# Principles and Methods of Toxicology

*Second Edition*

A. Wallace Hayes

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Editor

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# Preface

The First Edition of this textbook was designed primarily for courses dealing with an evaluation of toxicologic data with a particular emphasis on those methodologies used in toxicology. This Second Edition has been expanded to include a more systematic approach to toxicology without losing its methodological basis. This edition describes current testing procedures, offers useful guidelines on data interpretation, and highlights major areas of controversy. Every effort has been made to keep the book simple and suitable for use as a textbook for graduate teaching.

Since toxicology is the study of the harmful action of chemicals on biologic tissues, it necessitates an understanding of biologic mechanisms as well as the methods employed to examine these mechanisms. However, the vastness of the field of toxicology and the rapid accumulation of data preclude the possibility of any one individual absorbing and retaining more than a fraction of these techniques. There are, however, specific methods that are applicable to a large number of chemicals. An understanding of the principles underlying these methods is not only manageable but essential. Thus, individuals who are not directly involved with the day-to-day activity of toxicology, or who have not yet entered a specialized field in toxicology, will find this book a valuable resource in acquiring a broad understanding of toxicological approaches available.

This volume has been designed to serve as a textbook for, or adjunct to, courses in general as well as advanced toxicology. The overall framework of the Second Edition follows that of the initial volume with the exception that major sections on principles related to toxicology have been added. A number of new authors have been added to this edition to broaden input and provide coverage of the ever-changing field of toxicology. New chapters have been added on metabolism, food-borne toxins, solvents, pesticides, and on the regulatory process as it relates to toxicology.

The only true "facts" in biology are the results of individual experiments carried out under control conditions by carefully defined methodology. Although it is not the purpose of this volume to catalog or to discuss these biologic "facts," it is the purpose of this book to present those methodologies which can generate these facts. Achievement of this goal requires the more or less arbitrary resolution to select methods and testing protocols from the current literature. The bibliography of each chapter will carry the reader beyond the techniques and methods presented in the book.

This volume has been organized to best facilitate its use. The first section covers basic toxicologic principles including the philosophies underlying testing strategies. The second section covers basic toxicologic testing methods and includes most of the testing procedures now required to meet regulatory standards. The third section deals with specific organ systems and contains chapters on kinetics and effects on cellular organelles and target organs. Each method or procedure is discussed from the standpoint of technique and interpretation of data. A state-of-the-art approach is emphasized as are the various problems and pitfalls encountered. Each chapter contains information that allows a person to perform an experiment or test a protocol, and also provides insight into the rationale behind the experiment.

*Principles and Methods of Toxicology, Second Edition,* will be useful as both a text for introductory courses in toxicology and as a valuable, timely review for the practicing toxicologist. Research scientists who have used the first edition as a reference source will find updated material in areas of their special or peripheral interest.

*A. Wallace Hayes*

# Preface to the First Edition

This volume has been designed primarily as a textbook for courses dealing with an evaluation of toxicologic data. It provides a thorough, systematic introduction to toxicology. It describes the most current testing procedures, offers useful guidelines on data interpretation, and highlights major areas of controversy. Every effort has been made to keep the book simple and suitable for use as a textbook for graduate teaching.

Since toxicology is the study of the harmful action of chemicals on biologic tissues, it must of necessity involve an understanding of biologic mechanisms, which can only be accomplished through an understanding of the appropriate methods. However, the vastness of the subject, as well as the rapid accumulation of data, precludes the possibility that any one individual can absorb and retain more than a small fraction of these techniques. It is evident, however, that certain specific methods are applicable to a large number of chemicals, and an understanding of the principles underlying each method is essential. For those individuals not directly involved with the day-to-day activity of toxicology or who have not yet entered a specific field in toxicology, this book will be a valuable resource to select from the many representative approaches available.

The only true "facts" in biology are the results of individual experiments carried out under control conditions by carefully defined methodology. Although it is not the purpose of this book to catalog and discuss these biologic "facts," it *is* the purpose of this book to present those methodologies that can generate these facts. Achievement of this goal requires the more or less arbitrary resolution to select from the current literature the methods and testing protocols that will allow this goal to be readily achieved. In addition, the bibliography of each chapter will carry the reader beyond the techniques and methods presented in the book.

