisms involved in the toxicant causing disease in the animals.

A key feature of the exposure–response experimental design illustrated in Figure 1.7 is the use of multiple sacrifice times for all exposure levels. In some cases it may be possible to evaluate the functional status of organs at these times, i.e. pulmonary function. In animals with inhalation exposure, when a respiratory tract response is of concern, it may be feasible to collect bronchoalveolar lavage fluid samples for analysis of biochemical and cellular parameters. Most importantly, tissue samples can be collected for histopathological evaluation. The information obtained from the serially sacrificed animals, combined with that obtained from the terminal sacrifice animals, can provide valuable insight into the progression of disease processes over the course of the study. Without question, insight into the pathogenesis of toxicant-induced disease processes will be much more complete when serial sacrifices are conducted than that obtained only from an evaluation of the terminal sacrifice animals. Another option in the design of exposure–response studies is to include a group of animals at each level that are removed from further exposure at one or more times post-inhalation of exposure for maintenance without further exposure. These animals may be euthanized at later times and evaluated for evidence of recovery or reversibility of earlier toxicant-induced changes.

The basic guidance for using multiple exposure (dose) levels and making experimental observations at multiple times is as applicable to the conduct of studies examining hypotheses on the mechanisms of action of toxicants as it is to studies developing information for regulatory decisions. I remain disappointed at the number of published articles on mechanisms of action of specific toxicants that fail to use multiple exposure (dose) levels and multiple observation times. It is only when exposure (dose) level and duration of exposure are included as experimental variables that a true understanding of the mechanisms of toxicity for an agent can be elucidated. Mechanisms are frequently exposure (dose) level and exposure duration dependent.

As the science of toxicology has advanced, increasing attention has been given to developing specialized approaches for evaluating toxicity induced in different organ systems. The various guidelines developed by the USEPA, FDA and NTP are useful references for these specialized approaches. For example, the EPA has published guidelines for evaluating carcinogenicity (EPA, 1996a), gene mutation (EPA, 1996b), reproductive toxicity (EPA, 1996c), developmental toxicity (EPA, 1991) and neurotoxicity (EPA, 1995). The EPA is continually reviewing and updating its guidelines for toxicity testing. Forty-nine harmonized health effects test guidelines used in the testing of pesticides and toxic substances have been developed and can be found on the EPA Office of Prevention, Pesticides and Toxic Substances website (EPA/OPPTS, 2006).

The FDA has provided specific guidance for evaluating the safety of compounds used in food-producing animals (FDA, 1994) and principles for evaluating the safety of

food ingredients (FDA, 2000). The EPA has provided guidelines for evaluating the safety of products intended for use with cats and dogs (EPA, 1998) and domestic livestock (EPA, 1996d).

The various guidelines are useful for planning safety evaluation studies. However, the guidelines should not be used as a substitute for the use of professional judgment in planning, conducting and interpreting toxicological investigations. As noted earlier, toxicology is not a "cookie cutter" or "check the box" science.

TOXICOLOGICAL DESCRIPTORS

Toxicology rooted in observations

The results of toxicological investigations, either from clinical case observations or planned experimentation, involve describing the exposure, the dose, the response and interrelationships between these parameters. Exquisite knowledge of exposure or dose or response is not sufficient. Ultimately, it is necessary to understand their interrelationships. With clinical case observations, the initial emphasis is on the clinical findings – what is the response and the need, on the basis of a differential diagnosis, to establish that a toxicant is or is not involved. The evidence for a specific toxicant may be based initially on clinical findings complemented by gross necropsy findings potentially buttressed by histopathological findings. The differential diagnosis of a toxicosis may be strengthened by evidence of a marker of dose, i.e. urine, blood or tissue levels of suspected toxicant. The diagnosis may be further strengthened with evidence of exposure, i.e. the presence of the toxicant in the feed or identification of a poisonous plant. At each step, the qualitative evidence of a toxicosis and a specific toxicant is enhanced as qualitative findings are supplemented by quantitative findings. The analysis is not completed there, though. Other reasonable differential causes of the same or similar clinical signs must also be "ruled out" if the animals or humans are in a real world or field setting.

Quantifying exposure

Quantitation is paramount in evaluating exposure. In the experimental setting, quantitation is considered beginning with the design of the study and continued through all aspects of the experimentation. To the extent feasible, exposure to the toxicant should be rigorously characterized. This starts with physical and chemical characterization of the test material, be it an alleged pure compound or a mixture, including identification of any contaminants. The exposure circumstances need to be rigorously characterized. This, of course, is easiest to do when the test material is administered by injection. Even with injection, care must be taken to ascertain that the desired quantity of toxicant was actually injected. The quantity administered is typically related to the body weight of the subjects.

With administration by routes other than injection, the situation becomes more complicated. This may involve providing the experimental subjects' feed to which the toxicant has been added. If this approach is used, samples of the contaminated feed should be collected periodically for analysis of the test agent. In some cases, the concentration of the test agent in the feed will be used as a measure of the exposure. To accurately quantify exposure, it will be necessary to know the concentration of the test agent in the feed and also determine the quantity of the contaminated feed containing the test agent that has been ingested. For dermal administration, it is necessary to know the concentration of the test agent in the liquid media applied to the skin and the quantity of the media applied to the skin.

The situation is much more complex for a test agent in the air, whether it is a diluted gas or suspended particulate material. In both cases, it will be necessary to sample and measure the concentration of the test agent in the air at a location as close to the breathing zone of the experimental subjects as possible. For both particulate material and reactive gases, there may be substantial loss of the test agent in the delivery system between the generator used to create the test atmosphere and the breathing zone of the subject(s). Care needs to be taken to minimize such losses. For a toxic agent in a particulate matter form, it is essential to know not only the concentration of the test agent, but also the size distribution of the particulate matter since the aerodynamic particle size distribution will influence the fraction of the inhaled material that will be deposited and where it deposits in the respiratory tract. In some experiments, it may be possible to use a plethysmograph to measure respiration of individual subjects during inhalation exposure. This is most readily accomplished when the exposure period is relatively brief as in a study of the toxicokinetics of the agent. The total quantity of test agent inhaled can be estimated from knowledge of the air volume inspired and the concentration of the test agent in the air. In many studies the air concentration of the test agent may be used as a surrogate measure of exposure. As indicated earlier, exposure and dose are not synonymous. However, in many studies it may be necessary to use the concentration of the test agent in the feed, water or air as a surrogate measure of dose.

Describing absorption, distribution, metabolism and excretion

A number of different parameters may be evaluated in assessing the kinetics of a test agent (recall Figure 1.6). Some

of the common parameters and terms used are shown in Table 1.1 adapted from Spoo (2004). The four key events involved are absorption, distribution, metabolism and excretion. It is important to recognize that species differences may exist for each of these events. Absorption is the amount of the material that enters the body. As already discussed, the concept is simple. However, in reality it becomes complex as one moves from parenteral administration to oral intake, to dermal uptake or inhalation exposure. Distribution of the material will be influenced by the route of entry and the physicochemical properties of the test agent. Metabolism for compounds varies dependent on the physicochemical properties of the material. In some cases, the material may be very inert and simply be transferred mechanically within the body with some portion excreted over time. In other cases, especially with organic compounds, the metabolism may be quite complex and result in metabolites that are either more toxic, less toxic or have toxicity similar to the parent compound.

Excretion or elimination of the material and its metabolites, if metabolized, may occur via the kidney (urine), gastrointestinal tract (feces) or the lungs (exhalation of volatile compounds). In addition, the agent or metabolites may appear in tears, sweat or exfoliated skin. Some species, such as the rat, may engage in coprophagy, ingestion of feces, such that the test material in the feces is ingested and some portion passes through the body multiple times. Animals may be euthanized at various times during the course of the study and samples of various tissues collected and analyzed for the test agent or metabolites. With small experimental subjects, it may be possible to analyze all the tissues and obtain an estimate of the total body burden of the test agent and metabolites.

In some short-term studies it may be possible to collect and analyze excreta and expired air, if the compound is metabolized to a form that will be present in expired air. This information, along with the results of tissue analyses, can provide an estimate of the total quantity in the body, excreta and expired air for comparison with an estimate of the quantity administered. This kind of mass balance approach is obviously most feasible when radioactive or stable isotope tracers are used. One should not be surprised to find the estimated quantity recovered varying from 75% to 125%; there will be a high degree of experimental variability when multiple samples are being collected and analyzed. Obviously, one should view with suspicion data tables showing recovery of exactly 100% of the administered dose. Such values are typically the result of an over zealous investigator normalizing the data to 100% recovery. For chronic exposure studies, it may be possible to use kinetic modeling to estimate the quantity of the test agent or metabolites present in the experimental

TABLE 1.1 Common terms used to describe the ADME characteristics of chemicals (Adapted from Spoo, 2004)

Term	Abbreviation	Definition
Concentration	C_p	Concentration of a chemical in plasma (p) at a specific time (t)
Time	t	Chronological measurement of a biological function
Half-life	$t_{1/2}$	Time required for exactly 50% of a drug to undergo some defined function (i.e. absorbed, distributed, metabolized or excreted)
Volume of distribution	V_d	Unitless proportionality constant that relates plasma concentration of a chemical to the total amount of that chemical in the body at any time after some pseudoequilibrium has been attained
Volume of distribution (steady state)	$V_{d(ss)}$	Same as V?, except measured when the chemical has reached a steady state in the body
Area under the curve	AUC	Total area under the plasma chemical concentration curve from $t = 0$ to $t = \infty$ after the animal receives one dose of the chemical
Body clearance of a chemical	Cl_B	The sum of all types of clearance from the body
Renal clearance of a chemical	Cl_R	Volume of chemical that is completely cleared by the kidneys per unit of time (ml/min/kg)
Non-renal clearance of a chemical	Cl_{NR}	Volume of chemical that is completely cleared by organs other than the kidneys per unit of time (ml/min/kg)
Dose	D	The amount of chemical that is administered to an animal; can be further defined as the total dose, that total dose the animal was exposed to, or the absorbed (effective) dose, that being the fraction of the total dose that was actually absorbed by the animal
Bioavailability	F	Also known as systemic availability of a chemical. The quantity of percentage portion of the total chemical that was absorbed and available to be processed (CME) by the animal, in the case of intravenous administration, $F = 100\%$

ADME: absorption, distribution, metabolism and excretion; CME: chemical metabolism and excretion.

subjects at each exposure concentrations at various times after initiation of exposure.

