Toxicology and Risk Assessment

Principles, Methods, and Applications

edited by

Anna M. Fan
Louis W. Chang

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To
Rocky Cheuk
and
Jane C. Wang-Chang

our spouses, for they are like the wind beneath our wings, giving us constant and much needed support.

Anna M. Fan
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Foreword

The problem with toxicology is not the practicing toxicologists, but chemists who can detect precisely toxicologically insignificant amounts of chemicals.
(René Truhaut, Late Professor of Toxicology, University of Paris, 1909–1994)

Our theories are the mirrors in which we see ourselves. (Unknown)

There have been monographs dealing with toxicology in which risk assessment played an incidental role. There have been other books and reviews on risk assessment in which the question of the underlying toxicological phenomena was not the main emphasis. The current monograph, to be published toward the end of this century, combines—rightfully so—the essentials in toxicology logically extending into risk assessment.

Although the concept of toxicology is ancient, in practice, the field of toxicology was a specialty within the discipline of pharmacology. It was only about 1960 that toxicology began to establish itself as a field in its own right.

Overall, toxicology attempts to define possible adverse effects in humans through laboratory research, or to review and explore in the field observations of certain toxic or adverse effects in humans. These can be quite varied, from the occurrence of poisoning from overdosages of drugs, of alcoholic beverages, or from exposure to certain products at the place of work, or combinations thereof. A major early concern, therefore, was in occupational toxicology.

Professional pursuits, and also widespread media attention, in recent decades, have singled out the observation and evaluation of chronic chemical exposures leading to cancer, allergies, neurotoxicity, or to effects on the immune system. In many instances, it is the question of cancer that has caught the imagination of the public, with no discrimination of whether justified, or scientifically unjustified, allegations were raised of cancer risks from environmental chemicals. That chemicals could cause cancer was first observed at the workplace, especially at the end of the last century and in the first half of this century. Such observations, involving relatively few

cases, were made in many of the industrialized countries, and public attention was fostered by extensive publicity. In turn, this public knowledge led to the generalization, in the 1940s, that the existing cancer burden, affecting several 100,000s patients per year, was related to exposures to chemicals. The obvious candidates for suspicion in the general population were chemicals in the food chain as additives or contaminants. After relatively brief hearings, the Congress of the United States amended the existing food and drug laws by addition of the Delaney Clause in 1958, which stipulated that carcinogens, as documented in humans or in animals, could not be added to foods. One might say that this clause was justified, based on knowledge existing at the time. This understanding was meager indeed in the area of the mechanisms of carcinogenesis, or that of causes of major types of cancer in humans.

Beginning with that period, concern with health in general, and cancer in particular, has dramatically enlarged research funding through the National Institutes of Health and other public health service agencies, and also other voluntary societies, such as the American Cancer Society, the American Heart Association, and other disease-related groups.

These funds have been a splendid investment. The base of knowledge on causes of major chronic diseases, heart disease, stroke, diabetes, many types of cancer and, importantly, the underlying mechanisms have increased dramatically. Even more relevant are the substantial advances in fundamental knowledge in the basic sciences, including those associated with toxicology. The genetic apparatus and DNA were virtually unknown 50 years ago, whereas currently studies on the gene are common and, in fact, are the basis of a new exciting industry that is based on biotechnology.

On the other hand, legislation and regulatory actions by varied agencies in the United States have not taken advantage of the factual knowledge and mechanistic understanding achieved. Yet, the time is opportune to consider mechanisms in evaluating and defining environmental problems, especially those relating to cancer, allergies, the immune system, or the nervous system. We have introduced the term *genotoxic* to denote a reactive form or metabolite of a chemical that can act as an electrophilic reactant, or can generate reactive oxygen compounds. Such specific reactive chemicals can interact with the genetic apparatus to yield somatic mutations, the fundamental change eventuating in cancer, or those that can modify DNA or proteins, including specific receptor proteins, that would eventually be expressed in virtually all other adverse effects. In many instances, cells carrying abnormal DNA, or others with abnormal proteins, need to duplicate to express the initial changes. Thus, any activity affecting cell duplication rates necessarily will be reflected in the ultimate outcome.

A number of nongenotoxic chemicals play a major role in controlling DNA synthesis and cell duplication. However, for nongenotoxic mechanisms, dose–response action must be considered in applying any results to public health activities. In fact, high dose levels of nongenotoxic chemicals have displayed a variety of adverse effects, including cancer, in laboratory animals. For that reason, such chemicals were labeled carcinogens. In turn, this evaluation has led to regulatory actions, or even public pressures, that given an understanding of the underlying science, are not well justified, in my opinion. For example, there is widespread fear of environmental contamination with a group of chlorinated chemicals known as dioxins. At high dosages in animals, dioxins have induced cancer. However, in studies involving a number of dosages, a low level was found that failed to induce a significant number of specific cancers under the conditions of the test. After high-level human exposure during industrial accidents in the United States and in Italy, the affected individuals displayed chloracne, but observation of the individuals affected has not produced evidence of cancer, except a few select cases, for whom other factors may have been involved. On the other hand, dioxin is a potent enzyme inducer, even at low levels. The enzymes induced are not only those of the cytochrome P-450 system, but also phase II detoxification enzymes. Studies in animal models with low level dioxins and

a carcinogen show inhibition of the action of the carcinogen through such mechanisms. The data from the extensive contamination of people in Seveso, Italy, begin to show that the breast cancer rate in the exposed population may be lower than in uncontaminated control groups. Chemical procedures can accurately measure tiny amounts of environmental dioxins. The question arises of whether these are really health risks, or perhaps, might even be beneficial. Recently, it was proposed that hospital incinerators be shut down because of emissions of dioxins. This raises the key problem of the safety to ship and bury hospital waste, which contains hazardous bacterial and viral contaminants, including HIV. I believe that traditional high-temperature destruction of any wastes by local incineration is the safest, most effective, and most economic means. This also applies to solid waste incineration by energy plants, which is occasionally not supported by lay groups with a different interpretation and understanding of the toxicology and objectives, and who often emphasize the potential risks from dioxins. Overall, experienced toxicologists should serve as a sound, objective, information resource on such questions.

Pharmacokinetic parameters are important controlling elements in the disposition and metabolism of xenobiotics and endogenous products. One reason dioxin displays prolonged activity is the slow elimination of this chemical and, in addition, it binds to the Ah receptor, extending its action on several physiological and pharmacological effectors. In contrast, ethanol is metabolized rapidly and its effect at several target sites evanescent. Metabolic and other pharmacological elements are frequently modified quantitatively by chemicals. Thus, it is important to consider not only the action of individual chemicals, but also of realistic mixtures of chemicals. Furthermore, it is clear that chemicals usually do not act in a qualitative, absolute way, but that quantitation is most important. One can state that an individual who smokes 40 cigarettes per day is at a high risk of heart disease or of specific cancers. In contrast, the effect in individuals smoking three to five cigarettes per day is hard to define. The question of risk assessment in relation to evaluation of toxicological data is critical. This is especially so for chemicals forming DNA-reactive metabolites that are labeled, thus, genotoxic. In the past, many scientists and regulatory agencies commonly used the linear extrapolation without threshold for all chemicals. Yet, other scientists hold that mechanistic considerations would suggest that the linear extrapolation should be applied only to DNA-reactive chemicals. Even in this instance, there may be deviations from linearity at low doses or exposures, and consideration needs to be given to practical thresholds for this class of chemicals. Indeed, there are mechanisms for removal of damaged DNA through processes such as DNA repair. Damaged cells can be eliminated through cell death or through the phenomenon of apoptosis. The mycotoxin aflatoxin B_1 is a powerful genotoxic chemical in the human dietary environment. It was discovered to be a carcinogen in 1962, and the FDA and USDA established regulations on the maximal amount of aflatoxin in foods for human consumption. The action level selected, 20 ppb, was appropriate, based on practical considerations of ensuring an adequate food supply, even though in rats, this dose level displays active carcinogenicity. In all species tested, aflatoxin B_1 causes liver cancer. This disease has a low incidence in the United States, but a high incidence in equatorial Africa, where the level of food contamination is 100–500 times higher, and the people are more likely to carry the hepatitis antigen. This might suggest that there is a no-effect level for this powerful genotoxic carcinogen. The regulatory action reflected the proper decision, displaying reasoning and approaches based on sound toxicological considerations.

There are also many nongenotoxic carcinogens, and we emphasize carcinogens mainly because, in the context of environment and health, the question of cancer causation and prevention is a field of general broad interest. Early developments in risk assessments for such chemicals assumed that they were no different from genotoxic chemicals. Such cases have not considered that nongenotoxic chemicals function by totally different mechanisms from those applicable to genotoxic carcinogens. Increased support is given to the operation of nongenotoxic

mechanism, as evidenced by sound laboratory research and considerations of human epidemiological studies, establishing that these agents present a nonlinear dose–response, with a threshold. Thus, prevailing environmental concentrations below the threshold should have no adverse effects. Furthermore, it has been demonstrated that mixtures of such chemicals affecting distinct target organs would act independently. Yet, failure to consider these facts can lead to costly proposals to completely eliminate such chemicals, for example, from drinking water. Parts per billion of chloroform and similar halogenated compounds stem from the chlorination of water, an important and, in fact, essential health-preserving process. Chloroform can be measured very precisely through accurate chemical techniques. Nonetheless, the amounts usually present in water have no toxicological significance, given their mechanism of action. However, debate still continues over the adequacy of existing or needed evidence to support a threshold phenomenon for nongenotoxic carcinogens.