This volume has been organized to best facilitate its use. The first section covers basic toxicologic testing methods and includes most of the testing procedures now required to meet regulatory standards. The second section deals with specific organ systems and contains chapters on kinetics, metabolism, and effects on cellular organelles and target organs. Each method or procedure is discussed from the standpoint of technique and interpretation of data. The "state-of-the-art" approach is emphasized as are the various problems and pitfalls encountered. Each chapter contains information that not only allows a person to perform an experiment or test a protocol, but also provides insight into the rationale behind the experiment.

*Principles and Methods of Toxicology* will be useful as both a text for introductory courses in toxicology and as a valuable, timely review for the practicing toxicologist.

*A. Wallace Hayes*

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# Contents

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CHAPTER 1

# The Use of Toxicology in the Regulatory Process

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## BACKGROUND

Since ancient times, man has attempted to reduce or eliminate the risk of disease from toxic materials. Early regulatory authorities focused much of their attention on adulterated food and drugs. Undoubtedly, this reflected the relative ease in associating acute health effects, such as food poisoning, with exposure to contaminants in the diet or in medications. Thus Hutt (59) quotes Pliny the Elder, writing in the 1st century A.D., as stating, "So many poisons are employed to force wine to suit our taste—and we are surprised that it is not wholesome!"

Recently, particularly during the last 20 years, government agencies have increased their efforts to develop and implement legislation to protect people from toxic chemicals. The increase in effort is due to several factors:

1. The realization that the presence of environmental chemicals is pervasive. The publication of *Silent Spring* by Rachel Carson in 1962 (26), detailing the presence of the pesticide DDT in the environment and its effects on wildlife, has had a major influence here.
2. The additional realization of the vast number of environmental chemicals to which man has been exposed, especially since World War II. Of the more than 5,000,000 known chemicals, approximately 70,000 are in commercial use today (45).
3. The establishment of a causal relationship between certain diseases and environmental exposure. The asbestos-induced diseases—asbestosis, lung cancer, and mesothelioma—are examples of the types of debilitating illnesses that are attributed to environmental exposure.
4. The reduction in microbial disease, which is a result of improved sanitation, has altered the focus of society to other causes of ill health. As the standard of living has improved, the public has demanded greater protection from chemically induced disease than in the past.

At the federal level in the United States, four agencies bear most of the direct responsibility for the regulation of toxic chemicals—the Food and Drug Administration (FDA), the Occupational Safety and Health Administration (OSHA), the Consumer Product Safety Commission (CPSC), and the Environmental Protection Agency (EPA). Table 1 describes the acts that empower these and several other agencies.

It is clear from Table 1 that there is a broad range of chem-

**TABLE 1.** *Federal laws related to exposures to toxic substances*

| Legislation | Agency | Area of concern |
|---|---|---|
| Food, Drug and Cosmetics Act (1906, 1936, amended 1958, 1960, 1962, 1968, 1976) | FDA | Food, drugs, cosmetics, food additives, color additives, new drugs, animal and food additives, and medical devices |
| Federal Insecticide, Fungicide and Rodenticide Act (1948, amended 1972, 1975, 1978) | EPA | Pesticides |
| Dangerous Cargo Act (1952) | DOT, USCG | Water shipment of toxic materials |
| Atomic Energy Act (1954) | NAC | Radioactive substances |
| Federal Hazardous Substances Act (1960, amended 1981) | CPSC | Toxic household products |
| Federal Meat Inspection Act (1967); Poultry Products Inspection Act (1968) | USDA | Food, feed, color additives, and pesticide residues |
| Egg Products Inspection Act (1970); Occupational Safety and Health Act (1970) | OSHA, NIOSH | Workplace toxic chemicals |
| Poison Prevention Packaging Act (1970, amended 1981) | CPSC | Packaging of hazardous household products |
| Clean Air Act (1970, amended 1974, 1977) | EPA | Air pollutants |
| Hazardous Materials Transportation Act (1972) | DOT | Transport of hazardous materials |
| Clean Water Act (formerly Federal Water Control Act; 1972, amended 1977, 1978) | EPA | Water pollutants |
| Marine Protection, Research and Sanctuaries Act (1972) | EPA | Ocean dumping |
| Consumer Product Safety Act (1972, amended 1981) | CPSC | Hazardous consumer products |
| Lead-based Paint Poison Prevention Act (1973, amended 1976) | CPSC, HEW (HHS), HUD | Use of lead paint in federally assisted housing |
| Safe Drinking Water Act (1974, amended 1977) | EPA | Drinking water, contaminants |
| Resource Conservation and Recovery Act (1976) | EPA | Solid waste, including hazardous wastes |
| Toxic Substances Control Act (1976) | EPA | Hazardous chemicals not covered by other laws, includes premarket review |
| Federal Mine Safety and Health Act (1977) | DOL, NIOSH | Toxic substances in coal and other mines |
| Comprehensive Environmental Response, Compensation, and Liability Act (1981); Superfund Amendments and Reauthorization Act (1986) | EPA | Hazardous substances, pollutants and contaminants |