Toxicant–induced responses

The types of studies typically used by toxicologists to investigate exposure–response relationships can be placed in four categories related to the duration of the studies: acute, sub-acute, sub-chronic and chronic (recall Figure 1.7). Acute studies are usually of a day or less and may involve intraperitoneal, intravenous or subcutaneous injection, gavage, dermal application or inhalation. Injections may be given once or several times in the 24-h period. Acute inhalation exposures are typically 4-6 h in duration. In all cases, the observations are made over a 24-h period. Sub-acute studies typically involve repeated exposures made on a daily, or 5 days/week, basis for 2-4 weeks with observations over the same period of time. Sub-chronic studies are usually conducted over a period of 1-3 months. In the case of inhalation exposures, these are typically conducted for 4-6 h/day, 5 days/week. Chronic studies are usually conducted for more than 3 months and, most typically, for 2 years. I personally view the use of the terms acute, sub-acute, sub-chronic and chronic as jargon and prefer to communicate the duration of studies in a specific manner, i.e. number of days or months, or as short or long term. I prefer to use the terms acute, sub-acute or chronic as descriptors of a medical condition.

The kinds of toxicant-induced responses that may be encountered are broad, essentially mirroring the range of disease processes that may occur in humans and other animal species. In any well-conducted toxicity study, the investigator should use as broad an array of observational techniques as are reasonably available to characterize the pattern of morbidity and mortality that may develop. Inevitably, cost constraints will influence the choice of endpoints evaluated. It will be useful to prioritize the potential endpoints as to their likely value in terms of the information gained. It is crucial that detailed necropsies be conducted on subjects euthanized at prescribed times and at termination of the study. Tissues should be collected from any gross lesions and tissues identified in the protocol as likely target tissues and processed for histopathological evaluation. It is now routine to establish a defined set of criteria for evaluating the various tissues and characterizing lesions. This approach allows the quantitative evaluation of any pathological findings on a group basis rather than restricting the evaluation to qualitative descriptions of responses in individual subjects.

Toxicity studies to evaluate exposure (dose)–response relationships may extend from minutes to hours when biochemical and physiological responses are being evaluated, to hours to days when acute morbidity and mortality are being assessed, to weeks to months and finally to a significant portion of the lifespan of the species, e.g. 2 years for mice and rats when chronic effects, including cancer induction, are being evaluated. With increased attention given to animal welfare considerations, emphasis is being given to using as few animals as possible to define the acute morbidity and mortality of test materials. Rather than use a traditional approach to attempt to precisely define a lethal dose 50% (LD_{50}), it has become customary to use approaches with many fewer animals to define an approximate LD_{50}. In some cases, it may be desirable to determine the concentration of a test agent in water or air that produces 50% lethality over a defined period of time, a lethal concentration, LC_{50}. This approach remains in common use when studying aquatic organisms.

In modern toxicology, increasing attention is given to conducting studies with exposures that are defined by the anticipated conditions of use of the test material. This may involve initially conducting a study of 2-week duration, perhaps with up to five exposure levels anchored by a level related to anticipated use. The results of this study are then used to select exposure levels, perhaps three or four, and to sharpen the focus of a 90-day study. The results of the 90-day study, in turn, are used to select the exposure levels and sharpen the focus of a study of 2-year duration. Although it has become customary to conduct chronic exposure or 2-year studies with three exposure levels, it should be recognized that use of a control group and three exposure levels spanning a range of concentrations differing by a factor of 2, i.e. 1, 1/2 and 1/4, or a factor of 3, i.e. 1, 1/3, and 1/9, does not provide a robust data set for characterizing the shape of the exposure (dose)–response relationship. On the other hand, the use of exposure levels differing by a factor of 10, i.e. 1, 1/10 and 1/100, may provide an excessively broad range of exposure levels for identifying a lowest observed adverse effect level (LOAEL) or no observed adverse effect level (NOAEL) as will be discussed later.

In chronic studies, major attention is directed to evaluating any toxicant-induced changes in animals at the several exposure levels compared to controls over a 2-year period or until a defined mortality level is reached, such as 20% surviving. Any changes in various indices of morbidity or pathological alterations will be evaluated compared to controls as well as tested for trends across the exposure levels. In many cases, the primary endpoint of concern will be cancer which should include evaluation of all stages of tumor development up to sarcomas and carcinomas. It has become customary to use life table statistical methods such as that of Kaplan–Meier (1958) to evaluate the incidence of key changes. This approach allows for the use of data not only from the survivors at the end of the study, but also animals that have died or been euthanized at interim times. This situation is analogous to that encountered in human epidemiological studies when subjects may be lost to follow up.

It has become customary when the results of chronic studies will be used for regulatory purposes to convene a pathology peer review panel of expert veterinary pathologists, typically Diplomates of the American College of Veterinary Pathology (ACVP), to evaluate histological specimens from representative cases and the diagnoses of the original pathologist to verify that the diagnoses are appropriate and consistent with the scientific norm. As an aside, I encourage veterinary toxicologists to personally review the pathology findings in studies with the study pathologist so as to be familiar with the nature of the pathology findings. However, I discourage veterinary toxicologists from taking on a dual role of toxicologist and pathologist for a study. Indeed, this approach would be unacceptable for a study to be submitted for regulatory purposes unless the toxicologist was also an ACVP Diplomate.

Describing exposure–response relationships for non-cancer endpoints

It is appropriate to now consider how the data generated from toxicological investigations can be used. Let us first examine a threshold exposure-response relationship as shown in Figure 1.3 and shown now in an expanded form in Figure 1.9. The first step is to examine the data set from critical exposure–response studies to identify key parameters to be used to describe the results. Key determinations are the no observed effect level (NOEL), the highest exposure level for which no effects are observed and the NOAEL, the highest exposure level that produces no adverse effects. Obviously, characterization of an effect as adverse or not adverse is a matter of professional judgment. For example, in a cholinesterase inhibitor study, is a reduction in blood cholinesterase in the absence of salivation or other clinical signs an adverse effect or merely an effect?

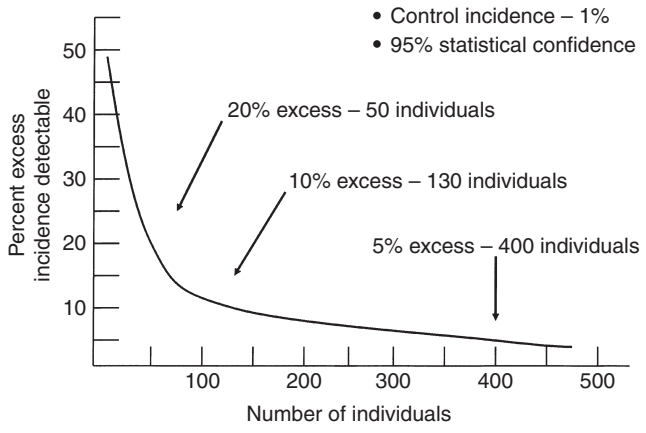


FIGURE 1.9 Relationships between number of subjects required to detect excess risk and the level of detectable excess risk.

In the absence of the identification of an NOAEL, there is a need to identify the LOAEL, the highest exposure level at which an adverse effect is observed. The specific NOAEL and LOAEL that can be identified are a function of the exposure levels originally selected for studies. To state the obvious, observations can only be made at the exposure levels studied. For example, if the exposure levels studied did not extend to a sufficiently low level, the lowest level might produce an effect thereby precluding observation of an NOAEL. Alternatively, the study might be designed with three exposure levels separated by a factor of 10 with the lowest exposure level identified as an NOAEL and the next higher exposure level identified as producing some modest adverse effects and, thus, identified as the LOAEL. In retrospect, in such a study it is not known whether the "true" LOAEL might have been a factor of 3 or 5 above the NOAEL since these levels were not investigated.

Another consideration is the nature of the effects identified at the NOAEL, was there evidence of enzyme induction or hyperplasia, hypertrophy or atrophy with no evidence of a change in organ weight? Likewise, at the LOAEL was hyperplasia, hypertrophy or atrophy present resulting in modest or substantial changes in organ and body weight? Were histological changes observed that were reversible? Were the changes sufficiently profound that the level would be identified as a functional effect level (FEL)? These questions serve to emphasize the extent to which professional judgment is involved in interpreting the results of all toxicological investigations.

