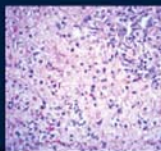
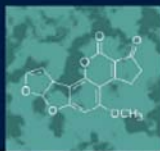


EDITED BY  
RAMESH C. GUPTA



# VETERINARY TOXICOLOGY

BASIC AND  
CLINICAL PRINCIPLES



# VETERINARY TOXICOLOGY

*This book is dedicated to my daughter Rekha, wife Denise, and parents the late Chandra and Triveni Gupta*

# VETERINARY TOXICOLOGY

## Basic and Clinical Principles

---

Edited by

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*There are in fact two things, science and opinion; the former begets knowledge, the latter ignorance*

Hippocrates (460BC–377BC)

# 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

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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

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# Part 1

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## General

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# 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



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](http://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](http://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](http://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](http://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

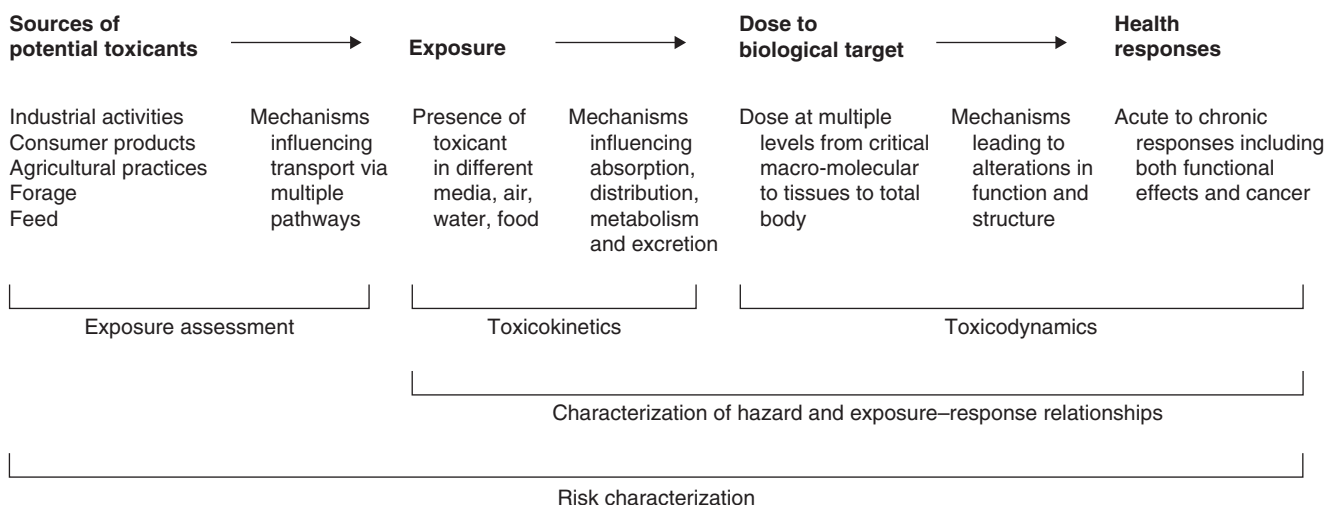


FIGURE 1.1 Critical linkages for integrating information from sources of toxicants to the development of adverse health effects.

