EDITED BY

MORTON LIPPMANN | GEORGE D. LEIKAUF

ENVIRONMENTAL TOXICANTS

HUMAN EXPOSURES AND THEIR HEALTH EFFECTS

FOURTH EDITION

WILEY

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Fourth Edition

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MORTON LIPPMANN
GEORGE D. LEIKAUF

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PREFACE

This fourth (2019) edition of *Environmental Toxicants* reflects the evolution of the field of environmental health science. Most of the 26 chapters review the health effects associated with exposures of human populations to chemical toxicants encountered in the course of routine activities in and around their homes, while commuting, and in recreational microenvironments. They do not emphasize the generally higher levels of exposure to some of the same toxicants that can occur in occupational settings, which are the focus of other multi-author reference works. However, two of the chapters address very high exposures that were intended to terrorize military and/or civilian populations.

Chapter 8 covers chemical and biological agents that may be used to kill, harm, or terrorize noncombatant people. Chapter 25 describes the results of a terrorist attack on September 11, 2001, that brought down the twin towers of the World Trade Center in New York City. The resulting dense cloud of highly alkaline inhalable dust caused acute respiratory irritation among residents and recovery workers immediately, as well as chronic respiratory effects among those disturbing and re-entraining the settled dust in the months that followed.

Other new chapters include Chapter 7, "Acrolein and Unsaturated Aldehydes," wherein health risks have attained new prominence, and Chapter 19, which covers the risks of inhaling relatively low concentrations of a new type of man-made particulate matter that is increasingly incorporated into a wide range of industrial and consumer products, that is, particles with diameters <50 nm. They are known as nanoparticles and represent a new challenge to our biological defense mechanisms.

Some of the differences between traditional occupational and other environmental toxicant exposures, and any adverse health effects that may result from such exposures, are affected by temporal exposure patterns, as well as by activity levels. Occupational exposures are generally limited to 8 h per day but often involve higher levels of minute ventilatory volumes and dermal exposures than those in most of their other microenvironments. In contrast, nonoccupational toxicant exposures usually involve low concentrations of more diverse chemical mixtures over longer time periods. For such exposures, it is often

more difficult to detect increased rates of mortality, morbidity, or losses of function. In this volume, most of the chapters deal with exposures to complex chemical mixtures and/or multiple chemicals having similar biological impacts or common sources. Other chapters deal with either specific metals in their various chemical forms or the classes of gaseous ambient air pollutants that are regulated under the U.S. Clean Air Act and other U.S. governmental entities.

Some chapters that appeared in previous editions have been dropped on the basis that nonoccupational exposures of populations likely to cause adverse health effects have substantially diminished (sick building syndrome, benzene, and dioxins). Others (microwaves, ionizing radiation, ultraviolet radiation, noise, and drinking water) were dropped because the size of the volume was getting too great and/or they were not focused on chemical toxicants.

Some of the chapters that reappear have new authors or coauthors who have brought their topics up-to-date with new information and insights, acquired in the intervening decade, on exposures, biological responses, and their mechanisms.

BACKGROUND

The origin of the first edition of this reference book was the result of a visit to my office at NYU School of Medicine by Bob Esposito, who then worked at Van Nostrand Reinhold (VNR). He had seen the Lippmann comprehensive critical review paper on ozone, which appeared in the *Journal of the Air Pollution Control Association,* and was interested in gathering together a series of such reviews on other environmental toxicants for a new title for VNR. I said I would think about it and let him know.

Before responding, I sounded out some professional colleagues who were experts on a variety of environmental toxicants, and, somewhat to my surprise, many were willing to prepare a chapter-length critical review. On that basis, I signed a contract with VNR to produce the first version of the volume (containing 23 chapters), which was published in 1992. When Esposito, by then working for Wiley, which had acquired VNR, called again, in 1988, about preparing a second edition, I agreed. The second edition, with 30 chapters, appeared in 2000. The third edition, also published by Wiley, with 30 chapters, appeared in 2009.

When Esposito recently indicated that Wiley was interested in a fourth edition, I was hesitant to pull it all together again as a single editor. However, I was able to recruit one of my former NYU doctoral students, George D. Leikauf, by then a professor at the University of Pittsburgh School of Public Health, to work with me as co-editor for the fourth edition. It would be up to Professor Leikauf to carry it forward should there be any subsequent editions.

A PERSONAL NOTE FROM THE SENIOR EDITOR (ML)

I began my professional career, in 1954, by enrolling in an MS program in Industrial Hygiene at Harvard. I studied there under Philip Drinker and Leslie Silverman, who were, along with Ted Hatch, leading academic pioneers in occupational health and aerosol science. At that time, there was not yet an academic program in environmental health science at Harvard, or elsewhere.

Upon completion of my MS, I joined the U.S. Public Health Service (PHS) in 1955 to work at the Occupational Health Program in Cincinnati; I met colleagues who investigated not only occupational exposures to workplace toxicants but also the lethal smog exposures in 1948 in Donora, PA. While in Cincinnati, I also had the opportunity to get to know Herb Stokinger, the chief toxicologist of the PHS, and to work with him in refining the design of animal chambers for inhalation exposures of toxicants.

When I moved on, in 1957, to work in the Industrial Hygiene Branch of the Health and Safety Laboratory (HASL) of the Atomic Energy Commission (AEC) in New York City, I got to work with the lab director, Merrill Eisenbud, and my branch chief, Bill Harris, and became familiar with their work with their HASL colleagues in assessing stratospheric samples of radioactive aerosols and worldwide fallout from atmospheric testing of A-bombs and H-bombs. I also helped to develop early versions of particle size-selective air samplers that could separate particles, during the sampling process, into those depositing within respiratory tract regions having different particle deposition and particle clearance dynamics.

I moved on to NYU in 1964 to pursue a doctoral research project under the supervision of Dr. Roy E. Albert, which was to define quantitative aspects of regional particle deposition and bronchial airway particle clearance in the human respiratory tract, along with a parallel and simultaneous effort in laboratory animals. My doctoral thesis was on the effect of particle size on regional deposition and particle clearance dynamics in human airways.

In retrospect, I recognize how fortunate I was to get to know personally and to appreciate the historic work of my mentors. They were pioneers who created so much of the knowledge base that I relied upon in pursuing my own subsequent research career.

After completing the PhD program at NYU in 1967, the Department Chair, Dr. Norton Nelson, arranged for me to be appointed to the faculty, where I remained for the next 50 years, pursuing research projects involving human exposure assessment, inhalation toxicology, and occupational and environmental epidemiology. This varied experience, along with my service on review and advisory committees for EPA, the National Institute for Occupational Safety and Health (NIOSH), the National Institute of Environmental Health Science (NIEHS), the World Health Organization (WHO), and other academic institutions, broadened my horizons, and enabled me to (1) focus my specific research proposals on key issues that were in most need of resolution, (2) help in guiding the regulatory and research programs elsewhere to make more effective use of their own resources, and (3) have the broad perspective in environmental health science to guide the organization and overall perspective for this unique book. I now pass the task of maintaining this unique reference book on to Dr. Leikauf.

The inclusion of chapters on the criteria air pollutants for which I was the senior or coauthor, those on airborne fibers, and on the dust generated by the collapse of the World Trade Center towers on September 11, 2001, reflects my own strong research interests, as well as my service on external scientific advisory committees dealing with such toxicant exposures to substantial populations. For the other toxicant classes covered in the other chapters, we are grateful to the expert authors, their excellent contributions, and their willingness to provide the complementary content on the other toxicant groupings.

My research career began at a time that occupational health science was nearing maturity and was beginning to broaden into environmental health science. In occupational health, it was possible and reasonable to focus research on the identification of specific toxic agents or elements as the or at least major causal components of an exposure-related occupational disease or disability in a working adult population. As my research focus

shifted, at least in part, to environmental health science, it became clear that the identification of specific toxic agents or elements as one or more major causal components of an exposure-related environmental disease or disability in a community-based population was a more complex and difficult task. The populations at risk for adverse environmental exposures can be much larger. The exposures include those in microenvironments other than occupational, such as *in utero*, residential, schoolroom, recreational, commuting, and retirement communities. They can occur over 24 h each day for people with highly variable activity patterns and minute volumes, ethnicities, dietary patterns and deficiencies, smoking and health histories, and preexisting diseases. Furthermore, even when the relative risks in a general population are typically much lower than those seen in the past for toxicant-exposed occupational populations, there can be many more people at risk and therefore much larger numbers of affected people.

Based on these differences in the total numbers of people exposed, and the combinations of specific ambient air toxicant exposures with personal exposures to indoor radon, tobacco smoke, kitchen effluents, other combustion effluents, or the various pesticides in common usage, it is often much more difficult to assign causality for an environmental disease or disability to a specific environmental toxicant. In view of such complexities, most of the chapters in this book cover the exposures and reasonably likely adverse health effects that have been associated with such exposures while recognizing that other components in the environmental mixtures may also be contributing to the observed effects.

While the caveats outlined above indicate that caution is still needed in our chapter authors' conclusions on likely causality, we also need to recognize that our research tools are much more powerful than they were in previous decades and that they continue to improve. These tools include (1) those in our laboratories for chemical identification and quantitation, cellular and genetic factors and their alterations resulting from toxicant exposures, and access to *in vitro* cellular and *in vivo* animal models for effects bioassays and (2) more powerful biostatistical models for identifying susceptibility and risk factors in epidemiological studies.

We also need to recognize that environmental interventions in recent decades have been remarkably effective in reducing environmental pollution in many parts of the richer countries, as well as in some parts of the developing world. This creates opportunities to document improvements in environmental quality and in any associated improvements in public health. Such documentation could provide additional public support for further gains in environmental quality and public health.

1

INTRODUCTION AND BACKGROUND

MORTON LIPPMANN AND GEORGE D. LEIKAUF

This book identifies and critically reviews current knowledge on the health effects of human exposure to selected chemical agents and physical factors in the ambient environment. It provides a state-of-the-art knowledge base essential for risk assessment for exposed individuals and populations to guide public health authorities, primary care physicians, and industrial managers having to deal with the consequences of environmental exposure.

Aside from professionals in public health, medicine, and industry who may use this book to guide their management functions, the volume can also be used in graduate and postdoctoral training programs in universities and by toxicologists, clinicians, and epidemiologists in research as a resource for the preparation of research proposals and scientific papers.

The subject is focused on those environmental toxicants, that is, chemical or physical agents released into the general environment that produce adverse health effects among large numbers of people. Such effects are usually subclinical, except when cumulative changes lead to chronic effects after long exposure. Short-term responses following acute exposures are often manifest as transient alterations in physiological function that may, in some sensitive members of the population, be of sufficient magnitude to be considered adverse. Each of the specific topic chapters has a thorough discussion of the extent of human exposure as well as of toxic responses. The four chapters on the uses of the data for risk assessment, risk management, clinical applications, and industrial operations provide guidance for those performing individual and/or collective population hazard evaluations. The first provides individuals and public agency personnel with a basis for decisions on risk avoidance and relative risk assessment. The second outlines the operational philosophies and techniques used by environmental engineers in scoping and managing environmental risks. The third enables the primary care physician to recognize diseases and symptoms associated with exposures to environmental toxicants and to provide counsel to patients. The fourth assists decision makers in industry in evaluating the potential impacts of their plant operations and products on public health.

Environmental Toxicants: Human Exposures and Their Health Effects, Fourth Edition.
Edited by Morton Lippmann and George D. Leikauf.

Although many books provide brief reviews of hundreds of chemicals encountered in the work environment at levels that can cause demonstrable health effects, both acute and chronic, they contain relatively little information on the effects of low-level exposures on large populations of primary interest in environmental health and risk assessment. This book is designed to provide in-depth, critical reviews of the environmental toxicants of contemporary public health concern.

1.1 CHARACTERIZATION OF CHEMICAL CONTAMINANTS

1.1.1 Concentration Units

In environmental science, confusion often arises from the use of the same or similar sounding terms having different meanings in different contexts. This is especially true in describing the concentrations of water or air contaminants. For water contaminants, solutes are expressed frequently in parts per million (ppm) or parts per billion (ppb). However, when used for air contaminants, the units are molar or volume fractions, whereas when used for water contaminants, they are weight fractions. This problem can be avoided by expressing all water contaminant concentrations as the weight of contaminant per unit volume (e.g., m^3 or L) of fluid. In air, the units generally used are mg/m^3 or $\mu g/m^3$, whereas in water they are most often mg/L or μg/L.

1.1.2 Air Contaminants

At normal ambient temperatures and pressures, chemical contaminants are dispersed in air in gaseous, liquid, or solid forms. The latter two represent suspensions of particles in air and were given the generic term “aerosols” by Gibbs (1924) based on analogy to the term “hydrosol,” used to describe disperse systems in water. On the contrary, gases and vapors, which are present as discrete molecules, form true solutions in air. Particles consisting of moderate- to high-vapor-pressure materials tend to evaporate rapidly, since those small enough to remain suspended in air for more than a few minutes (i.e., those smaller than about 10 μm) have large surface-to-volume ratios. Some materials with relatively low vapor pressures can have appreciable fractions in both vapor and aerosol forms simultaneously.

1.1.2.1 Gases and Vapors Once dispersed in air, contaminant gases and vapors generally form mixtures so dilute that their physical properties, such as density, viscosity, enthalpy, and so on, are indistinguishable from those of clean air. Such mixtures may be considered to follow ideal gas law relationships. Vapors are not practically difference than gases, except that vapors are generally considered the gaseous phase of a substance that is normally a solid or liquid at room temperature. While dispersed in the air, all molecules of a given compound are essentially equivalent in their size and probabilities of contact with ambient surfaces, respiratory tract surfaces, and contaminant collectors or samplers.

1.1.2.2 Aerosols Aerosols, being dispersions of solid or liquid particles in air, have the very significant additional variable of particle size. Size affects particle motion and, hence, the probabilities for physical phenomena such as coagulation, dispersion, sedimentation, impaction onto surfaces, interfacial phenomena, and light-scattering properties. It is not possible to characterize fully a given particle by a single size parameter. For example, a

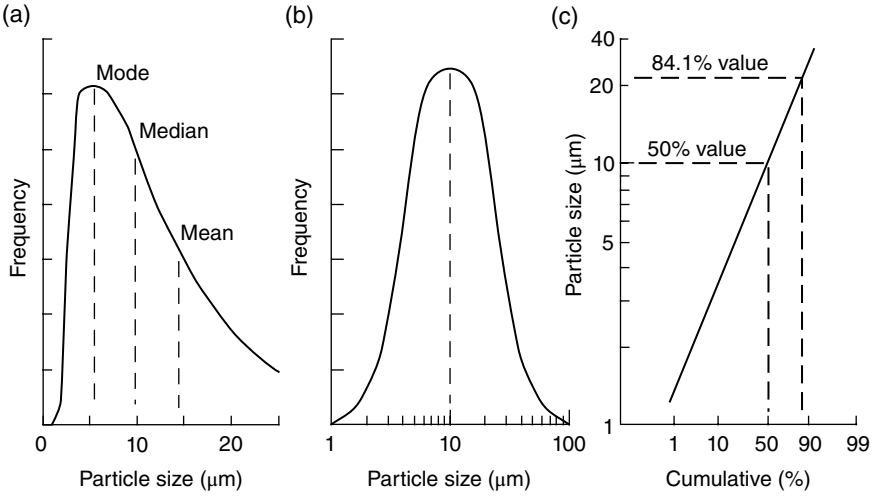


FIGURE 1.1 Particle size distribution data. (a) Plotted on linear coordinates. (b) Plotted on a logarithmic size scale. (c) In practice, logarithmic probability coordinates are used to display the percentage of particles less than a specific size versus that size. The geometric standard deviation (s_g) of the distribution is equal to the 84.1% size/50% size.

particle's aerodynamic properties depend on density and shape as well as linear dimensions, and the effective size for light scattering is dependent on refractive index and shape.

In some special cases, all of the particles are essentially the same in size. Such aerosols are considered monodisperse. Examples are natural pollens and some laboratory-generated aerosols. More typically, aerosols are composed of particles of many different sizes and hence are called heterodisperse or polydisperse. Different aerosols have different degrees of size dispersion. Therefore, it is necessary to specify at least two parameters in characterizing aerosol size: a measure of central tendency, that is, the mean or median diameter, and a measure of dispersion, that is, the arithmetic or geometric standard deviation.

Particles generated by a single source or process generally have diameters following a lognormal distribution; that is, the logarithms of their individual diameters have a Gaussian distribution. In this case, the measure of dispersion is the geometric standard deviation, which is the ratio of the 84.16 percentile size to the 50th percentile size (Fig. 1.1c). When more than one source of particles is significant, the resulting mixed aerosol will usually not follow a single lognormal distribution, and it may be necessary to describe it by the sum of several distributions.

1.1.3 Particle Characteristics

Many properties of particles, other than their linear size, can greatly influence their airborne behavior and their effects on the environment and health. These include the following:

Surface: For spherical particles, the surface area (A) varies as the square of the radius (r) (i.e., $A = 4\pi r^2$). Importantly, for an aerosol of given mass concentration, the total aerosol surface increases greatly with decreasing particle size. Airborne particles have much greater ratios of external surface to volume than do bulk materials, and, therefore, the particles can dissolve or participate in surface reactions largely than

would massive samples of the same materials. Furthermore, for nonspherical solid particles with internal cracks or pores, the internal surface area can be much greater than the external area.

Volume: Particle volume (V) varies as the cube of the radius (r) (i.e., $V = 4/3\pi r^3$). Therefore, a few large particles in an aerosol tend to dominate its volume concentration and therefore can contribute more to the overall mass concentration.