For non-cancer effects a reference dose (RfD) for oral intake or a inhalation reference concentration (RfC) for airborne materials is calculated using the NOAEL or LOAEL as a starting point (Jarabek *et al.*, 1990; Jarabek, 1994). An RfD or RfC may be defined as an estimate (with uncertainty spanning perhaps an order two magnitude) of a continuous oral or inhalation exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious non-cancer effects during a lifetime. The RfD and RfC values are developed from the experimentally determined NOAEL or LOAEL values as shown in Figure 1.9 (Jarabek, 1994) and normalized to continuous exposure. For a more complete description of the process, the reader is referred to a recent book chapter by McClellan *et al.* (2006). The EPA maintains an Integrated Risk Information System that includes comprehensive summaries of the toxicological information available on specific chemicals including RfD and RfC values and estimates of cancer-causing potency. These profiles are available on line (EPA/IRIS, 2006).

A somewhat similar approach for non-cancer effects has been used by the ACGIH to develop TLVs (ACGIH, 2006). A TLV is defined as airborne concentrations of substances that represent conditions under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse health effects. Since the ACGIH TLVs

apply to healthy workers they may not always incorporate an SF or UF of 10 for human variability. The exposure duration for TLVs is based on a 40-h/work week and, thus, the results of animal studies will be normalized to 40 h/week.

The Agency for Toxic Substances and Disease Registry (ATSDR) develops minimal risk levels (MRLs) using a similar methodology. An MRL is an estimate of the daily human exposure to hazardous substance that is likely to be without appreciable risk of adverse non-cancer effects over a specified duration of exposure. For example, MRLs are derived for acute (1–14 days), intermediate (14–365 days) and chronic (365 days and longer) exposure durations. The MRLs are intended to serve as a screening tool to help public health professionals decide to look more closely at particular exposure situations. The ATSDR has prepared toxicological profiles on many chemicals including their MRLs. More than 200 profiles are available on line (ATSDR, 2006).

The NIOSH develops recommended exposure levels (RELs). RELs are set at levels such that virtually all persons in the working population (with the possible exception of hypersensitive individuals) would experience no adverse effects. The Occupational Safety and Health Administration (OSHA) sets permissible exposure levels (PELs) based on consideration of the NIOSH RELs. However, the OSHA values are legally enforceable limits unlike the NIOSH RELs which are guidance.

The International Program on Chemical Safety (IPCS) prepares authoritative reviews on the environmental health impact of various chemicals. The reports are available on line (IPCS, 2006). The exposure limiting values developed by the IPCS are guidance values and not legally enforceable limits. The United States makes extensive use of legal enforceable exposure limits. Many other countries emphasize the use of guidance values. This distinction is important when comparing standards versus guidance originating from different countries.

In considering all of the foregoing guidance or regulatory levels, it is important to recognize that they are set to control exposures for workers or the general public. In each case, they are set to be health protective and, thus, are set at levels below where human effects have been observed or are expected to occur. These values should not be interpreted as being equivalent to levels producing adverse effects in humans.

Cancer as an endpoint

For cancer as an endpoint, animal exposure–response studies may provide two kinds of input. First, the results may be used in carcinogen classification processes such as those of the IARC, the EPA or NTP. As discussed earlier, these are hazard-based classification schemes – Is a given agent capable of causing human cancer without consideration of the potency of the agent? These schemes have been described elsewhere (McClellan, 1999; McClellan *et al.*, 2006).

If a positive cancer outcome is observed in animal studies, the quantitative exposure–cancer response data may be used in a second way – to develop a risk coefficient, lifetime cancer risk per unit of exposure, for the potency of the agent for causing human cancer. Such extrapolations typically involve linear statistical extrapolations from high levels of exposure used in the animal studies to potential human exposure levels several orders of magnitude lower (recall Figure 1.3). In addition, they may purposefully be calculated based on upper 95% confidence limit on some level of risk, for example, with a probability of a one in one million occurrence. In my opinion, these extrapolated values are highly uncertain. It is quite possible that for some agents classified as possibly or probably carcinogenic to humans based on high exposure level animal study results there is no added cancer risk at very low levels of exposure (Gold *et al.*, 2003). The EPA (2005a) has recently issued guidance for alternative approaches to estimate cancer risks when information is available on the mode of action of the agent, for example, if the cancer arises as a result of the toxicity and secondary cell proliferation rather than a direct effect of the chemical or metabolite on DNA. For example, chloroform has been shown to cause cancer by this mode of action (Butterworth *et al.*, 1995). The EPA (2005b) has also provided guidance for considering the impact of susceptibility of early life exposures for causing cancer.

Information on the cancer-causing potential of various chemicals is included in the material summarized in the USEPA's Integrated Risk Information System (EPA/IRIS, 2006). The IARC monographs on the evaluation of carcinogenic risks to humans are all available on line (IARC, 2006). The monographs cover the carcinogen classification reviews of over 800 compounds. The NTP publishes, on a biannual basis, a *Report on Carcinogens*. The 11th report contained 246 entries, 58 of which were listed as "human carcinogens" with the remaining 188 being listed as "reasonably anticipated to be human carcinogens" (NTP, 2005). The potency of the various agents for causing cancer is quite varied. When examining this literature, many in the public, including some scientists, are surprised to learn how few agents have been conclusively identified as "human carcinogens." The facts stand in sharp contrast to the view conveyed in the popular media and some scientific publications that people live in a "world of carcinogens."

New potential endpoints

In recent years, the expansion of knowledge at the molecular and cellular level has provided the opportunity for considering the addition of a myriad of new endpoints to toxicological evaluations. This includes an array of new molecular biomarkers which have received substantial

attention. Although biomarkers are frequently discussed as new approaches, it is well known to the veterinary clinician and toxicologist and to physicians that biomarkers have been used in both human and veterinary medicine for centuries.

In some cases, measurement of the biomarkers present in body fluids, urine or exhaled breath serves as an indicator of exposure or, even, dose of a toxicant. Recall the report of the individual arrested for "driving while intoxicated" based on a breathalyzer test for exhaled alcohol which has been converted to a blood alcohol level. In other cases, the biomarker is an indicator of a disease process. Recall individuals being evaluated for prostate cancer based on an elevated level of prostate specific antigen in serum samples.

New biomarkers of exposure will continue to be proposed. For each potential biomarker of exposure, it will be necessary to conduct experiments to validate the utility of the biomarker. A special challenge relates to recognizing the dynamics of the toxicokinetics of various toxicants and establishment of quantitative relationships between exposure and dose at any particular time over the course of the intoxication.

The potential list of biomarkers for toxic responses is seemingly endless. In all fields of medicine, from different kinds of cancer to various functional diseases of every organ system, new molecular markers are being identified on a regular basis. The challenge for toxicologists is to consider from among this array of opportunities which biomarkers are sufficiently well validated with regard to their linkage to diseases and sufficiently reasonable in cost to warrant their use in exposure–response studies. This includes consideration of the new and highly sophisticated genomic tools. There is a special challenge in designing validation studies to make certain that the experimental design is directed toward identifying specific disease-related endpoints or toxicant-related effects rather than merely being another, albeit more sophisticated, marker of non-specific toxic effects. A serious issue in many previous validation studies has been the use of a single high exposure level and a few short-term observation times. Such studies are unable to evaluate exposure-related changes in biomarkers and may not be able to identify toxicant specific changes.

CONCLUSIONS AND SUMMARY

Veterinary toxicology is a multi-faceted hybrid that draws on and contributes to the veterinary medical profession, the scientific field of toxicology and, broadly, to medical science. Some have characterized toxicology as a distinct scientific discipline. I view toxicology as an applied area of science addressing important societal issues by drawing on multiple scientific disciplines and professions. Veterinary toxicology, as a sub-specialty in veterinary medicine, had a very applied origin – the diagnosis and treatment of toxicoses in domestic animals and companion animals. That important role continues today. However, the field has broadened to include concern for contaminants in human food products originating from animals and for contributing to the conduct and interpretation of safety/risk evaluations for pharmaceuticals, food additives, consumer products and specific chemicals. Veterinary toxicologists who understand both normal and disease processes extending from the molecular level to the integrated mammalian organism and, indeed, populations, have an array of opportunities for making significant contributions to society. The prospects for the future of veterinary toxicology and the opportunities for veterinary toxicologists have never been brighter.

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CHAPTER

2

Pharmacokinetics and toxicokinetics: fundamentals and applications in toxicology

Rakesh Dixit

INTRODUCTION

The combined and well coordinated processes of the absorption (A) of a drug or a xenobiotic into the systemic circulation, its distribution (D) to organs and tissues, metabolism (M) to other active or inactive chemical species, and its elimination (E) from the body is collectively known as ADME (Gibaldi and Perrier, 1982; Voisin *et al.*, 1990; Shargel and Yu, 1993; Rowland and Tozer, 1995; Medinsky and Valentine, 2001). The pharmacokinetics refers to the kinetics of ADME processes employed at relevant low pharmacological doses where pharmacokinetics generally follow first-order kinetics and kinetic processes are expected to be linear (Gibaldi and Perrier, 1982; Shargel and Yu, 1993; Rowland and Tozer, 1995). Toxicokinetics unlike pharmacokinetics represent the study of kinetic processes of ADME under the conditions of preclinical toxicity/safety testing where depending on the doses employed both first- and zero-order kinetics are expected and kinetic process can substantially change between low and high doses (Medinsky and Valentine, 2001; Dixit *et al.*, 2003). During the last 20 years, the application of toxicokinetics has evolved in the pharmaceutical and chemical industry and toxicokinetics often refer to exposure assessment in drug or chemical safety assessment studies. In contrast to pharmacokinetics, the pharmacodynamics refer to effects elicited by the drug and active metabolites at relevant pharmacological doses while toxicodynamics refer to toxic effects related to doses (systemic exposures) used under the conditions of toxicity testing (Gibaldi and Perrier, 1982; Shargel and Yu, 1993; Rowland and Tozer, 1995).