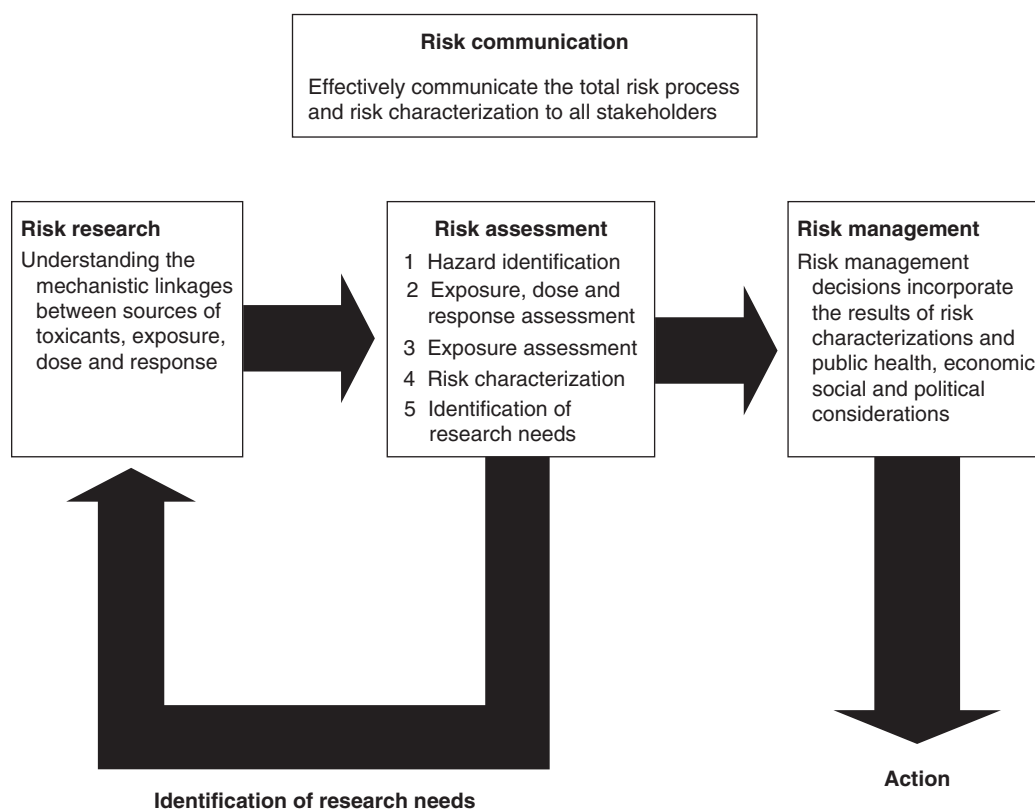


FIGURE 1.2 Risk paradigm for evaluating potential health impacts of a toxicant.

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

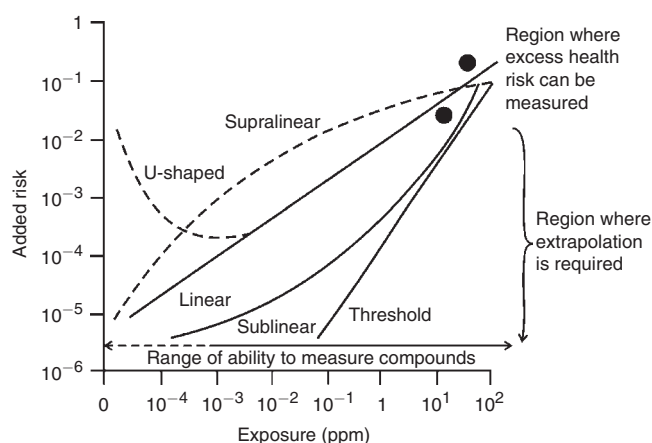


FIGURE 1.3 Schematic rendering of exposure–response relationships for various toxicants.

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.

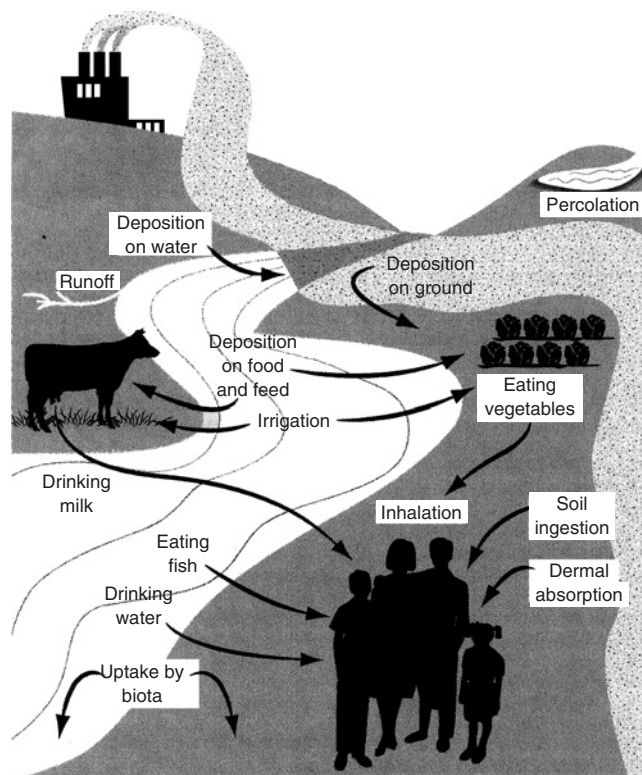
## A FRAMEWORK FOR ACQUIRING INFORMATION

### 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

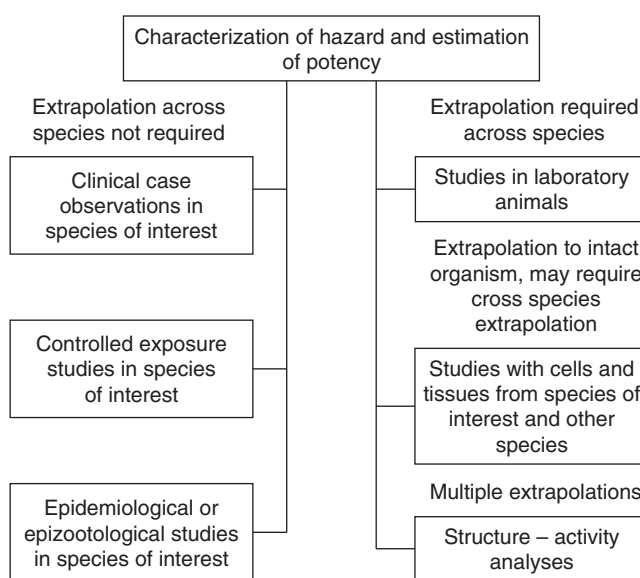
more specialized with many focusing their clinical skills on a single species. This book does not include a section addressing the toxicology of individual species. To have done so would have substantially increased the size of this text. However, chapter authors have endeavored to discuss species variations 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.

