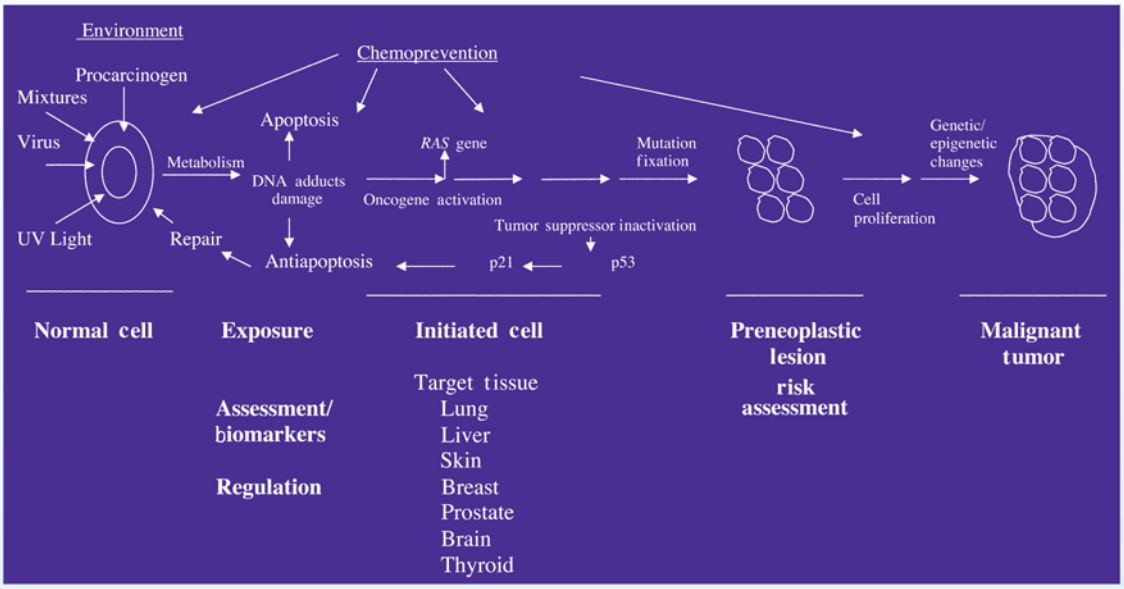
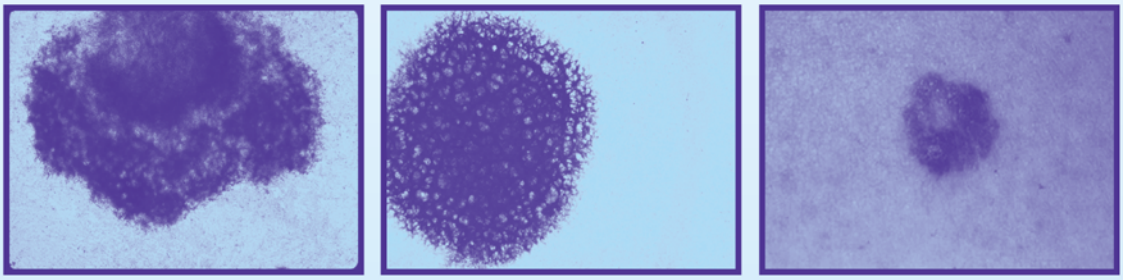
MOLECULAR CARCINOGENESIS AND THE MOLECULAR BIOLOGY OF HUMAN CANCER





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DAVID WARSHAWSKY

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Preface

There is a strong need for a basic graduate textbook in molecular carcinogenesis. Dr. David Warshawsky has taught a comprehensive course in chemical carcinogenesis at the College of Medicine in the University of Cincinnati (UC) for many years. The colleagues of Dr. Warshawsky from UC who contributed to this textbook include Drs. Glenn Talaska (carcinogen biomarkers), George Leikauf and Jay Tichelaar (lung cancer), Mario Medvedovic (bioinformatics), Susan Heffelfinger (breast cancer), Zalfa Abdel-Malik (skin cancer) and Eula Bingham (carcinogen regulation). Hence, this book is the result of a strong USC–UC collaboration of Dr. Joseph Landolph, Jr. and Dr. Warshawsky and the colleagues of Professor Melvin Calvin at the University of California at Berkeley (Dr. Andrew Salmon) and in the laboratory of Professor Charles Heidelberger at the University of Wisconsin, Madison (Dr. Steven Nesnow). Colleagues of Dr. Landolph recruited from USC who have also contributed to this textbook are Drs. James Ou (Hepatitis B virus), Colin Hill (radiation carcinogenesis), and Nouri Neamati (proteomics). We have, in addition, recruited many experts and their collaborators from many other universities, research institutes, and regulatory bodies: Dr. Ole Laerum and Johan R. Lillehaug (cancer of the brain), University of Bergen; Dr. Dan Djakiew (prostate cancer), Georgetown University; Dr. Helmut Zarbl (oncogenes), Fred Hutchinson, Cancer Research Center; Dr. Bernard Weissman (tumor suppressor genes), University of North Carolina; Dr. Wynshaw-Boris (transgenic and knockout mice in carcinogenesis), University of California at San Diego; Dr. Jeff Ross (carcinogen DNA adducts), USEPA; and Dr. Gary Stoner (chemoprevention), Ohio State University.

Dr. Landolph has taught a similar course on chemical carcinogenesis at the Keck School of Medicine of the University of Southern California (USC) since 1982. Part I covers the basic science of cancer and includes lectures on cancer pathology, the epidemiology of cancer, stress responses, DNA methylation and cancer, oncogenes, tumor suppressor genes, DNA repair, chemical mutation, chemical carcinogenesis, viral carcinogenesis, and radiation carcinogenesis. Part II covers cancers of various organ systems and some treatments for these cancers, including breast cancer, prostate cancer, lung cancer, bladder cancer, colon cancer, leukemia, cancer immunology and immunotherapy, and gene therapy of cancer.

The collaborations between Dr. Landolph and Dr. Warshawsky go back to l973, and have led directly to the writing of this book. They first met when Dr. Landolph was a graduate student (l971–l976), and Dr. Warshawsky was a postdoctoral fellow (1973–l976), in the chemical biodynamics laboratory of Professor Melvin Calvin at the University of California at Berkeley, California. Dr. Warshawsky received training in chemical carcinogenesis from Professors Calvin, Orchin, and Bingham. Similarly, Dr. Landolph received training from Professors Charles Heidelberger and Calvin.

This book is divided into two parts, similar to the organization of the two courses at USC and UC in the molecular biology of cancer. Part I covers the basic science of cancer, including an historical overview of cancer and chemical carcinogenesis, chemical carcinogenesis, viral carcinogenesis, and radiation carcinogenesis, oncogenes, tumor suppressor genes, and genomics and proteomics approaches to understanding the molecular nature of cancer. Part II covers an overview of human cancer induction and human exposure to carcinogens, complex mixtures of chemical carcinogens, and tumors of various human organs, including breast cancer, prostate cancer, skin cancer, cancer of the brain, and cancer of the thyroid. Part II continues with chemoprevention of chemical

carcinogenesis and human cancer, exposure assessment and biomarkers, carcinogen risk assessment, and the regulation of carcinogens. Written by authors who are all experts in their chosen fields, this book should give the reader an overview of chemical, viral and radiation carcinogenesis, the carcinogenicity of complex mixtures and chemicals that cause human cancer, the proportional causes of human cancer, and the cell and molecular biology of specific important human tumors. This textbook is most appropriate for first and second year Ph.D. or M.D./Ph.D. students and postdoctoral fellows and is suitable for either a one or two semester course. However, this book should also be useful to advanced undergraduate students, to scientists moving into cancer research from other areas, to scientists teaching graduate courses in cancer biology and molecular carcinogenesis, to regulatory scientists, and to attorneys practicing law in the area of toxic torts.

We would like to thank two people without whom this book would not have been possible: Veronica Ratliff and Sireesha Kandula. Their collective skills were necessary to complete the book in a timely manner.

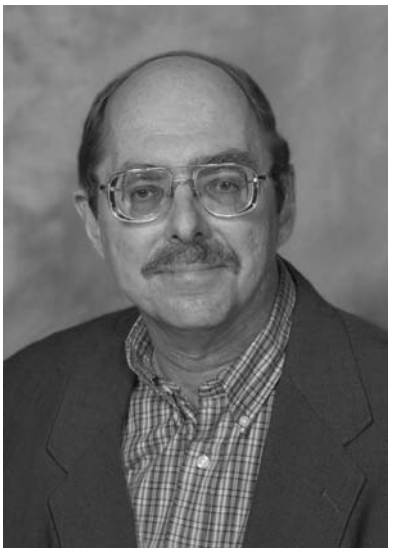
Dr. Warshawsky would like to thank his wife, Susan, and children Lisa and Bart, and Daniel and Deborah for their patience and understanding and insistence that this book be written to fill a need in molecular carcinogenesis. Dr. Landolph would like to thank his family, Alice and sons Joe III and Lewis, for their compassion and understanding in the writing of this book.

Editors

Dr. David Warshawsky is a professor of environmental health in the Division of Environmental Genetics and Molecular Toxicology in the College of Medicine at the University of Cincinnati. He has published over 120 papers in journals such as *Cancer Research, Chemical Research in Toxicology, Carcinogenesis, Molecular Carcinogenesis, Proceedings of the National Academy of Sciences, and Applied Environmental Microbiology.* Dr. Warshawsky has been funded by the NIH, USEPA, and US DOE. He received his B.S. with Robert Coates in chemistry from the University of Illinois, his M.S. in biochemistry from Rutgers University, his Ph.D. in organic chemistry with Milton Orchin from the University of Cincinnati, and did his postdoctoral training at University of California, Berkeley with Melvin Calvin, and research collaborations with Eula Bingham, Glenn Talaska, and Roy Albert at the University of Cincinnati. He has completed sabbaticals with Mina Bissell at Lawrence Berkeley Laboratory at Berkeley California, Steve Nesnow at Research Triangle Park at North Carolina, and Gary Stoner at Ohio State University. He is on the editorial board of *Toxicological Sciences* and has been on numerous review panels and has reviewed numerous manuscripts and proposals. He is a member of the Society of Toxicology, the American Association for Cancer Research, the American Chemical Society, and the American Association for the Advancement of Science.

Dr. Warshawsky has been a University of Cincinnati and State Representative on environmental issues for the Ohio Advisory Board from 1993 to 1996. He received an Outstanding Achievement Recognition Award from the department of environmental health, University of Cincinnati in 1995. He was also elected as Fellow of the Graduate School of the University of Cincinnati in 1998. Recently, he organized the 18th International Symposium on Polycyclic Aromatic Compounds that was held in Cincinnati in 2001 and he is the chair of the Oversight Committee for the University Mass Spectrometry Facility. He has been a technical consultant for OSHA. He was the Elected Councillor for the Society of Toxicology, Carcinogenesis Specialty Section, 1999 to 2000. He organized, with Dr. Landolph, a symposium in March 1998 at the Society of Toxicology meeting on the subject of molecular and cellular biology of chemical carcinogenesis.

Dr. Warshawsky's research focuses on the early events in the carcinogenic process for polyaromatic compounds and mixtures thereof including metals, the development of biomarkers of exposure, and methods for the microbial biodegradation of these recalcitrant compounds. He has investigated mechanisms of chemical carcinogenesis in target tissues, liver, skin, lung, and breast. This research involves the metabolism, binding to DNA and protein, gene expression, and growth factor regulation. The relationship of DNA adducts with specific mutations in the activated *RAS* oncogene and *p*53 tumor suppressor gene in target tissues is being assessed. His laboratory is studying mammary tumor prevention using angiogenic inhibitors. The role of angiogenesis in noninvasive tumors is largely unexplored. Although chemoprophylaxis in women at increased risk for developing invasive breast disease is a topic of national attention; all current approaches utilize hormone manipulation. This laboratory is the first to be able to delineate and characterize the degradation pathway of benzo[*a*]pyrene, a potent carcinogen. Work is underway to characterize the genes involved in the pathway. Based on the long history of this department on studying complex mixtures, studies are focused on the effects of a binary mixture on lung tumors, K-ras mutations, and DNA adducts. Dr. Warshawsky served on the National Academy of Sciences Committee on Complex Mixtures from 1986–1988.



Dr. Joseph R. Landolph, Jr., is currently associate professor of molecular microbiology and immunology and pathology and a member of the University Southern California (USC)/Norris Comprehensive Cancer Center, in the Keck School of Medicine, and associate professor of molecular pharmacology and toxicology, in the School of Pharmacy, with tenure, at the University Southern California in Los Angeles, California. Dr. Landolph received a B.S. degree in chemistry from Drexel University in l971 and a commission as a 2nd Lieutenant in the U.S. Army through ROTC. He received a Ph.D. in chemistry from the University of California at Berkeley in l976. At the University of California Berkeley, Dr. Landolph studied under the guidance of professor Melvin Calvin, and conducted research on the metabolism of the chemical carcinogen, benzo[*a*]pyrene (BaP), and its ability to induce cytotoxicity in cultured mouse liver epithelial cells and mechanisms of resistance to the cytotoxicity of BaP. He also studied BaP-induced cytotoxicity and morphological transformation in Balb/c3T3 mouse fibroblasts. Dr. Landolph did his postdoctoral study in chemically induced morphological and neoplastic cell transformation, chemically induced mutagenesis, and chemical carcinogenesis at the USC/Norris Comprehensive Cancer Center at the School of Medicine in the University of Southern California, under Professor Charles Heidelberger, from l977 to l980.

Dr. Landolph was appointed assistant professor of pathology in l980, and associate professor of microbiology, pathology, and toxicology at USC in l987. At USC, Dr. Landolph lectures to medical and Ph.D. students. These lectures include medical microbiology, prokaryotic molecular genetics, the molecular biology of cancer, the cytochrome P450 gene family and xenobiotic metabolism, and the role of oxygen radical generation in chemical carcinogenesis and tumor promotion. Dr. Landolph's research interests and activities include studies of the genetic toxicology and carcinogenicity of carcinogenic insoluble nickel compounds, carcinogenic chromium compounds, carcinogenic arsenic compounds, and carcinogenic polycyclic aromatic hydrocarbons. His laboratory studies the ability of these carcinogens to induce morphological and neoplastic transformation of C3H/10T1/2 mouse embryo cells and the cellular and molecular biology of the transformation process. His laboratory currently studies the ability of carcinogenic nickel compounds to induce activation of expression of oncogenes and inactivation of expression to tumor suppressor genes by mRNA differential display

in cell lines transformed by insoluble carcinogenic nickel compounds, such as nickel subsulfide, crystalline nickel monosulfide, and green (high temperature) and black (low temperature) nickel oxides. His laboratory is also studying the molecular biology of chromium compound-induced cell transformation. Dr. Landolph is an expert in chemically induced morphological and neoplastic transformation and chemically induced mutation in murine and human fibroblasts. He is the author of 56 scientific publications and has held peer-reviewed research grant support from the U.S. Environmental Protection Agency (USEPA), the U.S. National Cancer Institute, and U.S. National Institute of Environmental Health Sciences.