With a full understanding of dose response, including dose administered and circulating drug levels (systemic exposure) and their relationship to toxicity, the toxicity and safety of xenobiotics, including drugs can be better assessed. In contrast to pharmacokinetics studies where ADME processes are generally first order and linear, toxicokinetics especially at higher doses encompass zero-order and non-linear processes. Non-linearity in toxicokinetics typically result from saturable metabolic clearance processes, saturable transporters, drug-specific biopharmaceutical factors, and toxicodynamics. Biopharmaceutical factors may include alterations in drug absorption at different doses (e.g. from low solubility of drug), differences in blood or tissue distribution (e.g. due to saturable protein binding, changes in tissue pathology), differences in metabolism (e.g. saturable metabolic enzymes kinetics), and in drug elimination (e.g. urinary and fecal excretion) (Medinsky and Valentine, 2001; Dixit *et al.*, 2003). In practice, the most practical surrogate or measure of dosimetry is the determination of the time course of drug and its major metabolite(s) in easily accessible body fluids, including blood or plasma or urine. With a good quantitative understanding of the time course of plasma drug concentration, one can gain information on the kinetics of absorption, distribution, metabolism, and elimination of a given drug. Because of the difficulty in quantifying drug and metabolites concentrations in target organs of toxicity, it is expected at the steady state the concentrations of a drug in blood or plasma are likely to be in equilibrium with concentrations in tissues. At the steady state, the plasma/blood can be considered a reasonable practical

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surrogate for tissue(s) exposure to drug and changes in plasma drug concentrations may reflect changes in tissue drug concentrations over time, and relatively simple pharmacokinetic calculations or models can be extremely useful to describe the behavior of that drug in the body. In order to integrate preclinical animal toxicology data into human risk assessment, it is imperative to have some comparative human pharmacokinetics data at relevant exposures. In veterinary risk assessment, toxicokinetics/pharmacokinetics data from multiple species need to be incorporated into risk assessment.

The fundamental objective of the toxicokinetics is to obtain information on the relationship between the dosage administered and circulating levels of xenobiotics (systemic exposure) under the conditions of toxicity testing. Toxicokinetics data when appropriately obtained may provide the following additional useful information:

1 Relationship between increasing doses and exposures attained (linear, non-linear, or plateau).
2 Sex differences in exposures and their relationship to any potential sex-related differences in toxicity.
3 Effect of repeated administration on exposures and if increase or decrease in toxicity is related to changes in ADME toxicokinetics.
4. Safety of the proposed initial doses in clinical trials or proposed acceptable daily intake (ADI), tolerable daily intake (TDI), or reference dose (RfD).
5 Support dose escalation in subsequent clinical trials.

In pharmaceutical risk assessment, the most important use of the toxicokinetics data has been in assessing safety margins based on interspecies comparison of plasma *AUC* or C_{max} at no-observed adverse-effect level (NOAEL) and observed adverse-effect level from animal toxicology studies and expected/observed exposures at relevant clinical doses. Unlike the pharmaceutical industry, the human risk assessment of exposures to chemicals present in food, water, or air has traditionally relied on safety or uncertainty factor approach. The ADI or TDI or RfD on mg/kg basis is determined by dividing the NOAEL in mg/kg/day from most sensitive animal species by a factor of 100 which includes a factor of 10 each for extrapolation of safe doses: interspecies (animals to humans) and intraspecies (within humans). The use of this approach has often given low ADI or TDI and RfD for chemicals which are not easily achievable or enforceable. In recent years, there has been an increased emphasis on the use of toxicokinetics to reduce uncertainty in extrapolation of doses across species.

This chapter provides a general introduction to toxicokinetics, description of toxicokinetics parameters, and their assessment using simple equations. The chapter also discusses the applications of toxicokinetics in veterinary risk assessment and drug development.

FUNDAMENTALS OF XENOBIOTIC DISPOSITION

Animals and humans may be exposed to xenobiotic chemicals present in our air, water, and food through multiple routes, including oral, dermal, and pulmonary. Intentional therapeutic drug exposure may occur through multiple routes, including oral, intravenous, intraperitoneal, subcutaneous, intramuscular, buccal, pulmonary, ocular, and direct regional administration. Therefore, a good understanding of the fundamental processes of ADME processes is critical to understanding toxicokinetics.

ABSORPTION

Absorption is collectively defined as all processes that comprise the transfer of an unchanged xenobiotic introduced into the body into systemic circulation or site of measurement may be exposed to xenobiotic chemicals present in our air, water, and food through multiple routes, including oral, dermal, and pulmonary. Intentional therapeutic drug exposure may occur through multiple routes, including oral, intravenous, intraperitoneal, subcutaneous, intramuscular, buccal, pulmonary, ocular, and direct regional administration. Therefore, a good understanding of the fundamental processes of ADME processes is critical to understanding toxicokinetics. Some basic concepts in absorption following extravascular routes of administration are demonstrated in Figure 2.1.

Gastrointestinal absorption

Oral route is the predominant route of exposure to drugs and chemicals. A prerequisite for the absorption through membrane is that the chemical must be dissolved in gastrointestinal (GI) fluids, have enough lipophilicity, and lack charge to pass through the lipid layers. Chemicals that are water soluble, metabolically resistant, and have sufficient lipid solubility are generally rapidly absorbed with a rapid peak concentration. If a chemical is not appropriately soluble in GI fluids, it will have difficulty in making it available to membranes and may face slow and/or sustained dissolution-limited absorption. This may occur when sparingly soluble chemicals/drugs are administered as suspensions. From biopharmaceutical perspectives, drug absorption across biological membranes is governed largely by the p*K*a, pH at the site of absorption, molecular size, molecular weight, and dissociation constant, degree of ionization, aqueous and lipid solubility, partition coefficient, chemical reactivity. Other factors that may impact the biological absorption process include

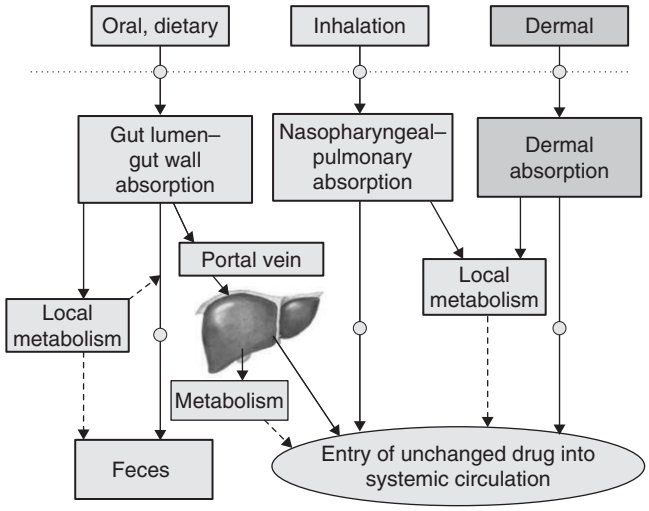

FIGURE 2.1 *Concepts in extravascular absorption.* When a xenobiotic such as a drug is administered via non-intravenous route, the absorption at the local site is the key process in the systemic delivery of the active substance. Some of the most important extravascular routes include oral/dietary, inhalation (nasopharyngeal–pulmonary), and dermal. With extravascular routes of administration, significant portion of drug can be lost as a result of local site metabolism. In addition, when a drug is administered through oral route, hepatic first-pass metabolism plays a pivotal role in controlling the systemic availability of drug (see text for additional discussion).

gastric emptying time, intestinal transit time, presence or absence of food, gut microflora, and specific drug transporters (Dethloff, 1993).

Route-specific/site of delivery-specific factors includes cell types, surface area at the absorption site, blood flow to and from site of absorption, pH at site of absorption, and site-specific metabolic/transporter effects (Dethloff, 1993). Chemicals, including drugs, are absorbed across lipid-rich membranes principally by (a) diffusion, (b) membrane pores (aqueous channels) and (c) energy-dependent active carrier mediated saturable processes. The majority of xenobiotics pass through membranes through a diffusion process, a process largely controlled by Fick's law. Very small molecules (chemicals up to 4×10^{-4} μm in diameter with a molecular mass of less than approximately 200 Da) can filter through the membrane pores without much difficulty.

According to Fick's law (Dethloff, 1993), the rate of diffusion is proportional to concentration gradient ($C1-C2$) across the membrane, the surface area available for diffusion (a), and a diffusion constant (k). Overall the diffusion rate is inversely proportional to the thickness of the membrane (d):

$$\text{Diffusion rate} = k \times \frac{a(\text{C1} - C2)}{d}$$

Most chemicals or drugs are weak acids or weak bases and therefore exist as ionized or non-ionized species. Because non-ionizable form is lipid soluble, it facilitates diffusion through lipid membranes. In contrast, the ionizable form is poorly lipid soluble and is generally unable to pass through the lipid membranes.