Adapted from ref. 86.

ical exposures with which regulatory authorities are concerned. Chemicals may be regulated by an environmental medium (e.g., air and water), by usage (e.g., food and consumer products), and by the type of environment (e.g., workplace). While the acts in Table 1 represent 80 years of legislative history, 14 of the 19 have been written since 1970.

The language, philosophy, and historical context of the different acts have had an important influence on how agencies function in establishing exposure limits for environmental chemicals or other activities relevant to their mission (e.g., setting priorities for hazardous waste-site clean-up). For example, Section 109 of the EPA's Clean Air Act, which forms the basis for setting ambient air quality standards, focuses solely on the protection of public health. Section 109 states that the EPA must set "ambient air quality standards the attainment and maintenance of which in the judgment of the Administrator, based on such criteria and allowing an *adequate margin of safety,* are requisite to protect the public health" (emphasis added) (116). Congress did not define "an adequate margin of safety" nor did it specify that the EPA should consider feasibility or costs in standard setting.

In contrast, Section 6 of the Toxic Substances Control Act (TOSCA; 18) states that for chemicals which "present(s) an unreasonable risk of injury to health or the environment" the EPA needs to "protect adequately against such risk using the least burdensome requirement." Here, the focus is not only on the protection of public health, but also on the costs associated with such protection.

While "adequate margin of safety" and "unreasonable risk of injury to health" have not been clearly defined, over the past six or seven years agencies have generally interpreted this language as requiring a qualitative, and frequently quantitative, estimate of the health risks associated with an exposure and the reduction in risks resulting from regulatory action. A major factor in the increased use for risk analysis was the Supreme Court decision in 1980 in the case of Industrial Union Department, AFL-CIO v. American Petroleum Institute. In this case, OSHA proposed lowering the

occupational standard for benzene from 10 to 1 ppm on the basis that benzene was a carcinogen, that any reduction in exposure would result in a reduction in risks, and that 1 ppm was technologically feasible. The Supreme Court did not find for the Union, stating that "Before he can promulgate any permanent health or safety standard, the Secretary [of Labor] is required to make a threshold finding that a place of employment is unsafe—in the sense that *significant risks* are present and can be eliminated or lessened by a change in practices" (emphasis added) (59a). The Court left the decision of what constitutes a "significant risk" to OSHA. This landmark decision has had a major impact on agencies in addition to OSHA, resulting in an increase in the development and use of tools to quantify risks from exposure to environmental chemicals.

The primary focus of this chapter is on the use of regulatory toxicology at the federal level. However, recent activities in the state of California regulating exposure to carcinogens and reproductive toxins in food, drinking water, and other media merit discussion. In 1986, California passed the "Safe Drinking Water and Toxic Enforcement Act of 1986," commonly referred to as Proposition 65. This act contains two major provisions—one prohibiting the "discharge or release [of] a chemical known to the state to cause cancer or reproductive toxicity into water" and the other, a labeling requirement, mandating that no person expose another individual to any carcinogen or reproductive toxin without providing "clear and reasonable warning." Exemptions for carcinogens are provided for exposure levels that pose "no significant risk assuming lifetime exposure" and, for reproductive toxins, for exposures at 1/1000 the observed effect level.

The broadness of the language of the act has created much concern among both the environmental community and the regulated industries. Issues include the information required to label a chemical as a carcinogen or a reproductive toxin, distinctions between naturally occurring and added toxins, and the regulatory authority of the state versus the federal government. In theory, the regulation could be applied to a very large number of chemicals under many exposure scenarios. However, the latitude with which the act will be interpreted and its eventual magnitude of impact are still unclear. Many of the issues raised are similar to those debated in the federal regulatory arena, for example, regarding the relevance of animal response to human response and the definition of significant risk levels.