Shape: A particle's shape affects its aerodynamic drag as well as its surface area and therefore its motion and deposition probabilities. In addition, particle shape can influence the biological disposition of particles, for example, influencing the ability of macrophages to internalize particles via actin-driven movement of the macrophage membrane.

Density: A particle's velocity in response to gravitational or inertial forces increases as the square root of its density.

Aerodynamic diameter: The diameter of a unit density (1 g/cm^3) sphere has the same terminal settling velocity, as the particle under consideration is equal to its aerodynamic diameter. Terminal settling velocity is the equilibrium velocity of a particle that is falling under the influence of gravity and fluid resistance. The actual particle size, the particle density, and an aerodynamic shape factor determine aerodynamic diameter.

1.1.3.1 Types of Aerosols Aerosols are generally classified in terms of their processes of formation. Although the following classification is neither precise nor comprehensive, it is commonly used and accepted in the industrial hygiene and air pollution fields:

Dust: An aerosol formed by mechanical subdivision of bulk material into airborne fines having the same chemical composition. A general term for the process of mechanical subdivision is comminution, and it occurs in operations such as abrasion, crushing, grinding, drilling, and blasting. Dust particles are generally solid and irregular in shape and have diameters greater than 1 μm.

Fume: An aerosol of solid particles formed by condensation of vapors formed at elevated temperatures by combustion or sublimation. The primary particles are generally very small (less than 0.1 μm) and have spherical or characteristic crystalline shapes. They may be chemically identical to the parent material, or they may be composed of an oxidation product such as a metal oxide. Because they may be formed in high number concentration, they often rapidly coagulate, forming aggregate clusters of low overall density.

Smoke: An aerosol formed by condensation of combustion products, generally of organic materials. The particles are generally liquid droplets with diameters of less than 0.5 μm.

Mist: A droplet aerosol formed by mechanical shearing of a bulk liquid, for example, by atomization, nebulization, bubbling, or spraying. The initial droplet size can cover a very large range, usually from about 2 mm to greater than 50 μm.

Fog: An aqueous aerosol formed by condensation of water vapor on atmospheric nuclei at high relative humidity. The droplet sizes are generally greater than 1 μm.

Smog: A popular term for a pollution aerosol derived from a combination of smoke and fog. It is now commonly used for any atmospheric pollution mixture.

Haze: A submicrometer-sized aerosol of hygroscopic particles that take up water vapor at relatively low relative humidity. Typically, these aerosols are in the size range that interferes with visibility.

Submicrometer aerosol: Particles with a median aerodynamic diameter that are less than 1.0 μm and typically ≥0.1 μm.

Nanoparticles: Particles with a median aerodynamic diameter of less than 0.1 μm (i.e., ≤100 nm)

Aitken or condensation nuclei (CN): Very small atmospheric particles (mostly smaller than 0.1 μm) formed by combustion processes and by chemical conversion from gaseous precursors.

Accumulation mode: A term given to the particles in the ambient atmosphere ranging from 0.1 μm to about 1.0 μm and extending up to 2.5 μm for hygroscopic particles in humid atmospheres. These particles generally are spherical, have liquid surfaces, and form by coagulation and condensation of smaller particles that derive from gaseous precursors. Being too few for rapid coagulation, and too small for effective sedimentation, they tend to accumulate in the ambient air.

Coarse particle mode: Ambient air particles larger than about 2.5 μm and generally formed by mechanical processes and surface dust resuspension.

1.1.3.2 Aerosol Characteristics Aerosols have integral properties that depend upon the concentration and size distribution of the particles. In mathematical terms, these properties can be expressed in terms of certain constants or "moments" of the size distribution (Friedlander, 1977). Some integral properties such as light-scattering ability or electrical charge depend on other particle parameters as well. Some of the important integral properties are as follows:

Number concentration: The total number of airborne particles per unit volume of air, without distinction as to their sizes, is the zeroth moment of the size distribution. In current practice, instruments are available that count the numbers of particles of all sizes from about 0.005 to 50 mm. In many specific applications, such as fiber counting for airborne asbestos, a more restricted size range is specified.

Surface concentration: The total external surface area of all the particles in the aerosol, which is the second moment of the size distribution, may be of interest when surface catalysis or gas adsorption processes are of concern. Aerosol surface is one factor affecting light-scatter and atmospheric-visibility reductions.

Volume concentration: The total volume of all the particles, which is the third moment of the size distribution, is of little intrinsic interest in itself. However, it is closely related to the mass concentration, which for many environmental effects is the primary parameter of interest.

Mass concentration: The total mass of all the particles in the aerosol is frequently of interest. The mass of a particle is the product of its volume and density. If all of the particles have the same density, the total mass concentration is simply the volume concentration times the density. In some cases, such as "respirable," "thoracic," and "inhalable" dust sampling (Vincent, 1999), the parameter of interest is the mass concentration over a restricted range of particle size. In these applications, particles outside the size range of interest are excluded from the integral.

Dustfall: The mass of particles depositing from an aerosol onto a unit surface per unit time is proportional to the fifth moment of the size distribution. Dustfall has long been of interest in air pollution control because it provides an indication of the soiling properties of the aerosol.

Light scatter: The ability of airborne particles to scatter light and cause a visibility reduction is well known. Total light scatter can be determined by integrating the aerosol surface distribution with the appropriate scattering coefficients.

1.1.4 Water Contaminants

Chemical contaminants can be found in water, in solution, or as hydrosols; the latter are immiscible solid or liquid particles in suspension. An aqueous suspension in liquid particles is generally called an emulsion. Many materials of low aqueous solubility will be found in both dissolved and suspended forms.

1.1.4.1 Dissolved Contaminants Water is known as the universal solvent. Although there are many compounds that are not completely soluble in water, a few do not have some measurable solubility. In fact, the number of chemical contaminants in natural waters is primarily a function of the sensitivity of the analyses. For organic compounds in rivers and lakes, it has been observed that as the limits of detection decrease by an order of magnitude, the numbers of compounds detected increase by an order of magnitude, so that one might expect to find at least 10–12 g/L (approximately 1010 mol/L) of each of the million organic compounds reported in the literature (NIEHS, 1977). Similar considerations undoubtedly apply to inorganic chemicals as well.

1.1.4.2 Dissolved Solids Water quality criteria generally include a nonspecific parameter called "dissolved solids." However, it is customary to exclude natural mineral salts such as sodium chloride from this classification. In addition, water criteria for specific toxic chemicals dissolved in water are frequently exceeded without there being excessive total dissolved solids content.

1.1.4.3 Dissolved Gases Compounds dissolved in water may also exist in the gaseous phase at normal temperatures and pressures. Some of these, such as hydrogen sulfide (H_2S), and ammonia (NH_3) that are generated by decay processes, are toxicants.

Oxygen (O_2) is the most critical of the dissolved gases with respect to water quality. It is essential to most higher aquatic life forms and is needed for the oxidation of most of the organic chemical contaminants to more innocuous forms. Thus, a critical parameter of water quality is the concentration of dissolved oxygen (DO). Another important parameter is the extent of the oxygen "demand" associated with contaminants in the water. The most commonly used index of oxygen demand is the 5-day biochemical oxygen demand (BOD after 5 days of incubation). Another is the chemical oxygen demand (COD).

1.1.4.4 Suspended Particles A nonspecific water quality parameter that is widely used is "suspended solids." The stability of aqueous suspensions depends on particle size, density, and charge distributions. The fate of suspended particles depends on a number of factors, and particles can dissolve, grow, coagulate, or be ingested by various life forms in the water. They can become "floating solids" or part of an oil film, or they can fall to the bottom to become part of the sediments.

Natural waters contain many kinds of suspended particles, and not all of them are contaminants. Any moving water will have currents that cause bottom sediments to become resuspended. In addition, natural runoff will carry soil and organic debris into lakes and streams. In any industrialized area, such sediment and surface debris will always contain some chemicals considered contaminants. However, a large proportion of the mass of such suspended solids would usually be "natural" and would not be considered as contaminants.

The suspended particles can have densities that are less than, equal to, or greater than that of the water, so that the particles can rise as well as fall. Furthermore, the effective density of particles can be reduced by the attachment of gas bubbles.

Gas bubbles form in water when the water becomes saturated and cannot hold any more of the gas in solution. The solubility of gases in water varies inversely with temperature. For example, oxygen saturation of freshwater is 14.2 mL/L at 0°C and 7.5 mg/L at 30°C, and in seawater, the corresponding values are 11.2 and 6.1 mg/L.

1.1.5 Food Contaminants

Chemical contaminants of almost every conceivable kind can be found in most types of human food. Food can acquire these contaminants at any of several stages in its production, harvesting, processing, packaging, transportation, storage, cooking, and serving. In addition, many naturally occurring toxicants in foods as well as compounds can become toxicants upon conversion by chemical reactions with other constituents or additives or by thermal or microbiological conversion reactions during processing, storage, or handling.

Each food product has its own natural history. Most foods are formed by selective metabolic processes of plants and animals. In forming tissue, these processes can act either to enrich or to discriminate against specific toxicants in the environment. For animal products, where the flesh of interest in foods was derived from the consumption of other life forms, there are likely to be several stages of biological discrimination and, therefore, large differences between contaminant concentrations in the ambient air and/or water and the concentrations within the animals.

1.2 HUMAN EXPOSURES AND DOSIMETRY

People can be exposed to chemicals in the environment in numerous ways. The chemicals can be inhaled, ingested, or taken up by and through the skin. Effects of concern can take place at the initial epithelial barrier, that is, the respiratory tract, the gastrointestinal (GI) tract, or the skin, or can occur in other organ systems after penetration and translocation by diffusion or transport by blood, lymph, and so on. As illustrated in Fig. 1.2, exposure and dose factors are intermediate steps in a larger continuum ranging from release of chemicals into an environmental medium to an ultimate health effect.

Exposure is a key step in this continuum and a complex one. The concept of total human exposure, or exposome, has developed in recent years as essential to the appreciation of the nature and extent of environmental health hazards associated with ubiquitous chemicals at low levels (Rappaport and Smith, 2010; Escher et al., 2017). The exposome is defined as the measure of all the exposures of an individual in a lifetime and how those exposures relate to health. An individual's exposure begins before birth and includes insults from environmental and occupational sources. It provides a framework for considering and

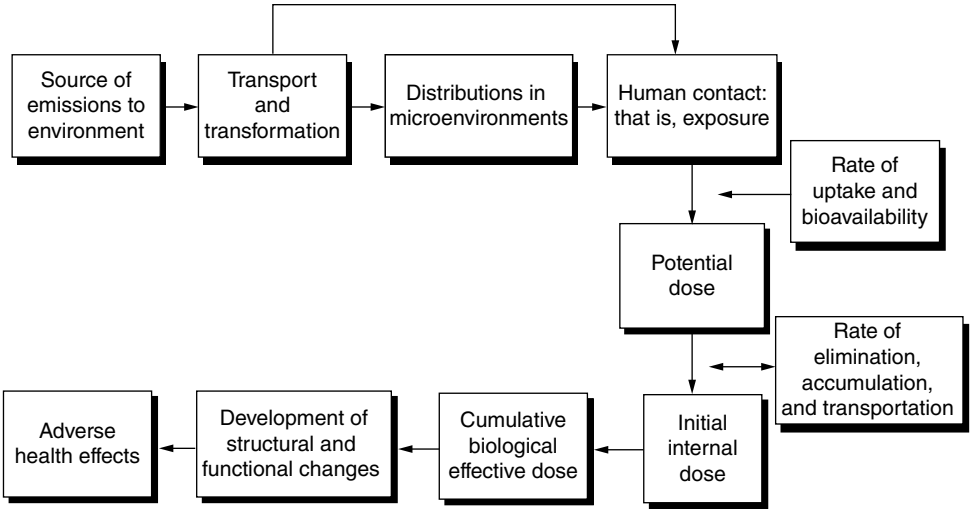


FIGURE 1.2 Environmental and biological modifiers of human exposure and health responses.

evaluating the contribution to the total insult from dermal uptake, ingestion of food and drinking water, and inhaled doses from potentially important microenvironments such as workplace, home, transportation, recreational sites, and so on. More thorough discussions of this key concept have been prepared by Sexton and Ryan (1988), Lioy (1990), and the National Research Council (NRC, 1991). Guidelines for Exposure Assessment have been formalized by the U.S. Environmental Protection Agency (U.S. EPA, 1992).

1.3 CHEMICAL EXPOSURES AND DOSE TO TARGET TISSUES

Toxic chemicals in the environment that reach sensitive tissues in the human body can cause discomfort, loss of function, and changes in structure, leading to disease. This section addresses the pathways and transport rates of chemicals from environmental media to critical tissue sites as well as retention times at those sites. It is designed to provide a conceptual framework as well as brief discussions of (1) the mechanisms for—and some quantitative data on—uptake from the environment; (2) translocation within the body, retention at target sites, and the influence of the physicochemical properties of the chemicals on these factors; (3) the patterns and pathways for exposure of humans to chemicals in environmental media; (4) the effects of chemicals at the cellular and organ levels; and (5) the influence of age, sex, size, habits, health status, and other factors.

An agreed on terminology is critically important when discussing the relationships between toxic chemicals in the environment and human health. The terms used in this book are defined below:

Exposure: Contact with external environmental media containing the chemical of interest. For fluid media in contact with the skin or respiratory tract, both concentration and contact time are critical. For ingested material, concentration and amount consumed are important.

Deposition: Capture of the chemical at a body surface site on the skin, respiratory tract, or GI tract.

Clearance: Translocation from a deposition site to a storage site or depot within the body or elimination from the body.

Retention: Presence of residual material at a deposition site or along a clearance pathway.

Dose: Amount of chemical deposited on or translocated to a site on or within the body where toxic effects take place.

Target tissue: A site within the body where toxic effects lead to damage or disease. Depending on the toxic effects of concern, a target tissue can extend from whole organs down to specific cells to subcellular constituents.

Exposure surrogates or indices: Indirect measures of exposure, such as (1) concentrations in environmental media at times or places other than those directly encountered; (2) concentrations of the chemical of interest, a metabolite of the chemical, or an enzyme induced by the chemical in circulating or excreted body fluids; or (3) elevations in body burden as measured by external probes.

In summary, exposure represents contact between a concentration of an agent in air, water, food, or other material and the person or population of interest. The agent is the source of an internal dose to a critical cell, organ, or tissue. The magnitude of the dose depends on a number of factors: (1) the mass (concentration × volume) inhaled or ingested; (2) the fractions of the inhaled or ingested material transferred across epithelial membranes of the skin, the respiratory tract, and the GI tract; (3) the fractions transported via circulating fluids to target tissues; and (4) the fractional uptake by the target tissues. Each of these factors can have considerable intersubject variability. Sources of variability include activity level, age, sex, size, and health status as well as genetic variabilities (e.g., pharmacogenomics variability in absorption, distribution, metabolism, and excretion).

With chronic or repetitive exposures, other factors affect the dose of interest. When the retention at or effects on the target tissues are cumulative and clearance or recovery is slow, the dose of interest can be represented by cumulative uptake. However, when the agent is rapidly eliminated, or when its effects are rapidly and completely reversible on removal from exposure, rate of delivery may be the dose parameter of primary interest.

1.4 CONCENTRATION OF TOXIC CHEMICALS IN HUMAN MICROENVIRONMENTS

The technology for sampling air, water, and food is relatively well developed, as are the technologies for sample separation from co-pollutants, media, and interferences and for quantitative analyses of the components of interest. However, to collect data relevant to the exposures of interest is difficult because it requires knowledge of when, where, how long, and at which rate and frequency to sample. It also requires knowledge of temporal and spatial variability of exposure concentrations. Unfortunately, we seldom have enough of this information to guide sample collections. In addition, the sampling method depends on the environmental distribution of the toxicant of interest and thus is compound specific. Many of these factors are discussed in detail in the chapters that follow as they apply to the specific environmental toxicants being discussed.

1.4.1 Water and Foods

Concentrations of environmental chemicals in food and drinking water are extremely variable. Further, the amounts consumed are variable because of the extreme variability in dietary preferences and food sources. The number of foods for which up-to-date concentration data for specific chemicals are available is extremely limited. Relevant human dietary exposure data are sometimes available in terms of market basket survey analyses. In this approach, foods for a mixed diet are purchased, cleaned, processed, and prepared as for consumption, and one set of specific chemical analyses is done for the composite mixture that is consumed.

The concentrations of chemicals in potable piped water supplies depend greatly on the source of the water and its treatment history. Surface waters from protected watersheds generally have low concentrations of both dissolved minerals and environmental chemicals. Well waters usually have low concentrations of bacteria and environmental chemicals but often have high mineral concentrations. Poor waste disposal practices may contribute to groundwater contamination, especially in areas of high population density. Treated surface waters from lakes and rivers in densely populated and/or industrialized areas usually contain a wide variety of dissolved organics and trace metals, the concentrations of which vary greatly with season (because of variable surface runoff), with proximity to pollutant sources, with upstream usage, and with treatment efficacy.

Uptake of environmental chemicals in bathing waters across intact skin is usually minimal in comparison with uptake via inhalation or ingestion. It depends on both the concentration in the fluid surrounding the skin surface and the polarity of the chemical, with more polar chemicals having less ability to penetrate the intact skin. Uptake via skin can be significant for occupational exposures to concentrated liquids or solids.

1.4.2 Air

Although chemical uptake through ingestion and the skin surface is generally intermittent, inhalation provides a continuous means of exposure. The important variables affecting the uptake of inhaled chemicals are the depth and frequency of inhalation and the concentration and physicochemical properties of the chemicals in the air.