Dr. Landolph has served as grant reviewer for the USEPA Health Effects Panel, for special Requests for Applications (RFAs) for the National Institute of Environmental Health Sciences (NIEHS), and as an ad hoc member of the Chemical Pathology Study Section and the Al-Tox-4 Study Section of the National Institutes of Health (NIH). Dr. Landolph is also a member of the Carcinogen Identification Committee of the Scientific Advisory Committee of the Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency (EPA) (l994 to present). Dr. Landolph has served as a member of the Human Health Strategies Review Committee of the USEPA and is currently a member of the Scientific Review Panel for Toxic Air Contaminants of the California EPA, and is a member of the Science Advisory Board, the Drinking Water Committee, and the Science and Technology Achievement Award Committee of the USEPA. He is the recipient of numerous awards, including the Merck Award in Chemistry and the Superior Cadet Award in Reserve Officer Training Corps (ROTC) from Drexel University in l971, an American Cancer Society Postdoctoral Fellowship from l977 to l979, the Edmundson Teaching Award in the Department of Pathology at USC in l985, and a Traveling Lectureship Award from the U.S. Society of Toxicology in l990.

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Contents

1 Carcinogens and Mutagens

David Warshawsky

CONTENTS

SUMMARY

For residents in the United States, the probability of developing cancer at some point during the course of their lifetime is approximately one out of two among men and one out of three among women [1]. Nearly everyone's life has been directly or indirectly affected by cancer. Most cancer researchers believe that many of the cancers are associated with the environment in which we live and work. According to well-documented reports on association between occupational exposures and cancer, an estimated 40,000 new cancer cases and 20,000 deaths due to cancer in the United States each year are attributable to exposure to carcinogens in the occupational environment [2].

1.1 THE ENVIRONMENT, WORKPLACE, AND CANCER

1.1.1 Historical Perspective

Until the late Middle Ages the environment in the workplace did not receive much attention from the medical profession. Due to the increased demands for gold, silver, iron, copper, and lead during the 15th century, miners and metal workers were among the earliest groups to be studied for occupationally related diseases. Ulrich Ellenberg, a German physician from Augsburg, published a pamphlet in 1472 entitled, "On the Poisons, Evil Vapors, and Fumes of Metals" detailed the irritating effects of the fumes of lead and mercury on goldsmiths. *De Re Metallica* was published by the Georgium Agricola in 1556 on the accidents and diseases among miners and smelters of gold and silver. He recommended the use of facial masks and ventilation for their chronic lung disease attributed to inhalation of dusts [3].

In 1700, Bernardino Ramazzini published the first edition of *Discourses on the Disease of Workers* (*DeMorbis Artificum Diatriba*). This work established the field of occupational medicine. This publication contained a survey of the existing knowledge of the nature of diseases thought to be associated with a particular profession or workplace environment [3,4].

TABLE 1.1
Early Landmarks — Physical, Chemical, and Infectious Carcinogens in Environmental and Occupational Settings [4,5]

Principal investigator(s)	Causative agent	Date	Type of cancer
Pott	Soot	1775	Scrotum
Ayrton	Arsenic containing material	1822	Skin
Thiersch	Sunlight	1875	Skin
Manourriez	Coal tar	1876	Skin
Harting and Hesse	Some fractions from distillation of crude petroleum	1879	Skin
Unna	Solar radiation	1893	Skin
Rehn	Manufacture of aniline dyes	1895	Bladder
Frieben	X-rays	1902	Skin
Ferguson	Egyptian peasants infected with parasite *Schistosoma haematobrium*	1911	Bladder
Davis	Pipe smokers and betel nut chewers	1915	Lip and mouth
Leitch and Seguina	Radium radiation	1920	Skin
Delore and Bergamo	Benzene	1928	Leukemia
Stephens	Nickel	1932	Lung
Alwens	Chromium compounds	1932	Lung
Wood and Gloyne	Arsenicals, beryllium, and asbestos	1934	Lung
Neitzel	Mineral oil mists and radiation	1934	Lung
Kawahata	Coal tar fumes	1936	Lung

In 1775, Percival Pott of London described the increased incidence of cancer of the scrotum among chimney sweeps and attributed this to their contact with soot. This was the first clinical report of occupational chemical carcinogenesis. Since then a number of physical, chemical, and infectious carcinogens in environmental and occupational settings have been reported (Table 1.1). Increased incidences of skin cancer are associated with exposure to chemicals generated from materials containing coal, petroleum, shale, and arsenic as well as from radiation. Bladder cancer in the workplace was associated with the manufacture of aniline dyes, such as magenta and auramine. A number of compounds containing metals, such as nickel, chromium, beryllium, and arsenic, coal and petroleum products, and asbestos, were thought to be responsible for the higher incidence of lung cancer in occupational environments. Similarly, leukemia was associated with benzene and ionizing radiation generated from radon and radium [3–5].

Early chemical carcinogenesis experiments were performed in the beginning of the 20th century (Table 1.2). In 1915, skin cancer was induced on rabbit ears by painting them with coal tar. Similar results were obtained with other animal species using a variety of mixtures from coal and petroleum. Benzo(a)pyrene (BaP), a strongly carcinogenic compound that has been used as an indicator of carcinogenic potency, was isolated from coal tar in 1933. A number of experiments were carried out in the 1930s and 1940s using polycyclic aromatic hydrocarbons (PAHs) and aromatic amines, which produced cancer in a variety of animal species. In 1941, Berenblum et al. proposed the two-stage mechanism in skin cancer using coal tar and BaP (Table 1.2) [6].

TABLE 1.2
Early Landmarks in Experimental Chemical Carcinogenesis [4,6]

Date	Early landmarks	Principal investigators
1915–1918	Induction of skin cancer in rabbits and mice by coal tar	Yamagiqa, Ichikawa, and Tsutsui
1930	Tumor induction by the first pure chemical carcinogen dibenz[a,h]anthracene	Kennaway and Hieger
1933	Isolation of the carcinogen benzo[a]pyrene from coal tar	Cook, Hewett, and Hieger
1933–1936	Induction of liver cancer in rats by *o*-aminoazotoluene and by *p*-dimethylaminoazobenzene	Yoshida and Kinosita
1937	Induction of urinary bladder cancer in dogs by 2-naphthylamine	Hueper, Wiley, and Wolfe
1941	Initiation and promotion stages in skin carcinogenesis with tar and benzo[a]pyrene	Berenblum, Rous, MacKenzie, and Kidd

1.1.2 PRESENT DAY KNOWLEDGE

Since these early observations on environmental carcinogenesis and experimental animal carcinogenesis, the number of identifiable carcinogens has increased. However, the number of new synthetic chemicals has been increasing even more rapidly. In the United States, there are more than 80,000 commercial chemicals registered and an estimated 2,000 new ones are introduced annually for use in everyday items such as foods, personal care products, prescription drugs, household cleaners, lawn care chemicals etc. [7]. These large number of chemicals are deposited into and distributed within the environment during their manufacture, distribution, use, and disposal. Reportedly, only $<2\%$ of chemicals in commerce have been tested for carcinogenicity [2].

Although relatively few chemicals are thought to pose significant risk to human health, it is important to identify the toxicological/carcinogenic effects of these chemicals as well as the levels of exposure at which they become hazardous to humans. People worldwide became more and more concerned about the relationship between their environment and cancer and they wanted additional information and knowledge on important public health issues. Since 1960, research on cancer and carcinogenesis has substantially increased, due to support and organization from governments, academia, and industrial firms. As a part of the World Health Organization (WHO), the International Agency for Research on Cancer (IARC) in Lyon, France, was created. From 1972 to 2002, it has evaluated 885 agents (chemicals, group of chemicals, complex mixtures, occupational exposures, cultural habits, biological, or physical agents) in the first 82 volumes of IARC Monographs series [8]. In accordance with the procedures adopted as standard IARC practice, the agents, mixtures, and exposures as evaluated are classified into four groups: (1) carcinogenic to humans, (2)(a) probably carcinogenic to humans; (b) possibly carcinogenic to humans, (3) not classifiable as carcinogenic to humans, and (4) probably not carcinogenic to humans. Of the total 885 agents, mixtures, and exposures in the last update [9] on December 4, 2002, 88 items (63 agents and groups of agents, 12 mixtures, 13 exposure circumstances) are listed in Group 1, 64 ($55 + 5 + 4$ in each category) in Group 2(a), 236 ($220 + 12 + 4$ in each category) in Group 2(b), 496 ($477 + 12 + 7$) in Group 3, and 1 item in Group 4. From earlier IARC Monographs [10], 34 industrial chemicals or processes subdivided into three groups are reported here in Table 1.3. It should be noted here that some of the compounds are grouped together, such as arsenic and certain arsenic compounds, chromium and certain chromium compounds, and soot, tars, and mineral oils and others by the process, manufacture of, refining and mining. This compilation of information is very useful in the legislative process, as well as to those monitoring or working in an occupational setting.

In the United States, the National Institute for Occupational Safety and Health (NIOSH) was created in the Department of Health and Human Services in the 1970s as a federal agency responsible

TABLE 1.3
Evaluation of the Carcinogenic Risk of Industrial Chemical Process to Humans [10]

1. Carcinogenic to humans
   - 4-Aminobiphenyl
   - Arsenic and certain arsenic compounds
   - Asbestos
   - Manufacture of auramin
   - Benzene
   - Benzidine
   - *bis*-Chloromethylether
   - Chloromethyl methyl ether
   - Chromium and certain chromium compounds
   - Underground hematite mining
   - Manufacture of isopropyl alcohol by strong acid process
   - Mustard gas
   - 2-Naphthylamine
   - Nickel refining
   - Soots, tars, and mineral oils
   - Vinyl chloride
2. Probably carcinogenic for humans
   - Acrylonitrile
   - Amitrole
   - Auramine
   - Beryllium compounds
   - Carbon tetrachloride
   - Cadmium and certain cadmium compounds
   - Dimethyl sulfate
   - Ethylene oxide
   - Nickel and certain nickel compounds
   - Polychlorinated biphenyls
3. Not classifiable as to carcinogenicity to humans
   - Chlordane-heptachlor
   - Chloroprene
   - DDT
   - Dieldrin
   - Hematite
   - Isopropyl oils
   - Lead and certain lead compounds
   - Trichloroethylene

for conducting research and making recommendations for the prevention of work-related injury and illness. Identifying occupational carcinogens is one of the priorities of NIOSH. NIOSH published a list of chemicals identified as Suspected Carcinogens. A sub-file of Toxic Substances was first published in 1974 and updated periodically. The latest list [11] of 132 substances that NIOSH considers to be potential occupational carcinogens was made public in 2005. This information on the workplace carcinogens can be further assessed by listing the carcinogens, suspected or confirmed by target organ (Table 1.4); particular target organs, that is, liver, nasal cavity and sinuses, lung, bladder, and bone marrow are associated with not only the carcinogens but also the occupations (Table 1.5).

TABLE 1.4
Confirmed and Suspected Occupational Carcinogens by Target Organ [12]

Target organ/tissue	Confirmed carcinogen	Suspected carcinogen
Bone		Beryllium
Brain	Vinyl chloride	
Gastroenteric tract	Asbestos	
Hematopoietic tissue (leukemia)	Benzene, ionizing radiation styrene butadiene and other rubber manufacture substances	
Kidney	Coke oven emissions	Lead
Larynx	Asbestos, chromium	
Liver	Vinyl chloride	Aldrin, carbon tetrachloride chloroform, DDT dieldrin, heptachlor PCB's, trichloroethylene
Lung	Arsenic, asbestos, *bis*-(chloromethyl)ether, chloromethyl methyl ether, chromates, coke oven emissions, mustard gas, nickel, soots and tars, ionizing radiation, vinyl chloride	Beryllium, cadmium, chloroprene, lead, hematite
Lymphatic tissue		Arsenic, benzene
Nasal cavity	Chromium, isopropyl oil, nickel, wood dusts	Leather dusts
Pancreas		Benzidine, PCBs
Pleural cavity	Asbestos	
Prostate		Cadmium
Scrotum	Soots and tars	
Skin	Arsenic, Coke oven emissions, cutting oils, soots and tars	Chloroprene
Urinary bladder	4-Aminobiphenyl, benzidine, β-naphthylamine	Auramine, 4-nitrodiphenyl, magenta

The pertinent information for each of the carcinogens listed in Tables 1.3 to 1.5 are described in detail in Table 1.6. Key animal studies are noted, particularly where these tests rather than human data are the primary basis for the carcinogenic potential of a compound. In some cases, the animal data were so convincing that widespread human exposure was averted. In some instances the work environment and activities in which exposure occurs is widespread while for other agents the exposure is questionable.

The route of absorption of an agent is dependent on both its physical and chemical properties and the route of exposure. Inhalation is the most frequent route of exposure to vapors and fumes while skin is the route of absorption for both liquids and gaseous agents. Ingestion can also occur, particularly with agents in the solid phase. It should be noted here that exposure to a carcinogen is confined to a limited number of target organs; in different species the same organs may not be affected by the same agent.