Risk assessment, thus, needs well-informed individuals to consider its use for risk management decision making. One noteworthy point is that risk management is often performed by scientifically lay people, and it often involves social, economic, legal, and political considerations, sometimes responding to public pressures, and cannot be totally oriented to health promotion. A more efficient use of public and private funds would be to develop more scientifically sound approaches to risk reduction and disease prevention, that are understood and accepted by everybody. Risk managers can best use the toxicological data base to inform and educate the public, so that options are clearly understood, and decisions can be made by all concerned that conform to a reasonable and sound toxicological evaluation and risk assessment. For example, relative to concerns with hazards attached to exposure to electromagnetic radiation from electric wiring, different opinions are held among some toxicologists, and thus the general public, concerning the associated resource priority.

Much has been learned through research about the causes of major diseases affecting people worldwide. In contrast with the views prevailing at the beginning of this century, current evidence, although not totally conclusive, shows that environmental contamination by chemicals play a smaller role than previously thought, at least in North America. In any event, environmental contamination should be avoided through risk reduction and pollution prevention.

Importantly, the locally prevailing lifestyle is associated with major public health problems. This includes the use of tobacco and, particularly, smoking of cigarettes, associated with a high risk of cardiovascular diseases and specific types of cancer. Excessive drinking of alcoholic beverages, meaning more than two glasses per day, is hazardous in some specific way, either as such or through interaction with other factors such as cigarette use. Traditional nutritional habits—high in fat and salt and too low in vegetables and fruits—account for a large fraction of heart disease, cancer, stroke, diabetes, and even premature aging, as well as obesity. Greater efforts are needed to inform people of the need to change their lifestyles, and to educate the younger generations toward health-promoting personal habits. Those controlling public opinion and political actions need to be aware that legislation and regulations on toxic materials and ensuing risk control will have little influence on the current high expenditures associated with the burden of chronic disease diagnosis and treatment. Active health promotion related to proper, low-risk lifestyles needs to be implemented, to ensure a healthy public through disease prevention.

Humans are entitled to clean water, clean air, and clean foods, and sociable personal interactions make life worth living. Great progress has been made to ensure clean air and water that in the 1990s is better, in many instances, than it was in the 1930s. The public has to understand the differences between theoretical and predicted risk, or the perceived and the real risk. Unfortunately, the media often seem to emphasize the few cases of criminal activities and play up the low, uncertain risk of disease stemming from exposure to trace amounts of chemicals

in the environment. It is important that the public be informed and educated about the major, proved, definitive risks of lifestyle-associated premature killing or maiming diseases.

The current volume illustrates a number of these points with reports on chemicals and mixtures with varied toxic actions, the underlying mechanisms, and, eventually, the quantitative aspects expressed as risk assessment. It is a relevant and contemporary standard for teaching and research. At the same time, it is hoped that those utilizing this volume would incorporate in their educational approaches some thoughts on interactions of toxicological processes and personal lifestyles in disease causation and prevention.

John H. Weisburger

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Preface

Recent advances in toxicology have brought us from the period of qualitative evaluation of toxicological effects of hazardous substances to the new era of quantitative assessment and prediction of the health risk from exposure to these agents. Classical toxicology has progressed from trying to answer the question, "Is it toxic?" to modern toxicology that attempts to address the concern, "How toxic is it?" The emphasis on the quantitative assessment of the probability of health risk, supported by qualitative evaluation, provides the basis for logical risk assessment. This information is useful to characterize the health risk and provide guidance for regulators and decision makers to develop regulatory and risk management options, especially those relating to setting priorities for managing environmental health problems.

In the 1960s, the book *Silent Spring* by Rachel Carson brought to our attention the toxic properties of pesticides. Other major environmental contaminants identified include: polychlorinated biphenyls (PCBs) and methylmercury in fish, dioxins in various environmental media, arsenic in drinking water, and lead in old homes from leaded paint. Occupational exposures of various agents related mesothelioma from asbestos, male reproductive toxicity from dibromochloropropane, and angiosarcoma of the liver from vinyl chloride. Identification of the agents in association with human disease conditions has led to the attempts to control and regulate environmental chemicals in order to reduce exposure drastically to these agents, and to eliminate or minimize the diseases resulting from exposure.

Efforts to control and regulate chemicals to prevent excessive human exposure have led to the perplexing question "How safe is safe?" Typical actions include developing drinking water and air standards and issuing health advisories for toxic chemicals in fish. These actions are based on risk assessment approaches leading to decisions on the levels of restrictive chemical intake. But the process involved and the considerations included are not simple or straightforward. We have gone through concerns and debates relating to the benzene ruling and the Delaney Clause, and arguments regarding insignificant risk level and voluntary versus involun-

tary risk. Development of more sensitive analytical methods has led to the capability of detecting lower and lower levels of chemicals and, at times, corresponding lower chemical standards. The concepts of threshold and no threshold for chemicals, especially carcinogens, have generated debates and different approaches for risk assessment. Mathematical models and statistical approaches are continuing to be developed to address the need to analyze data and conduct high to low dose extrapolation in order to support assessment of human health risk.

In the 1980s, we saw risk assessments receiving national attention. Ethylene dibromide, a fumigant originally thought not to leave a residue because of its high volatility, was found in cereal grains and bakery products. A mathematical model that incorporated exposure early in life was used to address the concern of infant or childhood exposure in the risk assessment. Following was the growth regulator daminozide used on apples. The risk assessments focused on the potential carcinogenicity of 1,1-dimethyl hydrazine, resulting from hydrolysis of daminozide. This product concentrated in apple juice following food processing, and again the major concern was the health effects in young children who consume apple juice. The development of the regulatory decisions in these two cases was the subject of intensive debate and discussions. Dietary exposure to pesticides has been brought to public attention in two recent reports by the National Academy of Sciences, and the related concerns are receiving programmatic attention at the federal level. Decisions for effective control measures for naturally occurring (versus intentionally used) substances or environmental byproducts are also difficult to make. Examples are arsenic, disinfectant byproducts and nitrate in drinking water, and methylmercury in fish.

Not all agencies that need the results of risk assessment to support their activities have the capability or resources to conduct risk assessment. In this regard, the U.S. Environmental Protection Agency has made available to the public and other agencies results of their chemical-specific risk assessment for applications in local programs. The need for more trained toxicologists is recognized, and educational programs for such purposes have steadily increased. Risk assessment is now often included as an important aspect of a modern toxicology training program, but availability of educational and training materials to meet the training needs has not been encouraging.

We have frequently been approached by professors, instructors, students, environmental consultants, attorneys, environmental health scientists, risk managers and those interested in risk assessment to help identify a specific useful reference source on risk assessment. We soon came to realize that very useful information was available in journal articles, independent publications, and books on special topics. There was not a single publication, however, that readily integrated all the useful, related information into one independent volume, and one was desperately needed. It became apparent to us that this was the opportunity to develop one. An outline for the book on principles and methods was developed, plus aspects to be considered for practical applications that, from practical experience, one would need to know and explore to be a toxicologist and to perform risk assessment. This book is our first attempt to provide an answer to all those who had asked for a textbook or reference book, all in one volume, eliminating the need to go to a diversity of resources to get an overall view and perspective. Much effort was made to make this a comprehensive compilation; however, due to the vast knowledge developed in the fields of toxicology and risk assessment, it is not possible to be exhaustive or complete in scope and coverage. In this regard, readers are encouraged to obtain more detailed information by using the references provided at the end of each chapter.

For those who intend to pursue professional development in toxicology and risk assessment, it is important to get a formal education in basic toxicology to understand the toxicological principles and not to just mechanistically follow the methodological steps in risk assessment. There are limitations and uncertainties attendant to the risk assessment methodology, and much

is gained from understanding the principles and issues continuously being debated. Current debates or considerations include the issues surrounding the following: interspecies scaling for body surface area, maximum tolerated dose, bolus dose (overdosing) compared to continuous dosing, benchmark dose, pharmacokinetic modeling, uncertainty factors, mechanism of toxic actions of chemicals [particularly those of genotoxic versus nongenotoxic (epigenetic) carcinogens], threshold versus nonthreshold models for carcinogens, specific cancer sites such as male rat kidney tumors and mouse liver tumors, multiple chemical sensitivity, and toxicity equivalent factors (e.g., dioxins and dioxin-like PCB congeners), among others. Those who use risk assessment for making risk management decisions often need to include social, economic, and technical feasibility considerations; above all expert advice from the toxicologists and risk assessors, or regulatory toxicologists, would be required.

The future of toxicological risk assessment is likely to include emphasis on special sensitive populations (e.g., infants and children, the elderly, ethnic groups), multiple chemical exposures, reducing uncertainties, multimedia exposure, exposure distribution analysis, and default assumptions. Reproductive/developmental toxicity and carcinogenicity are critical endpoints currently considered for environmental regulation. Immunotoxicity, neurotoxicity, and behavioral/developmental toxicity are getting increasing attention. Endocrine effects and ocular toxicity demand more information. Improved data bases on human activities (often termed lifestyle), chemical occurrence and monitoring, and overall exposure and toxicological data for chemicals are needed to adequately conduct risk assessments. Harmonization of risk assessment is an objective among different agencies and countries, and refinement of techniques is a goal among scientists. Coordination between researchers generating data and risk assessors using the data is important for the further advancement of risk assessment. Related areas with increasing attention are risk communication, ecological risk assessment, and environmental justice.