Exposures to airborne chemicals vary widely among inhalation microenvironments, the categories of which include workplace, residence, outdoor ambient air, transportation, recreation, and public spaces. Wide variations exist in exposure within each category, depending on the number and strength of the sources of the airborne chemicals, the volume and mixing characteristics of the air within the defined microenvironment, the rate of air exchange with the outdoor air, and the rate of loss to surfaces within the microenvironment.

1.4.3 Workplace

Exposures to airborne chemicals at work are extremely variable in terms of composition and concentration, depending greatly on the materials being handled and the process design and operation. Occupational exposures are also influenced the kinds and degree of engineering controls applied to minimize release to the air, work practices followed, and personal protection provided. Workplace air monitoring often involves breathing

zone sampling, generally with passive samplers for gases and vapors or with personal battery-powered extraction samplers for both gases and particles; these operate over periods of 1–8 h. Analyses of the samples collected can provide accurate measures of individual exposures to specific air contaminants.

Workplace air monitoring also is done frequently with fixed-site samplers or direct reading instruments. However, air concentrations at fixed sites may differ substantially from those in the breathing zones of individual workers. The fixed-site data may be relatable to the breathing zone when appropriate intercomparisons can be made, but otherwise they represent crude surrogates of exposure. The characteristics of equipment used for air sampling in industry are described in detail in Air Sampling Instruments (ACGIH, 2001).

1.4.4 Residential

Airborne chemicals in residential microenvironments are attributable to their presence in the air infiltrating from outdoors and to their release from indoor sources. The latter include unvented cooking stoves and space heaters, cigarettes, consumer products, and volatile emissions from wallboard, textiles, carpets, and so on. Personal exposures to chloroform, largely from indoor residential sources, are illustrated in Fig. 1.3, and the influence of smoking in the home on indoor exposures to respirable particulate matter is illustrated in Fig. 1.4. Indoor sources can release enough nitrogen dioxide (NO_2), fine particle mass (FPM), and formaldehyde (HCHO) that indoor concentrations for these chemicals can be much higher than those in ambient outdoor air. Furthermore, their contributions to the total

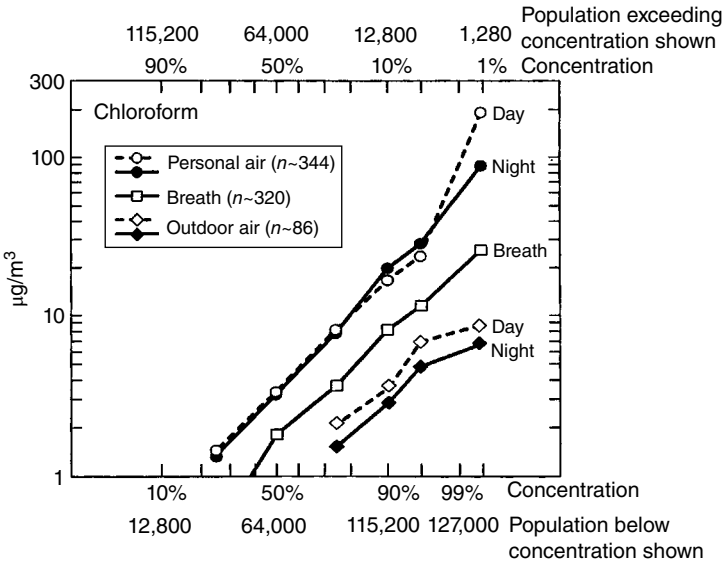


FIGURE 1.3 Estimated frequency distributions of personal air exposures to chloroform: outdoor air concentrations and exhaled breath values in Elizabeth-Bayonne, NJ area. *Note*: Air values are 12-h integrated samples. Breath value was taken following the daytime air sample (6 : 00 a.m. to 6 : 00 p.m.). Outdoor air samples were taken near participants' homes. *Source*: Wallace et al. (1985).

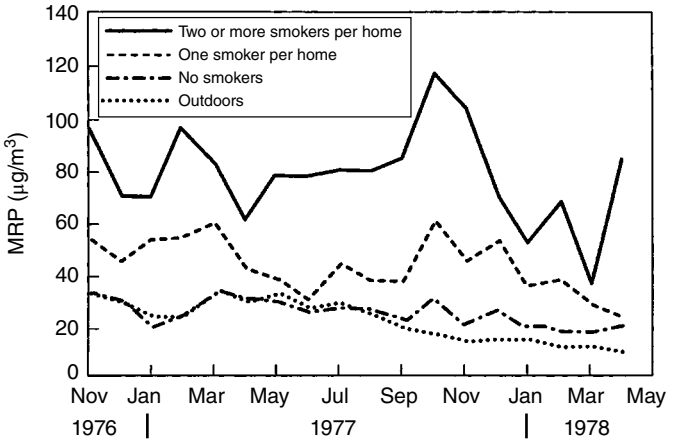


FIGURE 1.4 Respirable particle concentrations, six U.S. cities, November 1976 to April 1978. *Source*: National Academy of Science (1981).

human exposure are usually even greater, since people usually spend much more time at home than in the outdoor ambient air.

1.4.5 Outdoor Ambient Air

For pollutants having National Ambient Air Quality Standards (NAAQS), monitoring is conducted by an extensive network of fixed-site monitors, generally on rooftops. Although these devices generate large volumes of data, the concentrations at these sites may differ substantially from the concentrations that people breathe, especially for tailpipe pollutants, such as carbon monoxide (CO), and reactive chemicals, such as ozone (O_3) and sulfur dioxide (SO_2). Data for other toxic pollutants in the outdoor ambient air are not generally collected on as routine a basis.

1.4.6 Transportation

Many people spend from 1/2 to 3 h each day in autos or mass transport as they go to work, to school, or shopping. Inhalation exposures to CO in vehicles and garages can represent a significant fraction of total CO exposures. Persons employed in a transportation industry, for example, bus drivers, or on or near roadways, for example, traffic police or road maintenance worker, also may have extensive exposures to CO, diesel exhaust, and other vehicle emissions.

1.4.7 Recreation and Public Spaces

Recreational exposure while exercising may be important to total daily exposure because the increased respiratory ventilation associated with exercise can produce much more than proportional increases in delivered dose and functional responses. Spectators and athletes in closed arenas can be exposed to high concentration of pollutants. For example, Spengler et al. (1978) documented high exposures to CO at ice rinks from exhaust discharges by the ice-scraping machinery.

1.5 INHALATION EXPOSURES AND RESPIRATORY TRACT EFFECTS

1.5.1 Deposition and Absorption

The surface and systemic uptake of chemicals from inhaled air depend on both their physical and chemical properties and on the anatomy and pattern of respiration within the respiratory airways. The basic structure of the respiratory tract is illustrated in Fig. 1.5. The following discussion outlines some of the primary factors affecting the deposition and retention of inhaled chemicals. Discussions that are more comprehensive are available in reviews (ICRP, 1994; U.S. EPA, 1996; NCRP, 1997). Figure 1.6, from the 1994 ICRP Report, summarizes the morphometry, cytology, histology, function, and structure of the human respiratory tract, while Fig. 1.7 shows the compartmental model developed by ICRP (1994) to summarize particle transport from the deposition sites within the respiratory tract.

Gases and vapors rapidly contact airway surfaces by molecular diffusion. Surface uptake is limited for compounds that are relatively insoluble in water, such as O_3. For such chemicals, the greatest uptake can be in the lung periphery, where the residence time and surface areas are the greatest. For more water-soluble gases, dissolution and/or reaction

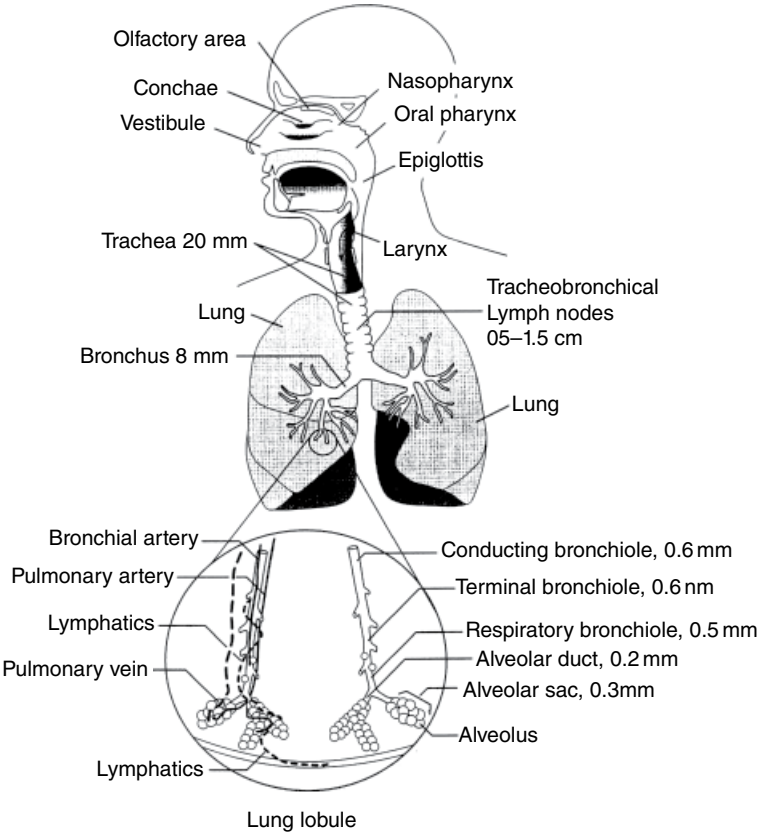
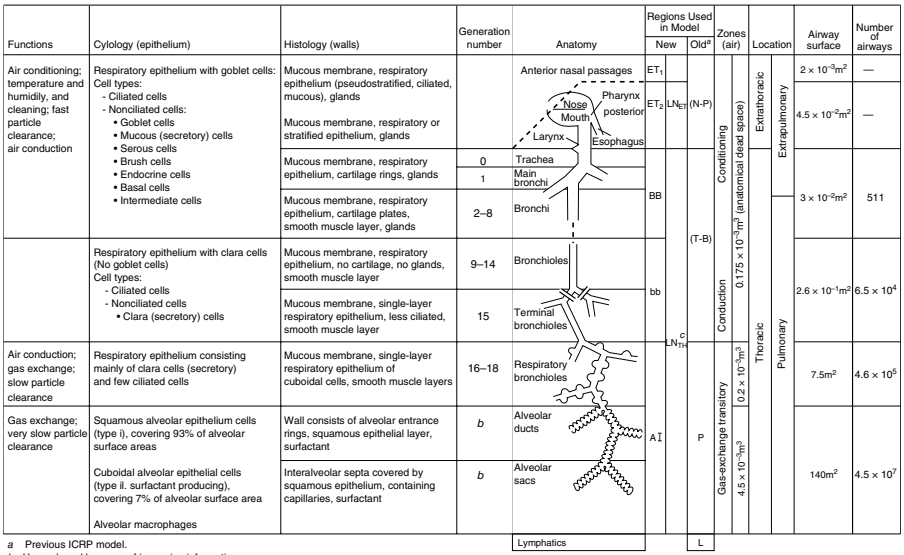


FIGURE 1.5 Structure of the respiratory tract. *Source*: From National Research Council (1979).



a Previous ICRP model.
b Unnumbered because of imprecise information.
c Lymph nodes are located only in BB region but drain the bronchial and alveolar interstitial regions as well as the bronchial region.

FIGURE 1.6 Morphometry, cytology, histology, function, and structure of the respiratory tract and regions used in the 1994 ICRP dosimetry model.

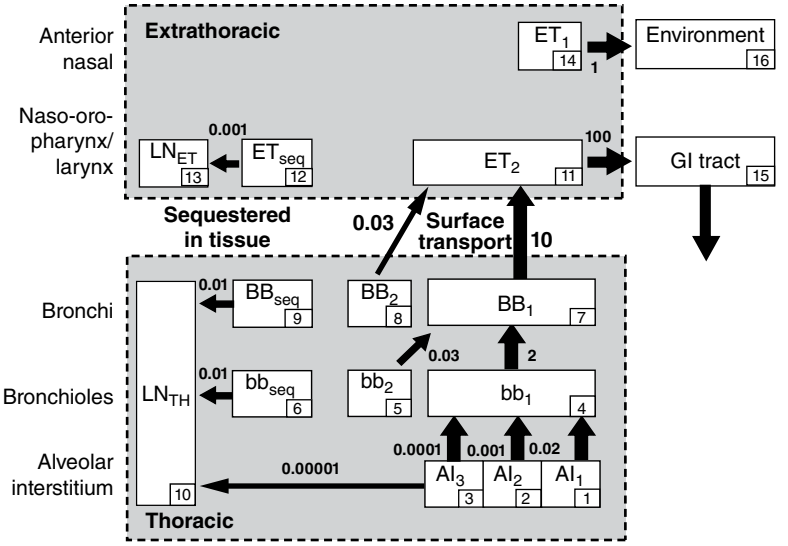


FIGURE 1.7 Compartment model to represent time-dependent particle transport from each region in 1994 ICRP model. Particle transport rate constants shown beside the arrows are reference values in d^{-1}. Compartment numbers (shown in the lower right-hand corner of each compartment box) are used to define clearance pathways. Thus, the particle transport rate from bb_1 to BB_1 is denoted $m_{4,7}$ and has the value $2\,d^{-1}$.

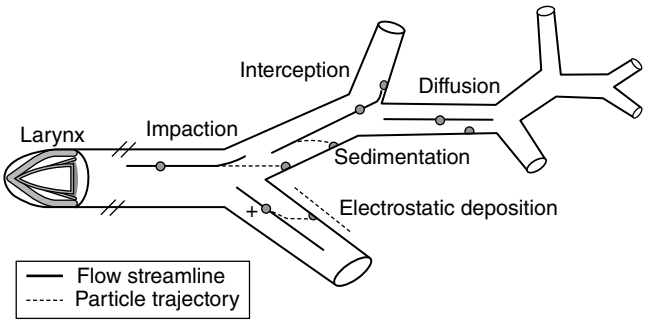


FIGURE 1.8 Schematic of mechanism for particle deposition in respiratory airways. *Source*: From Lippmann and Schlesinger (1984).

with surface fluids on the airways facilitates removal from the airstream. Highly water-soluble vapors, such as SO_2, are almost completely removed in the airways of the head, and very little of them penetrates into lung airways.

For airborne particles, the most critical parameter affecting patterns and efficiencies of surface deposition is particle size. The mechanisms for particle deposition within respiratory airways are illustrated in Fig. 1.8. Almost all of the mass of airborne particulate matter is found in particles with diameters greater than 0.1 μm. Such particles have diffusional displacements many orders of magnitude smaller than those of gas molecules, and they are small in relation to the sizes of the airways in which they are suspended. Thus, the penetration of airborne particles into the lung airways is determined primarily by convective flow; that is, the motion of the air in which the particles are suspended.

Some deposition by diffusion does occur for particles <0.5 μm in small airways, where it is favored by the small size of the airways and the low flow velocities in such airways. For particles >0.5 μm, deposition by sedimentation occurs in small to midsized airways. For particles with aerodynamic diameters >2 μm, particle inertia is sufficient to cause particle motion to deviate from the flow streamlines, resulting in deposition by impaction on surfaces downstream of changes in flow direction, primarily in mid- to large-sized airways, which have the highest flow velocities. The concentration of deposition on limited surface areas within the large airways is of special interest with respect to dosimetry and the pathogenesis of chronic lung diseases such as bronchial cancer and bronchitis.

Although particle inertia accounts for much of the "hot-spot" deposition on the trachea below the laryngeal jet and at the bifurcations of large lung airways, some of the concentrated deposition is attributable to inertial airflow, which directs a disproportionately large fraction of the flow volume toward such surfaces and, at the same time, lessens the boundary layer thickness. Thus, there is some preferential deposition of submicrometer-sized particles and gas molecules at small airway bifurcations.

Quantitative aspects of particle deposition are summarized in Figs. 1.9–1.12. It can be seen that deposition efficiencies in the major structural–functional regions of the human respiratory tract are both strongly particle size dependent and highly variable among normal humans. Additional variability results from structural changes in the airways associated with disease processes. Generally, these involve airway narrowing or localized constrictions, which act to increase deposition and concentrate it on limited surface areas.

All of the preceding was based on the assumption that each particle has a specific size. The surface of the airways is moist and inhaled air is saturated with water within the first airway bifurcation. This, for particles that are hygroscopic, considerable growth in size can occur as they take up water vapor in the airways. Some hygroscopic growth curves for acidic and ambient aerosols are illustrated in Fig. 1.13.

Materials that dissolve into the mucus of the conductive airways or the surfactant layer of the alveolar region can rapidly diffuse into the underlying epithelia and the circulating

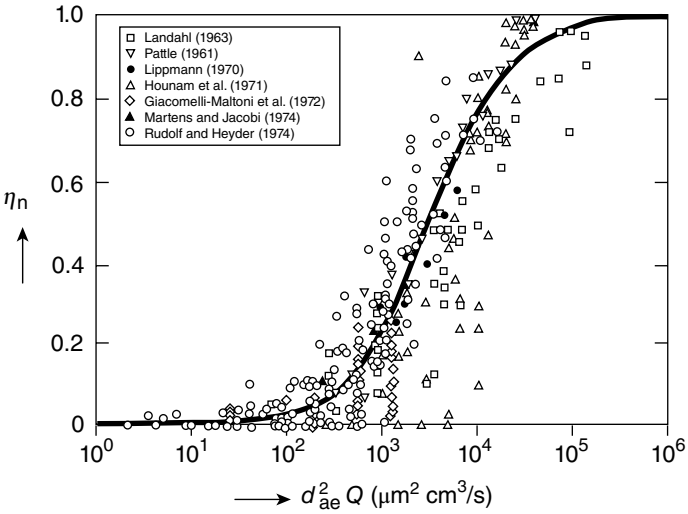


FIGURE 1.9 Inspiratory deposition of the human nose as a function of particle aerodynamic diameter and flow rate ($d_{ae}^2 Q$). *Source*: From U.S. EPA (1996).