Among those chemicals and mixtures, a total of 32 listed in Table 1.7 are federally regulated under the Occupational Safety and Health Act [16]. Additionally, in 1978 the Department of Health and Human Services established the National Toxicology Program (NTP) consisting of relevant toxicology activities of federal health research and regulatory agencies to evaluate agents of public health concern. With assistances from other agencies and nongovernmental institutions, the NTP prepares the *Annual Report on Carcinogens* (RoC), which has been changed to a biennial report since 1993. The most recent RoC, the Eleventh Edition [17], was released publicly during January 2005. The Tenth RoC lists 49 substances as Known to be a Human Carcinogen and 174 substances as

TABLE 1.5
Occupations Associated with an Excess Risk of Cancer [13]

Occupations	Confirmed and suspected carcinogen	Site of cancer
Tanners, smelters; vineyard workers; plastic workers	Vinyl chloride	Liver
Glass, pottery, and linoleum workers; nickel smelters, mixers and roasters; electrolysis workers; wood, leather, and shoe workers	Chromium, isopropyl oil, nickel, wood, and leather dusts	Nasal cavity and sinuses
Vintners; miners; asbestos users; textile users; insulation workers; tanners; smelters; glass and pottery workers; coal tar and pitch workers; iron foundry workers; electrolysis workers; retort workers; radiologists; radium dial painters; chemical workers	Arsenic, asbestos, chromium, coal products, dusts, iron oxide, mustard gas, nickel, petroleum, ionizing radiation, *bis*-chloromethylether	Lung
Asphalt, coal tar, and pitch workers; gas stokers; still cleaners; dyestuffs users; rubber workers; textile dyers; paint manufacturers; leather and shoe workers	Coal products, aromatic amines	Bladder
Benzene, explosives, and rubber, cement workers; distillers; dye users; painters; radiologists	Benzene, ionizing radiation	Bone marrow (leukemia)

Reasonably Anticipated to be Human Carcinogen (Table 1.8) and gives a detailed profile for each substances listed. Referring to the latest list of Potential Occupational Carcinogens completed by NIOSH [11], 79 of total 132 substances are listed in the Tenth RoC, and asterisked in Table 1.8. Among them, 26 substances are in the Known to be Human Carcinogen category and 53 items are in the Reasonably Anticipated to be Human Carcinogen category.

For many years, researchers have studied various substances in order to identify those that may cause cancer. Much of this information on specific chemicals or occupational exposures has been published in the scientific literature or in publicly available and peer-reviewed technical reports. It provides meaningful and useful data on (1) the carcinogenicity, genotoxicity, and biologic mechanisms of these substances in people and in animals, (2) the potential for human exposure to them, and (3) Federal regulation to limit exposure.

1.2 CARCINOGENS AND MUTAGENS

1.2.1 DEFINITIONS

Cancer can be defined as an unregulated growth of cells arising from one cell. The scientific or medical term for cancer is malignant neoplasm, which is defined as a relatively autonomous growth of tissue not subject to the rules and regulations of normal growing cells. Tumor is a general term indicating any abnormal mass or growth of tissue. Therefore, a neoplasm is a tumor. Major features of benign tumors are encapsulation, slow growth, and non-invasion of surrounding tissue; that is, lack of metastasizing ability. Malignant tumors grow rapidly, are not encapsulated and invade surrounding tissue and metastasize. Benign growths generally have a normal complement of chromosomes, exhibit good differentiation, and have rare cell division. The opposite is characteristic of malignant neoplasms [18].

At the turn of the 20th century, cancer was the eighth leading cause of death due to diseases in the United States and heart disease was ranked fourth. Cancer caused 16% of the total deaths in the United States. Since 1990, about 16 million cancer cases have been diagnosed in the United States. In 2002, 1,284,900 new cancer cases were diagnosed and 555,500 Americans died of cancer, which contributed to a quarter of the total disease-associated deaths [1]. Ranking only behind the heart

TABLE 1.6
Occupational Carcinogens [3,10,13–15]

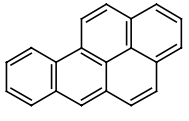
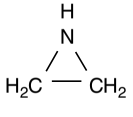
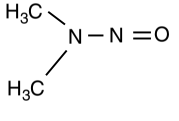
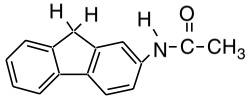
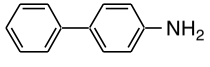
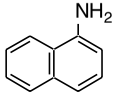
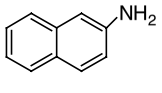
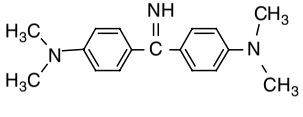
Agent	Chemical structure	Animal studies: Species	Animal studies: Route of exposure	Animal studies: Target organ	Human data: Route of exposure	Human data: Target organ	Human data: Latency period (years)	Occupational exposure
A. Organic agents								
1. Aromatic hydrocarbons								
Benzene		Mouse	Topical, S.C. injection	Inadequate	Inhalation, skin	Hemopoietic system, bone marrow leukemia	6–14	Benzene or rubber cement workers, distillers, dye users painters
Coke oven emissions (Benzo[a]pyrene)		Multiple	Topical, intratracheal	Lung	Inhalation, skin	Lung, kidney, skin	9–23	Coke oven workers
Soot, tars, oils-coal tars and pitch creosote oils, shale oils, cutting oils, coal tar fumes, mineral oils, coal soot		Mouse, rabbit	Topical, S.C. injection	Skin	Inhalation, skin	Skin, lung bladder, gastrointestinal tract, scrotum	9–23 or 12–30	Coal gas, coke and petroleum industry workers, miners, chimney sweeps, coal tar and pitch workers, textile weavers, rubber fillers
2. Amines								
Aliphatic amines, ethyleneimine		Rat, mouse	S.C. injection, P.O.	Sarcoma, kidney, Liver, lung	Inhalation, skin			Effluent treaters, organic chemical synthesizers, paper makers, textile workers; no documented human cases
N-nitrosodimethyl-amine		Rat	Inhalation P.O.	Liver, kidney Lung	Inhalation, skin			Dimethyl hydrazine makers

TABLE 1.6
(Continued)

Agent	Chemical structure	Animal studies: Species	Route of exposure	Target organ	Human data: Route of exposure	Target organ	Latency period (years)	Occupational exposure
3. Aromatic amines								
2-Acetylaminofluorene		Rat Dog Guinea Pig	P.O. P.O. P.O.	Liver Bladder, liver Negative	Inhalation, skin			Carcinogenic data averted commercial production
4-Aminobiphenyl		Mouse, rabbit, dog	P.O.	Bladder	Inhalation, skin	Bladder	15–35	Dye stuffs manufacturers and users, rubber workers, textile dyers
		Newborn mouse	S.C. injection	Liver				
		Rat	S.C. injection	Mammary gland Intestine				
1-Aminonaphthalene		Dog, mouse	P.O.	Inconclusive or negative	Inhalation, skin	Bladder	22	Dye stuffs manufacturers and users, rubber workers, activity has been attributed to contamination with 2-aminonaphthalene
2-Aminonaphthalene		Dog, hamster, monkey	P.O.	Bladder	Inhalation, skin	Bladder	16	Dye stuffs manufacturers and users, rubber workers
		Mouse	P.O.	Liver				
Auramine		Rat, mouse	P.O.	Liver	Inhalation, skin	Bladder	13–30	Dye stuffs manufacturers and users; auramine manufacture has been associated with increase in bladder cancer — the actual compound has not been identified

(continued)

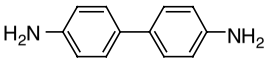
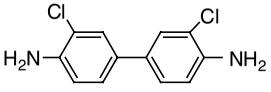
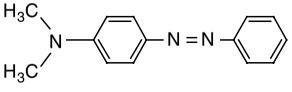
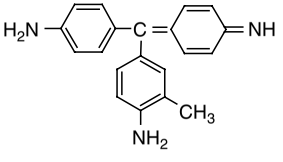
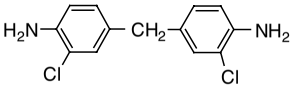
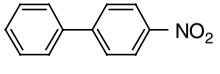
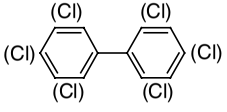
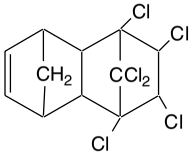
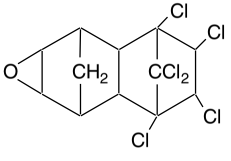
Benzidine		Rat, hamster Mouse, rat Dog	P.O. S.C. injection P.O.	Liver Liver, ear duct in rat Bladder	Inhalation, skin	Bladder	16	Dye stuff manufacturers and users, rubber workers; medical laboratory personnel and researchers use benzidine and derivatives
3,3′-Dichlorobenzidine		Rat, hamster	P.O.	Bladder	Inhalation, skin			Dye stuff manufacturers and users, cases have occurred with simultaneous exposure to benzidine
4-Dimethylaminoazobenzene		Rat, mouse	P.O.	Liver	Inhalation, skin			Carcinogenic animal data averted commercial production, no documented human cases
Magenta		Rat	S.C. injection	Local sarcomas	Inhalation, skin	Bladder		Dye stuff manufacturers and users, in 1895 Rehn's description of aniline tumors involved magenta manufacturers
4,4′-Methylene-*bis*-(2-chloroaniline) MOCA		Rat	P.O.	Liver, lung	Inhalation, skin			Elastomer makers, polyurethane foam workers no documented human cases
4. Biphenyls								
4-Nitrobiphenyl		Dog	P.O.	Bladder	Inhalation, skin			Research workers, carcinogenic animal data averted commercial production, exposure to compound occurred concurrently with 4-aminobiphenyl

TABLE 1.6
(Continued)

Agent	Chemical structure	Animal studies: Species	Animal studies: Route of exposure	Animal studies: Target organ	Human data: Route of exposure	Human data: Target organ	Human data: Latency period (years)	Occupational exposure
Polychlorinated biphenyls PCB		Mouse, rat	P.O.	Liver	Skin			Electric equipment makers and associated industries — capacitor producers and transformer workers. Dye stuffs manufacturers and users, plasticizer and resin makers, and wood preservers; slight increase in the incidence of cancer particularly melonoma of the skin reported in small groups of men exposed to mixture of PCB, Arochlor 1254, in occupational setting
5. Chlorinated hydrocarbons								
Aldrin/dieldrin	 	Dieldrin — mouse Dieldrin — rat, dog, monkey Aldrin — rat, mouse	P.O. P.O. P.O.	Liver Negative or inconclusive Negative or inconclusive	Inhalations, skin			Agricultural workers and insecticide manufacturers; Aldrin is metabolized to Dieldrin — involved too few subjects and insufficient follow up time to draw conclusion

(continued)

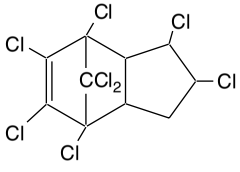
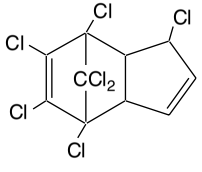
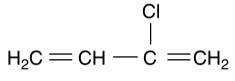
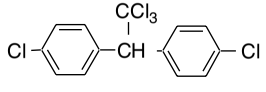
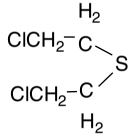
Carbon tetrachloride	CCl_4	Mouse, rat, hamster	P.O., inhalation	Liver	Inhalation, skin	Chemists, fluorocarbon skin makers, rubber workers, insecticide, refrigerant, lacquer and propellant makers, metal cleaners, three cases reported with liver tumors associated with cirrhosis following exposure
Chlordane/heptachlor	 	Mouse Rat	P.O. P.O.	Liver Inconclusive	Inhalation, skin	Agricultural workers and insecticide manufacturers; these compounds are considered together because similar in structure and often contaminated with one another; 5 out of 14 children with neuroblastoma had prenatal and postnatal exposure to chlordane, three persons with acute leukemia had been exposed to chlordane (3 to 7% heptachlor)
Chloroform	$HCCl_3$	Mouse	P.O.	Liver	Inhalation, skin	Chemists, fluorocarbon makers, solvent workers, lacquer workers; human data too limited

TABLE 1.6
(Continued)

Agent	Chemical structure	Animal studies			Human data			Occupational exposure
		Species	Route of exposure	Target organ	Route of exposure	Target organ	Latency period (years)	
Chloroprene		Mouse Rat	Skin S.C. injection, ingestion	Negative Negative	Inhalations, skin			Synthetic rubber makers; epidemiological studies inconclusive, one case report of angiosarcoma of liver in worker exposed
DDT		Mouse Rat Rat, guinea pig, dog, monkey	P.O. Ingestion P.O.	Liver Liver (nonmetastasizing tumors) Negative or inconclusive	Inhalations, skin			Agricultural workers, insecticide manufacturers; epidemiological studies inadequate
Ethylene dichloride		Rat Mouse	P.O. P.O.	Stomach, skin, breast Breast, uterus, lung	Inhalations, skin			Chemical makers, adhesive makers, plastic and solvent workers, degreasers, dry cleaner and insecticide makers; human data not available
Mustard Gas		Mouse	I.V. injection or inhalation, S.C. injection	Lung Local sarcoma	Inhalation	Respiratory tract	10–25	Mustard gas workers associated with chronic exposure

(continued)

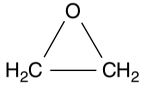
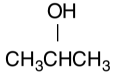
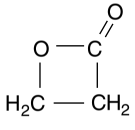
Trichloroethylene	$ClCH=CCl_2$	Mouse	P.O.	Liver, lung	Inhalations, skin			Anesthetic workers and users, cleaners, degreaser, dry cleaners, printers, resin workers, rubber cementers and solvent workers; no human data available
Vinyl chloride	$CH_2=CHCl$	Mouse, rat hamster	P.O., inhalation	Several sites including angiosarcoma of liver	Inhalation, skin	Angiosarcoma of liver and lung, brain, hemo-lymphopoietic system	20–30	Polyvinyl resin makers and rubber workers
6. Ethers								
Bichloromethyl ether (BCME)	$ClCH_2OCH_2Cl$	Mouse	Inhalation, topical, S.C. injection	Lung, site of application	Inhalation, skin	Lung	5+, 10–15	Ion exchange resin workers, small cell carcinoma
		Rat	S.C. injection	Site of application				
Chloromethyl methyl ether (CMME)	$ClCH_2OCH_3$	Mouse	Inhalation, topical, S.C. injection	Lung, site of application	Inhalation, skin	Lung	5+, 10–15	Chemists, ion exchange resin workers, small cell carcinomas, exposures generally involve CMME contaminated with BCME
		Rat	S.C. injection	Site of application				
7. Miscellaneous								
Acrylonitrile	$CH_2=CHCN$	Rat	P.O. injection	Brain, forestomach, zymbal gland	Inhalation	Colon, lung	20–25	Acrylic fiber and resin lung makers — epidemiological studies lack smoking history, exposure to other chemicals, and incomplete follow-up
Ethylene oxide		Mouse Rat	Topical S.C. injection	Inadequate Inadequate	Inhalation	Leukemia, gastric cancer		Ethylene oxide workers exposed to other chemicals cancer as well

TABLE 1.6
(Continued)