There are four main agencies with authority to regulate chemicals at the federal level, but other governmental and nongovernmental agencies can influence the regulatory process as well. The American Conference of Governmental Industrial Hygienists (ACGIH) sets exposure limits based solely on health protection for approximately 600 workplace chemicals. These exposure limits, known as Threshold Limit Values or TLVs (4), do not carry any regulatory weight, but it is not uncommon for workplaces to adhere to the TLVs for chemicals that OSHA does not regulate or which have an exposure limit that has not been revised since the inception of OSHA in 1970. The TLVs have also been used by several state environmental agencies to derive acceptable ambient levels for toxic air pollutants (119).

Agencies in the Department of Health and Human Services that influence the regulatory process include the National Cancer Institute; the National Institute of Environmental Health Sciences, in particular the National Toxicology Program; the National Institute for Occupational Safety and Health (NIOSH) and the Center for Environmental Health (part of the Centers for Disease Control, CDC); and the Agency for Toxic Substances and Disease Registry (114). These agencies affect the process in several ways, ranging from decisions on which chemicals to test in long-term cancer bioassays to defining principles for evaluating carcinogens, and to site-specific (as with a hazardous waste site) and chemical-specific risk assessments. International organizations such as the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) also have had a significant role in the use of information by regulatory agencies.

## INFORMATION USED IN THE REGULATORY PROCESS

Three categories of scientific information are employed by agencies in the evaluation and regulation of toxic chemicals in the environment: (a) epidemiology, (b) controlled clinical exposures, and (c) animal toxicology.

*In vitro* studies and evaluation of structure–activity relationships are also employed by regulatory agencies, although to a lesser extent than the three main categories cited above. *In vitro* studies and structure–activity relationships are often used to support the interpretation of information from the three major categories.

Epidemiology, studies of clinical exposures, and animal toxicology provide qualitatively different information, with unique advantages and limitations. Environmental epidemiology studies, which attempt to associate disease or other adverse outcomes with an environmental exposure, have the advantage of measuring an effect in humans at exposure conditions that are by definition realistic. The first demonstration that benzene was a carcinogen came from epidemiological studies of rubber workers (60). It wasn't until several years after these studies (68) that benzene was shown to cause cancer in animal studies. Studies of the London smog pollution episode in 1952 demonstrated that high levels of pollution from coal combustion could cause mortality, particularly in the very young, the elderly, and those individuals with pre-existing cardiopulmonary disease (66). Evaluation of similar effects in animal studies would be difficult, given the complexity of the exposure in London and the lack of good animal models for certain susceptible populations, such as asthmatics. In general, epidemiology has been particularly helpful in the evaluation of working environments or other environments where exposure concentrations are relatively high.

Several factors limit the use of epidemiological studies by regulatory agencies. One of the major limitations is the lack of good exposure information, for both chemical species and for actual concentrations. The lack of good exposure information limits the ability to quantify the effects of ambient air pollution in the United States. The Harvard University Six City Study has shown that outdoor $NO_2$-monitoring de-

vices are inadequate in accurately assessing exposure to $NO_2$, due to the importance of indoor exposure (95).

Another limitation is that epidemiological studies of worker populations may be unsuitable for prediction of health effects in the general population. The general population is more heterogeneous than the worker population and, for some pollutants, may exhibit a greater range in susceptibility. Thus, studies of lead workers would greatly underestimate the toxicity of this pollutant in the population of concern for ambient exposures, namely children under the age of 5 years (120).

Epidemiology studies are frequently limited by the need for a relatively large increase in disease incidence (twofold or more), given the sample sizes generally available to such investigations. For example, Enterline (41) notes that it would require a large population (1000 deaths using the Peto model) to detect a 50% excess in deaths from lung cancer at an asbestos level of 2 f/cm$^3$.

Lastly, epidemiological investigations can show a correlation between an exposure and an effect in the population only after the harm has occurred. From a public health perspective, this is disadvantageous, since such studies would not prevent the occurrence of disease. Recent efforts to utilize biological markers of exposure or effect of exposure, such as DNA adducts and urinary mutagens (91), as part of epidemiology studies may result in preventive actions before the occurrence of disease.