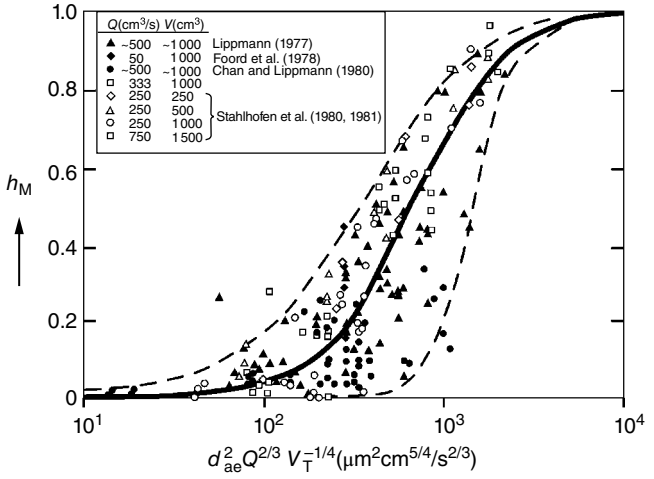


FIGURE 1.10 Inspiratory extrathoracic deposition data in humans during mouth breathing as a function of particle aerodynamic diameter, flow rate, and tidal volume ($d_{ae}^2 Q^{2/3} V_T^{-1/4}$). *Source*: From U.S. EPA (1996).

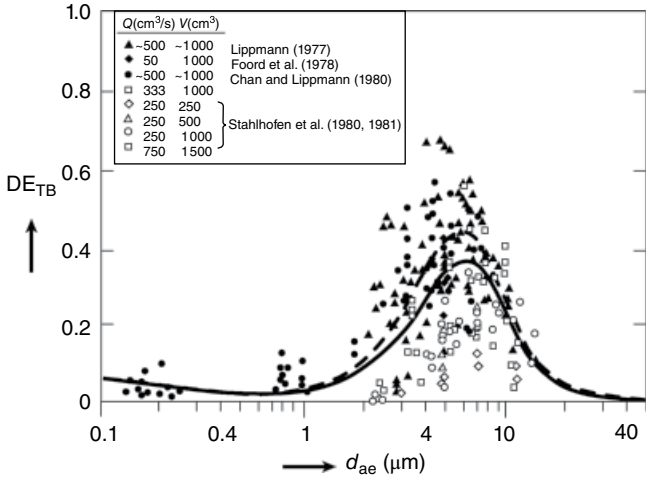


FIGURE 1.11 Tracheobronchial deposition data in humans at mouth breathing as a function of particle aerodynamic diameter (d_{ae}). The solid curve represents the approximate mean of all the experimental data; the broken curve represents the mean excluding the data of Stahlhofen et al. (1980, 1981). *Source*: From U.S. EPA (1996).

blood, thereby gaining access to tissues throughout the body. Chemical reactions and metabolic processes may occur within the lung fluids and cells, limiting access of the inhaled material to the bloodstream and creating reaction products with either greater or lesser solubility and biological activity. Few generalizations about absorption rates are possible.

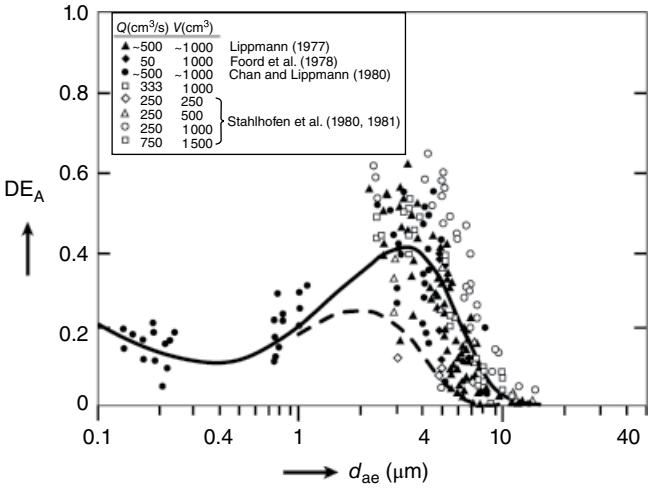


FIGURE 1.12 Alveolar deposition data in humans as a function of particle aerodynamic diameter (d_{ae}). The solid curve represents the mean of all the data; the broken curve is an estimate of deposition for nose breathing by Lippmann (1977). *Source*: From U.S. EPA (1996).

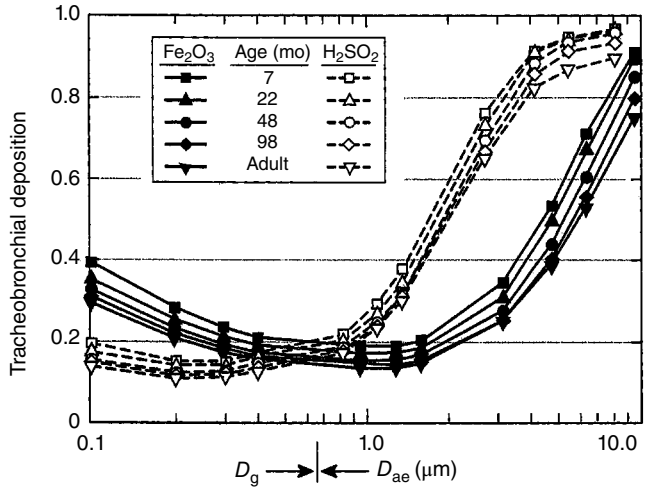


FIGURE 1.13 Tracheobronchial particle deposition as a function of particle size at various ages for both stable iron oxide particles and hygroscopic sulfuric acid droplets that grow in size in the warm moist respiratory airways. *Source*: From Martonen (1990).

1.5.2 Translocation and Retention

Particles that do not dissolve at deposition sites can be translocated to remote retention sites by passive and active clearance processes. Passive transport depends on movement on or in surface fluids lining the airways. There is a continual proximal flow of lung surfactant from alveolar epithelial cells to and onto the mucociliary escalator, which begins at the terminal bronchioles, where it mixes with secretions from Clara and goblet cells in the airway

epithelium. Within midsized and larger airways, there are additional secretions from goblet cells and mucus glands, producing a thicker mucous layer having a serous subphase and an overlying more viscous gel layer. The gel layer, lying above the tips of the synchronously beating cilia, is found in discrete plaques in smaller airways and becomes more of a continuous layer in the larger airways. The mucus reaching the larynx and the particles carried by it are swallowed and enter the GI tract.

The total transit time for particles depositing on terminal bronchioles varies from ~2 to 24 h in healthy humans, accounting for the relatively rapid bronchial clearance phase. Macrophage-mediated particle clearance via the bronchial tree takes place over a period of several weeks. The particles depositing in alveolar zone airways are ingested by alveolar macrophages within about 6 h, but the movement of the particle-laden macrophages depends on the several weeks that it takes for the normal turnover of the resident macrophage population. At the end of several weeks, the particles not cleared to the bronchial tree via macrophages have been incorporated into epithelial and interstitial cells, from which they are slowly cleared by dissolution and/or as particles via lymphatic drainage pathways, passing through pleural and eventually hilar and tracheal lymph nodes. Clearance times for these later phases depend strongly on the chemical nature of the particles and their sizes, with half-times ranging from about 30 to 1000 days or more.

All of the characteristic clearance times cited refer to inert, nontoxic particles in healthy lungs. Toxicants can drastically alter clearance times. Inhaled materials affecting mucociliary clearance rates include cigarette smoke (Albert et al., 1974, 1975), sulfuric acid (H_2SO_4) (Lippmann et al., 1982; Schlesinger et al., 1983), O_3 (Phalen et al., 1980; Schlesinger and Driscoll, 1987), SO_2 (Wolff et al., 1977), and formaldehyde (Morgan et al., 1984). Macrophage-mediated alveolar clearance is affected by SO_2 (Ferin and Leach, 1973), NO_2 and H_2SO_4 (Schlesinger et al., 1988), O_3 (Phalen et al., 1980; Schlesinger et al., 1988), and silica dust (Jammet et al., 1970). Cigarette smoke is known to affect the later phases of alveolar zone clearance in a dose-dependent manner (Bohning et al., 1982). Clearance pathways as well as rates can be altered by these toxicants, affecting the distribution of retained particles and their dosimetry.

1.6 INGESTION EXPOSURES AND GASTROINTESTINAL TRACT EFFECTS

Chemical contaminants in drinking water or food reach human tissues via the GI tract. Ingestion may also contribute to uptake of chemicals that were initially inhaled, because material deposited on or dissolved in the bronchial mucous blanket is eventually swallowed.

The GI tract may be considered a tube running through the body, the contents of which are actually external to the body. Unless the ingested material affects the tract itself, any systemic response depends on absorption through the mucosal cells lining the lumen. Although absorption may occur anywhere along the length of the GI tract, the main region for effective translocation is the small intestine. The enormous absorptive capacity of this organ results from the presence in the intestinal mucosa of projections, termed villi, each of which contains a network of capillaries; the villi result in a large effective total surface area for absorption.

Although passive diffusion is the main absorptive process, active transport systems also allow essential lipid-insoluble nutrients and inorganic ions to cross the intestinal epithelium and are responsible for uptake of some contaminants. For example, lead may be

absorbed via the system that normally transports calcium ions (Sobel et al., 1938). Small quantities of particulate material and certain large macromolecules, such as intact proteins, may be absorbed directly by the intestinal epithelium.

Materials absorbed from the GI tract enter either the lymphatic system or the portal blood circulation; the latter carries material to the liver, from which it may be actively excreted into the bile or diffuse into the bile from the blood. The bile is subsequently secreted into the intestines. Thus, a cycle of translocation of a chemical from the intestine to the liver to bile and back to the intestines, known as the enterohepatic circulation, may be established. Enterohepatic circulation usually involves contaminants that undergo metabolic degradation in the liver. For example, DDT undergoes enterohepatic circulation; a product of its metabolism in the liver is excreted into the bile, at least in experimental animals (Hayes, 1965).

Various factors can modify absorption from the GI tract by enhancing or depressing the mucosal barrier function. A decrease in GI mobility generally favors increased absorption. Specific stomach contents and secretions may react with the contaminant, possibly changing it to a form with different physicochemical properties (e.g., solubility), or they may absorb it, altering the available chemical and changing translocation rates. The size of ingested particulates also affects absorption. Because the rate of dissolution is inversely proportional to particle size, large particles are absorbed to a lesser degree, especially if they are of a fairly insoluble material in the first place. For example, arsenic trioxide is more hazardous when ingested as a finely divided powder than as a coarse powder (Schwartz, 1923). Certain chemicals, for example, chelating agents such as EDTA, also cause a nonspecific increase in absorption of many materials. Lastly, spastic contractions in the stomach and intestine may serve to eliminate noxious agents via vomiting or by acceleration of the transit of feces through the GI tract, which can act as a defense.

1.7 SKIN EXPOSURE AND DERMAL EFFECTS

The skin is generally an effective barrier against the entry of environmental chemicals. To be absorbed via this route (percutaneous absorption), an agent must traverse a number of cellular layers before gaining access to the general circulation (Fig. 1.14). The skin consists of two structural regions, the epidermis and the dermis, which rest on connective tissue. The epidermis consists of a number of layers of cells and has varying thickness depending on the region of the body; the outermost layer is composed of keratinized cells. The dermis contains blood vessels, hair follicles, sebaceous and sweat glands, and nerve endings. The epidermis represents the primary barrier to percutaneous absorption, the dermis being freely permeable to many materials. Passage through the epidermis occurs by passive diffusion.

The main factors that affect percutaneous absorption are degree of lipid solubility of the chemicals, site on the body, local blood flow, and skin temperature. Environmental chemicals that are readily absorbed through the skin include phenol, carbon tetrachloride, tetraethyl lead, and organophosphate pesticides. Certain chemicals, for example, dimethyl sulfoxide (DMSO) or formic acid, alter the integrity of the skin and facilitate penetration of other materials by increasing the permeability of the outer layer of epidermis, the stratum corneum. Moderate changes in permeability may also result following topical applications of acetone, methyl alcohol, and ethyl alcohol. In addition, cutaneous injury may enhance percutaneous absorption.

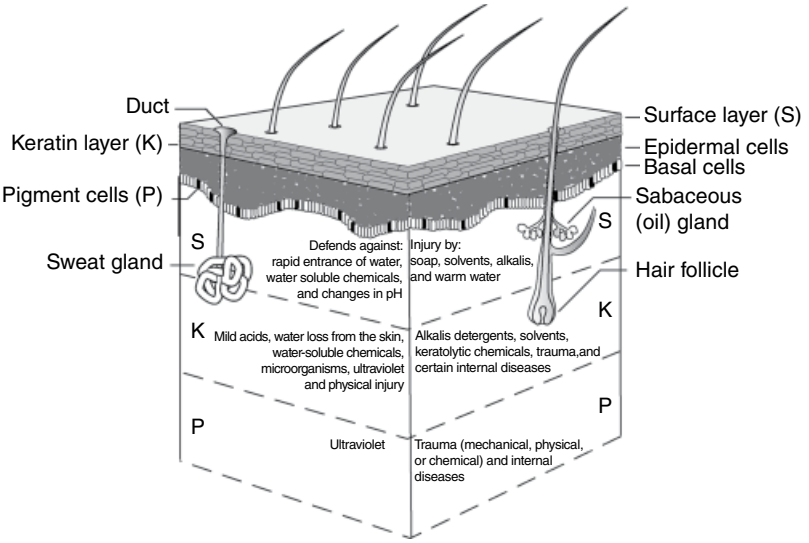


FIGURE 1.14 Idealized section of the skin. *Source*: From Birmingham (1973).

Interspecies differences in percutaneous absorption are responsible for the selective toxicity of many insecticides. For example, chlorinated hydrocarbons are about equally hazardous to insects and mammals if ingested but are much less hazardous to mammals when applied to the skin. This is because of their poor absorption through mammalian skin compared to their ready passage through the insect exoskeleton. Although the main route of percutaneous absorption is through the epidermal cells, some chemicals may follow an appendageal route, that is, entering through hair follicles, sweat glands, or sebaceous glands. Cuts and abrasions of the skin can provide additional pathways for penetration.

1.8 ABSORPTION THROUGH MEMBRANES AND SYSTEMIC CIRCULATION

Depending upon its specific nature, a chemical contaminant may exert its toxic action at various sites in the body. At a portal of entry—the respiratory tract, GI tract, or skin—the chemical may have a topical effect. However, for actions at sites other than the portal, the agent must be absorbed through one or more body membranes and enter the general circulation, from which it may become available to affect cells and internal tissues (including the blood itself). The ultimate distribution of any chemical contaminant in the body is, therefore, highly dependent on its ability to traverse biological membranes. This occurs by two main processes: passive transport and active transport.

Passive transport is absorption according to purely physical processes, such as osmosis; the cell has no active role in transfer across the membrane. Because biological membranes contain lipids, they are highly permeable to lipid-soluble, nonpolar, or nonionized agents and less so to lipid-insoluble, polar, or ionized materials. Many chemicals may exist in both lipid-soluble and lipid-insoluble forms; the former is the prime determinant of the passive permeability properties for the specific agent.

Active transport involves specialized mechanisms, with cells actively participating in transfer across membranes. These mechanisms include carrier systems within the membrane and active processes of cellular ingestion, that is, phagocytosis and pinocytosis. Phagocytosis is the ingestion of solid particles, whereas pinocytosis refers to the ingestion of fluid containing no visible solid material. Lipid-insoluble materials are often taken up by active transport processes. Although some of these mechanisms are highly specific, if the chemical structure of a contaminant is similar to that of an endogenous substrate, the former may be transported as well.

In addition to its lipid-solubility characteristics, the distribution of a chemical contaminant is also dependent on its affinity for specific tissues or tissue components. Internal distribution may vary with time after exposure. For example, immediately following absorption into the blood, inorganic lead is found to localize in the liver, in the kidney, and in red blood cells. Two hours later, about 50% is in the liver. A month later, approximately 90% of the remaining lead is localized in bone (Hammond, 1969).

Once in the general circulation, a contaminant may be translocated throughout the body. In this process it may (1) become bound to macromolecules, (2) undergo metabolic transformation (biotransformation), (3) be deposited for storage in depots that may or may not be the sites of its toxic action, or (4) be excreted. Toxic effects may occur at any of several sites. The biological action of a contaminant may be terminated by storage, metabolic transformation, or excretion, the latter being the most permanent form of removal.

1.9 ACCUMULATION IN TARGET TISSUES AND DOSIMETRIC MODELS

Some chemicals tend to concentrate in specific tissues because of physicochemical properties such as selective solubility or selective absorption on or combination with macromolecules such as proteins. Storage of a chemical often occurs when the rate of exposure is greater than the rate of metabolism and/or excretion. Storage or binding sites may not be the sites of toxic action. For example, CO produces its effects by binding with hemoglobin in red blood cells; on the contrary, inorganic Pb is stored primarily in bone but exerts its toxic effects mainly on the soft tissues of the body.