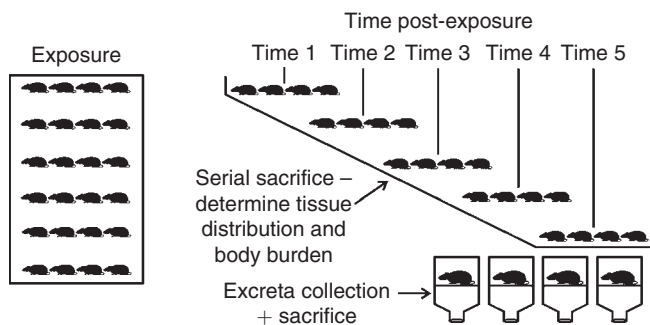
It is important to recognize that even with today's level of knowledge of these extrapolation issues, it is not possible to estimate, with absolute certainty, the precise numerical level of human exposure to a given agent that may be without any risk of potential harm or will produce a specific level 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.

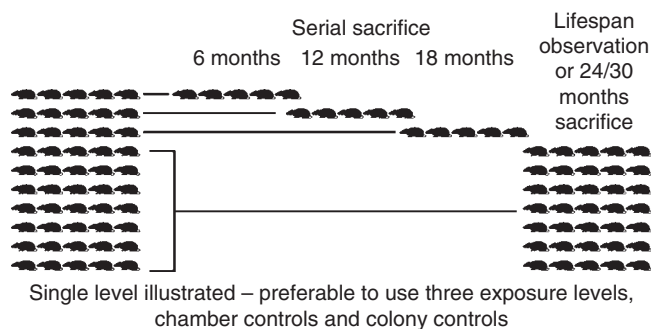
agent that is to be parenterally administered. However, the resulting data may be of limited relevance to other routes of intake. For example, it may not appropriately mimic oral intake since only a small fraction of some toxicants may be absorbed from the gastrointestinal tract. In short, the route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

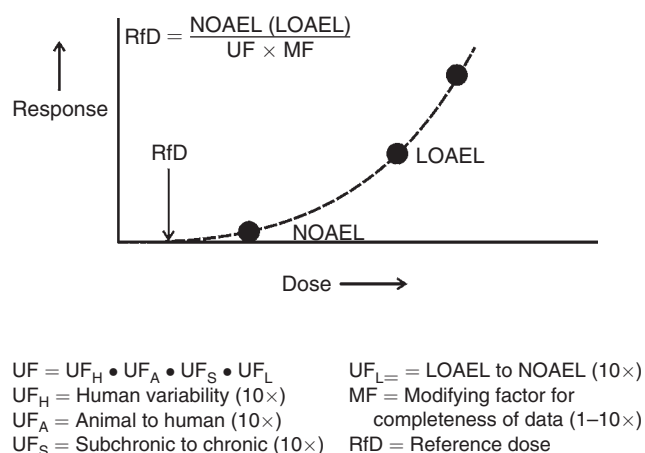


**FIGURE 1.7** Schematic rendering of an experimental design for evaluating exposure (dose)-response relationships for a toxicant.

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.



**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 $V_d$ , 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.

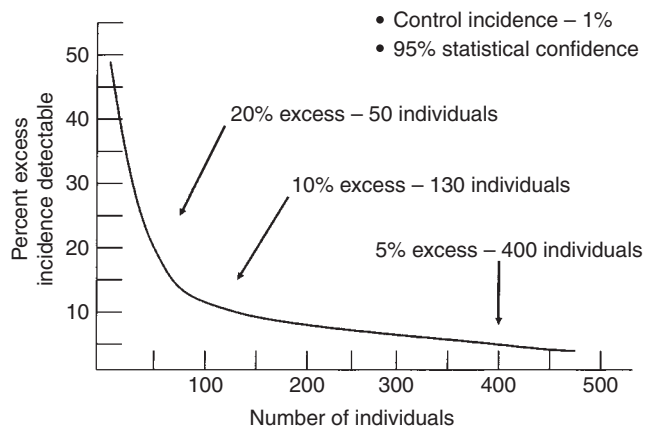
In chronic studies, major attention is directed to evaluating any toxicant-induced changes in animals at the several exposure levels compared to controls over a 2-year period or until a defined mortality level is reached, such as 20% surviving. Any changes in various indices of morbidity or pathological 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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