As is clear from the presentations in this volume, toxicological risk assessment is a complex and important science that will continue to guide and have an impact on future health risk prediction, public health protection, pollution prevention, and environmental regulations. In the twilight of the 20th century, we are proud of all the advancements made in the past decades. Looking into a new century, we can also see the dawn of new excitements and challenges ahead of us. The present volume, we hope, serves as a treatise reflecting the development, accomplishments, and current status in the science of toxicological risk assessment. We further hope that it will also serve as the stepping stone for a new generation of toxicologists to carry the torch into a new era of excellence.

Admittedly this accomplishment would not be possible without the refinement of the organization of the outline for the book and the diligent planning and coordination of the leading scientist(s) for each part of Parts I through VIII of the book, and the dedication of each author. Each is an eminent scientist in his or her area of expertise. Readers are strongly urged to refer to other publications and references provided by these authors in order to gain a better understanding of the relevant subject matters and issues described in their work. The review comments provided by peer reviewers, who are themselves authoritative experts, were invaluable to ensure the quality of this book. We acknowledge the important contributions of these individuals that made this book possible.

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Contents

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PART I
GENERAL TOXICOLOGY

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Fundamental to the conduct of risk assessment of environmental chemicals is the need to understand the principles of toxicology to provide a scientific basis for the use of toxicological data, whether derived from animal or human studies, for such an assessment. This section provides a discussion of the basic principles and modern concepts of toxicology, with a state-of-the-art coverage on specific disciplines, including general acute, subchronic, and chronic toxicity, carcinogenicity, genotoxicity, reproductive and developmental toxicity, neurotoxicity, and immunotoxicity. The mechanisms of action of specific chemicals and their toxic effects on specific organs are not explicitly discussed here, but examples are presented in other sections. Neurotoxicity and immunotoxicity are receiving increasing attention, and there are emerging concerns on endocrine effects and ocular toxicity. Readers are referred to Part II for discussions on toxicological testing to gain a more comprehensive understanding of the study of the toxic effects of chemicals and the types of effects that may result from chemical exposures. The pharmacokinetic principles, including the concepts of absorption, distribution, metabolism, and excretion, are presented. The importance of the dose–response relationship and cumulative effects are pointed out. All the toxicological principles form the basic foundation of knowledge for toxicologists who perform risk assessment, which would require sound judgments that are based on an ability to evaluate toxicological data, rather than a straightforward application of the methods used for risk assessment. It is this fundamental knowledge, coupled with a pertinent understanding of other related principles, issues, and perspectives described throughout this book, that enables toxicologists to become distinguished risk assessors.

All the factors and issues to be considered in toxicological risk assessment are not specified in any guidebooks or manuals, and the ability to interpret toxicological data for assessing human health implications is based on the education, training, and experience of the toxicologists who are the risk assessors. Considerable variations often exist in the interpretation of data and, for this reason, there are continuing debates on issues such as the significance of male rat kidney

tumors, mouse liver tumors, contact carcinogens, threshold versus nonthreshold phenomenon, blood cholinesterase inhibition, and the finding of teratogenicity in the presence of maternal toxicity. In the area of immunotoxicity, in spite of well-documented immunomodulative effects of some known chemicals, such as tetrachlorodibenzodioxin (TCDD) and its congeners and some pesticides, it has been difficult to relate these changes to a more definitive health risk or a disease process.

Acute toxicity has been evaluated under conditions of occupational exposures to toxic gases and solvents (chlorine), dietary ingestion of pesticides (aldicarb), and accidental releases of toxic chemicals (metam). Chronic health risks have been the focus of risk assessments for regulatory purposes, such as these for metals, pesticides, organic chemicals, and inorganic chemicals, in air, water, food, hazardous waste sites, and consumer products. Potential cumulative effects from long-term, low-level exposures and irreversible effects, such as neurotoxicity and carcinogenicity, are of great concern. Reproductive and developmental toxicity receive priority evaluation because of the possibility of a lifelong effect in offspring, especially when teratogenic effects can occur after a single exposure during a sensitive period of organogenesis during gestation. As the regulatory guidance for evaluating reproductive toxicity (effects on function or structure of male and female reproductive systems, fetotoxicity) is still undergoing review, the authors have focused on developmental effects (birth effects) in the present chapter. Hypersensitivity and multiple chemical sensitivity are often complaints received from the public. Often the cause-and-effect relationship is difficult to establish, and toxicology risk assessment is used to predict potential health outcomes. An understanding of the disposition and chemical reactivity of a chemical and its metabolites is necessary, and pharmacokinetic information is important in this prediction.

1
Principles and Highlights of Toxicology

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I. INTRODUCTION

Toxicology as an established science is relatively new, but poisons have been known since antiquity. Perhaps one of the earliest attempts to describe the field was in 1198 by the Spanish physician and philosopher, Maimonides, who published a book entitled, *Poisons and Their Antidotes*.

Toxicology is now defined as the study of the toxic properties, or adverse health effects, of agents or substances. In essence, modern toxicology encompasses two facets: qualitative evaluation, and quantitative assessment of toxicity. Qualitative evaluation here is the study of an agent, either chemical or physical, that can cause or have the potential to cause an adverse or harmful effect in living organisms, be it an intact human or animal, or some subcomponents of it. The quantitative assessment aspect is described by Philippus T.A.B. von Holenheim (1493–1541), who called himself Paracelsus and enunciated a dictum: *All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy.* In a more modern parlance, the statement is *the dose makes the poison*. In other words, not only is the "toxic culprit" with the capability of inducing harm of concern, but the amount of that agent needed that can cause the harm is equally important. This provides the basis for the concept of *dose–response* that is an integral part in understanding the principles of toxicology, and for the concept of *exposure*, an integral part of risk assessment.

Practically all phases of our culture is within the realm of the toxicologist who studies the adverse health effects of agents or substances. In the medical field, toxicity, diagnosis, treatment, and prevention are considered. In industries, workers are exposed to various agents in the form of gases, mists, or vapors; or particles such as metals, fibers, or dusts; or liquids such as organic solvents. In the food supply are fertilizers, pesticides, preservatives, and additives. In the ambient environment are criteria pollutants in air and contaminants in drinking water.

Following Paracelsus was the Italian physician, Bernardin Ramazzini (1633–1714), who

was concerned with the plight of the workers; he convinced the medical profession at that time of the importance of exposure of the workers to toxic chemicals in their occupation. He first described silicosis, and is considered to be the founder of occupational/industrial medicine; this is another important contribution to modern toxicology. In most cases, much higher levels of exposure occur in the workplace compared with those in the general environment (e.g., ambient air and water).

II. TOXICOLOGIST AS A PROFESSION

The variety of potential harmful effects that can be caused by a diversity of agents in our environment is legion. Some chemicals produce a general toxic action, whereas others appear to be organ-specific. These effects range from subtle, almost imperceptible effects, to gross pathology and even death of the exposed subject. Scientists in this field of endeavor must be conversant (but not necessarily an expert) in a broad range of related disciplines; this group of scientists who study and evaluate chemical toxicity are designated toxicologists. Being in a relatively new field, the toxicologists often come from a variety of scientific disciplines. Until the last few decades, there were no specific courses in toxicology per se offered in universities, nor was there a separate department within an academic institution devoted to this field. It is now possible to obtain formal training and a degree in toxicology from a university department, or training and a degree in a related subject area with an emphasis on toxicology. Previously, toxicologists were trained in a related or ancillary field. Many physicians specialize in toxicology; others come from fields of chemistry, biology, physiology, biochemistry, or pathology. Other scientists, such as some statisticians and mathematicians, are interested in applying statistical techniques and mathematical models to the toxicological data generated by toxicologists.

The field of toxicology encompasses such a vast variety of disciplines that, as a result, many toxicologists are very knowledgeable not only in toxicology, but serve as specialists in a particular subject area, such as reproductive and development toxicity (teratology), carcinogenesis, genotoxicity, immunotoxicity, and neurotoxicity. Each of these is discussed in more detail throughout Chapters 2–6 in this section. In addition to having opportunities in conducting laboratory research or experiments, toxicologists can apply their training to major practical applications of the science that will have an effect in environmental health protection; they may work in a poison control center or forensic laboratory, engage in regulatory functions, or serve as a consultant to the legal profession or to other industries or organizations. Thus, there are specialties in basic research and in environmental and applied toxicology that provide a variety of opportunities for professional development.

III. TOXICOLOGICAL INVESTIGATIONS

A. Acute, Subchronic, and Chronic Studies

Traditionally, the first measurements made by the toxicologists are the general *acute, subchronic, and chronic effects* of the agent under investigation in experimental animals. Human data are preferred, but these studies are difficult to conduct. Use of human data is discussed in a chapter that follows in Part VI. General toxic effects are of great and continued interest, but they do not dominate the field. Acute toxic effects are generally measured or noted as effects occurring within a few hours after a single exposure (or dose) or after short-term exposure to the agent. The observation period would depend on the type of endpoint evaluated. Often in experimental animal studies, the observation of the percentage of mortality in the exposed population is the

main object of the study. For these studies, the observation period is generally 2 weeks. By the graphic or statistical techniques, the relative (not absolute) values of the medium lethal dose or concentration (LD_{50} or LC_{50}) after oral, dermal, or inhalation exposure are among the first measurements made. Other studies include eye irritation, skin irritation, and sensitization studies.