Controlled clinical studies of humans exposed to pollutants address some of the difficulties of epidemiology studies. The exposures can be controlled and quantified, the effects are observed in humans, and the exposed population can be chosen to consist of susceptible individuals, such as asthmatics or exercising individuals. Thus, changes in airway resistance in asthmatics exposed to $SO_2$ during exercise (12,99) have been important in the EPA's evaluation of the National Ambient Air Quality Standard (NAAQS) for this pollutant, both in terms of the appropriate exposure concentration and the relevant averaging time. Given the subtlety of these changes (nonsymptomatic bronchoconstrictions) and the fact that they occur only in a selected subset of the general population (asthmatics constitute about 4% of the total population), these effects would not have been detectable in the general population.

However, one of the advantages of controlled clinical exposure studies is that they are performed with humans, and this is also their major limitation. Since these studies must be limited to short-term effects that are readily reversible, they cannot be used to evaluate the potential of a chemical to cause chronic disease. Furthermore, because of the mildness of the changes observed in these studies, one may question their clinical significance. For example, how does one interpret the change in resistance observed with $SO_2$ exposure, given that, if not perceptible, the relationship to physical performance may be questionable (51)? Also, although some susceptible populations, such as mild asthmatics, can be tested, individuals with a greater degree of impairment, such as asthmatics who require continual medication, are usually not considered to be appropriate subjects for these studies because of the greater potential for harm during exposure.

Animal toxicology studies constitute the third major source of information for assessing the toxicity of chemicals. Animal toxicology studies allow the investigator the greatest degree of control over the exposure conditions, the population exposed, and the effects measured. One can readily evaluate the very subtle effects of acute and chronic exposure. For example, recent studies demonstrated morphological and numerical changes in the pulmonary type I and type II cell populations in rats exposed to 0.12 ppm $O_3$ (32). It would have been very difficult to describe this effect with other approaches, and yet the effect is clearly of concern for humans who are exposed at comparable concentrations in the ambient environment, although for shorter time periods.

The ability to manipulate the experimental conditions permits the evaluation of many variables on the response to toxic chemicals. Thus, Elsayed and Mustafa (40) were able to demonstrate the protective effect of vitamin E on the acute toxicity of $NO_2$ in mice. The role of metabolism in susceptibility to polycyclic aromatic hydrocarbon-induced carcinogenesis has been evaluated in studies of genetic variants in mice (65). Such studies can be important in predicting modifiers of toxicity in humans and in predicting the susceptible human populations.

Lastly, animal toxicology studies are uniquely suited to the study of novel pollutants for which epidemiology studies are premature and for which clinical exposure studies would be unethical, due to uncertainties about the potential health effects. Using an animal bioassay, Beck and Brain (10) were able to demonstrate that emissions from residential coal-burning stoves were highly toxic and that emissions from wood-burning stoves were moderately toxic to the lungs as compared with the potent pneumotoxin, alpha-quartz.

The limitations of animal studies fall into two broad categories: (a) those due to difficulties in extrapolating from animals to humans, and (b) those due to difficulties in extrapolating from the high exposures in animal studies to the lower exposures typically experienced by humans. Interspecies extrapolation is complicated by the greater homogeneity of laboratory animals than humans, the controlled conditions of housing and diet, innate genetic factors, and other variables. The relevance of trichloroethylene (TCE)-induced hepatocarcinogenesis in the mouse to humans has been questioned on the basis of differences in peroxisomal proliferation in the liver in the two species (1,39). Similarly, it is possible that effects may occur at the high exposure concentrations typically used in animal studies that are not relevant to effects predicted at ambient exposure concentrations, where detoxification pathways are not saturated. Large collections of macrophages with unusual appearance are observed in rats exposed to high concentrations of diesel particulates (127), under conditions where particle clearance mechanisms from the lungs are probably overwhelmed. Such macrophages are not observed in rats at lower exposure concentrations, and their significance to humans who are exposed to diesel particulates in orders of magnitude lower than those employed in the animal studies is of question. A summary comparing the differences between epidemiology, controlled clinical exposure, and animal toxicology studies is provided in Tables 2–4.

We can conclude from the preceding discussion that there is no "best" source of information for regulatory agencies.