The pH and p*K*a on both sides of microenvironment largely determine the extent of ionization. The Henderson–Hasselbach equation to determine the extent of ionization can be as follows:

$$\text{pH} = \text{p}K\text{a} + \log \frac{[\text{conjugated base}]}{[\text{conjugated acid}]}$$

The pH at the absorption sites in relation to p*K*a has been exploited for improving the absorption and/or increasing urinary elimination of many drugs by reducing renal reabsorption of drugs. For example, when poisoned with salicylate, urinary excretion of salicylate can be substantially increased due to increased alkalinization of urine (from pH 6.5 to 8.0) by giving sodium bicarbonate. Because the p*K*a of salicylate is 3.5, the alkalinization of urine (pH 3.8) decreases the non-ionization form substantially (approximately 25-fold decrease) which reduces the renal reabsorption of salicylate and increases (approximately 5-fold) its urinary excretion. *It can be generally concluded that weak acids are likely to be better absorbed in the low pH of stomach whereas weak bases are likely to be better absorbed in the intestine.* It is not surprising to note that strong organic acids and bases are generally incompletely absorbed in the GI tract due to very strong ionization at all absorption sites. The absorption of most xenobiotics occurs in small intestine because this is well suited for absorption owing to its large surface area afforded by millions of villi and their specialized absorptive epithelium. The absorption through villus is also greatly supported by hepatic portal circulation and central lacteal (absorption into lymphatic system).

Factors impacting GI absorption

There are a large number of factors that limit the availability of xenobiotics. Extreme pH and hydrolytic enzymes (e.g. proteases and lipases) in the GI tract substantially impact the stability of the xenobiotics. The GI tract microflora is known to metabolize a large number of xenobiotics and this process can reduce the availability of xenobiotics absorption, and in some cases, may activate fairly benign xenobiotics into toxic metabolites. Despite very good absorption through the small intestine, many drugs fail to reach systemic circulation in sufficient amounts. Small intestine is very rich in both phase I and II metabolizing enzymes which can actively biotransform a

well absorbed drug. Overall, metabolism by the small intestine can prevent a well absorbed drug getting into portal circulation. In addition to the first-pass effects by intestine, the liver is the chief site of metabolism and contributes greatly to the first-pass metabolism leading to poor circulation of well absorbed drugs. Efflux and influx transporters present on the intestinal wall and liver can also modulate the systemic availability of drugs. Food present in the gut lumen can also substantially impact the absorption of drugs.

NON-ORAL ROUTES AND XENOBIOTIC ABSORPTION

Intravenous

For drugs that are poorly absorbed through oral route, intravenous route is often used to bypass absorption and deliver drug directly into systemic circulation. Intravenous route can also deliver very high peak concentrations very rapidly. Continuous intravenous infusion is often used to deliver the desired concentration in a well controlled manner.

Intramuscular, subcutaneous, and intradermal

These alternate routes are used to deliver drugs rapidly into systemic circulation because drugs delivered through these routes are not susceptible to first-pass metabolism. However, these different routes will deliver drugs with varying rates due to local site-specific absorption influenced largely by blood flow at local sites.

Buccal and sublingual

Oral mucosa has significant ability to absorb certain drugs. Because these routes are convenient ways to attain desired plasma concentrations, efforts have been made to actively exploit these routes for rapid delivery of drugs into systemic circulation. These routes offer a distinct advantage in delivering drugs into systemic circulation because they can bypass first-pass hepatic metabolism. Major limitations for these routes are local irritation and limitations of the doses.

Intraperitoneal

The advantage of the intraperitoneal route is that poorly absorbed drugs can be directly delivered to liver by bypassing intestinal absorption and without being subjected to intestinal first-pass metabolism. Drugs when given by the intraperitoneal route can attain a very rapid absorption when compared to the oral route.

Dermal

For environmental and industrial chemicals, the dermal route is a significant route of exposure. When compared to the oral route, the skin serves as an important barrier for absorption because of the substantially lower surface area, membrane thickness, and poor blood flow. Despite these limitations, many chemicals when presented at large doses can be substantially absorbed. Outer layers of skin (epidermis or stratum corneum) provide a significant barrier to absorption; however, hydration, epidermal erosion, or abrasion can greatly enhance absorption. Unlike the outer layers of skin, the inner layers such as dermis are well perfused. One additional advantage of the dermal route is that drugs given by the dermal route are not subject to significant metabolism, though epidermis has been shown to metabolism certain xenobiotics.

INHALATION

The inhalation route is one of the major routes of xenobiotic exposure. In recent years, the pulmonary delivery of drugs has become an important route of drug delivery. Lungs receive 100% of the cardiac output and the repetitive branching of the airways from trachea to terminal alveoli provides an enormous surface area for absorption. Absorption from lungs is quite rapid because there is little barrier (alveolar region thickness is only 0.2 μm) and up to 90% of the alveolar surface is exposed to capillary bed. Inhaled drugs are deposited on lungs epithelial surface and deposition of aerosols is influenced by lung-specific anatomical regions. Particles less than 1 μm are typically excluded from alveolar absorption. In certain regions such as nasopharyngeal, tracheal, bronchial and upper bronchiolar, xenobiotics are likely deposited by impactation while in deeper regions of lungs, xenobiotics are deposited by diffusional and sedimentation processes. Passive diffusion plays an important role in xenobiotic absorption and the alveolar region because its enormous surface area coupled with high blood flow is the major site for pulmonary absorption. Although the lung has capacity to metabolize chemicals which will likely decrease the systemic availability, chemicals delivered through lung absorption process bypass hepatic first-pass metabolism and directly enter systemic circulation through heart.

SPECIES DIFFERENCES IN ABSORPTION

This topic has been discussed in great depth by Beasley (1999). Of over 4000 species of mammals, there are large numbers of species differences in absorption. The food habits of carnivores, herbivores, and omnivores differ greatly and this can be responsible for differences in bioaccumulation of potentially toxic chemicals. It is not too surprising that the accumulation of fat-soluble xenobiotics is higher in carnivores than in herbivores and the extent of accumulation of food chain derived xenobiotics or bioaccumulation factor (concentration in animal tissues divided by concentration in environment/food chain) may vary a log order of magnitude. Ruminants show age-dependent absorption of xenobiotics. Young calf and lamb behave like monogastric animals until maturity when they adapt to high roughage diet. These species also dilute xenobiotics exposure with a longer GI transit time. Additionally consumption of foods rich in fiber tends to lower the bioavailability of toxic compounds. Anaerobic environment in the rumen tends to reduce xenobiotics much more efficiently than in non-ruminants.

Monogastric animals, including carnivores and omnivores, have a lower stomach pH. For example, the gastric pH in dogs and pigs is between 1 and 2. Unlike other species, horses have a higher gastric pH of about 5.5 and their stomach size is smaller in relation to their overall body size. This means they need to eat more often.

DISTRIBUTION

Organ distribution of xenobiotics is mainly controlled by three different factors: (1) diffusion rate, (2) perfusion rate, and (3) relative affinity to various components (e.g. enzymes, receptors, transporters) in a given organ. When the diffusion rate across membrane is poor, the physicochemical properties of xenobiotics control the rate of tissue penetration. The perfusion rate (rate of delivery to an organ) becomes rate limiting when diffusion is rapid. The well perfused organs include lungs, liver, kidneys, heart, intestines, and brain. Poorly perfused organs include skin, skeletal muscle, connective tissue, and fat. Compounds that are highly plasma protein bound may show a lower tissue distribution and vice versa; however, it is to be stressed that it is the balance between the relative affinity of a chemical to tissue components and protein binding association and dissociation rates that control overall tissue distribution. Protein binding alone does not necessarily control the entire tissue distribution. For example, beta blocker propranol shows a high protein binding and yet has a high tissue distribution because it has a higher affinity for many tissues. Extensive plasma protein binding may decrease the unbound fraction available for tissue distribution; however, in rapidly perfused organs this can increase the diffusion rate from blood to organs. This may happen due to an increase in the concentration gradient due to build up of the protein bound drug in blood which in turn can increase the off rate of protein bound drugs/chemicals to release more drug for tissue distribution. This illustrates that protein binding is a dynamic process and highly protein bound may indeed show high tissue distribution if relative affinities to tissue components are high.

METABOLISM AND ELIMINATION

Metabolism of xenobiotics is very complex and diverse. It shows differences in occurrence, function, and rates. Species differences in quantitative metabolism are fairly common and it is important to appreciate this when interpreting toxicology data from one species to another species. For a detailed review of metabolism, refer to DeBethizy and Hayes (2001). Primitive species like microbes also have significant metabolism capacity, though microbes generally have very different pathways than mammals and may modify drug toxicity in an unexpected manner. Metabolism generally occurs through both phase I and II pathways. Phase I reactions result in functionalization, which result in the addition or uncovering of functional groups that are needed for subsequent metabolism by phase II pathways. Phase I reactions include oxidation (e.g. cytochrome P-450 (CYP) isoenzymes, xanthine oxidase, peroxidases, amine oxidase, monoamine oxidase, dioxygenases), reduction (e.g. CYP isoenzymes, ketoreductase, glutathione peroxidases), hydration (epoxide hydrolase), and dehydrogenases (alcohol dehydrogenase, aldehyde dehydrogenase). Phase II reactions are biosynthetic in nature and a common goal of all phase II reactions is to make xenobiotics more water soluble, more polar, and more easily excretable. Phase II enzymes include glutathione-*S*-transferases, UDP-glucuronyltransferase, thioltransferase, sulfotransferase, amide synthesis transacylase, *O*-, *N*-, *S*-methyltransferase, acetyltransferases, and thiosulfate sulfotransferase (rhodanese).

Excretion of xenobiotics and their metabolites usually occurs via urine, feces, and expired air for volatile substances. Three major processes within kidney control urinary excretion. These processes are glomerular filtration, reabsorption, and tubular excretion. Glomerular filtration because of the limits of pore size of 70–80 Å filters anything smaller than molecular weight of 20,000 Da.