From the studies of acute toxicological action the initial concept of dose–response emerges. The *dose–response* relationship is an expression of the graded magnitude of response (or an effect) corresponding to the incremental increase in intensity of the dose and frequency of dosing (or exposure). This relationship is actually evaluated in more extensive longer-term studies, with refinements in the dosing regimen that incorporate a range of dose levels. In acute studies, however, a high-dose level of the test chemical is usually given, and the major target organ(s) of toxicity identified. The data generated help provide guidance on selecting dose levels and focusing on special toxicological endpoints for further toxicological evaluations. The various health effects that may result from acute exposures are evaluated in acute studies. From the information obtained from acute animal toxicity studies, the appropriate dose range is derived for further toxicity studies.

Subchronic studies are usually conducted for a duration of 30, 60, or 90 days. For these studies, more detailed pathological changes in organs or tissues, and other physiological and biochemical changes are evaluated. The results of these studies provide better insight into the toxic properties of the agent under study, and more information for conducting long-term or chronic studies, which could have a duration of longer than 90 days, or last the lifetime of the test animals. Detailed pathological, biochemical, or physiological effects are then determined in a chronic study. Toxicokinetics play an important role in the design of the chronic study. A chronic study can be combined with a carcinogenicity study (further described in Chapter 9 on carcinogenicity testing).

A detailed discussion of the potential health effects of substances to be predicted from animal studies is provided in Part II, *Toxicological Testing*, which describes the tests specifically designed to evaluate these effects. The mechanism of toxic action of chemicals can vary and for many chemicals it is not clearly understood. The biological basis of toxicity for specific chemicals is reflected in other chapters throughout this book. The use of testing data for risk assessment is discussed in Part III, *Risk Assessment*.

B. Experimental Systems

The nature of the investigations conducted by modern toxicologists encompasses a wide spectrum. Some studies involve the intact subject (in vivo), be it a human or animal, or a member of an alternative species. Others may study a specific and isolated tissue or organ (in vitro), such as lungs, brain, liver, or muscle. Assays or systems are developed with organ or cell culture, or with components of the cell, such as mitochondria or enzymes. Attention has been given to the interaction of agents with the ultimate genetic information found in nucleic acids, the DNA or RNA, and the proteins elaborated by them. There is no limit to the interests of toxicologists in the study of some living or near-living systems. The in vivo and in vitro testing and the associated assay systems are discussed in Part II, *Toxicological Testing*.

C. Factors Affecting Toxicity

The investigation of the adverse health effects of chemical in exposed biological systems is extremely complicated. Both absorption mechanisms and rates must be considered. The effects resulting from exposure to a substance is more closely related to the "effective dose" than the administered dose. Once in the bloodstream, the agent is usually carried by some component of the blood, be it a protein or the red blood cells. After passing through the liver, the agent can be

metabolized to a product that can be more toxic than the original parent chemical, or the agent can be metabolized (detoxified) to a less active or toxic agent. The mode, rate, and route of distribution and excretion, all may play a role in the final evaluation of the effects of the material on the subject exposed. These natural biological events are lacking in in vitro test systems, to which experimentally derived metabolism activation is sometimes added. *Cumulative effects* may result in toxicity being seen in chronic studies that is not seen in acute and subchronic studies. More details relating to the interplay of these aspects are provided in Chapter 7 on pharmacokinetics and in related aspects in later chapters on pharmacokinetic modeling.

In the study and evaluation of chemical toxicity, emphasis has been placed on the use of data relevant to human exposure, as the route of exposure can affect the final toxicity. These data would involve major exposure routes such as ingestion, inhalation, or dermal absorption, or combinations thereof (see Part III, *Risk Assessment*). However, data have also been generated from studies in which the route of administration is not of major importance to humans. For intact animals, every conceivable route has been employed; just about every organ in the body has been injected and the agent under investigation has been deposited at the site. Thus, information is now available on chemicals following implantation in the brain, the lungs, the eye, the liver, the kidney, the spleen, the muscle, the testes, the ovary, and the subcutaneous tissue.

Toxicological endpoints from the various studies constitute a wide spectrum of observable effects, such as behavior modification, alterations in respiration, change of color of the eyes of rodents or the condition of the fur, and quantitative recordings of activities and other physiological events. Pathological and biochemical changes are often measured. Endpoints can range from the most subtle changes, to total oblivion, death. Many toxicologists are mostly involved with the observational part of the science, others are mainly engaged in elucidating the mechanism of action of the chemical producing the effects observed with the "toxic" agent or studying the toxicokinetics of the agent. Factors such as sex, age, species, or strain differences; nutritional status; and multiple chemical interactions, among others, may affect the toxicity observation following chemical exposure. More details on these considerations are provided in Part VI on issues concerning the use of human data and on extrapolating data from animals to humans.

IV. THE MANY USES OF TOXICOLOGY

By understanding how an agent produces its toxic effects, it may be possible to predict the potential toxicity of other related compounds based on the *structure–activity relationships*. This can lead to developing alternate agents that can have the same beneficial or pharmacological effect, but with much less detrimental side effects on the exposed population. Some materials that appear innocuous because of their low acute toxicity can have surprisingly profound pathological consequences; the thalidomide tragedy is a case in point. The understanding of the types of potential health effects and mechanism of action can also help identify potential chemicals of health concern so actions can be taken to prevent unnecessary exposure.

Data generated by the toxicological investigations also can result in a great variety of social actions (or even inactions). Some experiments result in pure academic exercises; it is never possible, however, to predict when esoteric results find a "practical" application. Information can be used to suggest further research, or can result in practical applications such as decisions to clean up a toxic waste site, development of testing requirements to ensure safe use of chemicals or establishment of environmental standards to limit chemical exposure (see Part VIII, *Risk Assessment and Risk Management*). Some research results in identifying logical antidotes for some toxic materials.

An entire group of toxicologists is concerned with the application of data generated by

various toxicological investigations to make judgments about the risk to an individual or a population who may be exposed to toxic chemicals; these are the risk assessors. One major objective of a subgroup of these toxicologists is to protect the exposed individual or population from harmful effects of agents in the environment by minimizing exposure to the agents through technical support for the formulation of logical environmental regulations. At all times it is necessary to make educated judgments based on high-quality toxicological and exposure data. To this end, increasing attempts are made to quantitate, through the process of risk assessment, the potential or actual harmful effects of chemicals to those exposed or potentially exposed. Special considerations are now being given to protecting the more sensitive fraction of the population: infants, the elderly, and those in the population who are exquisitely sensitive. Mathematical and biological models and statistical approaches are being developed for data analysis and manipulation. Some formulas and computer-generated models are useful in attempting to examine high-dose exposures, and to extrapolate information from one or more data points, or from high doses (from experimental studies) to low doses, such as those found in the human ambient environment. These are discussed in Parts IV and V in this book in more detail. The specialty of risk assessment has evolved to evaluate toxicity and characterize the associated health risk of chemicals, or to predict the potential health risk and the associated probability that harm will result from such chemical exposure. Finally, of interest is that the science of toxicology is one of the very few disciplines that are concerned with protection of the general public from harmful substances. Increased use of risk assessment is also evident for management of health issues in the occupational environment.

As yet to be completely evaluated are the toxic effects of chemical mixtures; in such mixtures, the resulting toxicity can be additive, antagonistic, or synergistic. Every living organism in the universe is exposed to various complex mixtures of chemicals; this field still needs many more intensive investigations. Exposure to chemicals from multiple media also deserves increased attention. These are discussed in Part III and Part IV.

The key to performing adequate risk assessment is the availability of the needed information. More data are needed on toxicology of chemicals, exposure patterns in humans, and data on the issues receiving increasing attention, as noted in the foregoing. The regulatory basis for risk assessment and resources for providing existing available information is discussed in Part VII. Examples of the use of this information in risk assessment and risk management are discussed in Parts III and VIII.

V. SUMMARY

Following the basic principles and highlights presented in this chapter on general toxicology, the remaining chapters of Part 1 present in-depth details of the various disciplines of toxicology. Each chapter discusses the principles and concepts with a state-of-the-art coverage of the current knowledge and status of research development, combined with excellent, pertinent references to that subject. Each of the following chapters has provided excellent references, including those relating to all aspects discussed in this chapter; the compilation is comprehensive and the topics specific. Therefore, readers are referred to the references found in all these chapters, as they will serve as a complete compilation for this chapter.

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Carcinogenesis: Basic Principles

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I. INTRODUCTION

In multicellular organisms, cell growth is generally a well-regulated process that responds to specific needs of the organism. Occasionally, however, normal regulation of cellular proliferation is lost, and a cell can replicate in excess of those needs. If daughter cells retain the property of unregulated growth, a clone of cells with unlimited growth potential, or neoplasm, can be formed. This chapter concerns malignant transformation of normal cells and the ability of chemicals to participate in that process.