If the storage site is not the site of toxic action, selective sequestration may be a protective mechanism, because only the freely circulating form of the contaminant produces harmful effects. Until the storage sites are saturated, a buildup of free chemical may be prevented. On the contrary, selective storage limits the amount of contaminant that is excreted. Because bound or stored toxicants are in equilibrium with their free form, as the contaminant is excreted or metabolized, it is released from the storage site. Contaminants that are stored (e.g., DDT) may remain in the body for years without effect. On the contrary, accumulation may produce illnesses that develop slowly, as occurs in chronic Cd poisoning. In addition, storage in specific compartments can change during physiological processes, for example, loss of fat during dieting or loss of bone during pregnancy or breastfeeding.

A number of descriptive and mathematical models have been developed to permit estimation from knowledge of exposure and one or more of the following factors: translocation, metabolism, and effects at the site of toxic action. The use of these models for airborne particulate matter generally requires knowledge of the concentration within specific

particle size intervals or of the particle size distribution of the compounds of interest. Simple deposition models break the respiratory tract into regions (summarized by Vincent, 1999):

Head, nasopharynx, extrathoracic airways: nose, mouth, nasopharynx, oropharynx, laryngopharynx.

Tracheobronchial airways: larynx, trachea, bronchi, bronchioles (to terminal bronchioles).

Gas exchange, pulmonary, alveolar: respiratory bronchioles, alveolar ducts, alveolar sacs, alveoli.

Size-selective aerosol sampling can mimic the head airways and tracheobronchial airway regions so that airborne particle collection can be limited to the size fraction directly related to the potential for disease. More complex models requiring data on translocation and metabolism have been developed for inhaled and ingested radionuclides by the International Commission on Radiological Protection (ICRP, 1966, 1979, 1981, 1994).

1.10 INDIRECT MEASURES OF PAST EXPOSURES

Documented effects of environmental chemicals on humans seldom contain quantitative exposure data and only occasionally include more than crude exposure rankings based on known contact with or proximity to the materials believed to have caused the effects. Reasonable interpretation of the available human experience requires some appreciation of the uses and limitations of the data used to estimate the exposure side of the exposure–response relationship. The discussion that follows is an attempt to provide background for interpreting data and for specifying the kinds of data needed for various analyses.

Both direct and indirect exposure data can be used to rank exposed individuals by exposure intensity. External exposure can be measured directly by collection and analysis of environmental media. Internal exposure can be estimated from analyses of biological fluids and *in vivo* retention. Indirect measures generally rely on work or residential histories with some knowledge of exposure intensity at each exposure site and/or some enumeration of the frequency of process upsets and/or effluent discharges that result in high-intensity short-term exposures.

1.10.1 Concentrations in Air, Water, and Food

Historic data may occasionally be available on the concentrations of materials of interest in environmental media. However, they may or may not relate to the exposures of interest. Among the more important questions to be addressed in attempts to use much data are as follows:

(1) How accurate and reliable were the sampling and analytical techniques used in the collection of the data? Were they subjected to any quality assurance protocols? Were standardized and/or reliable techniques used?

(2) When and where were the samples collected, and how did they relate to exposures at other sites? Air concentrations measured at fixed (area) sites in industry may be

much lower than those occurring in the breathing zone of the person close to the contaminant sources. Air concentrations at fixed (generally elevated) community air sampling sites can be either much higher or much lower than those at street level and indoors because of strong gradients in source and sink strengths in indoor and outdoor air.

(3) What is known or assumed about the ingestion of food and/or water containing the measured concentrations of the contaminants of interest? Time at home and dietary patterns are highly variable among populations at risk.

1.10.2 Biological Sampling Data

Many of the same questions that apply to the interpretation of environmental media concentration data also apply to biological samples, especially quality assurance. The time of sampling is especially critical in relation to the times of the exposures and to the metabolic rates and pathways. In most cases, it is quite difficult to separate the contributions to the concentrations in circulating fluids of levels from recent exposures and those from long-term reservoirs.

1.10.3 Exposure Histories

Exposure histories *per se* are generally unavailable, except in the sense that work or residential histories can be interpreted in terms of exposure histories. Job histories, as discussed below, are often available in company and/or union records and can be converted into relative rankings of exposure groups with the aid of long-term employees and managers familiar with the work processes, history of process changes, material handled, tasks performed, and the engineering controls of exposure.

Routine steady-state exposures may be the most important and dominant exposures of interest in many cases. On the contrary, for some health effects, the occasional or intermittent peak exposures may be of primary importance. In assessing or accumulating exposure histories or estimates, it is important to collect evidence for the frequency and magnitude of the occasional or intermittent releases associated with process upsets.

1.11 CHARACTERIZATION OF HEALTH

1.11.1 Definitions of Health

There is no universally accepted definition of health. Perhaps the most widely accepted one today is that of the World Health Organization, which describes health as a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity. Unfortunately, by a strict interpretation of this rather idealistic definition, very few people could be considered healthy. The discussion to follow is limited largely to physical well-being. The adverse health effects discussed are those that can be recognized by clinical symptoms (i.e., subjective evidence that are reported by subjects), signs (measurements that are consistent with an adverse condition), or decrements in functional performance. Thus, for all practical purposes, in this volume we consider health to be the absence of measurable disease, disability, or dysfunction.

1.11.2 Adverse Health Effects

Recognizable adverse health effects in populations are generally divided into two categories: mortality and morbidity. The former refers to the number of deaths per unit of population per unit time and to the ages at death. Morbidity refers to nonfatal cases of reportable disease.

Accidents, infectious diseases, and massive overexposures to toxic chemicals can cause excess deaths to occur within a short time after the exposure to the hazard. They can also result in residual disease and/or dysfunction. In many cases, the causal relationships are well defined, and it may be possible to develop quantitative relationships between dose and subsequent response.

The number of people exposed to chemical contaminants at low levels is often greater than the number exposed at levels high enough to produce overt responses. Furthermore, low-level exposures are often continuous or repetitive over periods of many years. The responses, if any, are likely to be nonspecific and often can be an increase in the frequency of a chronic disease that is also present in nonexposed populations. For example, any small increase in the incidence of heart disease or lung cancer attributable to a specific chemical exposure could be difficult to detect, because these diseases are present at high levels in nonexposed populations. In smokers, these conditions could be influenced more by cigarette exposure than by the chemical in question.

Increases in the incidence of diseases from low-level long-term exposure to environmental chemicals invariably occur among a very small percentage of the population and can only be determined by large-scale epidemiological studies (epidemiology is the study of the distribution and frequency of diseases in a specific population) involving thousands of person-years of exposure. The only exceptions are chemicals that produce very rare disease conditions, where the clustering of a relatively few cases may be sufficient to identify the causative agent. Notable examples of such special conditions are the industrial cases of chronic berylliosis caused by the inhalation of beryllium-containing dusts, a rare type of liver cancer that resulted from the inhalation of vinyl chloride vapors, and pleural cancers that resulted from the inhalation of asbestos fibers. If these exposures had produced more commonly seen diseases, the specific materials might never have been implicated as causative agents.

Low-level chemical exposures may play contributory, rather than primary, roles in the causation of an increased disease incidence, or they may not express their effects without the co-action of other factors. For example, the excess incidence of lung cancer is very high in uranium miners and asbestos workers who smoke cigarettes but is only marginally elevated among nonsmoking workers with similar occupational exposures. For epidemiological studies to provide useful data, they must take appropriate account of smoking histories, age and sex distributions, socioeconomic levels, and other factors that affect mortality rates and disease incidence. In addition, environmental toxicants can exacerbate an underlying condition rather than be the sole cause of a disease. For example, ambient air pollutant exposure can be associated with increases in pediatric emergency department visit for asthma.

1.11.2.1 Mortality In industrialized societies, there is generally good reporting of mortality and age at death but, with few exceptions, quite poor reporting of cause of death. In studies that are designed to determine associations between exposures and mortality

rates, it is usually necessary to devote a major part of the effort to follow-up investigations of cause of death. The productivity of these follow-ups is often marginal, limiting the reliability of the overall study.

1.11.2.2 Morbidity Difficult as it may be to conduct good mortality studies, it is far more difficult, in most cases, to conduct studies involving other health effects. Although there is generally little significant variability in the definition of death, there is a great deal of variation in the diagnosis and reporting of many chronic diseases. There are variations between and within countries and states, and these are exacerbated by the differences in background and outlooks of the physicians making the individual diagnoses. Furthermore, some important chronic diseases cannot be definitively diagnosed *in vivo*.

Many epidemiological studies rely on standardized health status questionnaires, and the success of these studies depends heavily on the design and validation of the questionnaires. Of equal importance in many studies are the training and motivation of the persons administering the questionnaires. Similar considerations apply to the measurement of functional impairment. The selection of the measurements to be used is very important; those functions measured should be capable of providing an index of the severity of the disease. Equally important here are the skills of the technicians administering these tests and their maintenance and periodic recalibration of the equipment.

Some studies try to avoid bias from the administrators of the questionnaires and functional tests by having the selected population enter the desired information themselves. They may be asked to make appropriate notations in notebook diaries or to call a central station whenever they develop the symptoms of interest. Other investigations use nonsubjective indices such as hospital admissions, clinic visits, and industrial absenteeism as their indicators of the adverse health effects to be associated with the environmental variables.

1.12 EXPOSURE–RESPONSE RELATIONSHIPS

Exposure–response relationships can be developed from human experience, but there are many chemicals that are known to be toxic in animals for which the extent of human toxicity, if any, is unknown. To use animal bioassay data for the prediction of human responses to environmental exposures, it is necessary to make two major kinds of extrapolation. One is determine or estimate the relative responsiveness of humans and the animal species used in the bioassays. The other is to extrapolate from the observed effects resulting from relatively high administered doses to the much lower levels of effects still of concern at much lower levels of environmental exposure.

To deal with interspecies extrapolation, estimates are made on the basis of whatever is known about differences in uptake from environmental media, metabolic rates and pathways, retention times in target tissues, and tissue sensitivities. As uncertain as these extrapolations are, they are more straightforward than the low-dose extrapolation.

The goal of the dose–response assessment is to predict what response, if any, might occur, 10- to 1000-fold below the lowest dose tested in rodents (this is more representative of the range of doses to which humans are usually exposed). Because it would require the testing of thousands of animals to observe a response at low doses, mathematical models are used (Munro and Krewski, 1981). To appreciate the level of uncertainty in the dose extrapolation process and the typical regulatory use of low-dose models, it is useful to

discuss the dose–response curve. However, reliance on the results of only one mathematical model is a potential pitfall in the dose–response assessment.

There are at least six different modeling approaches that may need to be considered when estimating the risks at low doses. These models include the probit, multihit, multistage, Weibull, one-hit, and the Moolgavkar–Knudson–Venzon (MKV) biologically based approaches (Moolgavkar et al., 1988). Nearly all of them can yield results that are plausible. No single statistical model can be expected to predict accurately the low-dose response with greater certainty than another. As discussed by Paustenbach (1990), one possible way to resolve this problem is to present the best estimate of the risk from the two or three models that are considered equally reasonable along with the upper- and lower-bound estimates. An alternate approach is to identify a single value based on the "weight of evidence," as the EPA did for dioxin (U.S. EPA, 1988)

Low-dose models usually fit the rodent data in the dose region used in the animal tests. However, they often predict quite different results in the unobserved low-dose region (Fig. 1.15). The results of the most commonly used low-dose models usually vary in a predictable manner because the models are based on different mathematical equations for describing the chemical's likely behavior in the low-dose region.

In general, the scientific underpinnings of the dose–response models are based on the present understanding of the cancer process caused by exposure to ionizing radiation and genotoxic chemicals (NRC, 1980). Both types of agents may well have a linear, or a nearly linear, response in the low-dose region. However, promoters and cytotoxicants (e.g., nongenotoxicants) would be expected to be very nonlinear at low doses and may have a genuine or practical threshold (a dose below which no response would be present) (Butterworth and Slaga, 1987; Squire, 1987). Thus, the linearized multistage model may be inappropriate for dioxin, thyroid-type carcinogens, nitrolotriacetic acid, and, presumably, similar

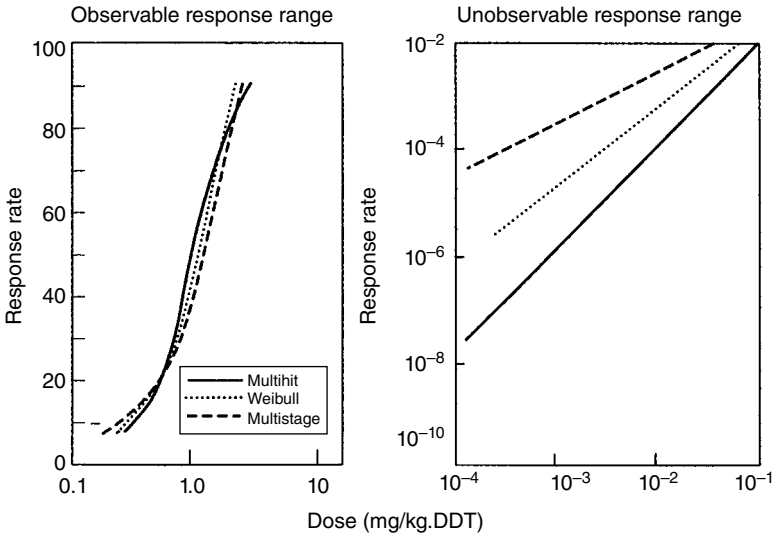


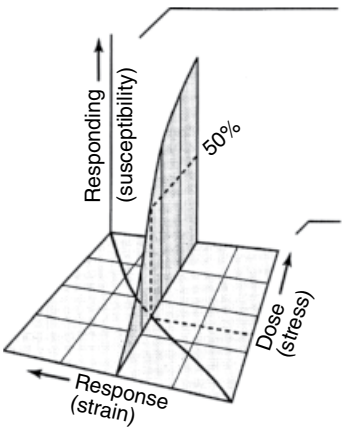
FIGURE 1.15 The fit of most dose–response models to data in the range tested in animal studies is generally similar. However, because of the differences in the assumptions on which the equations are based, the risk estimates at low doses can vary dramatically between different models. *Source*: From Paustenbach (1990).

nongenotoxic chemicals (Paynter et al., 1988; Andersen and Alden, 1989). For these types of chemicals, the MKV model, or one of the other biologically based models, could be more appropriate (Moolgavkar, 1978; Ellwein and Cohen, 1988).

1.12.1 Summary of Exposure- and Dose-Related Responses

Studies of the specific responses of biological systems to varying levels of exposure can provide a great deal of information on the nature of the responses, their underlying causes, and the possible consequences of various levels of exposure. However, it must be remembered that the data are most reliable only for the conditions of the test and for the levels of exposure that produced clear-cut responses.

Generally, in applying experimental data to low-level environmental exposure conditions, it is necessary to extrapolate to delivered doses that are orders of magnitude smaller than those are that produced the effects in the test system. Since the slope of the curve becomes increasingly uncertain the further one extends it beyond the range of experimental data, the extrapolated effects estimate may be in error by a very large factor. The basic dimensions of the dose–response relationship for populations were described by Hatch (1968), as illustrated in Fig. 1.16. Many factors affect each of the basic dimensions.



Population response scale

1. For test relationship
   Dose vs.% of test subjects responding, using sufficient numbers of appropriate subjects for valid statistical analysis
2. Application of results from one population (or species) to another
3. For practical dose–population–response relation
   Dose vs. probability of responding at different levels of effect for particular population at risk

Dose scale

1. For basic dose–response relation
   Quantitative scale of dose at critical site in body, expressed in terms of active form of stress agent at site
2. Intervening events between portal of entry into body and critical site, which mediate between external level of exposure and effective dose at critical site
3. Far practical dose–response relation
   Magnitude of exposure to external conditions that give rise to stress, measured in appropriate terms for translation into effective dose

Response scale

1. For basic dose–response relation
   Quantitative scale of response, expressed in terms of most basic event at critical site; response increments of equal value over entire scale
2. Consequent events, leading to ill health
3. For practical dose-response relation
   Magnitude of health risk (immediate or potential) to exposed individual

FIGURE 1.16 Dose (population)–response relationship with suggested distinction between basic (toxicological) and practical (health) scales on the three axes. The illustrative curve on the horizontal plane portrays the dose–response relationship for the middle (50%) of the exposed population; the curve on the vertical plane shows the percentages of population response of the indicated degree over the whole range of doses. The vertical line from the dose scale indicates the magnitude of dose needed to produce the indicated degree of response at the 50% population level. *Source*: From Hatch (1968).

1.12.1.1 Factors Affecting Dose The effective dose is the amount of toxicant reaching a critical site in the body. It is proportional to the concentrations available in the environment: in the air breathed, the water and food ingested, and so on. However, the uptake also depends on the route of entry into the body and the physical and chemical forms of the contaminant. For airborne contaminants, for example, the dose to the respiratory tract depends on whether they are present in a gaseous form or as an aerosol. For contaminants that are ingested, uptake depends on transport through the membranes lining the GI tract and, in turn, is dependent on aqueous and lipid solubility. For contaminants that penetrate membranes, reach the blood, and are transported systemically, subsequent retention in the body depends on their metabolism and toxicity in the various tissues in which they are deposited. In all of these factors, there are great variations within and between species and therefore great variations in effective dose for a given environmental level of contamination.

1.12.1.2 Factors Affecting Response The response of an organism to a given environmental exposure can also be quite variable. It can be influenced by age, sex, the level of activity at the time of exposure, metabolism, and the competence of the various defense mechanisms of the body. The competence of the body's defenses may, in turn, be influenced by the prior history of exposures to chemicals having similar effects, since those exposures may have reduced the reserve capacity of some important functions. The response may also depend on other environmental factors, such as heat stress and nutritional deficiencies. These factors must all be kept in mind in interpreting the outcomes of controlled exposures and epidemiological data and in extrapolating results to different species and across various age ranges, states of health, and so on.