All high molecular proteins and protein bound chemicals or their bound metabolites are not filtered and remain in blood and will likely be excreted through fecal excretion. Reabsorption of filtered components from urine to

blood occurs through specific active transport process occur in tubules. With the reabsorption of water urine may become concentrated and when this occurs, the reabsorbed components may diffuse back from tubules to blood. Many foreign compounds may also be secreted back into renal tubules against concentration gradient through both cation and anion carrier processes. Because these carrier processes are energy-dependent active process, they are saturable and with increasing doses renal secretion may get decreased causing accumulation of xenobiotic metabolites. Fecal excretion of xenobiotics and their metabolites is an important route of excretion. There are two sources for the excretion of compounds into feces. Unabsorbed drug and drug excreted through bile constitute the fecal excretion. For large molecular weight compounds and their metabolites biliary transport occurs through cationic transporters from hepatocytes into bile. Generally, large molecular weight drugs and their conjugative metabolites are excreted through bile. Based on the evaluation of molecular weight versus biliary excretion of a large number of molecules and their metabolites, it has been estimated that the molecular weight (Da) cutoff for the biliary excretion of chemical moieties in rats, guinea pigs, rabbits, and humans is 325 (±50), 440 (±50), 475 (±50), and 550, respectively (Hirom and Birch, 1976). Chemical moieties of molecular weights ranging from 350 to 450 Da are generally excreted via both urine and feces (Hirom *et al.*, 1972). The estimate of fecal excretion is often complicated by the fecal excretion of the unabsorbed drug when given by the oral route. Bile duct ligation studies can be helpful in evaluating the contribution of bile versus unabsorbed drug in fecal excretion of drug-related moieties.

CLASSICAL TOXICOKINETIC MODELS

The classical toxicokinetic models represent the most simple form of the pharmacokinetic models (Medinsky and Valentine, 2001; Dixit *et al.*, 2003). The principal component of a classical toxicokinetic model is a central compartment consisting of plasma (systemic) and tissues into which drug and its metabolites equilibrate. The basic principle of a simplistic one central compartment model is that it is assumed that all tissues, including both rapidly and slowly equilibrating tissues, and plasma will attain a rapid equilibrium and the kinetic profile of tissues can be described by measuring drug and its major metabolite(s) in plasma. Similarly, in the multiple compartmental models, drug is present into the central compartment and distributes rapidly between the central and peripheral compartments and the distribution between central and peripheral compartments follows a first-order process. In this model, drug elimination occurs from the central compartment, which is assumed to contain all major rapidly perfused drug eliminating tissues (e.g. kidneys and liver). The major advantages of the classical compartmental toxicokinetic models are that the models do not require information on disposition characteristics of drug-based tissue physiology or anatomical structure (Dethloff, 1993; Dixit *et al.*, 2003). These models, because of their simplicity, provide valuable information in describing and predicting the time course of drug concentrations in the systemic circulation at different doses, the extent of drug accumulation with multiple doses, and aid in selecting effective doses and dose regimens in efficacy and toxicity studies to achieve specific exposures.

One major disadvantage of classical models is their simplistic description of the kinetics of ADME processes in the body since these models are simple mathematic solutions for goodness of fit (Clewell and Andersen, 1985; Dixit *et al.*, 2003). Therefore, the classical models are not able to assess or reflect (1) the biology of specific tissues; (2) individual rates of transfer between compartments; (3) individual contributions and rates in routes of elimination; (4) the time course and exposure information in a specific tissue; and (5) specific drug disposition and elimination which involves dose-dependent non-linear and zero-order processes (Beasley, 1999; DeBethizy and Hayes, 2001).

FUNDAMENTAL NON-COMPARTMENTAL TOXICOKINETIC PARAMETERS

The non-compartment methods are simplistic and represent the most practical way to describe the kinetic behaviour of the drug. The most relevant pharmacokinetic parameters in the non-compartmental models typically include plasma or tissue area under the concentration versus time curve (AUC), maximum concentration achieved (C_{max}), time to maximum concentration (T_{max}), apparent volume of distribution (V_d), systemic clearance (CL_s), and terminal half-life ($T_{1/2}$). Figure 2.2 shows the visual concepts in toxicokinetics.

Area under the plasma/tissue concentration versus time curve

The AUC is considered the most important kinetic parameters in pharmacokinetics and is the quantitative measure of the exposure to drug over the sampling period. The linear trapezoidal rule is the most frequently used method to calculate AUC. Typically plasma concentration versus time curve is constructed. The overall curve is then divided into a series of trapezoids, typically indicated by observed time points and achieved concentrations. Overall AUC is calculated by the summation of the area within each individual trapezoid.

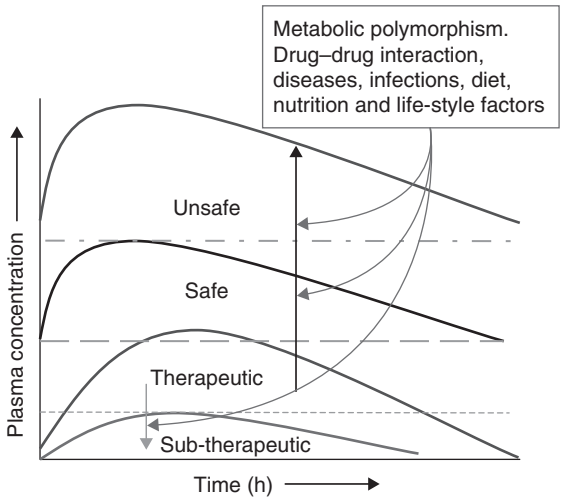

FIGURE 2.2 *Pharmacokinetics–pharmacodynamics and impact of intrinsic and extrinsic factors.* The use of pharmacokinetics data in clinical settings to maintain therapeutically efficacious concentrations. This is based on a thorough analysis of pharmacokinetics versus pharmacodynamics (effects associated with drug) during clinical trials. It is to realize that there are a large number of intrinsic (patient derived) and extrinsic factors that may increase drug levels which may be unsafe or decrease drug levels that may be sub-therapeutic and may decrease drug's efficacy (see text for additional discussion).

The area under each trapezoid can be calculated as follows:

$$\text{Area}_{\text{Trap}} = 0.5 \times (C_n + C_{n+1}) \times (t_{n+1} - t_n)$$

where C_n is the concentration at the earlier time; C_{n+1} is the concentration at the next later time; t_{n+1} is the later time; and t_n is the earlier time. The overall $AUC_{0-t_{\text{last}}}$ is then calculated as follows:

$$AUC_{0-t_{\text{last}}} = \sum 0.5 \times (C_n + C_{n+1}) \times (t_{n+1} - t_n)$$

The area calculated above is $AUC_{0-t_{\text{last}}}$ when the concentration at time zero is the first concentration (typically immediately prior to dosing); t_{last} is the time when the last sample was collected and concentration measured. In toxicokinetic studies supporting drug safety evaluation, $AUC_{0-t_{\text{last}}}$ is often measured from time 0 to 24 h. This is a measure of the daily systemic exposure during a repeated dosing. When steady-state toxicokinetics are expected (typically after attaining 5 half-lives), zero-hour concentrations are assumed to be equal to the 24-hour plasma concentrations. The measurement of AUC from time 0 to time $\propto$ represents the most accurate assessment of total systemic exposure following a single dose. When calculating, the remaining AUC from t_{last} to time $\propto$ is calculated with the terminal elimination rate constant, and is added to $AUC_{0-t_{\text{last}}}$. $AUC_{0-\infty}$ is then estimated as follows:

$$AUC_{0-\infty} = AUC_{0-t_{\text{last}}} + \frac{C_{\text{last}}}{k_{\text{el}}}$$

- C_{last}: Concentration measured at the last time point; k_{el} is the terminal elimination rate constant and is a measure of the fraction of drug removed from the site of collection per unit of time. The k_{el} has units of reciprocal time (e.g. min^{-1} and h^{-1}) and can be determined from the slope of the straight-line portion of the terminal phase of the concentration versus time curve when the concentration data are log-transformed as follows:

$$k_{\text{el}} = -2.303 \times slope$$

The multiplier 2.303 is a conversion factor from log units to natural log (ln). The first-order elimination rate constant k_{el} is independent of dose.