II. DISTURBANCES OF NORMAL CELL GROWTH

Normal cell replication and cancerous growth represent the two extremes of a continuum of growth patterns (reviewed in Lieberman and Lebovitz, 1990). In the adult, cell replication is generally limited to replacing cells lost through normal turnover. In addition, some tissues can regenerate an approximately normal structure through replication, which ceases after replacement of lost cells. *Hyperplasia,* an increase in a tissue or organ cell number, may increase the risk of neoplasia in an organ, especially if a chronic stimulus of cell division exists. Replacement of one cell type in a tissue with another is referred to as *metaplasia,* which can occur in response to different stimuli, including irritation. Since the replacement cells are morphologically normal, metaplasia is not usually considered a precancerous lesion, although occasionally, it may precede neoplasia. *Dysplasia* is characterized by morphologically atypical cells and a disorganized growth pattern. In dysplasia, cells are often pleiotropic and show an increase in the ratio of nucleus to cytoplasm, and severe dysplasia can be difficult to distinguish from carcinoma in situ, or preinvasive malignancy. A *neoplasm* (new growth) is defined as an abnormal mass of cells that exhibits uncontrolled proliferation and that persists after cessation of the stimulus (most often unknown) that produced it. Cells with proliferative capacity can give rise to

neoplasms, which, although they express varying states of differentiation, usually have sufficient normal characteristics that they can be classified according to the tissue and cell type from which they were derived. If a cause of neoplastic change can be identified, there is almost always a long delay, or latent period, between the causal event and the clinical manifestation of disease. *Benign* tumors remain localized in the area in which they arise, whereas *malignant* tumors, or cancers, have the ability to invade contiguous tissue and metastasize to distant sites where a subpopulation of cells can take up residence and continue unregulated growth. Cancerous cells, then, are characterized by lack of normal growth control, invasiveness, and metastasis, the underlying mechanisms of which are not yet completely understood.

A. Cellular Growth Control

Cell growth involves duplication of cellular contents, including DNA, and physical division of the cell into two daughter cells (reviewed in Murray and Hunt, 1993). These events can be used to describe a cell cycle, the ordered set of processes by which cells grow and divide. The cell cycle is divided into two fundamental parts, interphase and mitosis (M). Cells in mitosis, which includes the various stages of nuclear and cytoplasmic division, are easily recognized, as the replicated chromosomes condense and can be identified by light microscopy. Two types of processes occur during interphase: (1) continuous processes, such as ribosome, membrane, organelle, and (most) protein synthesis, which are collectively referred to as growth; and (2) stepwise processes, which occur once per cell cycle. DNA replication is an example of a stepwise process, and it is restricted to a specific part of interphase called S (synthesis) phase. Cells in S phase are readily visualized by a variety of techniques, including the use of radiolabeled DNA precursors and autoradiography. The remainder of interphase consists of G_1 phase, a gap between the previous cell division and S, and G_2 phase, a gap between DNA replication and mitosis. Cells in G_1 not yet committed to DNA replication can enter a resting state, referred to as G_0, distinct from proliferating cells in any stage.

The cell cycle is controlled by proteins that interact to induce and coordinate processes that duplicate and divide the cell contents (reviewed in Alberts et al., 1994). These proteins are regulated by signals from within the cell or from the environment that can stop or delay the cycle at multiple specific checkpoints. The cell cycle control system is primarily based on two families of proteins: the cyclin-dependent protein kinases (CDK) and the cyclins. Cyclin-dependent kinases are serine–threonine kinases capable of inducing downstream events. Cyclins, which build up during interphase and are degraded in mitosis by an ubiquitin-dependent pathway, are subunits that bind CDK molecules and regulate their catalytic activity. Animal cells have at least two CDK genes and multiple cyclins, referred to as G_1, S, and G_2, or mitotic, cyclins. Environmental signals generally act at one of two major check points, one in G_1 and the other in G_2. Mitotic induction, or passing the G_2 checkpoint, depends on Cdk_2 protein binding to cyclin B to produce a complex analogous to the yeast M–phase-promoting factor (MPF). When activated by phosphorylation, this complex triggers events that culminate in cell division. The G_1 checkpoint is the point at which the cell cycle control system can initiate DNA replication; when conditions are not favorable for cell division, cells may accumulate at this point. Formation of a CDK–G_1 cyclin (possibly a cyclin D) complex similar to MPF is thought to stimulate the events that lead to DNA replication. In addition to intracellular processes, positive signals, including protein growth factors, from other cells are generally required for cell growth and division in multicellular organisms. In the absence of these signals, which trigger intracellular signaling cascades to stimulate proliferation, cells can enter the G_0 phase. Negative-feedback signals are also important in ensuring that the cell cycle control system does not proceed until downstream events are completed. Another regulatory subunit family of CDK, the CDK

inhibitory proteins (CKI), play a role in stopping progression of the cell cycle (reviewed in Peter and Herskowitz, 1994). An example of feedback control is the system that operates to prevent cells with damaged DNA from entering S phase; a protein, *p53*, accumulates in cells with damaged DNA and seems to block progress of the cell cycle in G_1 by inducing transcription of the *p21* gene, which encodes a CKI protein.

Many genes implicated in neoplastic transformation encode proteins that are involved in regulating cell proliferation, either positively, by helping to promote growth and drive the cell past the G_1 checkpoint, or negatively, by stopping progression through the cell cycle and dismantling the control system. If a gene's product promotes proliferation and is expressed inappropriately, the altered gene is termed an *oncogene*, and the normal cellular counterpart is referred to as a *protooncogene*. If the genes' products restrain proliferation, they are referred to as *tumor suppressor genes*, as changes that inactivate these genes may also accelerate neoplastic transformation. In addition to cell proliferation, oncogenes and tumor suppressor genes have been implicated in the regulation of *apoptosis*, or programmed cell death (reviewed in Harrington et al., 1994), the inhibition of which may be involved in the growth of some malignant tumors. The role of oncogenes and tumor suppressor genes in carcinogenesis is discussed further later in the chapter.

B. Alterations in Cell-to-Cell Interactions

Invasiveness and metastasis confer the property of malignancy on a cell. To create a metastatic colony, cells must be able to leave the primary tumor, first enter the circulation, then leave it at some distant site, invade local tissue, and proliferate. Angiogenesis is also essential for both primary tumor and metastatic growth. These events appear to require a cascade of linked steps involving poorly understood multiple host–tumor cell interactions dependent on activation of several genes, some of which are distinct from those that regulate proliferation (reviewed in Liotta and Stetler-Stevenson, 1991).

The restriction of a normal cell type to a given tissue or organ is maintained by cell-to-cell recognition and by physical barriers, including the basal lamina that underlies layers of epithelial cells. Tumor cell binding to the basement membrane through both integrin- and nonintegrin-type cell surface receptors is an important step in invasion and metastasis, which also depend, in part, on the ability of tumor cells to digest their way through cell barriers. Several proteinases, which can disrupt the basal laminae, have been associated with the metastatic phenotype, including a plasminogen activator and metalloproteinases. Host proteinase inhibitors, including tissue metalloproteinase inhibitors, exist and may act to block metastasis; loss of genes encoding these proteins may favor tumor progression to metastasis. After disruption of the basal lamina, tumor cells must move into the interstitial stroma, a process that may be regulated by tumor cell cytokines and influenced by host chemoattractants. Invasion and metastasis, therefore, are facilitated by proteins that enhance binding of tumor cells to extracellular matrices and tumor cell proteolysis and locomotion. Other factors exist that act to block the production or activity of these proteins, and an imbalance in positive- and negative-control elements can result in acquisition of metastatic potential.

Other properties of malignant cells that may be due to alteration in cell-to-cell interactions are the ability of malignant cells to grow surrounded by cells with which they do not normally interact and the ability to elude the immune system. Reduced immunosurveillance may also be due to production of immunosuppressive agents by the cancer. It is evident that tumor cells can produce cytokines with immunosuppressive activity, but the extent to which immunosuppression might be responsible for the growth and spread of the tumors is unclear (reviewed in Sulitzeanu, 1993).

III. CARCINOGENESIS AS A MULTISTAGE PROCESS

Because all cancers share the properties of uncontrolled growth, invasion, and metastasis, a common mechanism for their origin has often been suggested. Various theories of carcinogenesis have been postulated to address a particular feature of the morphological, biochemical, or molecular aspects of the disease, but these have usually lacked general applicability. Among the suggested bases for cancer are selective deletion of certain protein species; failure of the immune system to recognize transformed cells; alterations in cellular membranes, including those of mitochondria, or of signal transducing pathways; and disruption of hierarchical relations within and among tissues. An early explanation of malignancy, still widely held, is the *somatic mutation theory,* which states that a tumor can arise by clonal proliferation from a somatic cell that has been transformed by acquired modification of its DNA base sequence (discussed in Crawford, 1985). Currently, the most commonly held view of carcinogenesis is that virtually all malignant tumors arise from single cells that retain proliferative capacity by a complex, multistage process, in which both genetic and epigenetic alterations are important (see, e.g., IARC, 1992). This view of cancer has evolved over many years, based on both pathological and epidemiological observations, as well as experimental studies of chemical carcinogenesis. As a result of these studies, the process of neoplastic development has been divided into operationally defined stages of initiation, promotion, and progression, each of which may also consist of multiple steps.

A. Initiation

Skin cancer studies provide support for the concept of carcinogenesis as a multistage process (reviewed in Hennings et al., 1993). Mouse skin tumors can be induced by applying initiators, that is, mutagenic agents, such as polycyclic aromatic hydrocarbons, directly to the skin. A single treatment of these agents does not typically give rise to a tumor, but may produce latent damage that can result in tumor formation following subsequent insult. The correlation between the ability to induce mutations and tumorigenesis is good for most chemical initiating agents, as well as ionizing radiation and viruses.