Agent	Chemical structure	Animal studies: Species	Animal studies: Route of exposure	Animal studies: Target organ	Human data: Route of exposure	Human data: Target organ	Human data: Latency period (years)	Occupational exposure
Isopropyl alcohol		Mouse	Inhalation, topical application, S.C. injection	Lung, inadequate	Inhalation	Nasal cavity, larynx	10+	Isopropyl alcohol makers isopropyl oils forms as by-product in manufacture of isopropyl alcohol by strong acid process
β-Propiolactone		Mouse Rat	Topical application, P.O.	Site of application Stomach Liver	Inhalation, skin			Chemists, plastic and resin workers; no skin cancer documented
B. Inorganic agents ***1. Metal and metal compounds***								
Arsenic and arsenic compounds	As, As_2O_3	Mouse Rat	P.O.	Negative/ inadequate	Inhalation, skin Oral	Lung Skin	10+ 15–35	Miners, smelters, dye stuff manufacturers and users, semiconductor compound makers; skin cancer associated with exposure to inorganic arsenic compounds; lung cancer increased in smelter workers exposed to arsenic trioxide

(continued)

Beryllium and beryllium compounds								
Beryl ore, beryllium sulfate, bertrandite, beryllium oxide	$3BeO\text{-}Al_2O_3\text{-}6SiO_2$, $BeSO_4$ $4BeO\text{-}2SiO_2\text{-}H_2O$, BeO	Rat Monkey	Inhalation Inhalation, intratracheal implantation	Lung Lung	Inhalation	Lung	10–15	Miners, smelters, refining alloy workers and users, metallurgists, electronic tube makers, nuclear reactor workers, rocket and aerosol research workers; other factors ruled out
Zinc beryllium silicate, beryllium, beryllium phosphate	$Zn\text{-}Be(SiO_2)_2$, Be, $Be_3(PO_4)_2$	Rabbit	I.V. injection	Bone tumors				
Chromium and chromium compounds								
Calcium chromate	$CaCrO_4$	Rat	Intratracheal implantation	Lung	Inhalation, skin	Nasal cavity	15–25	Producers, processors and users, metal workers and battery makers, increased incidence of lung cancer among chromate-producing industry and possibly chromate platers and chromium alloy workers; chromium compound(s) responsible — not known
Calcium chromate, strontium chromate and zinc chromate	$CaCrO_4$, $SrCrO_4$, $ZnCrO_4$	Rat	Topical application	Site of application		Sinuses, lung	10–30	
Barium chromate, lead chromate, chromic acetate, sodium chromate, and chromium carbonyl	$BaCrO_4$, $PbCrO_4$, $Cr(C_2H_3O_2)_3$, Na_2CrO_4, $Cr(CO)_6$	Rat, mouse	Inadequate					
Iron oxide and haemitite	Fe_2O_3	Hamster, mouse, guinea pig	Inhalation	Negative	Inhalation	Respiratory tract		Iron ore miners, metal grinders, and iron foundry workers; underground haemitite miners have high incidence of lung cancer, surface workers do not; excessing may be due to haemitite, radon, inhalation of ferric oxide or silica or combination of these

TABLE 1.6
(Continued)

		Animal studies			Human data			
Agent	**Chemical structure**	**Species**	**Route of exposure**	**Target organ**	**Route of exposure**	**Target organ**	**Latency period (years)**	**Occupational exposure**
Cadmium and cadmium compounds								
Cadmium chloride, cadmium oxide, cadmium sulfate, and cadmium sulfide	$CdCl_2$, CdO, $CdSO_4$, CdS	Rat	S.C. injection	Local sarcoma	Inhalation, skin			Miners, smelters, electroplaters, plastics alloy, solder, battery and insecticide workers, dye stuff manufacturers; increased risk to prostate, respiratory tract, and renal cancer due to Cd exposure perhaps CdO. The renal cancer risk doubled when cigarette smoking included in study
Cadmium powder, cadmium sulfide	Cd, CdS	Rat	I.M. injection	Local sarcoma				
Cadmium chloride, cadmium sulfate	$CdCl_2$, $CdSO_4$	Rat	S.C. injection	Testicular tumors				
Lead and lead compounds								
Lead and lead acetate	Pb, $Pb(C_2H_3O_2)_2$	Rat, mouse	P.O.	Renal tumors	Inhalation			Battery workers, gasoline additive workers, glass makers, insecticide makers, painters, plumbers, solderers, storage tank cleaners; no evidence of cancer in human
Lead acetate, lead subacetate, lead phosphate	$Pb(C_2H_3O_2)_2$, $Pb(C_2H_3O_2)_2$-$2Pb(OH)_2$, $Pb_3(PO_4)_2$	Rat	P.O., S.C., or I.P. injection	Renal tumors				

(continued)

Nickel and Nickel compounds								
Nickel sulfide Nickel powder, subsulfide, nickel oxide, carbonate, and nickelocene Nickel carbonyl	NiS Ni, Ni_3S_2, NiO, $NiCO_3$, $Ni(C_5H_5)$	Rat mouse, rat, hamster	Inhalation I.M.	Lung Local				Refiners, founders, smelters, dye stuffs manufacturers and users, battery, ceramic makers; increased incidence of cancer of nasal cavity, lung and larynx in nickel refiners — cannot tell which specific nickel compound is carcinogenic
	$Ni(CO)_4$	Rat	Inhalation	Lung	Inhalation	Nasal sinuses, lung, larynx	3–30	
2. Fiber								
Asbestos — all forms of commercial asbestos	$Mg_3Si_2O_5(OH)_4$, $(Fe^{++}Mg)_7Si_8O_{22}(OH)_2$, $(MgFe^{++})_7Si_8O_{22}(OH)_2$, $NaFe^{++}Fe^{+++}Si_8O_{22}(OH)_2$	Mouse, rat, hamster, rabbit	Inhalation, intrapleural, Intratracheal and I.P. administration	Mesothelioma, and lung	Inhalation, ingestion	Nasal sinuses, lung	4–50	Miners, millers, textile, insulation and shipyard workers; exposure to chrysotile, amosite, anthophyllite and mixtures containing crocidolite result in high incidence of lung cancer; tremolitic material mixed with anthophyllite and small amounts of chrysotile increase incidence of lung cancer, pleural and peritoneal mesotheliomas observed after exposure to crocidolite, amosite, chrysotile; gastrointestinal tract and larynx cancer increase for groups exposed to amosite chrysotile or mixed fibers containing crocidolite; mesotheliomas occur in individuals living near asbestos factories and crocidolite mine and in persons living with asbestos workers; smoking and asbestos work act multiplicatively

TABLE 1.6
(Continued)

Agent	Chemical structure	Animal studies			Human data			Occupational exposure
		Species	Route of exposure	Target organ	Route of exposure	Target organ	Latency period (years)	
3. Dusts								
Wood	—	Rodent	S.C.	Local sarcomas	Inhalation	Nasal cavity and sinuses	10–40	Wood workers; hardwood dusts
Leather	—				Inhalation	Nasal cavity and sinuses	40–50	Leather and shoe workers; carcinogen unknown
C. Physical agents								
1. Nonionizing radiation								
Ultraviolet rays	—	Mouse	Irradiation	Skin	Skin	Skin (varies with skin pigment and texture)	5–50	Farmers, sailors, researchers, chemists, welders, optical equipment workers
2. Ionizing radiation								
X-rays	—	Multiple	Irradiation	Skin, breast, thyroid, bone, lung	Skin	Skin, bone marrow and leukemia	10–25	Radiologists, medical personnel, high-voltage, vacuum tube makers and users
Uranium/radon	U/Rn	Rat	Inhalation	Lung	Skin	Small cell carcinoma of lung, skin, leukemia	10–15	Underground miners, inhalation of radon daughters with irradiation of bronchial tree
Radium	Ra				Ingestion	Bone sarcoma, leukemia, and lymphoma	10–15 10–50	Radiologists, miners, radium dial painters, radium chemists

TABLE 1.7
Federally Regulated Workplace Carcinogens under Occupational Safety and Health Act [16]

Name of carcinogen	Effective date	Latest amended date
1. Asbestos	June 2, 1972	January 8, 1998
2. 13 Carcinogens		
4-Nitrobiphenyl	June 27, 1974	April 23, 1998
α-Naphthylamine	June 27, 1974	March 7, 1996
Methyl chloromethylether	June 27, 1974	March 7, 1996
3,3′-Dichlorobenzidine	—	March 7, 1996
bis-Chloromethyl ether	—	—
β-Naphthylamine	—	—
Benzidine	—	—
4-Aminodiphenyl	—	—
Ethyleneimine	—	—
β-Propiolactone	—	—
2-Acetylaminofluorene	—	—
4-Dimethylaminoazobenzene	—	—
N-Nitrosodimethylamine	—	—
3. Vinyl chloride	April 1, 1975	June 18, 1998
4. Inorganic arsenic	August 1, 1978	June 18, 1998
5. Cadmium	December 14, 1992	January 8, 1998
6. Benzene	December 10, 1987	April 23, 1998
7. Coke oven emissions	January 20, 1977	June 18, 1998
8. 1,2-Dibromo-3-chloropropane	June 30, 1993	January 8, 1998
9. Acrylonitrile	November 2, 1978	April 23, 1998
10. Ethyleneoxide	August 21, 1984	November 7, 2002
11. Formaldehyde	February 2, 1988	April 23, 1998
12. Methylenedianiline	September 9, 1992	November 7, 2002
13. 1,3-Butadiene	November 4, 1996	November 7, 2002
14. Methylene chloride	April 10, 1997	March 22, 1999
Other compliance related safety and health topics		**Revised date**
15. Diesel exhaust		November 13, 2002
16. Isocyanates		January 21, 2003
17. Silica (crystalline)		December 16, 2002
18. Lead		February 4, 2003
19. Metalworking fluids		January 13, 2003
20. Synthetic mineral fibers		April 2, 2003

diseases, cancer is presently the second major cause of death. About 5 to 10% of cancers are hereditary. The remaining cancers result from damage to genes that occur throughout our lifetime either due to internal factors or external factors. According to late statistics, probably 80% of all human cancer deaths are related to external factors that can be controlled or prevented [1,19,20]. This includes use of tobacco and tobacco products, occupationally induced cancers, virally induced cancers, iatrogenically induced (medically induced) cancers, and cancers that arise from diet and lifestyle considerations.

A carcinogen is an agent that causes cancer. A carcinogen is also defined as a physical, chemical, or biological agent or a combination of agents that produces cancers in an organism, that is, it produces neoplastic or metastatic tumors in the animal host. Physical agents include ionizing radiation

TABLE 1.8
Carcinogens Listed in the Tenth Report on Carcinogens [17]

Part A. Known to be a human carcinogen
Aflatoxins
Alcoholic beverage consumption
4-Aminodiphenyl*
Analgesic mixtures containing phenacetin
Arsenic compounds, inorganic*
Asbestos*
Azathioprine
Benzene*
Benzidine*
Beryllium and compounds*
1,3-Butadiene*
1,4-Butanediol dimethylsulfonate
Cadmium and compounds*
Chlorambucil
1-(2-Chloroethyl)-3-(4-methyl cyclohexyl)-1-nitrosourea
bis-(Chloromethyl) ether and technical-grade chloromethyl methyl ether*
Chromium hexavalent* compounds
Coal tar pitch*
Coke tars
Coke oven emissions*
Cyclophosphamide
Cyclosporin A
Diethylstilbestrol
Dues metabolized to benzidine*
Environmental tobacco smoke*
Erionite
Estrogen, steroidal
Ethylene oxide*
Melphalan
Methoxsalen with ultraviolet A therapy
Mineral oils
Mustard gas
2-Naphthylamine*
Nickel compounds*
Radon*
Silica, crystalline*
Smokeless tobacco
Solar radiation
Soot
Strong inorganic acid mists containing sulfuric acid
Sunlamps and sun beds exposure to Thiotepa
Tamoxifen
2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD)*
Thorium dioxide
Tobacco smoking*
Vinyl chloride*
Ultraviolet Radiation, Broad spectrum
UV radiation
Wood dust*

Part B. Reasonably anticipated to be a human carcinogen
Acetaldehyde*
2-Acetylaminofluorene*
Acrylamide*
Acrylonitrile*
Adriamycin
2-Aminoanthraquinone
o-Aminoazotoluene
1-Amino-3-methylanthraquinone
2-Amino-3-methylimidazole [4,5-*f*]quinoline
Amitrole*
o-Anisidine Hydrochloride*
Azacitidine
Benz[a]anthracene
Benzo[b]fluoranthene
Benzo[j]fluoranthene
Benzo[a]pyrene
Benzotrichloride
Bromodichloromethane
2,2-*bis*-(Bromoethyl)-1,3-propanediol (technical grade)
Butylated hydroxyanisole
Carbon tetrachloride*
Ceramic fibers
Chloramphenicol
Chlorendic acid
Chlorinated paraffins (C_{12}, 60% chlorine)
1-(2-Chloroethyl)-3-cyclo-hexyl-1-nitrosourea
bis-(Chloroethyl)-nitrosourea
Chloroform*
3-Chloro-2-methylpropene
4-Chloro-*o*-phenylenediamine
Chloroprene*
p-Chloro-*o*-toluidine
Chlorozotocin
C.I. basic red 9 hydrochloride
Cisplatin
p-Cresidine
CupferronDacarbazineDanthron2,4-Diaminoanisoleo sulfate*
2,4-Diaminotoluene
Dibenz[a,h]acridine
Dibenz[a,j]acridine
Dibenz[a,h]anthracene
7H-Dibenzo[c,g]carbazole
Dibenzo[a,e]pyrene
Dibenzo[a,h]pyrene
Dibenzo[a,i]pyrene
Dibenzo[a,l]pyrene
1,2-Dibromo-3-chloropropane*
1,2-Dibromoethane*

(continued)

TABLE 1.8
(Continued)