1.12.1.3 Factors Affecting Individual Susceptibility The complete evaluation of the pathogenesis of human disease requires identification and assessment of the genetic, lifestyle, and environmental risk factors. Environmental factors are clearly critical to disease prevention because, at the societal level, they are determined by the most controllable processes. Thus, the overall purpose of environmental medicine is to improve our knowledge of the more commonly encountered environmental agents that present the greatest concern to human health.

In the past, diseases were categorized by the main etiological factor into either (a) genetic, (b) lifestyle-induced, or (c) environmental-induced disorders. Illustrative examples for each category include (a) alpha-1 antitrypsin [serine (or cysteine) proteinase inhibitor, clade A, member1] deficiency-induced chronic obstructive pulmonary disease, (b) cigarette-induced lung cancer, and (c) asbestos-induced mesothelioma, respectively. Environmental medicine almost exclusively focused on the latter category and has had its best impacts when armed with knowledge of quantifiable exposure (e.g., occupational diseases). Fortunately, additional cases of occupational diseases (e.g., mining-related coal workers pneumoconiosis or asbestosis) can be mitigated among the general population by improving work practices.

However, while categorizations based on etiological factors have allowed the development of therapeutic strategies to treat disease, they also may have limited past attempts to prevent disease. Disease prevention has a greater ability than most common treatment modalities to reduce incidence and to extend life expectancy. In the illustrative examples above, preventative approaches would include (a) genetic counseling and possibly gene-modification therapy, (b) public health education and smoking cessation pharmaceuticals, and (c) reduction or elimination (banning widespread usage) of asbestos

exposure. Each of these approaches has had tremendous value in focusing efforts on disease causalities and thus has had dramatic impacts on disease prevention.

Although the application of these simple approaches to disease prevention has been partially successful, further success will require more sophisticated approaches based on a deeper understanding of disease pathogenesis. With the widespread use of genetic screening, it soon became apparent that homozygotic recessive carriers (individuals inheriting both copies of the disease susceptibility alleles) often did not developed major signs and symptoms of the "genetic" disease. For example, homozygotic twins do not have 100% concordance in disease outcomes. Similarly, disease discordance is noted in lifestyle-induced diseases. For example, among individuals with exposures exceeding 50 pack-years, only four out of five cigarette smokers develop a tobacco-related disease, and these diseases vary in the population (i.e., multisite cancer, cardiovascular, and respiratory disease). Likewise, although diseases such as asbestosis, lung cancer, and mesothelioma are enriched in populations with excessive asbestos exposure, the incidence of affected workers given equivalent exposures is not 100%.

What can explain a lack of penetrance (affected individuals/genotype positive individuals) in equally susceptible, equally risky, equally exposed populations? It is the interactions of additional factors within and among each etiological category. Common pathological conditions are complex and involve multiple rather than a single gene(s). Single gene diseases with strongly expressivity typically appear early in life but due to a lack of complete penetrance affect only a few members of the population. Alternatively, most diseases involve multiple gene–gene interactions and are present in a large percentage of the population. Genetic polymorphisms that are strongly fixed in the genome probably did not arise from modern lifestyle or environmental factors (e.g., coal mining), but from ancient lifestyle factors or infectious agents.

Another reason why most common diseases are complex is that the selective advantage to a population that stabilizes a genetic polymorphism is likely to be dependent on multiple alterations in multiple genes. Many polymorphisms also may have been acquired from phylogenetic ancestry (which are likely to be greater than those that uniquely arise within a given species). Thus, the mechanisms by which bacteria combat virus, or how drosophila resist bacteria (Toll-like receptors), or how mice resist influenza may be conserved throughout species. This situation is further complicated by the fact that genes that impart sensitivity may also impart resistance to another disease. For example, the protection from tuberculosis may be advantageous to the population but may impart increased susceptibility to chronic inflammatory diseases, like arthritis, to a portion of the population.

From a solely genetic standpoint it would be advantageous if multiple genes contribute to a survival phenotype from a severe disease entity [i.e., the fully developed phenotype being dependent on gene–gene interactions (epistasis)]. Phenotypes with complex gene interaction require only a few members of an outbred population (humans) to carry the exact set of all the resistant alleles. Phenotypes dependent upon multiple genes thereby reduce the negative consequences of the combinatorial effects of multiple alleles to only a few individuals. Several other members of a population could inherit in partial combinations that would have little observable phenotypic expression. Thus, while only a few members of the population might survive an infectious epidemic, many members of population share the risky alleles.

With the initial assembling of the entire human genome by the Human Genome Project, substantial research has been invested in the identification of all disease causing genetic polymorphisms. However, individual susceptibility to common diseases is not solely controlled by multiple gene–gene interactions. Rather disease penetrance is also

influenced by multiple gene–lifestyle, gene–environment, lifestyle–environmental, and gene–lifestyle–environmental factors. Using our illustrative disease example, clear synergistic interactions occur among individuals who (a) have alpha-1 antitrypsin deficiency and smoke cigarettes (gene–lifestyle interaction), (b) have glutathione *S*-transferase pi 1 deficiency and are exposed to environmental tobacco smoke or to excessive air pollution (ozone and particulate matter) (gene–environment interaction), or (c) smoke and work with asbestos or radon (lifestyle–environment interactions).

1.12.2 Genomic Approaches to Understanding Gene–Lifestyle–Environmental Factors in Complex Disease Pathogenesis

Environmental health sciences have developed a wonderful cadre of tools to obtain global (nearly complete) evaluations of the genetic variants, transcriptional profile, protein usage and activation state, and the metabolic capability of individuals and populations following environmental stress. Using high-throughput microarray or microfluidic sequencing systems, genomics seeks to evaluate the entire genetic makeup of an individual and therefore identify candidate gene suspected to have a role in determining susceptibility. Genomics is based on known biological functional, cellular location, or pathophysiological roles of a gene and seeks to identify the allelic variants that associate with increased risk.

Identification of disease-associated region in DNA has been advancing rapidly in recent years with improvements and reduce of cost of DNA sequencing. Whole genome and whole exome sequencing can now be performed on large cohorts. In addition, the mapping of over 150,000 single nucleotide polymorphisms (SNPs) throughout the genome allows nearly complete coverage of all human variability and through linkage disequilibrium identification of small chromosomal region (areas of a few tens of thousands base pairs). Particularly attention is focused on nonsynonymous SNPs that result in alteration of RNA recognition codons and lead to amino acid changes in the predicted protein. Because regions of chromosomes are often inherited together, the number of independently inherited SNPs is reduced. Thus, the reduction to informative differences (tag-SNPs) makes these analyses even more powerful, and essentially entire genome coverage is possible. Supportive of genome-wide linkage analysis is transcriptomics, proteomics, and metabonomics.

Transcriptomics studies large sets of messenger ribonucleic acid (mRNA) molecules or transcripts produced by cells in culture (revealing cell-type specificity), single cells isolated from tissue, tissues, or a whole organism. The transcriptome, unlike the genome, which is fixed (excluding acquired mutations), can be altered by the environment and reflects the cell's attempt to acclimate to adverse conditions. The supportive high-throughput technology includes RNASeq or DNA microarrays that typically monitor steady-state transcript levels of 20,000 genes. This includes essentially all known genes and genes yet to be fully understood and annotated (e.g., predicted gene products and expressed sequence tags). Key targets identified are then confirmed by additional approaches including quantitative reverse transcription polymerase chain reaction, ribonuclease protection assay, and Northern blot.

Like transcriptomics, the proteomics is a global approach to evaluate altered protein usage and activation state induced by environmental signaling. In recent year, rather than be qualitative, proteomics has become quantitative and can provide novel insight in cellular responses to environmental perturbations. Proteomics may be closer than transcriptomics in revealing cellular function in that protein state varies to carry out various functions. For example, proteomics can measure many signaling peptides (e.g., kinases) that generate

amplifying cascades that alter cell functions including motility, transcription, cell–cell communication, proliferation, immunity, and apoptosis. The proteome includes nascent propeptides, mature inactive peptides, activated peptides, and peptide marked secretion or degradation. While the number of genes and possible transcripts are estimated to be 19,000–20,000 in humans, the proteome may have over 100,000 members, given that a single gene may produce as many as 100 protein variants. In addition, proteomics focuses on protein–protein and protein–macromolecular interactions, which adds to its functional significance. High-throughput technologies supporting proteomics include gas (gas chromatography), fluidic (high-pressure liquid chromatography), or gel (electrophoresis) separation and large-scale mass spectrometric protein identification. Key targets identified are then confirmed by immunological approaches including Western blot, antibody arrays, radioimmunoassay, or enzyme-linked immunosorbent assays (ELISA).

Also under rapid development, metabonomics (or metabolomics) is the global approach to the assessment of the metabolic response of living systems to environmental stimuli. Typically, the variety of small molecule metabolites generated by a living system is relatively small (<5000 compounds) when compared with the genome, transcriptome, or proteome. Metabonomic profiles reflect the nutritional status of the organism, and thus this approach is useful in determining lifestyle–environmental interactions. In addition, the metabolic consequences of genetic manipulation can be assessed using this approach (bypassing assessment of the transcriptome or proteome). Also of note is that metabonomics measures small molecules produced by the host microbiome. Each person is inhabited and surrounded by a unique signature of microbes. Diminished diversity of microorganisms is associated with diseases including allergy, diabetes, obesity, arthritis, inflammatory bowel diseases, and even neuropsychiatric disorders. Thus, an interaction of microorganisms with the host immune system is required for a healthy body. Exposure to microorganisms and environmental toxicants educates the immune system, induces adaptive immunity, and initiates memory B and T cells that are essential to combat various pathogens.

Metabonomics focuses on body fluids (serum or urine) and minimally invasive sampling (salvia or breath analysis). Although metabolic profiles may be shifted due to altered pathologies, metabolic capacities often can be predicted a very low doses of known toxicants (below the levels inducing any adverse response) or by surrogate that is handled much like a toxicant (e.g., caffeine). Supportive technologies include mass spectrometry and nuclear magnetic resonance (NMR). NMR is commonly preferred because this method does not require separation, can be performed with small volumes (0.01–0.1 mL), and does not destroy the sample.

1.13 STUDY OPTIONS FOR HEALTH EFFECTS STUDIES

In the discussion of the health effects in the chapters that follow, it is important to appreciate the strengths and limitations of the various kinds of studies that generated the data.

1.13.1 Controlled Human Exposures

For O_3, SO_2, concentrated $PM_{2.5}$, and CO, selected human volunteers have been exposed to the pollutant in purified lab air for specific time intervals ranging from minutes to 8 h. Most studies involve a series random order exposures to one or more concentrations as well as control exposure to purified air. Many of them involved prescribed periods and intensities

of exercise during the exposure interval. The most commonly measured pulmonary effects were changes in forced expiratory flow rates and volumes and/or changes in airway resistance and compliance. In addition, research bronchoscopy procedure has been safely performed that includes oral sampling, bronchoalveolar lavage, and endobronchial biopsy and brushing.

A broad variety of other pulmonary function tests require the inhalation of special breathing mixtures and hence more elaborate controls and protocol reviews. These include the inhalation of (1) a single breath of pure oxygen for the nitrogen washout test of small airway function (Buist and Ross, 1973), (2) 0.3% CO to determine diffusing capacity at the alveolo-capillary membrane (Crapo and Gardner, 1987), and (3) a low-density inert gas, such as helium (He), or a high-density gas, such as sulfur hexafluoride (SF_6), to measure inhomogeneity in flow distribution (Scott and Van Liew, 1983). Large-scale spatial inhomogeneity in ventilation can be detected using radioactive xenon (Xe) and external γ-emission-imaging equipment such as the Anger camera (Robertson et al., 1969).

Functional tests made before and after administration of bronchoactive agents can also be of diagnostic value. These can include bronchodilators such as isoproterenol, epinephrine, and atropine to measure reversible bronchoconstriction. They also include bronchoconstrictors, such as histamine, carbachol, methacholine, cold air, and SO_2, to detect airway hyperresponsiveness.

Other tests of demonstrated utility and/or with potential for supplying important diagnostic information can also be applied. The permeability of the respiratory epithelium can be determined from the externally measured rate of clearance from the lung of γ-emitting [^{99m}Tc] tagged diethylenetriaminepentaacetate (DTPA), inhaled as a droplet aerosol (Oberdorster et al., 1986).

Inert, insoluble, nonhygroscopic γ-tagged aerosols can be used to measure the regional deposition and clearance rates for inhaled particles. Thoracic retention of such aerosols after 1 day is considered to represent deposition in the nonciliated alveolar lung spaces, while the difference between the retention measured immediately after the particle inhalation and that at 1 day represents the aerosol that deposited on the conductive airways of the tracheobronchial tree (Lippmann, 1977). By appropriate control of particle size and respiratory parameters, the deposition efficiency data can be used to characterize airway obstruction (Chan and Lippmann, 1980). The rate and pattern of mucociliary particle clearance can be determined from serial measurements of thoracic retention during the first day, and the much slower rate of particle clearance from the gas exchange region can be determined from serial retention measurements made after the first day (Albert et al., 1969).

In other studies, cardiac function measurements, such as heart rate and heart rate variability, have been made following exposure to O_3 (Brook et al., 2002; Gong et al., 2003; Urch et al., 2004), SO_2 (Tunnicliffe et al., 2001), and concentrated $PM_{2.5}$ (Brook et al., 2002; Devlin et al., 2003; Urch et al., 2004).

The advantages of controlled human exposure studies are (1) the ability to carefully select and carefully characterize the subjects, whether they be healthy controls, atopics, asthmatics, smokers, and so on; (2) the willingness and ability of most volunteer subjects to perform various levels and durations of exercise during the exposures; (3) the ability to deliver and monitor the preselected challenge atmospheres during the exposure; (4) the ability of the subjects to reproducibly perform respiratory maneuvers required for some functional assays affected by the exposures and to provide information on mild symptomatic responses; and (5) the avoidance of the need to make interspecies extrapolations in evaluating human exposure–response relationships.

The limitations of controlled human exposures are that (1) the number of subjects that can be studied is limited by the generally large costs of performing the studies and/or by the availability of sufficient numbers of subjects with the desired characteristic, (2) the numbers of repetitive challenges and assays are limited by subject tolerance and cooperation, and (3) ethical constraints limit the challenges and effects assays that can be performed. In effect, challenges are limited to those producing only transient functional changes.

In summary, controlled human exposure studies are most useful for studying the nature and extent of transient functional changes resulting from one or a few brief controlled exposures. They can provide information on chronic pollutant effects only to the extent that prior exposures affect the transient response to single exposure challenges. Furthermore, interpretation of the results of such tests is limited by our generally inadequate ability to characterize the nature and/or magnitude of the prior chronic exposures.

1.13.2 Natural Human Exposures

Studies of the responses of natural populations to acute exposures to air pollutants have been particularly informative (Lippmann, 1989; Spektor et al., 1991; Thurston et al., 1997). Studying natural populations for evidence of acute health effects associated with exposures to ambient air pollutant is a challenging task. Among the more difficult challenges are (1) identifying an accessible population at risk whose relevant exposures can be defined and adequately characterized, (2) specifying measurable indices of responses that may be expected to occur as a result of the exposures of interest, (3) collecting an adequate amount of suitable quality-assured data on exposure and responses at times when exposures of magnitudes sufficient to elicit measurable responses actually occur, and (4) collecting sufficient data on identifiable host characteristics and environmental exposures to other agents that may influence the response variables and confound any of the hypothesized pollutant exposure–response relationships that may be present. In addition, one must also account for the usual operational problems encountered in performing population studies, especially studies in the field, such as maintaining (1) the motivation and skills of the field personnel for collecting reliable data, (2) the cooperation of the subjects in producing reliable data, and (3) the access to sufficient numbers of subjects with the preselected characteristics in each category as may be needed.

The basic design premise in field studies involving air pollutant exposures is to maximize the signal-to-noise ratio for the pollutant exposure versus response relationships. The noise on the response side of the relationships has been the focus of much work by others, and guidance on these aspects is available from the American Thoracic Society (1985). Focus is also needed on the reduction of the noise in the exposure variables. For example, the summer pollution haze is regional in scale and enriched in secondary air pollutants such as O_3 and H_2SO_4, both of which form gradually during daylight hours in air masses containing diluted primary pollutants transported over long distances from industrial, power plant, and motor vehicle sources, especially SO_2, NO_2, and hydrocarbons.

For the NYU field studies (Lippmann et al., 1983; Lioy et al., 1985; Spektor et al., 1988a, 1991; Thurston et al., 1997), populations of children attending summer camp programs were selected for main reasons: (1) cigarette smoking and occupational exposure to lung irritants would not be confounding factors, (2) the program of camp activities insured that they would be outdoors and physically active during the daytime periods when O_3 and H_2SO_4 exposures are highest, and (3) the cooperation of the camp staff provided effective

access to the children on a daily basis for the administration of functional tests and symptom questionnaires. Concentrations of O_3 and H_2SO_4 are usually higher outdoors than indoors. In addition, as regional-scale secondary pollutants, their concentrations do not vary greatly from site to site within the camp's activity areas or from those measured at nearby samplers or monitors. In addition, activity level among the children in the camp program was consistent.

The 1985 summer study (Spektor et al., 1988b) on the effects of the summer haze pollutants on respiratory function in healthy nonsmoking adults engaged in a regular program of outdoor exercise had a similar absence of confounding exposure factors as well as similar exposures to the ambient secondary air pollutants. Each of the adult volunteers maintained a constant daily level and duration of exercise, but they differed widely from one to another in these important variables. This not only increased the variability of the response among the population but also provided a means of studying the influence of these variables on the responses.