B. Promotion

Following initiation, subsequent application of certain substances, referred to as tumor promoters, to the skin can result in development of numerous benign papillomas. Tumor promoters have, in general, properties quite different from those of initiators (Pitot et al., 1992). First, promoters are not themselves mutagenic, that is, promotion is commonly an epigenetic phenomenon, which, like differentiation, involves changes in gene expression, not gene structure. Although promoting agents are incapable of directly inducing structural genetic changes, they may induce metabolic changes that lead to mutation. Specifically, the formation of active oxygen radicals that occurs as a consequence of exposure to various promoters can produce base modifications, DNA strand breaks, and chromosomal alterations. These secondary effects may accelerate the transition of cells from promotion to progression. Second, unlike most initiating agents, many promoters do not require metabolic activation, and several act through specific target cell receptors to enhance gene transcription. Whereas initiation is generally considered to be an irreversible process, promotion is not, so repeated exposure to promoters may be required for tumorigenesis. Possible mechanisms of tumor promotion, which need not be mutually exclusive, include induction of cell proliferation; inhibition of intercellular communication, which relieves initiated cells from restraint normally exerted by surrounding normal tissue; and immunosuppression.

C. Progression

The rate of conversion of papillomas to carcinomas, termed progression or malignant conversion, can be increased by treatment with some agents. Similar to initiation, progression is thought to have a genetic basis and be essentially irreversible. As aneuploidy (an abnormal number of chromosomes) is a common feature of cancer cells, it has been suggested that genomic instability itself could contribute to tumor progression (see, e.g., Nowell, 1991). A great number of genes code for proteins involved in maintaining genomic stability, including those involved in DNA replication and repair, mitosis, and control of the cell cycle. Mutations in these genes, which could decrease stability, would not necessarily produce the malignant phenotype directly, but would increase the likelihood of mutation throughout the genome, which could contribute to the evolution toward malignant behavior and heterogeneity characteristic of tumors.

D. Molecular Targets in Multistage Carcinogenesis

Heritable alterations that lead to altered expression or function of genes involved in regulation of proliferation and differentiation are important in carcinogenesis. Protooncogenes and tumor suppressor genes are two such gene classes. Protooncogenes are normal cellular genes that, when inappropriately activated by mutational events to oncogenes, alter regulation of growth and differentiation (reviewed in Cooper, 1990). Mutations of this sort have a dominant effect (i.e., only one affected allele confers the mutant phenotype on the cell). Many oncogenes have been identified through their presence in transforming retroviruses or by their association with chromosomal abnormalities. Protooncogene products include molecules implicated in all phases of cell signaling. Signaling elements encoded by oncogenes, with a representative gene given in parentheses, include growth factors (*sis*), membrane-associated tyrosine-specific kinases (*src*), GTP-binding proteins (*ras*), growth factor receptors (*erb* B), cytoplasmic tyrosine kinases (*fes*), steroidlike growth factor receptors (*erb* A), serine/threonine-specific protein kinases (*raf*), and nuclear proteins associated with gene expression (*myc*). Genetic mechanisms by which protooncogenes can become activated include insertional mutagenesis (proviral insertion or transposition into a defined host genomic locus), gene amplification, point mutation (base-pair substitutions, insertions, and deletions), and chromosomal rearrangement (deletions, inversions, and translocations). Alterations in protooncogene expression are not associated with all tumors, however, and it has been argued that generation of cancer genes by genetic transpositions, or recombination between largely nonhomogenous regions, may also be important in human disease (Cairns, 1981; Duesberg et al., 1991). Translocations can, if the breaks occur within genes on each involved chromosome, also result in creation of chimeric, or fusion proteins, which, like oncogene proteins, are often transcription factors and are commonly associated with tumors (Rabbitts, 1994).

Genetic alterations that inactivate tumor suppressor genes may also lead to loss of control of proliferative and differentiation processes and increase the likelihood of neoplastic transformation (reviewed in Knudson, 1993). As both alleles must usually be affected to alter phenotype, mutations in tumor suppressor genes have recessive effects on the cell. The best-studied tumor suppressor genes are the *Rb* gene and the *p53* gene. The *Rb* gene, associated with retinoblastoma, a rare human cancer, codes for a protein that, when not phosphorylated, appears to block passage from G_1 to S, apparently by complexing with a transcription factor. Individuals predisposed to the disease have experienced germline mutations inactivating one allele of the *Rb* gene, and cancers can develop if the remaining gene function is lost. Most genetic mechanisms that lead to inactivation of the second allele usually involve loss of flanking regions of the chromosome as well, and the resulting loss of heterozygosity of restriction fragment length polymorphisms is indicative of a cancer-dependent loss of function of a tumor suppressor gene (reviewed in

Dunlop, 1991). In addition, inactivation of one tumor suppressor gene allele through genomic imprinting, or differential expression of paternal and maternal genes, may be a relatively common phenomenon (Hochberg et al., 1994).

Mutations of the *p53* gene are the most common genetic lesions associated with human cancer. As with the *Rb* gene, people who inherit only one functional copy of the *p53* gene are predisposed to cancer development (the Li-Fraumeni syndrome) and, like the *Rb* gene product, the p53 protein acts to block cell replication. The p53 protein binds DNA and induces expression of a gene the product of which inhibits protein kinase activity of a CDK–cyclin complex. As previously noted, *p53* may function to halt proliferation in cells with damaged DNA, allowing the cells to repair damage before replication. Loss or inactivation of *p53*, then, may not only allow proliferation of initiated cells, but also generate further mutations when damaged DNA is replicated, contributing to the genomic instability that characterizes cancer cells. It has been technically easier to identify protooncogenes than tumor suppressor genes, so many more of the former (about 60) are currently known, whereas there are about 15 known or suspected tumor suppressor genes. The multiple tumor suppressor gene (*MTS-1*), that encodes the cell cycle regulatory protein, p16, and the *BRCA1* gene, implicated in some human breast cancers have been recently described, however, and it is likely that more genes of this type will soon be identified.

Many lines of evidence suggest that a single alteration is not sufficient to convert a normal cell into a malignant one, and it seems apparent that neoplastic disease development involves loss or inactivation of multiple tumor suppressor genes, or activation of protooncogenes, or a combination thereof, throughout the carcinogenic process. In addition to protooncogenes and tumor suppressor genes, other targets important for neoplastic transformation may exist. For example, transformation effector and suppressor genes have been described that are normal cellular genes which encode proteins that cooperate with, or oppose, oncogene functions, respectively (Boylan and Zarbl, 1991). Many other cancer-related gene targets have been proposed, including migration genes, metastasis and metastasis suppressor genes, genomic instability genes, immune tolerance genes, and epigenetic regulation genes (Cheng and Loeb, 1993), and cooperative interactions between various genes seems to be involved in acquisition of malignant properties.

E. Epigenetic Changes

Heritable alterations that are not genetic, that is, due to alterations in DNA sequence, or mutations, are referred to as *epigenetic*. Epigenetic changes involved in regulating gene expression include alterations in DNA methylation, transcription activation, translational control, and posttranslational modifications. These changes, which may be heritable and stable (Holliday, 1987), are not unique to carcinogenesis, but also occur during normal development and differentiation. It is also possible that mutations can result from interactions of xenobiotics with targets other than DNA, as shown, for example, by the ability of manganese ion to reduce the fidelity of DNA polymerase (Beckman et al., 1985). Nonheritable epigenetic changes, such as stimulation of cell proliferation through cytotoxicity or hormonal effects, may also contribute to neoplastic transformation (see, e.g., Melnick et al., 1993 and references cited therein). Cell division is essential for converting DNA damage into mutations and for selection of cells with altered phenotype. If an initiating event has occurred in a cell, clonal expansion also increases the likelihood of further genetic or epigenetic changes, and agents that induce cell division may influence each stage of carcinogenesis involving genetic change. An agent's ability to induce proliferation in a given tissue does not, however, unequivocally demonstrate its potential carcinogenicity, as the only relevant population to carcinogenesis is the initiated cell(s). Finally,

some investigators believe the somatic mutation theory may place undue emphasis on only one element of a multifaceted, dynamic process (see, e.g., Vasiliev, 1983; Farber, 1984; Epstein, 1986). One alternate view is that a hierarchy of morphogenic fields or tissue organizers are of primary importance in maintaining control of growth and differentiation (Rubin, 1985). This concept identifies epigenetic changes that alter tissue organization as the principal determinants of malignant transformation, and the chromosomal and other genetic modifications that occur are regarded as epiphenomena, or adaptive changes secondary to the primary events.

IV. CHEMICAL CARCINOGENESIS

The term *chemical carcinogenesis* is usually defined as the induction or enhancement of neoplastic disease, including both benign and malignant tumors, by xenobiotics. Chemical carcinogenicity can be manifested by (1) an increased frequency of tumors also seen in controls, (2) appearance of a type of tumor not seen in controls, (3) a decreased latent period before appearance of tumors, or (4) an increase in the number of tumors produced per animal (Lu, 1991). Epidemiological evidence for chemical carcinogenesis existed before animal models were developed, and both animal and human data are now used to classify compounds according to their carcinogenicity. Different risk assessment methodologies and regulatory approaches have been developed for environmental chemicals classified as carcinogens and those considered noncarcinogenic.