2,3-Dibromo-1-propanol
tris-(2,3-Dibromopropyl) phosphate
1,4-Dichlorobenzene*
3,3′-Dichlorobenzidine*
Dichlorodiphenyltrichloroethane (DDT)*
1,2-Dichloroethane*
1,3-Dichloropropene*
Diepoxybutane
Diesel exhaust particulate*
Diethyl sulfate
Diglycidyl ether (DGE)*; class, glycidyl ethers
3,3′-Dimethoxybenzidine
4-Dimethylaminobenzene*
3,3′-Dimethylbenzidine
Dimethylcarbomoyl chloride*
1,1-Dimethylhydrazine*
Dimethyl sulfate*
Dimethylvinyl chloride
1,6-Dinitropyrene
1,8-Dinitropyrene
1,4-Dioxane*
Disperse blue 1
Dyes metabolized to 3,3′-Dimethoxybenzidine
Dyes metabolized to 3,3′-Dimethylbenzidine
Epichlorohydrin*
Ethylene thiourea*
di(2-Ethylhexyl) phthalate
Ethyl methanesulfonate
Formaldehyde*
Furan
Glass wool (respirable size)
Glycidol
Hexachlorobenzene
Hexachlorocyclohexane isomers
Hexachloroethane*
Hexamethyl phosphoric triamide*
Hydrazine*
Hydrazobenzene
Indeno[1,2,3-cd]pyrene
Iron dextran complex
Isoprene
Kepone*
Lead acetate
Lead phosphate
Lindan
2-Methylaziridine
5-Methylcrysene
4,4′-Methylene-*bis*-(2-chloroaniline)
4,4′-Methylene*bis*(*N*,*N*-dimethyl)benzenamine
4,4-Methylenedianiline*
Methyleugenol
Methyl methanesulfonate
N-Methyl-*N*-nitro-*N*-nitroso-guanidine
Metronidazole
Michler's ketone
Mirex
Nickel and nickel compounds*
Nitrilotriacetic acid
o-Nitroanisole
6-Nitrochrysene
Nitrofen
Nitrogen mustard hydrochloride
2-Nitropropane*
1-Nitropyrene
4-Nitropyrene
N-Nitrosodi-*n*-butylamine
N-Nitrosodiethanolamine
N-Nitrosodiethylamine
N-Nitrosodimethylamine*
N-Nitrosodi-*n*-propylamine
N-Nitroso-*N*-ethylurea
4-(*N*-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone
N-Nitroso-N-methylurea
N-Nitrosomethylvinylamine
N-Nitrosomorpholine
N-Nitrosonomicotine
N-Nitrosopiperidine
N-Nitrosopyrrolidine
N-Nitrososarcosine
Norethisterone
Ochratoxin A
4,4′-Oxydianiline
Oxymetholone
Phenacetin
Phenazopyridine hydrochloride
Phenolphthalein
Phenoxybenzamine hydrochloride
Phenytoin
Polybrominated biphenyl
Polychlorinated biphenyl*
Polycyclic aromatic hydrocarbons
Procarbazine hydrochloride
Progesterone
1,3-Propane sulfone*
β-Propiolactone*
Propylene oxide*
Propylthiouracil
Reserpine
Safrole
Selenium sulfate
Streptozotocin
Styrene-7,8-oxide

(continued)

TABLE 1.8
(Continued)

Sulfallate	2,4,6-Trichlorophenol
Tetrachloroethylene*	1,2,3-Trichloropropane*
Tetrafluoroethylene	**Ultraviolet A radiation**
Tetranitromethane	**Ultraviolet B radiation**
Thioacetamide	**Ultraviolet C radiation**
Thiourea	Urethane
Toluene diisocyanate*	**Vinyl bromide***
o-Toluidine*	Vinyl cyclohexene diepoxide*
Toxaphene	**Vinyl fluoride**
Trichloroethylene*	

Bold entries indicate new or changed listing in the RoC, 10th edition.
*Substance are listed in the latest NIOSH Carcinogen List (2002).

(x-rays), nonionizing radiation (UV light), and particles (asbestos). Chemical agents include calcium chromate, benzidine, and complex mixtures such as cigarette smoke and coal by-products. Biological agents included viruses and parasites. Each of these types of agents may be found in the environment. It is often difficult, however, to ascribe a specific cancer to a particular agent beyond a certain point in the development of cancer. The probability of cancer due to association increases with the potency of the carcinogen, the degree and duration of exposure, and the length of time elapsed since the exposure. For example, mesothelioma of the pleura and peritoneum in humans can result from exposure to asbestos.

A mutagen is an agent that produces a genetic event resulting in a heritable change. The majority of known chemical carcinogens are also mutagens; for example polycyclic aromatic hydrocarbons, aromatic amines, and *N*-nitrosamines. Therefore, mutagenicity has been considered a reasonable endpoint to use to detect mutagens and mutagenic carcinogens by virtue of their mutagenicity, and to assess relative hazards in the environment. Besides the association with carcinogens, mutations may have other harmful effects. The integrity of the human gene pool can be compromised by any increase in the mutation frequency of the population. Apart from the direct impact to the quality of human life, one must consider the possible damage to the ecological balance of other species caused by increases in the mutation frequency. Thus, mutagenesis is a useful indicator of deleterious biological effects.

1.2.2 CONCEPTS BASED ON DEFINITIONS

The following factors must be considered when assessing a carcinogenic agent [21]:

1. *Latency period* — The time between exposure and the onset of cancer can be many years in human (sometimes 20 to 30 years or more).
2. *Metabolism of chemical agent* — The metabolism of chemical carcinogens is not necessarily the same in humans and lower animals and will differ for organs in the same animal. This will result in differences in target organs affected in different species of animals.
3. *Dose–response relationship* — The increase in dose or duration of exposure to a carcinogen will result in an increase in the probability of production of cancer. Therefore, it is prudent to limit exposure to carcinogens to either no exposure, or to that dose that the regulatory agencies permit based on risk assessment calculations. (See Chapter 23 by Dr. Salmon.)

4. *Benign tumor* — There is not always a sharp distinction between benign and malignant tumors. This makes it difficult to assess cancer formation. Therefore, in some instances benign tumors are used as an indication of potential hazard.
5. *Cocarcinogenesis* — Cocarcinogens are agents administered together with carcinogens. Cocarcinogens have no carcinogenic activity of their own, but enhance the carcinogenic potency of a carcinogen. This can be accomplished by shortening the latency period or increasing the number of tumors produced by the carcinogen alone. Promoters can also enhance the potency of a carcinogen but are administered after the application of a carcinogen. Ferric oxide and dodecane have been found to be cocarcinogens while phorbol esters and tobacco smoke condensate have been found to be promoters. These types of compounds are important in the study of and exposure to complex mixtures derived from fossil fuels.
6. *Susceptibility* — The response to a substance will vary depending on the species, strain, age, sex, diet, health, etc. of the experimental animals or humans.

1.2.3 Factors to be Considered in Handling Carcinogens

Most carcinogens have unique chemical and physical properties. For instance, carcinogens can be solids, liquids, or gases with low or high vapor pressures; photochemically reactive; direct acting (does not need to be metabolically activated to observe biological effects), or indirect acting compounds. Odors need not be present and particles or mists, if generated, may or may not be visible to the naked eye. Lastly, all of the compounds in the working environment need to be identified because of the synergistic effects of carcinogens with cocarginogenic or promoting agents.

In addition to the above considerations, the route of exposure is extremely important since carcinogens can be absorbed through the skin, eye, inhaled through the respiratory tract, or ingested through the gastrointestinal tract. The route of exposure could determine the target organ while the dose and length of exposure could affect the latency period.

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2 Whole Animal Carcinogenicity Bioassays

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CONTENTS

2.1 INTRODUCTION

Epidemiologists, physicians, and laboratory scientists all play a role in identifying agents that contribute to causation of cancer in humans. Two hundred and twenty-eight years ago, the British physician, Percivall Pott, determined that there was an association between occupational exposure of chimney sweeps to soot in the chimneys they cleaned and the induction of scrotal cancer in these workers. Since that time, epidemiological studies have shown associations between exposure to cigarette smoking and lung cancer, exposure to asbestos and mesothelioma, exposure to β-naphthylamine and several other aromatic amines and urinary cancer, and exposure to aflatoxin B_1 and hepatocellular carcinoma [1–6]. There are therefore a variety of chemicals in various occupational settings and in the environment that are human and animal carcinogens. To determine whether an agent is a potential human carcinogen, the strongest tools to use are epidemiological studies. However, these studies are difficult, since they must rely on natural, not experimental, conditions [1]. Further, epidemiological

studies are somewhat insensitive. It took many decades for epidemiologists to determine that cigarette smoke, a very strong carcinogenic mixture, was a cause of human lung cancer.

A second major group of tools in the area used to determine whether specific chemicals are carcinogens in humans are clinical observations, experimental long-term carcinogenicity bioassays in animals, initiation and promotion assays in animals, and short-term genotoxicity assays in the laboratory. The short-term genotoxicity assays include assays to detect chemically induced mutation in bacteria (*Salmonella typhimurium*, *Escherichia coli*), yeast, cultured mammalian cells, Drosophila, and in whole animals. Short-term genotoxicity assays also include assays to detect chemically induced DNA repair and chemical induction of chromosome aberrations, including micronucleus induction, in mammalian cells.

Among the laboratory studies that can be employed to detect carcinogens, the most important is the animal carcinogenesis bioassay. In many cases, specific agents were first found to cause cancer in animals, and then these agents were later found to induce cancer in humans. Experimental cancer useful to determine causes of human cancer research is based on the scientific assumption that substances causing cancer in animals will also cause cancer in humans [7]. Therefore, long-term carcinogenesis bioassay in animals, usually in two laboratory rodent species, mice and rats, over a range of doses for nearly the complete lifetime of the animals, are important assays that are used to detect chemical carcinogens. All experimental conditions are carefully chosen to maximize the likelihood of identifying any carcinogenic effects [8]. Although the responses of laboratory animals to agents may not always be exactly the same as for humans, animal studies remain the most valuable tool for determining the potential carcinogenicity of specific chemicals to humans [9,10]. In this chapter, we discuss the procedures used to conduct animal carcinogenicity bioassays, the variables that can affect the results of these assays, the interpretation of the data from these assays, and the utility of the animal carcinogenesis bioassay to detect chemical carcinogens.

2.2 EPIDEMIOLOGICAL STUDIES

Cancer epidemiology is very important because this approach provides direct evidence concerning factors involved in human cancer causation [11]. Epidemiological studies have shown that arsenic compounds are human carcinogens, even though arsenic compounds have not been shown to be carcinogenic alone in experimental studies [12].

Four basic epidemiological approaches have been used [11,13–15]. The informed hunch is used as a first step to develop a hypothesis concerning which type of exposure might be associated with a given cancer, for example, cigarette smoking and lung cancer. A second approach is the follow-up or prospective cohort study in which a large number of healthy people are followed for several years to determine which of them contract a specific cancer and die from that cancer. The logistical problems of prospective cohort studies, which require large numbers of healthy people to observe a statistically meaningful number of cases of cancer, prompted the development of case control or retrospective studies. Retrospective studies depend on locating cases with a particular disease as well as healthy or unrelated disease controls and asking them the same type of questions. The fourth approach, known as historical follow-up or retrospective cohort studies, is used to detect occupational causes of cancer. These studies exploit the follow-up approach, but here, the initial population of exposed workers, known as the cohort, is identified many years after the initial exposure.

When properly controlled retrospective or prospective studies indicate that an exposure to an agent increases the risk of cancer, it is accepted that the agent is carcinogenic. It should be noted that even though a working population develops cancer from exposure to an industrial carcinogen, the overall increase in worker death rate may not be great enough to exceed the death rate of a similar age group in the general population. Therefore, for any workforce under study, the control group of workers from other industries must be from industries where there is no exposure to the potential carcinogen. Negative results may not establish the noncarcinogenicity of agents. This may be because

either the sample size of the number of people involved or the number of cases is insufficient, or because the exposure may be too brief or too small to produce a carcinogenic effect.

Problems encountered with epidemiological studies are inadequate follow-ups due to the loss of a number of people in the studies and the long latency period of 5 to 30 years for the expression of cancer in humans following exposure to carcinogens. This presents problems in the development of good data bases and adequate registries. Due to the length of the latency period for cancer development in humans, it is difficult to determine retrospectively the chemical involved, the dose and duration of exposure, and the extent to which social and personal habits as well as diet is related to cancer [11,13–15].

In general, epidemiological studies are not predictive but rather depend on the occurrence of human cancer related to chemical exposures. These types of studies provide important evidence for associations rather than identification of specific causative factors. It is also important, however, to prevent the possibility of human exposure to potential carcinogens. This preventive medicine approach requires that both experimental animal bioassays and short-term tests be used to determine the carcinogenicity of a variety of compounds before usage.

2.3 ANIMAL CARCINOGENESIS BIOASSAYS

2.3.1 General and Theoretical Considerations

Long-term administration of chemical compounds to laboratory animals, usually rats or mice, is the best laboratory method for determining whether a compound is carcinogenic to lower animals, and therefore whether this specific compound may be carcinogenic to humans [7,11,15–19]. Bioassays of chemical carcinogens in whole animals involve administration of the candidate carcinogen by skin painting, inhalation, feeding, gavage, the drinking water route, and other modes of administration. For a critical appraisal of bioassay methodologies, the International Agency for Research on Cancer (IARC) book is an excellent reference [12].

Of course, human carcinogenesis testing is unethical and illegal. Futhermore, it is very difficult and inordinately expensive to induce tumors in primates with chemical carcinogens. Rodents are more sensitive to chemical carcinogens than primates and humans, and they also have a substantially shorter life span (2 years). Hence, rodents are substantially less expensive than primates as test animals. Rodents are therefore the animals of choice in most animal carcinogenesis bioassays. The primary bioassay for chemical carcinogens is currently conducted in both mice and rats.

Repeated applications of carcinogens to mouse skin give rise to papillomas and then carcinomas. It has never been proved whether papillomas progress to carcinomas or carcinomas arise independently (carcinomas are epithelial in origin). Locally, subcutaneous injection of carcinogens into mice and rats usually gives sarcomas (tumors of connective tissue). Systemic administration is usually accomplished through prolonged feeding experiments and usually results in liver tumors, but can also give many other types of tumors. Transplacental administration of the compounds to the pregnant mice results in tumors in the offspring. Rodents treated with carcinogens occasionally contract tumors in the F2 generation. Choriocarcinoma is an example of a tumor that arises in the fetus in women. Transplacental administration of carcinogens often results in tumors of the nervous system.

Carcinogenesis bioassays provide accurate information about dose and duration of exposure and are less affected than epidemiology studies by possible interactions of the substance with other chemicals or modifiers, since one compound can be tested at a time in a controlled fashion [8]. Thus, of known human carcinogens, only arsenic compounds have been found to be negative in classical rodent bioassays. However, recent studies from the laboratory of Dr. Michael Waalkes at the National Institute of Environmental Health Sciences (NIEHS) have shown that arsenic compounds are carcinogenic in transplacental studies. Occupational exposure of humans to benzene has resulted in induction of acute myelogenous leukemia (AML) as shown by epidemiological studies. However,

the induction of leukemia in lower animals by benzene has not been reported [20], but recent studies have shown that benzene induces solid tumors of other organs in humans [21]. Hence, there is often but not always a strict concordance between tumor sites in lower animals and humans exposed to the same carcinogen.

Although it has long been known that there are significant interspecies differences in the metabolism of xenobiotics and hence in the toxicities of xenobiotics, the metabolic activation pathways of carcinogens and the resultant carcinogen–DNA adducts are qualitatively similar among various animal species, including humans [22]. This scientific evidence thus supports the quantitative extrapolation of carcinogenesis data from laboratory animals to humans, when the carcinogenicity data in rodents is scaled for body surface area and metabolic rates in humans.