**TABLE 2.** *Advantages and disadvantages of epidemiological studies*

| Advantages | Disadvantages |
|---|---|
| Exposure conditions realistic | Costly and time consuming |
| Occurrence of interactive effects among individual chemicals | Post facto, not protective of health |
| Effects measured in humans | Difficulty in defining exposure, problems with confounding exposure |
| Full range in human susceptibility frequently expressed | Increase in risk must be ~2× to be detected |
| | Effects measured often relatively crude (morbidity, mortality) |

The rational approach is to examine all sources of information in the evaluation of toxic chemicals. Some kinds of information may be especially useful in *hazard identification,* the likelihood that a chemical will be toxic to humans, whereas other types of information will be more appropriately applied to the estimation of the exposure–response relationship.

## RISK ASSESSMENT PARADIGM

In response to a directive from the United States Congress, the FDA contracted with the National Research Council of the National Academy of Sciences to evaluate the risk assessment process in the federal government and to make recommendations on how the process could be improved. As a result of this effort, the Committee on the Institutional Means for Assessment of Risks to Public Health published a book in 1983 entitled *Risk Assessment in the Federal Government: Managing the Process* (78). The book summarizes past experiences, and although it does not propose new ways to evaluate environmental chemicals it has nevertheless had an important effect on the use of scientific information by regulatory agencies in its codification of the risk assessment process.

**TABLE 3.** *Advantages and disadvantages of controlled clinical studies*

| Advantages | Disadvantages |
|---|---|
| Well-defined, controlled exposure conditions | Costly |
| Responses measured in humans | Relatively low exposure concentrations and short-term exposures |
| Potential to study subpopulations (e.g., asthmatics) | Limited to relatively small groups (usually <50 individuals) |
| Ability to measure relatively subtle effects | Limited to short-term, minor, reversible effects |
| | Usually most susceptible group not appropriate for study |

**TABLE 4.** *Advantages and disadvantages of animal toxicology studies*

| Advantages | Disadvantages |
|---|---|
| Readily manipulated exposure conditions | Uncertainties in relevance of animal response to human response |
| Ability to measure many types of responses | Controlled housing, diet, etc., of questionable relevance to humans |
| Ability to assess effect of host characteristics (e.g., gender, age, genetics) and other modifiers (e.g., diet) of response | Exposure concentrations and time frames often very different from those experienced by humans |
| Potential to evaluate mechanisms | |

The report has been particularly influential in two areas: (a) the separation of the risk assessment process from the risk management process, and (b) the classification of the risk assessment process into four broad components—*hazard identification, dose–response assessment, exposure assessment,* and *risk characterization.*

The Committee defined risk assessment as the "characterization of the potential adverse health effects of human exposure to environmental hazard," and noted that risk assessment also involved "characterization of the uncertainties inherent in the process of inferring risk" (78). Given the uncertainties in the data base (e.g., the relevance of an animal model to humans or the choice of model for low-dose extrapolation), the Committee described the need to develop a risk assessment policy in the choice of "inference options," which represent scientifically plausible options for the interpretation and application of scientific data.

The risk management process is the mechanism whereby regulatory agencies evaluate alternative regulatory options and choose among them. Risk management utilizes the information derived from risk assessment, and it also incorporates "political, social, economic and engineering information in the decision process." The Committee noted that the distinction between risk assessment and risk management was critical. The influence of risk management issues, such as the economic significance of a product, on the risk assessment process would seriously undermine the credibility of the risk assessment. This concern is not novel, and is exemplified in the separation between NIOSH and OSHA. NIOSH is located in the Department of Health and Human Services and is responsible for recommending standards for workplace exposures to OSHA, located in the Department of Labor. As the federal agency responsible for setting standards for workplace exposures and for implementing them, OSHA also is required to consider feasibility in the choice of exposure limits. It is not uncommon to find that exposure levels permissible by OSHA are different from the NIOSH recommended exposure levels (115).

Of course, the distinction between risk assessment and risk management is not nearly so clear in practice. For example, the choice of a low-dose extrapolation model for carcinogens, which leads to a higher estimate of risk than other models, represents a risk management decision as much as a science

policy decision. That is, the approach is conservative and provides the regulator with a greater level of confidence that the true risk to the human population is likely to be less than that expressed through the model. This approach would be consistent with prudent public health policy.

The first component of risk assessment, *hazard identification,* involves an evaluation of whether a particular chemical can cause an adverse health effect in humans. The types of information used in hazard identification include all categories described in the previous section. In hazard identification, the risk assessor must evaluate the quality of the studies (choice of appropriate control groups, sufficient numbers of animals, etc.), the severity of the effect described, the relevance of the toxic mechanisms in animals to those in humans, and many other factors.