In evaluation of chemicals for carcinogenic potential by the International Agency for Research on Cancer (IARC), human data, usually from occupational or medical exposures, are given more weight than animal data, and evidence for carcinogenicity is considered stronger when malignant tumors are induced, when carcinogenicity can be demonstrated at low dose, in several species and strains, and if the chemical under consideration reacts with DNA. On the basis of these considerations, chemicals are placed in one of four categories: *group 1* includes those agents for which there is sufficient evidence to conclude they are carcinogenic to humans; agents in *group 2* are considered either probably (group 2a) or possibly (2b) carcinogenic to humans, depending on the strength of the supporting data; agents in *group 3* are not classifiable as to carcinogenicity; and agents in *group 4* are considered to be unlikely to be carcinogenic to humans. Presently, over 50 agents, mixtures, and occupational settings are considered to be carcinogenic to humans, and about 200 more are classified in group 2 (IARC, 1987). The compounds that have been identified as carcinogens are not believed to account for most human neoplastic disease, however, which appears to be associated with lifestyle, particularly diet, the use of tobacco products, and alcohol consumption (see, e.g., Weisburger, 1994b).

A. Mode of Action

1. Initiators

In the multistage paradigm of carcinogenesis, chemicals may act to increase the likelihood of cancers by initiating neoplastic transformation in a cell, promoting tumor formation, or conferring malignant properties on a neoplasm. Chemicals that can, by themselves, induce cancer are called *complete carcinogens*, which exhibit properties of all three (initiating, promoting, and progressor) agents (reviewed in Lu, 1991). Few agents are known that are pure initiators, without promoter or progressor capability, but many carcinogens act as initiators at low doses. Most initiating agents are genotoxic (i.e., they, or their metabolites, can react with DNA to produce adducts or other genetic lesions). Initiator-induced damage may be unrepaired, reversed through error-free DNA repair, or, if the DNA sequence is not exactly restored, misrepaired before S phase DNA replication, which may be blocked by some nonrepaired lesions, but that

can proceed past others. Following replication, then, misrepaired lesions would be expected to result in a high, and repaired lesions in a low, probability of mutation, whereas unrepaired lesions would be expected to lead to cytotoxicity or mutation with high probability. Carcinogenic initiation becomes essentially irreversible after the cell undergoes replication.

2. Promoters

Tumor promoters are known to produce a variety of effects on cells, ultimately leading to cellular proliferation. In skin cancer models, promoters increase the frequency of tumor formation markedly only when given after exposure to initiators and if sufficient exposure to promoter occurs. Phorbol esters, especially tetradecanoylphorbol acetate (TPA), are the best-studied tumor promoters (reviewed in Castagna, 1987). Cytosolic and membrane-bound protein kinase C (PKC) is a receptor for the phorbol esters, and their biological effects are probably produced by modulating PKC activity and the subsequent activation or inhibition by PKC of enzymes involved in cell proliferation. Other promoters that are structurally dissimilar to TPA, such as teleocidin and aplysiatoxins, may also produce their effects by interacting with PKC. Some cytotoxicants, such as nitriloacetic acid, and hormones, such as estradiol, do not interact with PKC, but act by increasing cell proliferation. If cell antioxidant defenses are overwhelmed, oxygen radicals can induce DNA damage and alter membrane-associated activities, such as signal transduction, and generation of free radicals may be involved in promoting effects of compounds such as chrysarobin, palytoxin, and peroxides. In contrast with promoters, co-carcinogens, such as ethanol, increase the carcinogenicity of simultaneously administered initiators. These compounds may increase the effective concentration of the ultimate carcinogen, for example, through effects on absorption or metabolism, but the agents alone are not considered to be genotoxicants.

3. Progressor Agents

Chemicals capable of inducing transition from the stage of promotion to that of progression are progressor agents. Since karyotypic alterations are a distinctive trait associated with progression, genotoxicants, especially clastogens, are potential progressor agents. The human carcinogens arsenic, asbestos, and benzene can induce chromosomal aberrations and may have progressor activity as well (Pitot et al., 1992), and it is possible that more progressor agents without significant initiating or promoting activities are yet to be discovered.

B. Chemical Classes of Carcinogens

A wide variety of chemical compounds, often with no obvious structural similarities, are carcinogenic (reviewed in Williams and Weisburger, 1991). A common mechanism for many diverse chemical agents has been proposed; namely, that compounds that are not themselves electrophilic reactants (*direct*, or *ultimate carcinogens*) must be metabolized to an electrophilic form that can react with nucleophilic moieties of cellular macromolecules (reviewed in Miller and Miller, 1981). Direct carcinogens are sometimes classified as genotoxic (DNA-reactive), whereas chemicals classified as nongenotoxic or epigenetic carcinogens do not damage DNA, but enhance the growth of tumors induced by genotoxic carcinogens. Chemicals may work through both genotoxic and nongenotoxic mechanisms, however, and it is not often easy to assign a chemical to a given category (Barrett, 1992). As most known chemical carcinogens are *procarcinogens*, which require metabolic intervention to become ultimate carcinogens either directly or through an intermediate stage, the *proximate carcinogens*, biotransformation is an important process in initiating chemical carcinogenesis and in determining the site of tumor formation. Xenobiotic metabolism, including that of carcinogens, is generally divided into *phase I* reactions, which include oxidations, especially those mediated by the cytochrome P-450 group

of enzymes, reductions, and hydrolyses, and *phase II* reactions, which involve conjugation of a number of substrates with the xenobiotic. Many agents require more than one enzymatic step for activation (i.e., they are converted first to proximate carcinogens then to ultimate carcinogens). The amount of ultimate carcinogen produced depends on the relative activities of the activation and detoxification pathways.

1. Polycyclic Aromatic Hydrocarbons

It is beyond the scope of this chapter to describe all known carcinogens and their metabolism, but some representative classes will be discussed. Some carcinogens, including *polycylic aromatic hydrocarbons* (PAH) can be produced by incomplete combustion of organic matter, including fossil fuels, and are widely distributed in the environment. A common source of human exposure to these agents is tobacco smoke. Many PAH, including benzo[*a*]pyrene, 7, 12-dimethylbenz[*a*]anthracene, and 3-methylcholanthrene, have been carcinogenic in animal studies. The metabolic activation of PAH requires a sequence of three reactions, catalyzed by enzymes of the cytochrome P-450 system, specifically CYP1A1, leading to generation of a dihydrodiol epoxide. Initially, it was felt that carcinogenicity was associated with K-region (i.e., the 9–10 phenanthrene-like double bond) epoxides, but it has since been shown that metabolites with epoxides adjacent to a bay region of the molecule are the active compounds. In vitro, dihydrodiol epoxides do not appear to be substrates for epoxide hydrolase, which may be important to their carcinogenicity. A class of sterically hindered bay region derivatives termed *fjord region* diol epoxides display marked genotoxic properties, together with resistance to hydrolysis, and may be important carcinogens, as well (see, e.g., Hecht et al., 1994). *Heterocyclic aromatic compounds* are a related group of carcinogens, which also can arise from combustion, and some members of this class, the heterocyclic aromatic amines, are pyrolysis products of amino acids and proteins and are found in cooked foods (reviewed in Sugimura and Wakabayashi, 1990). Representative members of this group include IQ, MeIQ, Glu-P-1, and Trp-P-1. Polycyclic aromatic heterocyclic agents undergo oxidation by another member of the cytochrome P-450 family, CYP1A2. Prostaglandin H synthase can, in the presence of arachidonic acid, generate free radical intermediates that also can bioactivate many chemical carcinogens, such as PAH and aromatic amines (reviewed in Eling et al., 1990). This pathway is probably of most significance in extrahepatic tissues with low monooxygenase activities.

2. Aromatic Amines and Azo Dyes

Unlike PAH, aromatic amines and azo dyes are not widely encountered in the environment, but individuals are exposed to these synthetic agents in certain occupational settings. Indeed, the initial observation that led to the discovery of this group of carcinogens was the finding of bladder cancer in aniline dye workers. The metabolism of the prototype aromatic amine, 2-napthylamine, also involves oxidation by cytochrome P-450 monooxygenases. One product, 2-napthylhydroxylamine, rapidly undergoes conjugation with glucuronic acid in the liver, and the unreactive conjugate is excreted in the urine. In the urinary bladder, however, low pH and the presence of a soluble β-glucuronidase regenerate the hydroxylamine, which can form the ultimate carcinogen. Other aromatic amine carcinogens, such as 2-acetylaminofluorene (AAF), also are converted to active *N*-hydroxyl compounds. Azo dyes undergo both reductive and oxidative metabolism, the latter catalyzed by both cytochrome P-450 and flavin-containing monooxygenases. Like aromatic amines, azo dyes are converted to *N*-hydroxyl derivatives that can be further metabolized to esters that serve as proximate carcinogens.