In summary, natural human exposure studies are most useful for studying the magnitude and extent of the acute responses to naturally occurring pollutants among people engaged in normal outdoor recreational activities. They provide little information on the possible influence of prior chronic exposures on acute responses to the exposure of the day or immediately preceding days. In addition, since the ambient mixture contains varying amounts of a variety of pollutants, it may sometimes be difficult to apportion the responses to one or more of the pollutants or to other uncontrolled variables such as temperature, humidity, and each individual's precise level of exercise or ventilation.

1.13.3 Population-Based Studies of Chronic Health Effects of Air Pollution

Since neither controlled human exposure studies in the laboratory nor natural human exposure studies in the field can provide any direct information on chronic effects of prolonged human exposures to air pollutants, the only way to get such information is to use the conventional epidemiological approach of comparing data on (1) reductions in lifespan and function and (2) increases in symptom frequency, lost activity days, hospital admissions, clinic visits, medical diagnoses, and so on in relation to estimates of chronic exposure intensity. Many confounding factors affect the indices of concern in such studies. The characteristics of the populations under study are highly variable in terms of age, sex, smoking history, cohabitation with smokers, health status, disease history, occupational exposures, hobby activities that generate air pollutants, the use of unvented stoves and heaters at home, and so on. Also, their exposures to outdoor pollutants are difficult to quantitate and are influenced by their proximity to the monitor that provides their exposure index, the time they spend outdoors, and whether this includes hours when the pollutant is high as well as the amount and duration of vigorous exercise during periods of high exposure.

Because of the large number of possible confounders and the difficulty of properly classifying exposures, very large populations must be studied to find significant associations between exposures and effects. Any significant effects that could be attributed to air pollution would tend to be underestimated because of the influence of the confounders. Alternatively, they could be spurious if the effects are really caused by variables that are colinear with the pollutant being studied.

In summary, epidemiological studies offer the prospect of establishing chronic health effects of long-term air pollution exposure in relevant populations and offer the possibility that the analyses can show the influence of other environmental factors on responses to

exposure. On the contrary, the strengths of any of the associations may be difficult to establish because of the complications introduced by uncontrolled cofactors that may confound or obscure the underlying causal factors.

1.13.4 Controlled Exposures of Laboratory Animals

The most convenient and efficient way to study mechanisms and patterns of response to pollutants and of the influence of other pollutants and stresses on these responses is by controlled exposures of laboratory animals. One can study the transient functional responses to acute exposures and establish the differences in response among different animal species and between them and humans similarly exposed. One can also look for responses that require highly invasive procedures or serial sacrifice and gain information that cannot be obtained from studies on human volunteers. One can expose the animals to a single pollutant or to a complex mixture as found in the environment or from a specific source. Finally, one can use long-term exposure protocols to study both short-term and cumulative responses and the pathogenesis of chronic disease in animals. Other advantages of studies on animals are the ability to examine the presence of and basis for variations in response that are related to age, sex, species, strain, genetic variations, nutrition, the presence of other pollutants, and so on. As in controlled human exposure studies, the concentrations and duration of the exposure can be tightly controlled, as can the presence or absence of other pollutants and environmental variables. Another important advantage of controlled animal studies is that relatively large numbers of individuals can be simultaneously exposed, creating the possibility of detecting responses that only affect a limited fraction of the population.

Among the significant limitations to the use of exposure–response data from animal studies in human risk assessments is our quite limited ability to interpret the animal responses in relation to likely responses in humans who might be exposed to the same or lower levels. Anatomical and physiologic differences between laboratory animals and human can also limit interpretations and must be accounted for in determining regional dose and the subsequent response to a toxicant. Controlled chronic exposure protocols can be very labor intensive and expensive, which tends to limit the number of variables that can effectively be examined in any given study.

1.13.5 Controlled Exposures *In Vitro*

For studies focused on the biochemical mechanisms of epithelial cells' responses to O_3, cells can be harvested from humans or animals and exposed to O_3 *in vitro*. Techniques have been developed for reasonably realistic O_3 exposures to cells and cell cultures *in vitro* (Valentine, 1985) for characterizing the release of eicosanoids from such cells (Leikauf et al., 1988) and for examining cell function (Driscoll et al., 1987). The main advantage of *in vitro* studies is their efficiency and relatively low cost. Interspecies comparisons of cellular response can often be made, and relatively few animals can provide much study material. In addition, because animal cells can be studied *in vitro*, the results can be compared with the *in vivo* results of studies performed on same species. However, our ability to interpret the results of *in vitro* assays in relation to likely effects in humans *in vivo* is often limited, even when the studies are done with human cells. The cellular response *in vitro* may differ from that of the same cells *in vivo*, and the *in vivo* controls on cellular metabolism and function, which may play a significant role in the overall response, are absent. In addition, *in vitro* studies can be lacking in cell–cell and cell–extracellular matrix interactions that modify responses *in vivo*.

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2

PERSPECTIVES ON INDIVIDUAL AND COMMUNITY RISK

JAMES P. FABISIAK

PREFACE

Previous editions of this textbook included versions of this chapter authored by Arthur C. Upton, MD (1923–2015) formerly professor of environmental medicine at the University of Medicine and Dentistry of New Jersey, chairman of the Department of Environmental Medicine at NYU School of Medicine, and director of the National Cancer Institute. As a renowned radiation biologist, he championed the incorporation of preventive oncology into the National Cancer Program and developed methods for multi-stakeholder involvement in risk assessment strategies at nuclear weapons sites. The present chapter here follows much of the same format and scope of that excellently crafted original text. Most of the original illustrations and tables are retained in that they serve as noteworthy resources that stand tall in the face of time. I have merely tried to put Dr. Upton's original thoughts and perspectives into my own voice and, when appropriate, attempted to reinforce the same points with more contemporary examples. I am grateful for having such a mindful template to follow in my preparation of this chapter, and any resemblance to the previous versions should be taken as nothing more than a sincere compliment to the original and outstanding work.

Throughout history mankind has always faced various dangers and sought ways to mitigate those hazards, often through technological innovation. The development of communal agriculture meant fewer encounters with large and ferocious beasts while hunting and greater constancy in food availability. The development of antibiotics dramatically lessened the risk of dying from bacterial infection. Oftentimes, these technological innovations themselves carried new risks. While application of pesticides and insecticides increases agricultural productivity and reduces vector-borne disease, some of those chemicals, such as DDT, are known to adversely affect reproduction in wildlife and perhaps even humans. Some highly sensitive individuals developed life-threatening allergic reactions to the antibiotic penicillin. The greatly increased life expectancy now enjoyed by populations

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in the industrialized world attests to the success with which modern civilization has been able to reduce certain risks to human health and safety.

This chapter discusses environmental risks to human health from two standpoints: (1) the risk to the individual and (2) the risk to the community. Considered in this context are scientific bases for assessing such risks and options for reducing risks at the individual and community levels. The relative magnitudes of different environmental risks, as evaluated by knowledgeable experts, along with the contrasting perspectives, in which risks may be perceived by different members of the public, are also discussed. Difficulties in risk communication have challenged societal efforts to protect health and the environment.

2.1 NATURE OF RISK

2.1.1 Probability of Effect

Risk is commonly defined as "possibility of injury or loss." A "risk" can be synonymous with "threat," "danger," "peril," or "liability." The term environmental risk to health is taken, herein, to mean the probability of an adverse effect on human health resulting from exposure to a particular environmental agent or combination of agents. For the purposes of this discussion, we consider the agent to represent some chemical to which people are exposed by air they breathe, food and water they consume, or as a contaminant or product they apply to their skin. One also needs to consider that there is a spectrum of adverse effects possible in response to an environmental chemical exposure. The most serious effects include cancer, coma, organ failure, and death. Examples of other, less severe, responses but nonetheless considered adverse include responses such as headache, skin irritation, fatigue, and loss of appetite. Some effects occur immediately or very soon after exposure, while others are delayed and only appear months or even years later. Local effects, such as the skin rash from poison ivy, are those primarily restricted to the initial site of exposure, while systemic effects, such as neurodevelopmental delay in children following lead (Pb) exposure, require absorption of the offending agent into the circulation and ultimate delivery to a specific target organ.

People often confuse the term *risk* of disease with *rate* of disease. Actual *rates* of disease are something that are or can be directly measured or observed such as by enumerating the number of coronary heart disease deaths in a specific county, whereas *risks* are estimated quantities or values that are based on accumulated scientific data drawn from other scenarios and rely on a variety of assumptions. Risks often exist at levels too small to be measured accurately without extremely large populations, very long time frames, and ways to account for large variations in individual exposure potential.

Once estimated, risks may be expressed in various ways, depending on the context in which they are considered: (1) average annual risk per individual, (2) average lifetime risk per individual, (3) expected number of individuals affected within a given population, or (4) average loss of life expectancy in affected individuals, to name a few. Apart from risks to human health and safety *per se*, such risks may also be expressed in terms of their economic impacts on the affected individuals, their families, their associates, and their communities, as noted below.

In some situations, risk can be expressed in terms of absolute risk, such as numbers of arsenic (As)-induced cancers from drinking contaminated water or number of premature cardiovascular deaths, per year, due to air pollutions. This is most useful when trying to ascertain the overall magnitude of disease burden on society arising from specific agents and

TABLE 2.1 Different Ways in Which the Carcinogenic Effects of 1 Sv Acute Whole Body Ionizing Radiation May Be Expressed in the Individual and in the Community

	Type of Cancer	
	Fatal Leukemia[a]	Other Fatal Cancer[a]
Risk to a population of 100,000 persons		
Annual number of excess deaths	9	70
Lifetime number of excess deaths	600	5,000
Cumulative person-year of life lost	12,000	75,000
Risk to the individual		
Increase in relative risk (%)	100	25
Excess annual risk of death ($\times 10^{-4}$)	1	7
Excess lifetime risk of death ($\times 10^{-3}$)	6	12
Attributable risk (%)	50	20
Years of life lost per attributable death	20	15
Years of life lost per exposed person	0.3	0.8

Source: UNSCEAR (2000) and NAS (2005).
[a]Values rounded.

exposures. The term relative risks (RRs) are useful in situations attempting to describe the relative contribution of specific defined environmental factors to multifactorial and common diseases within the population, when comparing an exposed and unexposed population or when comparing multiple risks to each other. For example, occupational exposure to benzene in excess of 40–100 ppm-years entails an RR of 1.9 of developing acute myelogenous leukemia, which translates to a 90% increase in the risk of leukemia compared with the unexposed population (Khalade et al., 2010). Depending on the baseline frequency, or background rate, a small increase in RR may translate to a large increase in the number of individuals affected. Conversely, merely a few additional cases of an otherwise rare disorder may result in a large increase in the RR, and thus a high attributable risk (i.e., risk from benzene relative to all risk factors), in the disorder. Table 2.1 shows a variety of the ways one might express the degree of risk from a single acute dose of radiation exposure.

2.1.2 Severity of Effect

The overall magnitude or importance of any risk is a complex function of both the severity and frequency of the effect in question. As mentioned above, adverse health effects cover a wide range of severity from severe (death, coma), moderate (nausea, vomiting), and even asymptomatic (mild changes in blood pressure). Moreover, the degree to which sublethal effects are reversible, progressive, painful, and disfiguring also contribute to the risk's relative importance. In addition, the frequency of the adverse event within the population also needs to be taken into account when prioritizing risks for mitigation strategies. Regulating a chemical contaminant with potential to cause two to five excess cancers per million people may take a back seat to controlling a water contaminant that produces gastrointestinal upset in nearly all of those exposed. Risks to children and pregnant woman often assume a higher priority to those of similar magnitude within the general population. In the broadest context, the measures of severity not only go beyond impacts on health *per se* but also relate to various esthetic, psychosocial, ethical, and economic factors (Hammond and Coppock, 1990; Arrow et al., 1996).

2.1.3 Psychosocial and Cultural Factors Influencing the Perception of Risk

The severity of risk perceived by the general public is frequently disparate in magnitude from that assessed in the technical estimation of risk by scientists. Smokers will willingly accept the approximate 1-in-a-1000 chance of lung cancer in their voluntary choice to smoke but would almost certainly find that same risk via an exposure to a carcinogenic dust like asbestos fibers unacceptable. Words like "natural" and "synthetic" are unfairly used to categorize something as safe or hazardous, respectively. Therefore, in addition to objective measures like frequency and severity, there are also many subjective and qualitative descriptors of risk as well, such as those listed in Table 2.2 (Raynor and Cantor, 1987; Fischoff et al., 1997; Omenn and Faustman, 1997).

Nonscientists often not only fail to understand the technical basis for evaluating a given risk but actually also distrust and reject it (Plough and Krimsky, 1987; Fischoff et al., 1997). Some have suggested that a more holistic definition of environmental risk should be expanded to include those subjective public perception or "outrage" factors, as well as the technical risk assessment estimates themselves (Fig. 2.1). The importance of such

TABLE 2.2 Psychosocial and Cultural Characteristics Affecting the Perception of a Risk

Characteristics of a Risk that Increase Its Acceptability	Characteristics of a Risk that Decrease Its Acceptability
Voluntary	Involuntary
Familiar	Unfamiliar
Immediate impact	Remote impact
Detectable by individual	Undetectable by individual
Controllable by individual	Uncontrollable by individual
Fair	Unfair
Noncatastrophic	Catastrophic
Well understood	Poorly understood
Natural	Artificial
Trusted source	Untrusted source
Visible benefits	No visible benefits
Well defined	Poorly defined
Predictable	Unpredictable

Source: Adapted from Slovic et al. (1979) and Plough and Krimsky (1987).

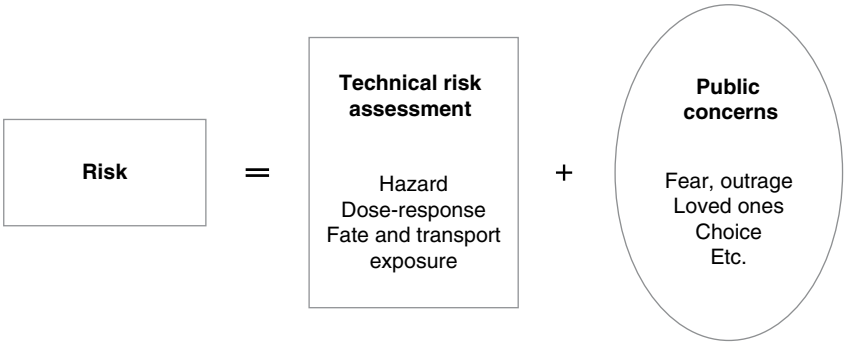

FIGURE 2.1 Holistic definition of risk. *Source*: Adapted from Allen (1992) and Sandman (1985).

TABLE 2.3 Comparison of "Perceived" to "Real" Risks Associated with Widespread Activities and Technologies

Activity or Technology	Technical Estimates (deaths/year)[a]	Geometric Mean Fatality Estimates, Average Year	
		LOWV[b]	Students[c]
Smoking	150,000	6,900	2,400
Alcoholic beverages	100,000	12,000	2,600
Motor vehicles	50,000	28,000	10,500
Handguns	17,000	3,000	1,900
Electric power	14,000	660	500
Motorcycles	3,000	1,600	1,600
Swimming	3,000	930	370
Surgery	2,800	2,500	900
X-rays	2,300	90	40
Railroads	1,930	190	210
General (private) aviation	1,300	550	650
Large construction	1,000	400	370
Bicycles	1,000	910	420
Hunting	800	380	410
Home appliances	200	200	390
Fire fighting	195	220	390
Police work	160	460	390
Contraceptives	150	180	120
Commercial aviation	130	280	650
Nuclear power	100[d]	20	27
Mountain climbing	30	50	70
Power mowers	24	40	33
School and college football	23	39	40
Skiing	18	55	72
Vaccinations	10	65	52

Source: Slovic et al. (1979).
[a] Based on assessments by technical experts.
[b] League of women voters.
[c] College students.
[d] Geometric mean of estimates, which ranged from 16 to 600 per year.

nontechnical factors is illustrated by the marked degree to which public perceptions of a given risk can be different from those of more informed experts (Table 2.3). The relatively recent Deepwater Horizon (DWH) Gulf oil spill serves as a case in point. The novel and unprecedented use of oil dispersants in this environment caused much fear and apprehension among the general public, fishermen, and cleanup workers largely because the populace was unfamiliar with dispersant application. In contrast, small oil spills and extremes in temperature are not uncommon in this environment (White, 2011). While some cleanup personal reported an increase in relatively minor symptoms associated with dispersant use (McGowan et al., 2017), the petroleum hydrocarbons, heat, and psychosocial stress remained priorities for risk assessment professionals (Fabisiak and Goldstein, 2011; Gohlke et al., 2011; Goldstein et al., 2011; Dickey and Huettel, 2016).

2.2 IDENTIFICATION AND QUANTIFICATION OF RISKS

Environmental risk assessment for human health involves a relatively defined sequence of interrelated steps (NAS, 1983; Omenn and Faustman, 1997). This process first begins with identification of postulated specific causative agents or exposure scenarios. Preexisting knowledge regarding disease or adverse effect association with the hazardous agent including quantitative dose–response relationships, along with exposure estimates within specific populations of interest, are then systematically integrated to produce estimations of the severity of the effects, such as number of persons affected (Fig. 2.2).