The carcinogenicity of an agent is established when the route of administration to animals is adequately designed and the experiments conducted result in an increased incidence of one or more treatment-related types of cancer. Increased incidences of tumors are regarded with greater confidence if positive results are observed in both sexes of one species of animals, in different species of animals, and also by different laboratories. Bioassay treatments are usually repeated and given for a long period of time. The time for appearance of tumors in 50% of the animals treated with a carcinogen following initiation of application of a carcinogen is defined as the latent period. The latent period for mice can take 10 weeks (Figure 2.1), whereas for humans, the latent period can be decades, as for induction of mesothelioma induction following asbestos exposure (5 to 50 years). Tumor incidences are very difficult to analyze theoretically. The incidence of tumors is variable. Strong carcinogens at high doses can induce a 100% incidence of tumors. Weak carcinogens can induce incidences of tumors $<50\%$ at the conclusion of the assay (Figure 2.1). For a strong carcinogen giving a 100% incidence of tumors at the end of the assay, the latent period is defined at the time at which 50% of the tumors appear (Figure 2.1). For a weak carcinogen, giving $<100\%$ incidence of tumors, the latent period is defined as the time at which half of the final tumor incidence occurs (Figure 2.1). In the typical carcinogen bioassay, the number of tumors per animal is recorded, as is the percentage of animals with tumors. To have a complete carcinogen bioassay, it is necessary to have a complete autopsy of the animals. The investigators then conduct histopathological analysis of all tissues, determine a histological diagnosis of all tumors, and record all types of tumors that appear — papillomas, sarcomas, leukemias, and carcinomas — and the organs in which they occur. The Iball index has been used to relate the dose, latent period, and incidence of tumors (Figure 2.1 and Figure 2.2). This is not universally accepted, nor is any other relationship. The TD50 or dose at which 50% of the tumors occur, is more commonly reported.

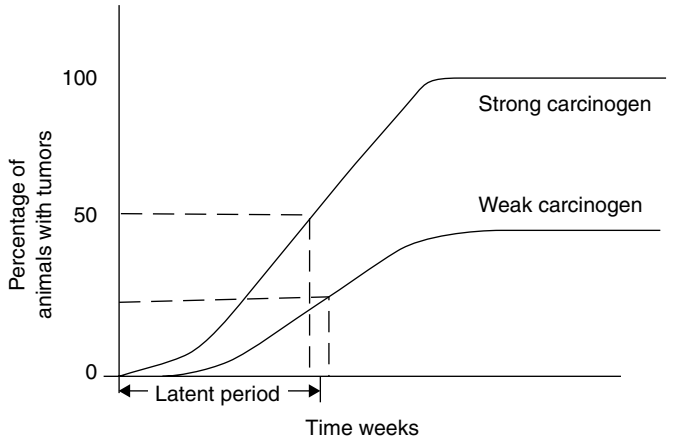

FIGURE 2.1 Idealized curves.

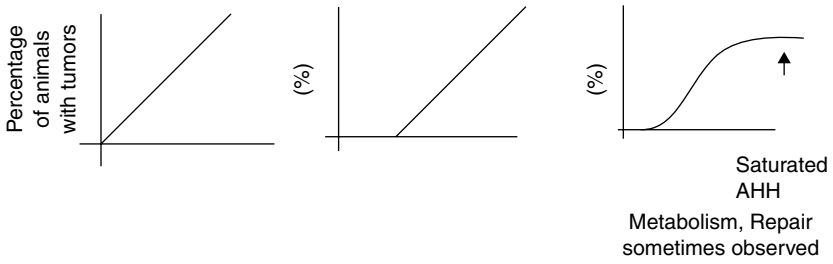

FIGURE 2.2 Dose linear curve (a) without threshold, (b) with threshold, and (c) linear above threshold saturating at high concentrator.

The evidence of carcinogenicity is strengthened if there is a dose response relationship, that is, the incidence of cancer increases monotonically with an increase in the dose of the compound being tested in the carcinogen bioassay. There are three general types of dose–response curves for carcinogenicity in animal experiments. The first curve is the linear, no threshold curve. This curve is observed with mutagenic or genotoxic carcinogens (Figure 2.2[a]). The second type of dose–response curve has a threshold concentration that must be exceeded before carcinogenicity manifests. Once the threshold is exceeded, the dose–response is then linear beyond the threshold concentration (Figure 2.2[b]). A third more complicated type of dose–response is a combination of the two threshold curves, which is linear above the threshold and then saturates at high concentrations (Figure 2.2[c]). The saturation in this curve may be due to a saturation of carcinogen-metabolizing enzyme activities beyond a specific dose of carcinogen.

A positive result in an animal carcinogen bioassay generally supersedes negative findings in epidemiological studies, due to the insensitivity of the epidemiological methods [11,13]. A negative result obtained in an animal bioassay conversely does not preclude the potential carcinogenicity of a compound in humans. In the animal bioassay, a negative result could be due to an inappropriate species having been chosen, too few animals having been tested, or too short an observation period having been used. It has been shown that there are significant variations between species of animals and strains within species in terms of their response to specific carcinogens. For example, 2-naphthylamine, an aromatic amine, does not induce bladder tumors in mice and rats, but does induce bladder tumors in hamsters, monkeys, and dogs, and also induces bladder tumors in humans. Extrapolation of carcinogenicity data in lower animals to humans is further strengthened when positive carcinogenicity results for a chemical are obtained in more than one species.

Note also that negative results in carcinogenicity bioassays obtained in one species do not detract from the significance of clearly positive results obtained in another species. Also, a lack of tumors in a carcinogenesis bioassay could easily result due to the utilization of too few animals. Therefore, to maximize the sensitivity of these bioassays to detect carcinogens, high doses of carcinogens, which are often many times the human exposure, are commonly employed to increase the sensitivity of the bioassay. In using such high doses, it is very important to utilize a dilution series, to determine whether a dose response for carcinogenicity exists for a specific chemical. If a dose–response can be found, then the results at high doses are considered valid. Genotoxic carcinogens yield a linear dose–response curve. This dose–response curve can be extrapolated from high doses to lower doses by a linear extrapolation. The carcinogenicity results can also be extrapolated to humans by use of appropriate scaling factors for surface area and metabolic rate [1,15–19]. Some nongenotoxic carcinogens may also follow a linear dose–response curve, while others that bind to specific biological receptors, such as hormone receptors, may exhibit a threshold in their dose–response curve for carcinogenicity (Figure 2.2[b]).

Extrapolation of carcinogenicity results from lower animals to humans for evaluating human risks entails uncertainties. An unresolved issue remains concerning whether carcinogens display

a threshold or no-effect dose level and the precise shape of the curve at low and high doses. Until substantial information is available that demonstrates the existence of a threshold, the position of many scientists and most regulatory agencies is that one must assume that no threshold exists and that the shape of the dose–response curve for carcinogenesis is linear for genotoxic carcinogens and for nongenotoxic carcinogens that do not bind to specific biological receptors (Figure 2.2). The official position of most regulatory agencies is that unless there has been found strong evidence for the existence of a threshold, a threshold is presumed not to exist (Office of Environmental Health Hazard Assessment, California Environmental Protection Agency).

2.3.2 The General Design of Standard Animal Carcinogenesis Bioassays

Animal bioassays must be well designed and each chemical agent evaluated individually [16–19]. Some of the important variables to be considered in the animal carcinogenesis bioassay are the route of administration, the species and strain of animal used, whether a dose–response relationship can be observed, the duration of dosing, metabolism of the carcinogen, the effect of modifying agents, and the dose of the agent to which humans are or will be exposed. Animal husbandry, diet, safety measures, and written protocols are important and are occasionally overlooked in design considerations. Data acquisition, data reduction, and statistical analysis of tumor induction data of the bioassay are important in estimating the potential human risk of carcinogens. There are some practical considerations that must also be taken into account in the bioassay testing, including the total number of animals, the length of the bioassay, its cost, dose levels, and the specific animal species used in the bioassay.

The standard rodent bioassays conducted by the National Cancer Institute (NCI) or by the National Toxicology Program (NTP) each require 600 animals per compound and require more than 2 years to complete. Each standard bioassay costs from $250,000 to $500,000 for skin studies and from $500,000 to $5 million for inhalation studies. This standard bioassay usually uses both B6C3F1 mice and F344 rats. This standard bioassay usually provides data at usually three doses, the maximum tolerated dose (MTD), $\frac{1}{2}$ the MTD, and $\frac{1}{10}$ the MTD (or some other fraction of the MTD) to determine whether a dose–response exists and to obtain statistically significant results if a dose–response does exist. At the end of the bioassay, a complete autopsy of the animal is conducted, with histopathology studies conducted on all tissues.

A number of practical considerations should also be used when selecting chemicals for carcinogenicity testing, particularly because these studies are very expensive and lengthy to conduct. The levels of production of a specific chemical, usage of this chemical, and occurrence of this specific chemical in the workplace, in commerce, and in the environment should be considered. Those chemicals with the highest production, use, and occurrence should be considered as high priority for bioassay testing. Those chemicals for which there is either the highest actual human exposure or the highest anticipated human exposure should also be considered a high priority for animal bioassay. Where there is epidemiological evidence to associate exposure to a specific chemical with a high incidence of cancer in humans, then this chemical should be considered a high priority for animal carcinogenicity bioassay. In addition, if a specific chemical is suspected to be a carcinogen due to its having a structural relationship with known carcinogens, or physical and chemical properties that suggest a potential carcinogenicity, then this chemical should also be considered a high priority for testing in animal carcinogenesis bioassays. Also, if a specific chemical has induced important deleterious biological effects in short-term assays, such as bacterial or mammalian cell mutagenesis assays, clastogenesis assays, or DNA repair assays, or has shown activity in other animal bioassays, then this chemical could also be considered a high priority for testing in animal carcinogenesis bioassays. If the specific chemical has shown biological interaction with tumor promoters or cocarcinogens, it could also be considered a priority for testing in animal carcinogenesis bioassays.

2.3.3 Specific Practical Factors that are Important in the Design of Animal Carcinogenesis Bioassays

2.3.3.1 Personnel

Planning of a long-term carcinogenicity study involves animal science, pathology, toxicology, and biostatistics. It may also involve analytical and clinical chemistry, biochemistry, epidemiology, pharmacokinetics, hematology, microbiology, nutrition, and computer science. Hence, personnel skilled in all these areas are important to the proper conduct of animal carcinogenesis bioassays.

2.3.3.2 Properties of Chemicals Tested for Carcinogenicity

Information on the chemical and physical properties of the test agent, in addition to its structure, molecular weight, and chemical formula should be obtained before initiating the long-term carcinogenicity bioassay. The purity of the compound should be determined as well as the identification of the impurities present. Small amounts of carcinogenic impurities can give an incorrect answer in a carcinogenesis bioassay, and this has been a problem in the past. In addition, the source, batch, date received, method of storage, probable daily exposure level to this potential carcinogen for humans, and any biochemical information are also important variables and must be reported [16–19].

2.3.3.3 Species and Strains of Test Animals

There is no ideal animal species known for carcinogenicity testing, one in which the biological response to the test agent is always identical to that of humans. Therefore, at least two different species are used, usually rats and mice [15–19]. Selection of a particular animal species may be used based on metabolic and pharmacokinetic data or their susceptibility to a particular class of carcinogens. The preferred species are the rat and mouse, and to a lesser extent the hamster. These species are used based on practical considerations of short life span (2 years), small size, availability, relatively small cost, and considerable biological and genetic knowledge of these species. Sex differences have been documented with respect to carcinogen susceptibility. Therefore, equal numbers of male and female animals are included in the carcinogenesis bioassays. Furthermore, when a given species of animals is chosen, the strains of that species of animals used in a particular laboratory are based upon the experience and background knowledge of the colony of animals over the years. The strain usually used is one with little or no incidence of spontaneous tumors and a high and specific susceptibility to many carcinogens. Certain strains will produce a biological response to a test carcinogen and others may not. For example, a single administration of 7,12-dimethylbenz[a]anthracene (DMBA) induces mammary tumors in Sprague-Dawley rats but not in Long-Evans rats [23]. Similarly, aflatoxin B_1 induces a high incidence of liver tumors in both Wistar and Fisher rats and a high incidence of kidney tumors in Wistar rats but not in Fisher rats [24].

2.3.3.4 Route of Administration of Test Chemicals

To ensure that the carcinogenicity bioassay results are as relevant to humans as possible, the test agent should be administered by a relevant route of administration, that is, one that approximates as close as possible to the route by which humans are exposed to the chemical in question [15–19]. The three main routes of exposure are oral, cutaneous, and inhalation or intratracheal instillation. Examples of oral administration of chemicals are mixing the chemical in the diet and feeding it to animals, or dissolving the chemical in the drinking water of the animals, or administering the chemical to the animal by gastric intubation. Gastric intubation, which is usually performed over a 1 day, 5 days, or 1 week period, is less desirable because high concentrations may result in severe local damage to the upper digestive tract. If the specific chemical is administered in the diet or in the drinking water the exposure is continuous but there is the possibility of contamination of the laboratory facilities and exposure of laboratory personnel to a potential carcinogen. The ultimate

choice of route of administration of the test chemical depends upon the volatility, solubility, stability, and palatability of the agent under test.

Cutaneous exposure is used to simulate dermal exposure, which is one major route of human exposure. Cutaneous exposure may also be used as a model system in animals to study induction of skin tumors. Using the cutaneous route, animals are usually dosed weekly twice until appearance of tumors or for the lifetime of the animals. A basic requirement for cutaneous studies includes clipping the hair to allow maximal skin absorption. As a skin tumor model, these assays may also be useful in initiation and promotion studies.

Inhalation through the respiratory tract is one of the main modes of human exposure for many substances, and therefore, is an important route of testing despite the large cost of inhalation carcinogenesis experiments. Properly designed exposure chambers and equipments for generation, sampling, and monitoring the test chambers are needed for studies involving vapors and aerosols [25]. Such inhalation carcinogenesis studies usually are carried out 4 to 6 h a day, 5 days a week or 22 h a day, 7 days a week. Aerosol exposure is generally not satisfactory for long-term exposures because of contamination of animal fur, resulting in undesired oral intake, or due to filtering of the air by the animals when they hide their noses in their own or in each other's fur. Individual housing and head and nose exposure chambers will reduce problems associated with aerosol exposure. Intratracheal instillation, which has been used in experimental respiratory tract carcinogenesis studies, is an alternative to inhalation for studying aerosols. Since humans are more likely to inhale air through the mouth than the rodents are, this type of instillation may mimic human exposure more closely. Intratracheal instillation of candidate carcinogens is usually administered to animals once or twice per week.