- C_{max}: This refers to the maximum drug concentration (C_{max}) attained during the time course of the measurement of drug levels. In the non-compartment methods, the information on C_{max} is often used to determine the extent of drug absorption.
- T_{max}: This refers to the time to attain maximum concentration. This parameter is often useful to assess the rate of drug absorption.
- *Trough levels* (C_{min}): This refers to the minimum drug concentration (C_{min}) attained during the elimination phase in time course of the plasma drug versus time curve. In the non-compartment methods, the ratio of trough level to maximum concentration can often provide valuable information on the rate of drug elimination.
- *Half-life* ($T_{1/2}$): It is the time required for the blood or plasma drug concentration to decrease by one-half, and can be determined from the terminal elimination rate constant by the following calculation:

$$T_{1/2} = \frac{0.693}{k_{\text{el}}}$$

The numerator 0.693 is the natural log of 2. It is to be emphasized that the accuracy of k_{el} and $T_{1/2}$ estimates is dependent on selection of time points. As a general rule, time points covering up to 5 half-lives should be taken. Both k_{el} and $T_{1/2}$ are dependent on both volume of distribution and clearance by the following relationship:

$$T_{1/2} = \frac{0.693 \times V_{\text{a}}}{CL_{\text{s}}}$$

Clearance

Drugs and their metabolites are removed from body via a variety of routes that may include fecal and urinary excretion, excretion in tears or sweat, metabolism in liver, kidneys, lungs, intestinal or other tissues, or by exhalation. Clearance is defined as the volume of drug removed from the body per unit of time with units as ml/min. For example, a CL_{s} value of 30 ml/min means that 30 ml of blood or

plasma containing drug is removed each minute. This parameter is often normalized to body weight, and thus clearance values are often reported in units of ml/min/kg. The CL_s means clearance as measured from the systemic circulation. CL_s is best calculated from concentration versus time data after an intravenous bolus or infusion dose because there is no absorption and nearly 100% of the drug is bioavailable following intravenous administration. Following extravascular administration (e.g. oral), estimates of clearance should be normalized to bioavailability and can be calculated as follows:

$$CL_s = \frac{F \times dose}{AUC_{0-\infty}}$$

where F is the fraction of the drug dose that entered the systemic circulation following extravascular administration. When constant intravenous infusion to steady state is used, CL_s may be calculated as a function of the infusion rate and the achieved steady-state concentration:

$$CL_s = \frac{k_0}{C_{ss}}$$

where k_0 is the rate of intravenous infusion and C_{ss} is the steady-state concentration.

Total body clearance can be estimated as the sum of clearances by individual eliminating organs such that:

$$CL_s = CL_r + CL_h + CL_i + \cdots$$

where CL_r is the renal clearance, CL_h the hepatic clearance, and CL_i the intestinal clearance. It is worthy to note that clearance of compounds from a particular organ cannot be higher than blood flow to that organ.

Volume of distribution

The apparent volume of distribution (V_d) relates the total amount of drug in the body to the plasma concentrations in the body. V_d is essentially the volume into which the drug distributes in the body. V_d is considered an indicator of extravascular distribution and has units of liters or liters/ kg of body weight.

V_d can be calculated as follows:

$$V_d = \frac{F \times dose}{k_{el} \times AUC_{0-\infty}}$$

where F is the fraction of dose that enters the systemic circulation. Following intravenous administration, F will have a value of 1 because the bioavailability is 100%.

V_d is considered the *"apparent" volume of distribution* and it has very little or no physiological significance because it usually does not relate to a real biological volume. V_d term is typically drug specific and represents the distribution of drug out of the central plasma compartment. In this context, a drug with a larger V_d will have high extrasystemic tissue distribution and if the drug binds to tissue extensively, V_d may exceed the tissue volume. Once the V_d for a compound is known, it can be used to estimate the amount of drug in the body as follows:

$$X_{drug} = V_d \times C_p$$

where X_{drug} is the amount of drug in the body and C_p is the plasma drug concentration.

BIOAVAILABILITY

The oral route is the predominant route of administration of pharmaceuticals and the fraction of dose that is available after absorption and first-pass clearance in the systemic circulation is termed *bioavailability* (*F*). Bioavailability can be essentially described as the amount of a drug that enters the systemic circulation and is considered a measure of drug absorption. The route of drug administration, intestinal first-pass effect, hepatic first-pass effect, transporters, formulations, and dissolution rate characteristics can greatly impact the bioavailability. In simplest terms, to determine bioavailability one needs to know the blood/plasma systemic exposure ($AUC_{0-\infty}$) values following intravenous and extravascular (e.g. oral, intramuscular, subcutaneous, intraperitoneal) dosing at the same doses; however, bioavailability can also be determined at varying doses provided the drug pharmacokinetics is linear and follows first-order pharmacokinetics. The bioavailability following an oral exposure is determined as:

$$F\% = \frac{dose_{iv} \times AUC_{0-\infty,ev}}{dose_{ev} \times AUC_{0-\infty,iv}} \times 100$$

$dose_{iv}$ is the intravenous dose; $dose_{ev}$ the extravascular dose administration; $AUC_{0-\infty,iv}$ the area under the curve after the intravenous dose; and $AUC_{0-\infty,ev}$ the area under the concentration versus time curves for the extravascular dose.

Relative bioavailability is often necessary to evaluate the impact of different dose forms (e.g. particle size, solubility, dissolution, vehicle delivery, etc.) on the systemic bioavailability of a drug. To assess bioavailability, intravenous data is not essential, as one extravascular dose form can be compared against another extravascular dose form, where one of the dose forms may be used as the reference material.

CLASSICAL PLASMA/TISSUE TOXICOKINETICS: APPLICATIONS IN DRUG DISCOVERY AND DRUG DEVELOPMENT

Prior to the testing of promising new therapeutics in healthy humans and/or sick human patients, non-clinical safety studies in laboratory animal species and/or appropriate *in vitro* models are conducted. Figure 2.2 shows a pharmacokinetics guided dosing strategy to improve therapeutic efficacy. Maintenance of plasma drug within a defined window is critical to the success of attaining the therapeutic efficacy. However, a large number of intrinsic and extrinsic factors, including metabolic polymorphism, drug–drug interaction, diseases, infections, diet, nutrition, and life-style factors could influence the therapeutically effective concentrations. Although it is not possible to evaluate many intrinsic and extrinsic factors in animal toxicology studies, it is believed that the use of safety factors/uncertainty factors (10 or greater) will allow the safe conduct of clinical studies.

Non-clinical toxicology studies when conducted properly in two species, typically in one rodent and one non-rodent, provide critical information on dose response in toxicity and its reversibility under a variety of exposure scenarios. The major objective of each toxicity study is to determine an NOAEL and adverse-effect level. Additionally, it is critical to know if toxicity is reversible or irreversible in nature and how long it will take to recover completely from toxicity. With the knowledge of quantitative species differences in ADME and susceptibility to toxicity, the risk assessment of preclinical safety data for human safety has been challenging. There has been a steady progress in integrating toxicity mechanisms, toxicology–pathology data, toxicokinetics, and ADME data into risk assessment. This has greatly helped to reduce the uncertainty regarding the extrapolation of animal toxicology data to estimate the probably of harm at relevant clinical exposures.

APPLICATIONS OF ADME AND TOXICOKINETICS IN DRUG DISCOVERY AND DEVELOPMENT

Early optimization of promising discovery candidates

Drug discovery process starts with a hypothesis, including the identification of biological target related to a given disease or illness, and strategies to block the specific target without ensuing toxic effects. A specific disease target may involve an up-regulated target enzyme or receptor, and its blockage may lead to effective treatments. Once a suitable target is characterized, molecules are screened to identify hits with desired biological activity. Prior to undertaking *in vivo* investigations, adequate *in vitro* and *in vivo* pharmacokinetics and metabolism characteristics are usually obtained.

Toxicokinetics information and its utility in toxicity studies

Because of the limitations associated with the frequency of sampling and total blood withdrawn in a given time, toxicokinetic information from an integrated toxicity-toxicokinetic protocol is limited to an assessment of *AUC*, C_{max}, T_{max}, C_{min}, and the ratio of C_{min}/C_{max}. Figure 2.3 shows the potential utility of toxicokinetics in toxicity studies. Below is a scientific discussion on the basics of toxicokinetic information (Figure 2.4).

Extent of drug absorption: rate and extent of drug exposure

The rate and extent of drug entering the blood stream following drug administration is extremely important. A drug may be very rapidly absorbed (rapid attainment of C_{max} with T_{max} of generally less than 2 h) at the low dose; however, solubility- or dissolution-limited drug absorption at

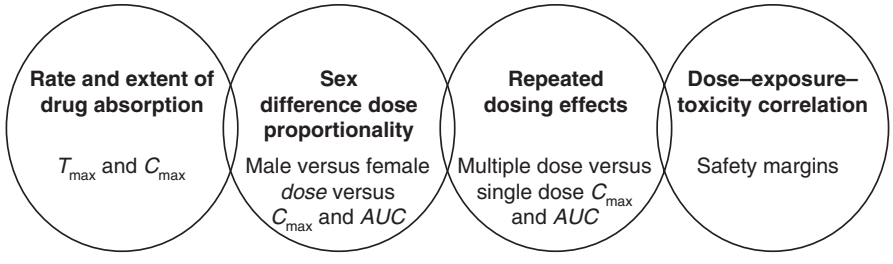

FIGURE 2.3 *Applications of toxicokinetics in non-clinical toxicology studies.* Preclinical toxicokinetics data when generated along with toxicology studies may have multiple applications. The toxicokinetics data have many applications including the following: (1) rate and extent of drug absorption by knowing how fast a drug enters plasma and to what extent it achieves maximal concentrations; (2) sex differences in exposures help to interpret any potential sex differences in toxicity or differences in dose proportionality in exposures; and (3) repeated dosing effects on exposures by comparing single and multiple dose (typically at presumed at steady state) toxicokinetics (e.g. C_{max} and *AUC*) (see text for additional discussion).