3. N-Nitroso Compounds

Many *N*-nitroso compounds are carcinogenic, producing tumors at a wide variety of sites. The prototype agent is *N*-nitrosodimethylamine, a symmetrical *N*-nitrosamine reported to be carci-

nogenic in all animal species tested. Other nitrosamines, including asymmetrical compounds, such as *N*-nitrosomethyl-*n*-propylamine, and cyclic compounds, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific compound, are also animal carcinogens. Humans can be exposed to certain of these agents (e.g., NNK) in the environment, and other compounds in this class may be generated in vivo through the reaction of nitrite ion with amines and amides. Nitrosamines undergo oxidation by several enzymes, including the cytochrome P-450 monooxygenases CYP1A2, CYP2A6, and CYP2D6. The resulting metabolites are converted nonenzymatically to the ultimate carcinogens, which may be diazonium compounds or carbonium ions. A subgroup of *N*-nitroso compounds, including alkylnitrosoureas, nitrosourethanes, and nitrosoguanidines, give rise to reactive intermediates without the intervention of cellular metabolism. The symmetrical hydrazines may be converted through a series of reactions to the same ultimate carcinogens that are produced from nitrosamines.

4. Other Carcinogens

Carcinogenic properties are also associated with some natural products, including aflatoxin B_1, formed by certain strains of *Aspergillus flavus*, safrole, cycasin, and isatidine. Halogenated aliphatic hydrocarbons, such as carbon tetrachloride, ethylene dibromide, and vinyl chloride are another class of carcinogens, and urethane and related compounds make up another small group. Inorganic chemicals, including some metals and metalloids (e.g., beryllium, chromium, nickel, and asbestos), and miscellaneous organics, including thiourea and thioacetamide, have also been implicated as carcinogens. Agents that increase the number of peroxisomes in tissues, although not considered genotoxic themselves, can produce tumors in rodents (reviewed in Gibson, 1993). These agents damage DNA through increased production in the cell of active oxygen species and can induce proliferation, oncogene activation, CYP4504A1 induction, and hepatomegaly. Examples of peroxisome proliferators include clofibrate, di(2-ethylhexyl)phthalate and 1,1,2-trichlorethane.

C. Anticarcinogens

Dietary constituents are known that inhibit carcinogenesis (reviewed in Weisburger, 1994a). Several antipromoters have been identified that are analogues of vitamin A, a retinoid essential for normal epithelial cell differentiation. Retinoids and other carotenoids appear to block the promotion–progression phase of carcinogenesis, as they are ineffective when given before or together with an initiating carcinogen, but can block the promoting effects of phorbol esters. Anticancer activity has also been demonstrated in some models with other antioxidants, such as vitamin E, selenium, and the polyphenol, epigallocatechin gallate. Sphingolipids, which are hydrolyzed to PKC inhibitors, and some fatty acids, such as conjugated linoleic acid and the ω-3 fatty acids, especially eicosapentaenoic acid and docosahexaenoic acid, which modify the conversion of arachidonic acid to prostaglandins, also show anticarcinogenic activity in some circumstances (Borek, 1993). Components of cruciferous vegetables, such as phenethyl isothiocyanate, inhibit production of lung cancer by a nitroso compound found in tobacco smoke; and ellagic acid, which inhibits CYP1A1 activity and reduces the incidence of PAH-induced carcinomas, are other examples of this group. Both synthetic and naturally occurring compounds with the ability to inhibit preneoplastic events of carcinogenesis have been employed in cancer chemoprevention studies (see, e.g., El-Bayoumy, 1994).

V. VARIABLES IN MULTISTAGE CARCINOGENESIS

Variation, both in number and site of tumors, has been noted in the response of different animal species and strains to the same chemical carcinogens. This variability may be related to endogenous factors, such as extent of metabolic activation and detoxification reactions, DNA repair capability, and capacity for cell proliferation.

A. Animal Studies

Over 400 long-term chemical carcinogenesis studies using rats and mice have recently been reviewed (Huff et al., 1991), and some similarities in incidence and site of tumor development were found. For example, in both species of rodents, liver is the most common tumor site, and, although mice are more likely to experience liver tumors, there is an 80% interspecies concordance for hepatocarcinogenicity. Other organ sites, such as lung, forestomach, and the hematopoetic system, also show a high interspecies correlation. Differences were also noted in response of the two rodent species. For example, female rats had the most chemically associated mammary tumors, whereas the male rat was most prone to chemically induced tumors of the kidney and pancreas. Furthermore, tumors at some sites were far more common in a particular species: for example, urinary bladder cancers occur more frequently in the rat, but harderian gland neoplasms are found mainly in the mouse.

Sites of tumor formation in humans show some similarities to those produced in rodent carcinogenicity bioassays (Huff et al., 1991). The lung, hematopoetic system, mammary gland, urinary bladder, and uterus are among the ten most frequent sites of tumor development in both the United States population and in rodent bioassays. Moreover, all agents for which there is evidence of carcinogenicity in humans cause cancer at a common site in at least one animal species. In contrast, the data for induction of human tumors by known animal carcinogens are much less consistent, perhaps because human data are lacking for some chemicals, or because of the difference between genotoxic and nongenotoxic carcinogens. Most known human carcinogens are genotoxicants, whereas about half known rodent carcinogens are of the nongenotoxic variety, which usually require long exposures to relatively high doses to cause their effect. The mechanism of tumorigenesis by these agents may thus be so different from that of genotoxic carcinogens that extrapolation to the low doses to which humans are exposed is questionable. Some chemicals, however, also produce tumors in humans in the same organs as found in rats or mice. Examples are aflatoxin and diethylstilbesterol, which were first shown to be carcinogenic in rodents.

B. Biotransformation

Many carcinogens must undergo biotransformation to produce the ultimate carcinogen, and some observed species differences in susceptibility to carcinogenesis have a metabolic basis. Many of the reactions that convert chemically stable procarcinogens to electrophilic, reactive agents are carried out by cytochrome P-450 enzymes. Multiple P-450 isozymes exist with different substrate specificities or differences in their distribution among organs, species, and individuals (Harris, 1991). The differential sensitivity of rodents and humans to vinyl chloride-induced liver tumors is one example of metabolic capacity determining tumor incidence (cited in IARC, 1992). Rats and mice oxidize vinyl chloride 12 and 15 times faster (normalized by body weight), respectively, than do humans, and the rodent sensitivity to vinyl chloride-induced liver cancer is

greater by approximately the same degree. There are also many instances in which species differences in carcinogenicity cannot be explained by metabolism: Cotton rats, for example, are resistant to the carcinogenic effects of AAF, although the compound is readily metabolized in vivo to genotoxic products.

In addition to species differences, xenobiotic-metabolizing activity can be modified by other variables, such as pharmacokinetic factors. The relatively high doses employed in testing regimens may saturate some metabolic reactions, whereas, at the lower doses to which humans are exposed, rates and pathways of metabolic processes may be qualitatively and quantitatively different. Nutritional factors, hormonal influences, or exposure to carcinogens or other drugs can also alter drug-metabolizing enzymatic activity. In animal studies that use reasonably homogenous populations, these factors can be controlled, and metabolic differences between individual animals are generally small. In humans, however, there can be considerable interindividual differences, which may be reflected in different risk of neoplastic disease. In addition to environmental or nutritional factors, genetic polymorphisms exist in several enzymes that catalyze carcinogen activation or detoxification (reviewed in Idle et al., 1992). Polymorphisms that may modulate chemical carcinogenesis are known for both phase I reactions, including those catalyzed by members of the cytochrome P-450 family—CYP1A1, CYP1A2, CYP2A6, CYP2D6, and CYP3A4—and phase II reactions, including UDP-glucuronosyltransferases, *N*-acetyltransferases, sulfotransferases, and glutathione *S*-transferases. Although these polymorphisms are well-established, epidemiological data linking a particular phenotype to increased or decreased cancer risk are often lacking. An association between the extensive metabolizer phenotype of debrisoquine-4-hydroxylase (CYP2D6) and increased lung cancer risk has been reported, and the tobacco-specific nitrosamine, NNK, is a substrate for this enzyme. Associations between arylhydrocarbon hydroxylase inducibility (CYP1A1) and lung and laryngeal cancer and between the slow-acetylator phenotype (*N*-acetyltransferase) and bladder cancer and the fast-acetylator phenotype and colon cancer have also been reported.

C. DNA Repair

The DNA molecules undergo frequent, potentially mutagenic alterations, including spontaneous deaminations, depurinations, and oxidative damage, as well as damage from xenobiotic exposure. Most alterations are quickly corrected by a variety of DNA repair processes, most of which depend on the existence of double-stranded DNA in the region of the damage (reviewed in Barnes et al., 1993; Sancar and Tang, 1993). Animal cells have pathways for direct reversal of DNA damage in a single enzymatic step, such as repair of alkylated bases or strand breaks, for both base and nucleotide excision repair and for mismatch repair. Recombinational repair of daughter strand gaps and inducible SOS repair response to severely damaged DNA exist in prokaryotes, and analogous processes are thought to operate in animal cells as well. The enzymes involved in DNA repair interact to form a network of reactions, such that alterations in a single component of the system might have a marked influence in overall repair capacity. In addition, some proteins involved in DNA repair processes are also involved in other cellular activities relevant to carcinogenesis, such as gene regulation and DNA replication (Hanawalt et al., 1994). The carcinogenicity of some chemical agents, such as arsenicals, may, at least partly, be due to inhibition of DNA repair processes.

Human mutagen–hypersensitivity syndromes provide evidence that defective DNA repair systems can increase the risk of cancer (reviewed in Heddle et al., 1983). Individuals with xeroderma pigmentosum develop skin cancer as a result of accumulated sunlight (UV)-induced DNA damage and are defective in the incision step of nucleotide excision repair. At least seven different gene products are associated with the disorder, which may reflect the need for