Subcutaneous, intraperitoneal, intramuscular, and intravenous injections are not used very often for testing potential carcinogens. Subcutaneous injection may be used when the agent is poorly absorbed after oral administration or to minimize contamination of the laboratory. The other types of injections may be used for testing for compounds readily absorbed by the digestive tract in humans and not mice or rats. The frequency of injections may be once or twice a week.

2.3.3.5 Dose Selection

An assay to determine carcinogenic activity may require only a high dose and a low dose [15–19]. However, generation of an accurate dose–response curve and use of carcinogen bioassay data in carcinogen risk assessment evaluations usually requires several dose levels with dose–response analyses, knowledge of the metabolism of the chemical being studied, and pharmacokinetic data on the specific chemical being studied. The high dose in many instances is the maximum tolerated dose (MTD), determined in a 90-day subchronic study. The MTD is used to maximize the sensitivity of the animal model to the chemical in order to determine whether a carcinogenic effect does exist. If the candidate chemical does exert carcinogenic effects, then lower dose levels are used to determine whether a dose–response exists (Figure 2.2), and to facilitate risk assessment evaluations. A major difficulty in these experiments is uncertainty about whether a threshold dose exists in these experiments. For genotoxic (mutagenic) carcinogens, and for many nongenotoxic carcinogens, it is widely believed that no threshold for carcinogenesis exists, and a linear, nonthreshold relationship is presumed to exist for the dose–response curve. For some nongenotoxic carcinogens, a threshold dose might exist, particularly if they exert their carcinogenicity by binding to biological receptors, such as hormone receptors.

To simply determine the potential carcinogenicity of a test agent, two dose levels plus the control treatment condition usually are sufficient. The high dose or MTD and two lower doses, usually $\frac{1}{2}$ the MTD and $\frac{1}{10}$ the MTD, are used to ensure that at least one group of animals can be compared meaningfully with the controls even if there was an error in the selection of the high dose, that is severe mortality or weight loss, and to evaluate whether a dose–response for carcinogenesis exists. Pharmacokinetic and metabolism data should be taken into consideration in selecting the lower

dose(s). When it is important to determine whether a dose–response relationship exists for a known or candidate carcinogen, three or more dose levels are used, scaled down by factors of 3 to 5 and possibly 10 from the highest dose (MTD, $\frac{1}{2}$ MTD, $\frac{1}{10}$ MTD). This type of study becomes important when evaluating the human risk to a carcinogenic exposure.

2.3.3.6 Inception and Duration of Exposure

Exposure of animals to the test agent begins a few weeks after weaning the animals [16–19]. The animals are normally shipped right after weaning (3–4 weeks for most rodents) and quarantined for 1–2 weeks. The animals are then treated with the candidate carcinogen from 5–6 weeks of age through the major portion of the animal's life span. Studies with low doses of carcinogen are usually terminated between 104 to 130 weeks of age for rats, 99–120 weeks of age for mice, and 80–100 weeks of age for hamsters. For those chemicals that may be carcinogenic to fetal tissues and possibly to newborn and adults, the pregnant mother is treated and the exposure of the offspring is continued during infancy and adult life. An example of this type of chemical is diethylstilbestrol (DES), which resulted in an increased risk of vaginal cancer in daughters of those pregnant women administered DES [6]. This same effect was produced in hamsters treated with DES in utero [13].

2.3.3.7 Numbers of Animals, Methods of Randomization, and Statistics of the Carcinogenic Response

Animals used in a carcinogenesis bioassay must be from the same colony and shipment. Randomization procedures are used to allocate animals to different groups, including the positive and negative control groups. The birth date and weight of each animal are recorded, and a number is assigned to each animal. The animals are then randomized and weight distribution is considered in this randomization.

To allow for accurate statistical and biological evaluation, 50 animals are used per group. With a group size of 50, in which 10% of the control animals possess the same tumor, 28% of the animals in the experimental group must have tumors in order to achieve a significance of $P = .05$. In cases where no spontaneous tumors occur, only 12% incidence of tumors is needed in order to achieve a significance at the $P = .05$ level [17]. Another important factor to be considered is the relative potency of a carcinogen. With a group size of 50 animals only certain strongly carcinogenic compounds may be detected and weaker carcinogens may be detected only by a group size of 100. An effect that occurs in only 1% of the test animals will be entirely missed 37% of the time even with 100 animals and a 0% incidence in the controls. Therefore, carcinogens that are responsible for perhaps a 1% incidence of cancer in human populations could escape detection in animal bioassays due to a lack of sensitivity of these assays in detecting such weak carcinogens. An example of this relative potency is illustrated by the following: 1 μg of aflatoxin B_1 given daily to animals for their life span will produce cancers in 50% of the animals, whereas it would require 10 g of saccharin to produce the same tumor effect [26].

2.3.3.8 Animal Husbandry and Animal Diet

Housing conditions, animal care facilities, diseases in the test animals, purity of animals' diets, purity of air and water, composition of the animals' diets, composition of the animals' bedding, occurrence of cannibalism in the experiment, and pesticide use during the experiment can all alter the outcome of animal carcinogenesis bioassays [16–19]. Animal rooms should be well ventilated, with controlled lighting, temperature, and humidity. Animals received from outside a facility must be quarantined up to 2 weeks to prevent the introduction of unwanted pathogens and to allow the animals to recover from the stress of shipment. Infection of the animals with hepatitis viruses or other viruses could affect the outcome of carcinogenesis experiments.

Cages, racks, and other equipment should be constructed of materials that allow convenient and frequent cleaning, minimum stress to animals, and convenient and accurate feeding. Solid bottom caging should be fabricated from stainless steel, polycarbonate, or polypropylene and can be used with filter tops, which decreases exposure to microorganisms and fugitive contamination but increases levels of humidity, ammonia, CO_2, and temperature. Stainless-steel wire-mesh cages are durable and provide for animals' visibility, but the animal is not protected from microorganisms, nor does this method maximize environmental contamination of the test chemical. These types of cages are used in inhalation chambers. Laminar flow caging systems provide unidirectional airflow after passage through a high efficiency particulate aerosol (HEPA) filter, reduces the variability in environment but does protect animals from volatile chemicals in the room. Animal bedding should be absorbent, dust free, sterilizable, and not contaminated with pesticides or fungal products that would produce physiological changes in animals during carcinogenesis experiments.

Single housing facilitates husbandry and avoids cannibalism and autolysis. Single housing of animals also eliminates positive and negative correlations between animals in the same cage. Both single and multiple housing produce stresses, that is, isolation versus aggressive behavior, respectively. Attending the animals on a daily basis will reduce the stress due to isolation. At present, it is not known whether the cost of single housing would be better spent in purchasing more animals for each group. Most laboratories at present, normally engage in multiple housing, with control and treated groups being housed in separate racks [16–19].

Nutritional factors can also play an important role in carcinogenesis [16–19]. Therefore, irrespective of the source of the diet, for example, commercial, natural ingredients, laboratory designed, or semi-synthetic purified diets, the concentrations of common dietary constituents and possible dietary contaminants, should be determined. Diets used in animal carcinogenesis bioassays are of constant quality and contain standard amounts of essential nutrients. Animal feed older than 3 months is not used due to loss of labile nutrients. Finally, concentrations of dietary constituents should be determined on a periodic basis and the results retained and included in the final report [16–19].

2.3.3.9 Written Protocols and the Safety Action Plan

For each bioassay conducted, a detailed written protocol should be prepared. This written protocol should delineate the responsibility of each individual researcher involved in the assay, and all the aspects of the design of the study described previously in this section. The protocol should also include procedures for data collection, such as time of sacrifice, body weight, feed consumption, necropsy procedures, and for data analysis.

In addition, each chemical compound tested is regarded as potentially carcinogenic. Therefore, precautions need to be taken to prevent inadvertent exposure of laboratory personnel and laboratory facilities to each test chemical. A safety action plan (Table 2.1) should be constructed, and should include a list of the personnel that are potentially exposed, and descriptions of work practices, monitoring procedures, decontamination procedures, and disposal and emergency procedures (Safety Action Plan presented in Table 2.1 is based on a plan designed for and presently used by The Department of Environmental Health in the College of Medicine of the University of Cincinnati since 1975. References 27 and 28). Each safety action plan is designed specifically for the particular compound being studied. There are no general overall procedures that can be used. Each compound has unique physical, chemical, and biological properties, which require different means of disposal and surveillance procedures.

Of all the measures that are required in a safety action plan, the monitoring, decontamination, disposal, and emergency procedures are the most difficult to initiate. Before an animal carcinogenesis study is undertaken, the literature is reviewed for procedures employed with similar compounds. A number of chemical companies make available to investigators the preferred methods of disposal for their products. Through the National Cancer Institute (NCI), safe handling procedures are being developed and made available for a variety of carcinogenic and potentially carcinogenic compounds.

TABLE 2.1
Important Factors to be Considered in the Safety Action Plan

1. Name of test chemical
2. Personnel potentially exposed to carcinogens
3. Structure of chemical
4. Physical and chemical properties of chemical
5. Toxicity data
6. Carcinogenicity data
7. Description of work practices
   a. Personal protection
   b. Type of use
   c. Location of use
   d. Transportation
8. Monitoring procedures
   a. Medical surveillance
   b. Personnel monitoring
   c. Surveillance procedures for environmental contamination
9. Decontamination and disposal procedures for chemical
10. Emergency procedures

For example, it has been found that chlorinated hydrocarbons and polycyclic aromatic hydrocarbons should be incinerated, while nitrosourea compounds can be decomposed in sodium hydroxide. Standard textbooks that deal with decontamination and disposal of chemical carcinogens are available [27,29,30].

Procedures to monitor the health of laboratory workers who conduct animal carcinogenesis bioassays are conducted on an individual basis. If surveillance and personnel monitoring procedures are not available, new methods involving gas or liquid chromatography may have to be developed to conduct such monitoring studies. Medical surveillance procedures too may be difficult to assess due to the lack of appropriate endpoints to observe changes, for example, in personnel urinalysis, sputum cytology, or hematological tests. The safety action plan is practical because each procedure can be conducted routinely. All procedures are tested on a trial basis before the actual carcinogenesis bioassays are conducted to ensure a safe plan of action.

2.3.3.10 Data Collection and Statistical Analysis of the Data

Routine observations are conducted on a daily basis to provide accurate indications of the health status of the animals and biochemical analyses are conducted to aid in diagnosing diseases in the test animals. At the completion of the carcinogenesis bioassay, a complete autopsy and histopathological examinations are performed on all of the tissues of all the animals. Gross pathology analyses are performed first and the gross lesions are sized in standard units. All lesions are recorded and autopsies are performed on the dead or killed animals. All organs and tissues are fixed in appropriate formaldehyde or formalin solutions. Daily worksheets are kept, and if possible, the data on induction of tumors stored on a computerized data storage and retrieval system.

There are several different types of cells in lower animals and in humans from which tumors can arise. Epithelial cells are those cells covering the organs and very often in contact with air. Connective tissues, muscles, and bones comprise mesenchymal cells. In humans, the most common general type of tumors are carcinomas, which are derived from epithelial cells and comprise 92% of human tumors. Leukemias, derived from white blood cells, plus sarcomas, derived from mesenchymal

cells, together comprise 8% of the total human cancer burden. The observed high cancer frequency in epithelial cells, which leads to the genesis of carcinomas in animals and humans, is a line of evidence that suggests environmental carcinogen may contribute to the human cancer burden.

Benign tumors are tumors which are locally encapsulated and that remain within the capsule. For instance, an astrocytoma is a benign brain tumor, but astrocytomas can kill the host by occupying space within the brain. Malignant tumors, such as carcinomas and sarcomas, invade into other tissues and can metastasize from the point of origin and spread to other tissues.

Statistical analysis of survival curves is used to determine the adequacy of the carcinogenesis bioassay, since loss of animals before completion of the assay can be a factor in the total tumor incidences. Other factors that are considered in the statistical analysis are the spontaneous incidences of tumors in animal strains, the total number of tumors in experimental groups versus controls, the latency periods, dose–response relationships, and differences in longevity in different experimental groups. The statistical methods are described elsewhere and are beyond the scope of this text. However, once the data has been analyzed, an evaluation of the bioassay can be undertaken [31]. Depending on whether the results are significant ($P = .01$) or marginally significant ($.01 < P < .1$ to .01), conclusions can be drawn as to whether the compound being assayed is carcinogenic or not in experimental animals.

Once the carcinogenic potential of a compound has been established in animals, extrapolations from these data to relatively safe levels and the probability of tumor induction in humans exposed to this chemical can be calculated [15]. The greatest difficulty with the extrapolation lies with the low end of the dose range and the potential of a threshold for a nongenotoxic carcinogen for the exposed population.

A number of mathematical models for statistical analyses of whole animal data have been used, such as the one hit (linear), multikit (k-hit), multistage, extreme value, and the log-probit. These models have been used in data evaluation and data extrapolation to humans. One other result of the extrapolation of carcinogenicity data from animal bioassays to humans is the incorporation of safety factors. These safety factors are used to calculate dose levels of agents that may be allowed in the environment and are considered safe for humans. A number of safety factors have been suggested: (1) to divide the observed experimental no observed effect level (NOEL) by 100; (2) to divide the lowest experimental effect level by 5,000 and (3) identification of a safe dose level at which the risk calculated for exposure to the carcinogen would not exceed very small level of 1 in 100,000,000 or 10^{-8} .

However, it should be noted that there is no way to demonstrate absolute safety of an agent on the basis of statistical analyses of data [15]. Pharmacokinetic and pharmacodynamic models, including physiologically based pharmacokinetic (PBPK) models have also been employed to enhance the precision of risk assessment procedures [32]. The problem, associated with extrapolation of lifetime exposure of animals to the MTD of an agent to exposure of humans to lower doses for shorter periods of time has been addressed by the EPA through the use of the Weibull Model [33].

Finally, in extrapolating animal carcinogenesis bioassay data to human risk, there are a number of factors that need to be considered. These include reproducibility of experimental data; dose-dependence of tumor incidence; experimental dose that approximates that of human exposure; availability of biochemical, metabolic, and pharmacokinetic data; relative potency of compounds as determined by human or experimental assays; the nature and quality of human exposure, and individual susceptibility [15–19]. It has been well-known for many years that there are quantitative differences in carcinogen metabolism and DNA adduct formation among different cell types, tissue types, and outbred individuals of the same species. A new research field in carcinogenesis, molecular epidemiology, was named for combined laboratory–epidemiological studies of human cancers [34]. The primary goal of molecular epidemiology is to identify individuals in human populations who are at the highest risk for contracting cancer. A second goal of molecular epidemiology is to determine the specific molecular mutations that occur in specific genes of specific tumors, and to identify the agents that have caused these tumors [35].