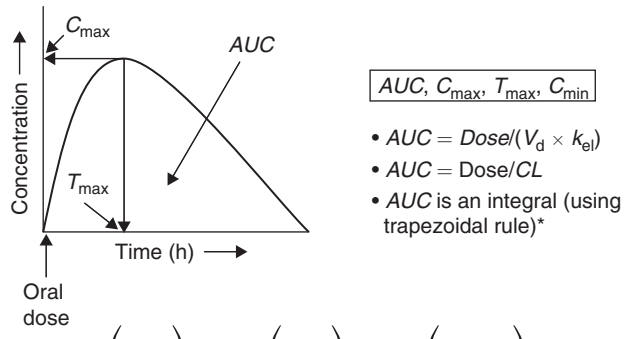

FIGURE 2.4 *Basic toxicokinetics parameters*. In toxicokinetics studies, the assessment of toxicokinetics is often limited to the assessment of some very limited parameters, such as C_{max}, T_{max}, and *AUC*. This is due to limited blood sampling along with toxicity evaluation. The *AUC* is the most important pharmacokinetic parameter which can be estimated by statistical methods (trapezoidal rule) or by equations using V_d, k_{el} (elimination constant), and drug clearance (*CL*). Because the correct estimate of k_{el}, V_d, and drug clearance is difficult to attain in oral toxicokinetics studies, it is a common practice to use trapezoidal rule to estimate the *AUC*.

the middle and high doses may lead to slower rise in concentration with longer T_{max} and sustained concentrations. The rate of drug exposure has significant impact on overall safety profile of a drug. There are a large number of biological and drug derived factors that may impact the overall process of drug absorption (Curatolo, 1998). The total amount of drug circulating through blood relative to the administered dose in mg/kg is critical in interpreting the dose response in toxicity and establishing thresholds for adverse effects. Drugs that are poorly or incompletely absorbed due to solubility/dissolution-related problems may leave a large amount of unabsorbed drug in the GI tract. Chronic accumulation of large amounts of unabsorbed drug in the intestinal tract following chronic repeated oral dosing may result in adverse effects on GI homeostasis (e.g. nutrient absorption, GI emptying, changes in GI microflora, chronic GI irritation, and inflammation) and toxicity may arise secondary to these local adverse effects. The local untoward effects due to unabsorbed drug may not be relevant for humans because most drugs are administered at very-low-dosage strengths. In oral studies conducted at high doses for safety testing it is not possible to obtain information on percent bioavailability because of the lack of exposure data at relevant intravenous doses. The extent of absorption can be roughly estimated by comparing the ratio of C_{max} to *AUC*; however, when the absorption is slow and sustained, it is difficult to know the extent of absorption. Drugs that have an effect on GI homeostasis, including pH, changes in gut microflora, gastric and intestinal secretions and enzymes, GI motility, and first-pass intestinal metabolism can substantially alter the rate and extent of drug absorption. Additionally, drug induced GI toxicity can also have a detrimental influence on the systemic availability of drug and its major metabolites. The time (T_{max}) to reach C_{max} may be used to monitor certain adverse effects which may be peak concentration dependent and may include CNS adverse effects (e.g. tremors, convulsions), cardiovascular effects (e.g. ECG changes, blood pressure changes), and certain hormonal effects. For certain target organ toxicities that require a sustained presence of drug-related substances, it is critical to fully establish plasma concentration versus time profile and its relationship to target organ toxicity. This information is extremely important when oral dietary route of administration is chosen. Differences in treatment regimens, dosing schedules, or oral routes can produce differences in toxicities because of the mechanistic differences in patterns of drug exposures and adverse effects. Because of the advances in high throughout screening and tendency to generate metabolically stable molecules, newer drug molecules tend to be bulky and poorly soluble in water. Increasingly it is being recognized that these large lipophilic molecules are often poorly absorbed by animal species providing very limited drug exposure for toxicity testing.

Increases in solubility with lipophilic vehicles, including polyethylene glycols (PEG) 300/400, Imwitor, propylene glycol, sorbitol, Tween (polysorbate 80), acidified carboxymethyl cellulose, hydroxypropylcellulose/sucrose/sodium lauryl sulfate (SLS), cremophor, cyclodextrin, and span (sorbitan monoester), have been successfully obtained (Dressman, 1989; Crowley and Martini, 2001). Many of these lipophilic vehicles have been successfully used to enhance absorption for poorly soluble drugs. It is to be emphasized when attempting to enhance exposure maximum caution must be taken to assure that the selected vehicles and their dosing volumes will be well tolerated by preclinical animal species and the toxicity of the formulated drug will not be enhanced by the direct adverse effects of formulation other than what is expected from the increase in exposures.

The rate of drug elimination has an important effect on the extent of drug exposure. Because of the difficulty in knowing when absorption is complete and when elimination begins after oral dosing, it is often difficult to precisely calculate elimination half-life. Drug elimination can be semiquantitatively estimated by examining the ratio of trough (C_{min}) and maximal concentration (C_{max}). Drugs with rapid elimination (e.g. short half-life, low C_{min}/C_{max} ratio) generally tend to be less toxic; however, drugs with slower elimination (e.g. longer half-life, high C_{min}/C_{max} ratio) can result in large accumulation of drug after repeated chronic dosing leading to unexpected toxicities. It is to be emphasized however, when a drug is largely cleared via metabolism and the

metabolite(s) is toxic, significant adverse effects can occur with a drug that has a short half-life.

Species-specific sex differences in metabolism leading to exposure differences

It is not an uncommon finding in toxicology studies to have males and females responding to a drug differently. Therefore, it is usually of significant interest to find out if differences in toxicity are due to toxicokinetics differences or related to differences in susceptibility. Sex-related differences in drug metabolism are generally more common in laboratory rats than in other species, including humans, mice, dogs, and monkeys (Skett, 1988; DeBethizy and Hayes, 1994; Shapiro *et al.*, 1995). As early as 1937, Holck *et al.* demonstrated that female rats slept longer than male rats when given hexobarbital (Holck *et al.*, 1937). Subsequently it was proven that prolonged sleeping time was due to a slow metabolism of hexobarbital in female rats than in male rats. Generally, male rats tend to have more CYP per gram liver and greater rate of CYP-dependent metabolism than female rats which may markedly impact the metabolic clearance and overall exposure of the drug. Sex-related differences are developmentally regulated (Dressman, 1989) and appear more frequently in sexually mature rats. Generally, male rats have higher activities of certain important most abundant CYP enzymes than females; however, female rats have higher activities of certain specific CYP enzymes than males (Lin and Lu, 1997). For example, CYP2A2, CYP3A2, and CYP2C11 are male-dominant; however, CYP2A1, CYP2C7, and CYP2C12 are generally female-dominant (Holck *et al.*, 1937; Skett, 1988; Dressman, 1989; DeBethizy and Hayes, 1994; Shapiro *et al.*, 1995). Differential expression of sex-dependent CYPs leads to sex differences in drug exposure. The sexual dimorphic secretion pattern of growth hormone (constant low-level secretion in females versus pulsatile secretion in males) and sex hormones (e.g. testosterone and estrogen) directly regulates the expression of certain hepatic CYPs (CYP2C11 and CYP2C12) in male versus female rats (Kato and Yamazoe, 1990; Legraverend *et al.*, 1992a, b; Waxman *et al.*, 1985, 1990). It is also important to note that as male rats age, their metabolism declines to resemble young female rats (i.e. sexual dimorphism declines). This is due to changes in growth hormone patterns with older male rats exhibiting a sustained release of growth hormone (versus pustule pattern) similar to young female rats. When considering the fact that male rats predominately have higher activity of many CYP enzymes, it is not too surprising that male rats tend to have lower drug exposures than female rats and may show a reduced toxicity.

Other safety species show substantially less and infrequent sex-related differences in metabolism, including species like mice (Macleod *et al.*, 1987), ferrets (Ioannides *et al.*, 1977), and dogs (Dogterom and Rothuizen, 1993). Humans tend to show a relatively less frequent sex difference in drug metabolism. When sex differences in pharmacokinetics are observed in humans, these differences appear to be specifically related to anatomical and physiological differences (e.g. body weight, height, etc.) that may indirectly impact the ADME processes.

Dose proportionality in toxicokinetics

A clear understanding of changes in systemic exposures with increasing doses is critical to interpreting the toxicity response. Given the species differences in rates and extent of ADME process, the comparison of systemic exposure versus doses administered is one of the most practical means of assessing margins of safety (exposure at NOAEL in animals/desired therapeutic exposure). In toxicity studies where doses may vary over several log orders of magnitude, the non-linearity in exposure with increasing doses is relatively a common finding. In interpreting dose proportionality, one also needs to carefully look at the variability across the mean or median values because preclinical animal species tend to vary a lot in ADME processes. These data are best compared when normalized for dose. For example, a dose comparison of AUC/dose will give the best assessment of dose proportionality.

Several possible scenarios may exist with regard to dose proportionality. In scenario A, the increase in exposure is proportional to increase in dose. This usually happens with drugs that follow a linear ADME process. A plot of dose versus AUC/dose will show no or little change with increasing doses. In case B, the increase in exposure is clearly greater than dose proportional and with increasing doses, AUC/dose will increase. This process generally occurs when the kinetics of the drug is dependent on one or multiple saturable processes. The saturable processes may include the following: (a) plasma and tissue protein binding; (b) metabolic enzymes, including saturation by substrate, depletion of cofactors, and product inhibition; (c) renal tubular secretion and tubular reabsorption; and (d) biliary excretion. All saturable processes typically follow Michaelis–Menton kinetics. With increasing concentrations the processes can get saturated (i.e. when concentration exceeds K_m (plasma concentration needed to cause 50% saturation)), and the elimination follows a zero-order reaction ($dC/dt = V_{max}$). Under these circumstances, drug accumulation with high trough concentration may occur with increasing doses. When absorption is limited by dissolution and the saturable clearance occurs in parallel, a sustained plasma concentration versus time profile is often observed with increasing doses. In case C, with increasing doses there is a less than dose proportional increase in exposure. A plot of dose versus AUC/dose will decrease with increasing dose. With increasing doses, the rate and extent of absorption decreases and/or rate of elimination increases. The