The result is a scientific judgment that the chemical can, at some exposure concentrations, cause an adverse health effect in humans. Usually the result is not a simple yes-or-no evaluation but a weight-of-evidence estimation of the likelihood that the particular chemical is toxic. For example, studies showing that ozone can suppress pulmonary defenses against microbial agents in several species of animals (70), and information on similarities in pulmonary defenses between humans and animals (49), would lead to the conclusion that ozone exposure in humans could, under certain conditions, result in an increased susceptibility to infection.

The hazard identification process has been codified mainly for carcinogens as exemplified in the classification schemes from a variety of agencies including IARC (62), the EPA (122), and OSHA (83).

*Dose–response evaluation,* the second component of the risk assessment process, involves the characterization of the relationship between the dose of a chemical administered or received and the incidence of an adverse health effect in the exposed population. Characterizing the dose–response relationship involves a determination of the importance of the intensity of exposure, concentration × time relationships, whether a chemical has a threshold, and the shape of the dose–response curve. The metabolism of a chemical at different doses, its persistence over time, and an estimate of the similarities in disposition of a chemical between humans and animals also are involved. While the National Academy of Science report considers dose–response estimates mostly in terms of carcinogens, the evaluation of the dose–response relationships has long been a key component of pharmacology and toxicology for many chemicals (78). For example, in Chapter 6 of this book, methods are described on the use of analytical techniques for describing $LD_{50}$ studies.

In *exposure assessment,* the third component of the risk assessment process, a determination is made of the amount of a chemical to which humans are exposed. Data are frequently very limited in exposure assessment. Measures of chemicals in environmental media, such as air or soil, or in food may be available; however, the extrapolation of those levels to a dose received by humans has many uncertainties. Models exist that can describe the movement of chemicals through a particular medium and assumptions can be made regarding inhalation, ingestion, or dermal contact rates and the bioavailability of the chemical. This information can then be used to derive an estimate of the dose taken up by humans. Host factors, such as exercise, the use of certain consumer products, or the consumption of particular foodstuffs, will complicate the exposure assessment as will concomitant exposure to chemicals that may interact with the chemical of concern. The use of biological monitoring—measurement of volatile organic chemicals in exhaled breath for example (125)—as well as personal sampling devices, such as respirable particulate monitors (112), represent new ways in which the uncertainties of exposure assessment can be reduced.

The last stage of the risk assessment process, *risk characterization,* involves a prediction of the frequency and severity of effects in the exposed population. Information should be provided on both the risk to individuals and the aggregate risk of the exposed population. It is critical that the risk characterization describes the biological and statistical uncertainties in the final estimation and identifies which component of the risk assessment process (hazard identification, dose–response, or exposure) involved the greatest degree of uncertainty. Unfortunately, there is a tendency to focus solely on the *number* in the risk characterization, such as 2000 cancer deaths from toxic air pollutants in the United States each year (52), and not on the uncertainties and assumptions used in the derivation of the number. In this example, the uncertainties include the use of the upper 95% confidence of the linearized multistage model for carcinogenesis, the assumption of a 24-hr/day lifetime exposure at a ventilation rate of 20 $m^3$/day, and other variables. Because the degree of uncertainty varies greatly among risk assessments for different chemicals, the lack of consideration of uncertainty can lead to inappropriate levels of concern for different chemicals.

## EVALUATION OF CARCINOGENS

### Background

The evaluation of carcinogens by regulatory and other agencies currently represents the most developed use of animal toxicology, as compared with the evaluation of systemic toxicants for either short- or long-term effects. This is a reflection of several factors, such as the availability of more sophisticated models for cancer than for noncancer effects and the response of regulatory agencies to the great public concern for carcinogens in the environment. In this chapter, we describe some of the key issues that agencies address in the interpretation and application of scientific data on carcinogens. These issues fall into the categories of hazard identification and dose–response assessment (78). Hazard identification for carcinogens addresses two questions: (a) What is the evidence that a particular chemical is an animal carcinogen? and (b) What is the likelihood that an animal carcinogen is a human carcinogen?

The evidence that a chemical is an animal carcinogen frequently derives from long-term animal bioassays. Such studies usually consist of exposing groups of about 50 animals (typically rats or mice) to two concentrations of a chemical over the lifetime of the animals. Sex- and age-matched unexposed animals constitute the control group. At termination of the bioassay, the animals are killed and the number of