TABLE 2.2
Two-Stage Carcinogenesis Systems [36–38]

Organ system	Initiator	Promoter
Mouse skin	Polycyclic aromatic hydrocarbons, urethane, direct-acting electrophiles	Croton oil, phorbol esters, (TPA) fatty acids and fatty acid esters, surface-active agents, linear long chain alkanes, benzoyl peroxide, tobacco smoke condensate and extracts of unburned tobacco
Rat and mouse liver	2-Acetaminofluorene, dimethylnitrosamine, 2-methyl-*N*,*N*′-dimethyl-4-aminoazobenzene	Phenobarbital, DDT, butylated hydroxy toluene (BHT), polychlorobiphenyls (PCB), tetrachlorodibenzodioxin (TCDD)
Mouse lung	Urethane, polycyclic aromatic hydrocarbons	BHT, Phorbol
Rat colon	Dimethylhydrazine	Bile acids, high fat diet, high cholesterol diet
Rat bladder	*N*-methyl-*N*-nitrosourea	Saccharin, cyclamate
Rat and mouse mammary gland	Polycyclic aromatic hydrocarbons	Hormones, high fat diet, phorbol
Rat stomach	*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine	Surfactants
Rat esophagus	Diethylnitrosamine	Diet
Mouse cell culture system	Polycyclic aromatic hydrocarbons, radiation	Phorbol esters, saccharin
Rat cell culture system	*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine	Phorbol esters

2.4 MODIFIERS OF CARCINOGENESIS — INITIATORS, PROMOTERS, COCARCINOGENS, AND INHIBITORS OF CHEMICAL CARCINOGENESIS

Initiators and promoters (Table 2.2), cocarcinogens (Table 2.3), and inhibitors will modify the strength of a carcinogen. Promoters and cocarcinogens can either shorten the latency period or increase the number of tumors per animal. On the other hand, inhibitors of carcinogenesis (Table 2.4) will increase the latency period or decrease the number of tumors caused by treatment of animals with a carcinogen [36–41]. The mechanisms of each of these processes and specific examples of each type of agent are discussed below.

The mechanisms of action of initiation and promotion, or two-stage carcinogenesis, were first studied by Peyton Rous and Isaac Berenblum, and later by Philip Shubik in mouse skin. These studies have since been extended to mouse liver, mouse lung, and mouse mammary gland. In addition, initiation and promotion have been demonstrated in rat liver, rat colon, rat bladder, rat mammary gland, rat stomach, and rat esophagus. Initiation and promotion of neoplastic cell transformation have also been demonstrated *in vitro* in mouse and rat cell culture systems (Table 2.2). Initiation occurs when a tissue is treated with low levels of a complete carcinogen or an initiator, and is an irreversible process (Figure 2.3). Initiators bind covalently to DNA. Many scientists believe that initiation is due to a mutation, likely in cellular proto-oncogenes. Treatment of initiated cells with

TABLE 2.3
Cocarcinogens in Various Organs/Tissues [36–38]

Skin	Catechol, pyrogallol, lauryl alcohol, decane, undecane, tetradecane, *n*-dodecane, pyrene, benzo[e]pyrene, fluoranthene, benzo[g,h,i]perylene, anthralin, croton oil, phorbol esters, phenols nicotine, surfactants, radiation, viruses
Lung	Asbestos, radiation, *n*-dodecane, ferric oxide, magnesium oxide, hypoxia, ethanol
Mammary gland	Hormones, estradiol, prolactin
Bladder	L-Tryptophan, saccharin, cyclamate
Cheek pouch	X-Radiation, croton oil
Liver	Cyclopropenoid, fatty acids, alcohol

TABLE 2.4
Examples of Compounds that Inhibit Carcinogenesis in Various Tissues [36–38]

Lung	Butylated hydroxy anisole (BHA), ethoxyquin, retinoids
Liver	BHT
Forestomach	BHA, BHT, disulfiram, benzylisothiocyanate, phenethylisothiocyanate, ethoxyquin
Breast	BHA, BHT, ethoxyquin, disulfiram, benzylisothiocyanate, phenethylisothiocyanate, phenylisothiocyanate, benzylthiocyanate, cysteamine hydrochloride, retinoids
Large intestine	Disulfiram, diethyldithiocarbamate
Skin	Cycloparaffins, esculin, quereetin, squalene, oleic acid, eugenol, resorcinol, hexadecane, hydroquinone, limonene, phenol, retinoids
Bladder	Retinoids

a tumor promoter, such as tetradecanoyl phorbol acetate (TPA), causes promotion or the growth of initiated cells. Promotion is reversible up to a point (Figure 2.4, step 1). If application of the promoter is stopped at this point, then the rate of cell death will equal the rate of cell growth, and the developing tumor will regress. After a certain number of repeated applications of promoter, the carcinogenesis process becomes irreversible, and the tumors become fixed and permanent (Figure 2.3 and Figure 2.4). In these initiation and promotion experiments on mouse skin, 92% of the tumors that appear are papillomas (benign tumors of the papillary glands) and 8% of the tumors are carcinomas

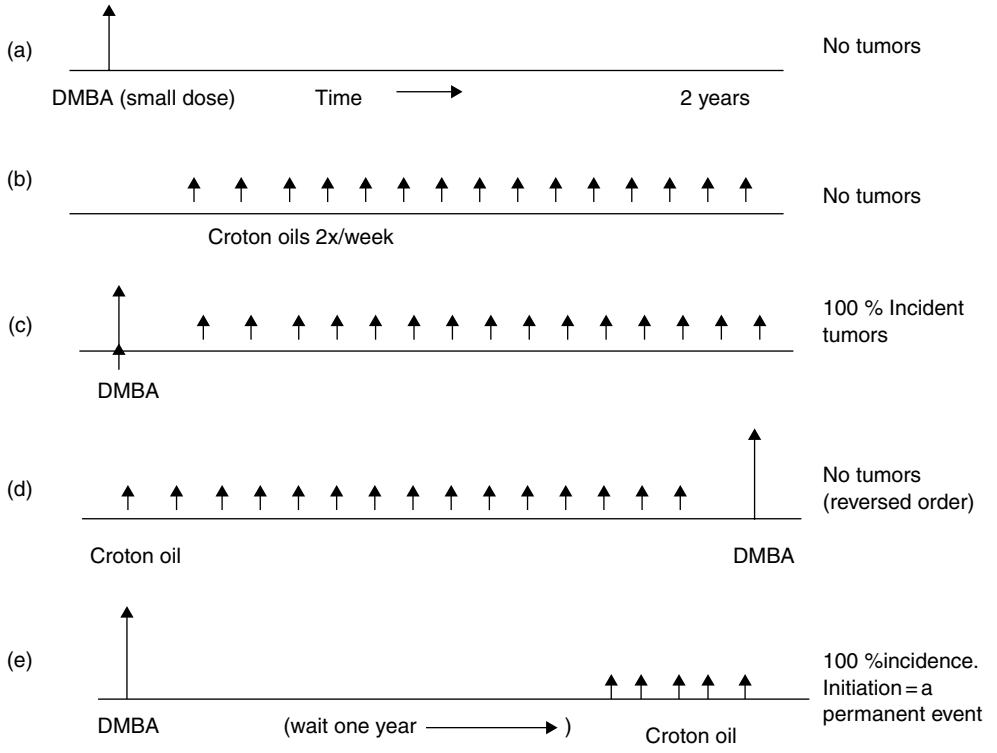

FIGURE 2.3 Initiation and promotion.

Initiation	Promotion	Progression
DMBA, low dose	Stage 1 TPA	Stage 2 Mezerein

FIGURE 2.4 Schematic of initiation, promotion, and progression.

(malignant tumors). Tumor promoters do not bind covalently to DNA, but bind to protein kinase C and stimulate the growth of initiated cells.

Cigarette smoke condensate also contains tumor promoters. Environmental promoters may also be important in human cancer causation. TPA is one of the most powerful tumor promoters known. Four situations that have been clearly identified and well-studied where initiation and promotion occur are (1) in mouse skin treated with DMBA and TPA, (2) in rat liver treated with acetylaminofluorene (AAF) and phenobartital, (3) in rat bladder treated with *N*-methyl-*N*-nitrosurea and promoted with either saccharin or cyclamate, and (4) in rat colon treated with dimethylhydrazine and bile acids. Pure tumor promotes are not tumorigenic themselves, except at very high concentrations, where they are likely to promote already initiated cells. Cigarette smoke has very powerful promoters in it. There is synergism between initiators and promoters, such that the carcinogenic effect of initiators (or low doses of complete carcinogens) is enhanced by promoters to a greater than additive extent. TPA is a naturally occurring phorbol ester derived form the irritant croton oil, synthesized by the plant, *Euphorbia lathyris*. TPA was purified and identified by

Dr. Eric Hecker of the German Cancer Research Center in Heidelberg, Germany. TPA is widely used for mouse skin tumor promotion studies. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), or dioxin, is formed as an impurity during the synthesis of trichlorophenol and also in fires. TCDD is one of the most effective promoter studies for rat liver carcinogenesis and is also effective as a tumor promoter in lung and skin [42].

Cocarcinogenesis is completely an operational definition. Cocarcinogenesis indicates that two agents are given together, simultaneously, and enhance the yield of carcinogenesis. In contrast, initiators and promoters are administered to animals at different times. An example of cocarcinogenesis is two carcinogens given together with a synergistic effect on carcinogenesis. Therefore, cigarette smoke contains cocarcinogens including catechols. A second example of cocarcinogenesis is intratracheal instillation of BaP together with ferric oxide in Syrian hamsters, which produced lung tumors, whereas the control, BaP, did not [43]. Similarly, cutaneous coadministration of BaP with *n*-dodecane to C3H mice resulted in an increase in skin tumors when compared to administration of BaP alone, providing another example of cocarcinogenesis [44]. Additional instances of cocarcinogenesis have been demonstrated to occur in skin, lung, mammary gland, bladder, the cheek pouch of hamsters, and in liver (Table 2.3).

Certain compounds have been shown to exert strong inhibitory effects on chemical carcinogenesis. For instance, BaP administered in the diet induces forestomach tumors in mice [45]. Addition of butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) together with BaP in the diet inhibits the formation of BaP-induced forestomach tumors in mice [45]. In the lung, BHA, ethoxyquin, and retinoids are inhibitors of chemical carcinogenesis. BHT inhibits chemical carcinogenesis in the liver and forestomach. In the breast, BHA, BHT, ethoxyquin, disulfiram, benzylisothiocyanate, phenethylisothiocyanate, phenylisothiocyanate, benzylthiocyanate, cysteamine hydrochloride, and retinoids inhibit chemical carcinogenesis. Disulfiram and diethyldithiocarbamate inhibit carcinogenesis in the large intestine. In the skin, cycloparaffins, esculin, quercetin, squalene, oleic acid, eugenol, resourcinol, hexadecane, hydroquinone, limonene, phenol, and retinoids inhibit chemical carcinogenesis. Retinoids also inhibit bladder carcinogenesis (summarized in Table 2.4).

Extensive examples of initiators, promoters, cocarcinogens, and inhibitors of carcinogenesis listed by target tissue are shown in Table 2.2 (initiators and promoters), Table 2.3 (cocarcinogens) and Table 2.4 (inhibitors of carcinogenesis) (reviewed in References 1, 36, and 46). As is indicated in Tables 2.2 to 2.4, these modifiers are ubiquitous in the environment and in particular in the occupational setting. Depending on the carcinogenesis bioassay protocol used, a compound may be a carcinogen or a promoter as indicated by skin painting studies, while phenol has been shown to be an inhibitor and a cocarcinogen on skin. It is clear that with the few exceptions where pure compounds have been implicated, mixtures containing carcinogens and modifying agents need to be examined more closely. It is unclear at this time what effects varying of the ratios of carcinogen to modifier will have on the carcinogenic response of mixtures. It may be that all that is necessary are very low doses of carcinogen in these mixtures of modifiers to produce detrimental effects in humans.

2.5 COMPLEX MIXTURES

The most common complex mixtures in the environment are those formed from the combustion of fossil fuels and tobacco smoke [47–52]. The conversion or processing of shale, coal, or petroleum involves elevated temperatures and elevated pressures. Certain compounds formed in these mixtures exhibit carcinogenic activities for a variety of organ sites in experimental animals, and epidemiological evidence implicates their role as carcinogens in humans. These mixtures contain carcinogenic and noncarcinogenic polycyclic aromatic hydrocarbons (PAHs), aromatic amines, nitrosamines, halogenated hydrocarbons, and metal compounds. In addition, these mixtures also contain cocarcinogens, tumor promoters, and inhibitors of carcinogenesis, such as straight aliphatic

hydrocarbons and cycloparaffins. Interactions between chemicals in the mixture may be additive, inhibitory, antagonistic, or synergistic [53].

It is difficult to assess the carcinogenic potencies of these mixtures based solely on their chemical composition. Investigators have attempted to determine the relative potency of these mixtures by determining the concentrations of indicator compounds, such as BaP and benz(a)anthracene, with some success. It has been shown, however, that mixtures or fractions of mixtures are highly carcinogenic or mutagenic even without the presence of BaP in these mixtures [54] and without the PAHs in the basic fractions [55,56]. It also has been noted that the BaP content cannot account for the relative potency of mixtures [47,48,54,57]. An additional complicating factor is the effects of sunlight or near ultraviolet light on the carcinogenic potential of mixtures. Under appropriate conditions, there can be an enhancement of the carcinogenic activity of BaP [58], asphalt, and coal tar in the presence of ultraviolet light or sunlight [47,59,60]. It has been also shown that coal tar pitch fume is more than 90% aromatic in content, while asphalt is more than 99% aliphatic and less than 1% aromatic in content. The pitch fume materials showed effects consistent with high BaP content. The asphalt fume materials showed a higher activity than would be expected based on their BaP or total PAH content, suggesting the presence of other compounds contributing to the carcinogenic response, that is the aliphatic content [61].

Based on this discussion, it is apparent that it would be difficult to assess complex mixtures solely by use of long-term skin carcinogenesis bioassay due to the high cost and long duration of the experiments. Short-term assays have been developed, in particular the Salmonella/microsome mutation assay, to assess the potential activity of these mixtures and to prioritize the mixtures for further testing [31,62,63]. For a further in-depth discussion of mixtures, please see Chapter 14 by Dr. Stephen Nesnow.

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