2.2.1 Hazard Identification

In its most simple description, the first step in risk assessment, hazard identification, is determining whether a specific agent, substance, or mixture possesses the potential to cause a defined human health effect. In most cases it likely involves prioritizing those multiple hazards among the combination to which the subject population is exposed in terms of severity and magnitude of risk regardless of the level of exposure. Also important is a description of what those most relevant toxicities are in terms of target organ systems and symptomatology. One of the first decisions to be made is often whether the agent in question is a carcinogen or produces some other noncancer endpoint, since this dichotomy produces differences in how risks are evaluated and described (U.S. EPA, 2002, 2005a) although attempts are being made to harmonize the process. Classically, the hazard identification process has relied upon the historical clinical and epidemiological evidence from human population studies. For most environmental agents of interest, however, data describing toxicity to humans is limited, at best, or unavailable at worst (NAS, 1989, 1994). Moreover, the concept of prevention is foundational to public health practice; hence risk

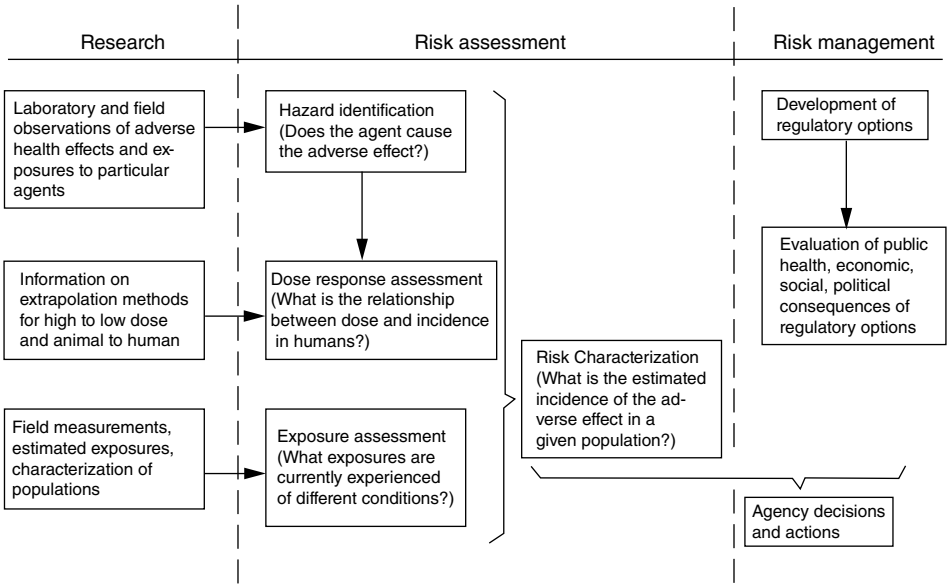

FIGURE 2.2 Steps in the risk assessment process and how it bridges research and risk management.

assessment should strive to prevent potentially adverse human exposures from occurring in the first place. Therefore, hazard evaluation must also depend on other approaches, including short- and long-term animal toxicity studies, *in vitro* bioassays, and systematic analysis of pertinent molecular structure–activity relationships (e.g., Tennant et al., 1987; Ashby and Tennant, 1988; IC-PEMC, 1988; Omenn and Faustman, 1997). While principles and methodology have been proposed using these alternative approaches to predict human toxicity, the current chemical universe, as well as the gamut of possible toxic reactions, is large and diverse. Moreover, uncertainties regarding the extrapolation of animal data to human responses also limit these approaches (e.g., Lave et al., 1988; NAS, 1994; Bailey et al., 2014). For most of the many thousands of chemicals in commercial production, insufficient toxicological data are available to adequately evaluate their toxic potential (NAS, 1984, 1994; U.S. EPA, 1987, 1990). In summary, hazard identification collectively evaluates all the existing scientific studies and information that specifically link an environmental agent to an adverse health effect and chooses those that best meet a set of standardized criteria for judging causality (Hill, 1965; Philips and Goodman, 2004).

2.2.2 Dose–Response Analysis

Based on Paracelsus's dogma of "Dose Makes the Poison," risk assessment requires the second step, dose–response analysis, as a way to quantify the relationship between the magnitude of exposure to the agent of interest and its likelihood of untoward health effects. In other words, it provides a standardized benchmark with which to then compare the myriad of exposure scenarios encountered in practice. Much of the time the same literature sources used in the hazard identification step will also contain useful dose–response information if performed in a comprehensive way. The sources of hazard and dose–response information are, however, frequently drawn from occupational settings or high-dose short-term animal studies that entail higher exposure levels than encountered in most ambient settings. Therefore, derivation of the desired risk estimates often requires the use of dose–response models of uncertain validity when extrapolated over a broad range of much higher doses often applied in nonhuman models (Omenn and Faustman, 1997).

A threshold model represents one typical dose–response model often used to establish "safe" vs. "hazardous" exposures to a given hazard especially for noncancer endpoints. Basically, a threshold dose (or concentration) is first determined from the available dose–response data [lowest observable adverse effect level (LOAEL); no observable adverse effect level (NOAEL)] and then adjusted downward in a systematic fashion that reflects varying levels of uncertainty in the available study data to obtain a benchmark reference dose (RfD). The RfD is considered to represent the minimally safe dose for all potentially exposed individuals in the population. Any given exposure level is then placed as a numerator over the RfD denominator to create a proportional hazard index (HI). The degree to which the HI deviates above or below unity then reflects the potential for harm or safety, respectively. However, this approach is not without its limitations. First the potential for harm or safety relates only to the specific health effect being measured in the study used to derive the original dose–response relationship. Attempts are made to exploit studies that use what is considered to be the most sensitive target organ toxicity; however, many adverse health effects lack appropriate animal models for screening, and some toxicities of specific chemicals may as yet remain relatively undescribed. Take for example the recent classification of some chemicals as "obesogens" (Gohlke and Allison, 2013). In addition, the HI provides little in the way to actually quantify the possible risk burden for any given

dose level such as number or percent of people potentially affected and may not adequately permit comparison of different chemicals in terms of the severity of their effects.

While the threshold concept is relevant for many hazardous agents, in some cases no threshold or a level without effect has been empirically established [such as exposure to Pb, ozone (O_3), and fine particulate matter ($PM_{2.5}$)] (see Chapters 9, 17, and 21). In other cases when considering the mutagenic mechanisms shared by ionizing radiation and carcinogenic chemicals (ICRP, 1991, 1997; UNSCEAR, 2000; NAS, 2005), it is assumed that even the smallest dose carries some hypothetical risk, albeit small. For these agents, dose response is then considered to follow a linear nonthreshold relationship. In these cases, risks on the order of even 100–1000 affected/million exposed, while considered significant from a risk management point of view, are still too small to usually be observed in conventional animal and human studies, especially for those with high background levels of the same disease. Assessing such risks then requires considerable extrapolation from the high-dose observations into the low-dose domain. However, the choice of the best dose–response models for extrapolation to low doses and of the models for extrapolation to the human species (Zeise et al., 1987; Lave et al., 1988; NAS, 2005) is the source of considerable uncertainty (NAS, 1994). Certain agents appear to exhibit biphasic dose responses or even a reversal of effect at low doses (hormesis) implying significant departure from linearity and even protection at low doses (Calabrese et al., 1999). While there may be a mechanistic basis in some circumstances (i.e., stimulation of DNA repair by low-dose mutagens) (Cohen, 2002; Scott, 2008), this concept remains controversial (NAS, 2005), and many times the beneficial and harmful effects involve distinct biological responses for which each exposure and individual may possess their own unique dose–response curve.

In contrast to controlled laboratory situations, human exposures in the real world generally occur in the context of mixtures of multiple chemicals. Thus the potential for complex chemical interactions to impact dose–response relationships is high and represents another source of uncertainty. Such interactions may manifest as additivity, synergy, potentiation, or even antagonism and thus complicate the prediction of the combined effects of mixtures of agents based on what is known about the toxicological effects of any given agent acting alone (NAS, 1988; Mauderly, 1993). For example, the acute CNS effects of a benzene and toluene mixture likely represent the additive effect of each agent alone, whereas toluene can markedly inhibit the hematological and immune toxicity from benzene presumably by competitive inhibition of benzene metabolism (Snyder, 1989).

Furthermore, individuals express highly variable degrees in susceptibility based on genetic background, age, gender, physiological state, diet, lifestyle, health habits, and concurrent chemical exposures (e.g., smoking history), among other variables. Therefore a risk assessment that is based on observations derived in a particular narrowly defined population cannot necessarily be assumed to apply to any given individual in the population or to any other populations as a whole (NAS, 1994, 2005; Aardema and MacGregor, 2002).

2.2.3 Exposure Assessment

Exposure assessment, the third step in risk assessment, is the process of estimating or measuring the magnitude, frequency, and duration of exposure to an agent along with the number and characteristics of the population exposed. Ideally it also describes the sources, pathways, and routes of exposure. Exposure assessment is probably the most challenging of all the risk assessment steps. It frequently relies on historical recall of determinants of

possible exposure, limited measurement of chemical concentrations in various media (food, water, air, soil), and sometimes nonvalidated models of exposure. First, it should be realized that a wide continuum exists, from an actual introduction of a contaminant into the environment and its subsequent achievement of a sufficient concentration at the target organ to produce a health effect. Thus, it is problematic to assign a unitary exposure metric to a large population (Lioy, 1990). Contaminants exhibit a large degree of spatial and temporal heterogeneity within the environment, making it difficult to interpret limited measurements taken at finite times and locations. Multiple routes of exposure for the same agent may occur, with each containing its own unique properties for systemic absorption. Imagine a contaminant in the municipal water supply that is ingested as drinking water, applied to the skin during handwashing, and even aerosolized and inhaled during showering. Variations in personal behavior, such as amount and source of fluid intake, consumption patterns of certain foods, and exercise-induced changes in respiratory exposure to air pollutants, may also complicate precise exposure estimation within populations. Many times population-based exposure assessment are based on nonvalidated exposure models based on untested and even questionable assumptions. Retrospective studies can be complicated by long lag times between exposures and ultimate health effects and be subject to recall bias and low response rates. Technologies leading to equipment to monitor personal exposure are advancing the field, especially in the area of air pollution (Larkin and Hystad, 2017). Biomarkers of exposure such as measurement of contaminants or their metabolites in biological samples also hold promise as means to assess individual exposures; however, these approaches are still limited by variability in timing between exposure and sampling, heterogeneity in human populations, multiple sources for the same chemicals, challenges in routinely obtaining appropriate biological samples in some populations, and choice of chemical species to analyze. These limitations notwithstanding, advances in analytical techniques and in the development of putative molecular biomarkers of effect, such as DNA adducts (NAS, 1987) and altered gene expression (Brandt-Rauf, 1997), have paved the way for the field of molecular environmental epidemiology (Wilson and Suk, 2002). Technological breakthroughs in GC-MS analysis and "big data" handling initially applied to metabolomics have sparked attempts to capture the entire xenobiotic chemical landscape or "exposome" within human populations (Haines et al., 2017; Steckling et al., 2018).

2.2.4 Risk Characterization

Finally, risk characterization represents an integration of the information obtained from the previous steps to derive some quantitative estimate of the magnitude or probability of risk. It contains a description of the types and severities of the anticipated health consequences and, at best, estimates of the number of individuals likely to be at risk. As an example, Table 2.4 ranks the cancer risk burdens for a variety of environmental exposure sources for the U.S. population. Sometimes such risks are expressed as a fraction of a denominator representative of a total population (i.e., the number of cancer cases expected per million people exposed). In addition, estimation of risk at the population level does not permit one to evaluate the risk to any one individual.

As already mentioned, there are varying and multiple levels of uncertainty inherent in all of the first three risk assessment steps. Therefore, the precision of the final risk characterization will always be constrained by the combination of the different ambiguities. A good risk assessment points out the most important sources of uncertainty and explains

TABLE 2.4 Cancer Risk Rankings Assigned to Various Environmental Sources in EPA's "Unfinished Business" Report

Environmental Source	Rank Order	Estimated Yearly Number of Cases of Cancer in the U.S. Population
Category I (high risk)		
Exposure of workers to chemicals	1	250, from only four of the many carcinogens in question; risks to individuals may be high
Indoor radon	1 (tied)	5000–20,000 (of lung); risks to individuals may be high
Pesticide residues in foods	3	6000, based on assessment of only 7 of 200 potentially carcinogenic pesticides
Indoor air pollution (non-radon)	4	3500–6500 (primarily from tobacco smoke): risks to individuals may be high
Exposure to consumer products	4 (tied)	100–135, from only 4 of the more than 10,000 chemicals in consumer products
Other hazardous air pollutant	6	2000, from only 20 of the many pollutants in air; risks to individuals may be high
Category 2 (medium to high risk)		
Depiction of stratospheric ozone	7	Possibly 10,000 annually by the year 2100
Hazardous waste sites (inactive)	8	More than 1000
Pollutants in drinking water	9	400–1000
Application of pesticides	10	100 in the small population exposed: risk to individuals may be high
Ionizing radiation	11	360 (largely from building materials): risks to individuals may be high
Other exposures to pesticides	12	150 (estimate highly uncertain)
Hazardous waste sites (active)	13	Probably fewer than 100; risks to individuals can be high
New toxic chemicals	15	No quantitative possible; risks judged to be moderate
Category 3 (low to medium risk)		
Municipal waste	16	40 (excluding municipal surface impoundments)
Contaminated sludge	17	40 (mostly from incineration and sludge)
Mining waste	18	10–20 (largely from arsenic); risks to individuals can be high
Storage tank releases	19	Less than one
Nonpoint source discharges to surface water	20	No quantitative estimate, but judged to be the most serious surface water category
Other groundwater contamination	21	Less than one
Criteria air pollutants	22	Carcinogenicity questionable, but exposure extensive
Category 4 (low risk)		
Direct point discharges to surface water	23	No quantitative estimate (excluding drinking water)
Indirect point discharges to surface water	24	No quantitative estimate
Accidental release—toxicant	25	No quantitative estimate
Accidental release—oil spills	26	No quantitative estimate
Category 5 (unranked)		
Biotechnology	–	No estimate
CO_2 and global warming	–	No estimate
Other air pollutants	–	No estimate

Source: U.S. EPA (1987).

TABLE 2.5 Estimated Risks of Human Bladder Cancer from Daily Ingestion of 0.12 g Saccharin, Based on Extrapolation from Oncogenic Effects Observed in the Rat

Method of High-to-Low-Dose Extrapolation	Lifetime Cases per Million Exposed
Rat dose adjusted to human dose by surface area rule	
Single-hit model	1200
Multistage model (with quadratic term)	5
Multihit model	0.001
Mantel-Bryan probit model	450
Rat dose adjusted to human dose by milligram chemical per kilogram body weight per day equivalence	
Single-hit model	200
Multihit model	0.001
Mantel-Bryan probit model	21
Rat dose adjusted to human dose by milligram chemical per kilogram body weight per lifetime equivalence	
Single-hit model	5200
Multihit model	0.001
Mantel-Bryan probit model	4200

Source: NAS (1978).

the rationale for the approach chosen and how the characterization could be modified by alternative inputs. Table 2.5 illustrates a risk characterization of human bladder cancer derived from experimental data obtained using laboratory rats. Note the extreme variation that can arise from differences in methods used to extrapolate high doses to the low-dose range and selection of an appropriate biological model of carcinogenesis. Therefore, because of biological complexity, data requirements, and resources required for each step, as well as the inherent uncertainties, performing an accurate risk assessment is challenging and explains why many potentially hazardous agents remain poorly characterized in terms of risk (e.g., Ames et al., 1987; U.S. EPA, 1987, 1996, 2005b; NAS, 1994).

2.3 RISK COMMUNICATION

2.3.1 Bridging Different Cultures

How individuals perceive risk is a significant determinant in how they respond to that risk (Fischoff et al., 1997). As noted earlier, perceptions of risk involve not only the scientific technical considerations (technical rationality) but also nontechnical considerations ("cultural rationality"). It is essential to realize that all of the various stakeholders involved in a potentially hazardous situation represent a diverse group of varying backgrounds and interests. Community members, parents, local politicians, and industry representatives all have unique sets of values, priorities, resources, educational backgrounds, and cultures with which to assimilate the technical considerations presented during a risk assessment. Effective risk communication should be inclusive of all stakeholders and consider those nontechnical societal factors that contribute to cultural rationality. Table 2.6 illustrates a number of these and how they often stand in opposition to those held by the technical experts (Fischoff et al., 1997; NAS, 1989). Therefore, it is safe to say that no single unified message is likely to be effective for all audiences.

TABLE 2.6 Comparison of Factors Relevant to the Cultural Rationality, as Opposed to the Technical Rationality, of Risk

Technical Rationality	Cultural Rationality
Trust in scientific methods, explanations, and evidence	Trust in political culture and democratic process
Appeal to authority and expertise	Appeal to folk wisdom, peer groups, and traditions
Boundaries of analysis are narrow and reductionist	Boundaries of analysis are broad and include the use of analogy and historical precedent
Risks are depersonalized	Risks are personalized
Emphasis on statistical variation and probability	Emphasis on the impacts of risk on the family and community
Appeal to consistency and universality	Focus on particularity; less concerned about consistency of approach
Where there is controversy in science, resolution follows expertise, status	Popular responses to science: the differences do not follow the prestige principle
Those impacts that cannot be measured are less relevant	Unanticipated or unarticulated risks are relevant

Source: Plough and Krimsky (1987).

Community issues arising during risk assessment during the DWH oil spill serve to illustrate a number of these issues (Safford, 2012; Lichtveld et al., 2016; Simon-Friedt, 2016). Community members at large, rather than trained professionals, are frequently recruited in early stages of spill response, and consequently, this group lacks training and expertise in oil spill cleanup. The large number of fisherman from Vietnam and other Southeast Asian countries limited the use of English as the preferred method of communication of hazards, risks, and protective actions. High levels of mistrust for government and regulatory agencies were observed, along with fear that consumption of seafood was unsafe despite professional assurance that contaminant levels on finfish and shellfish were routinely below established levels of concern. Tolerance of risk also varies greatly among individuals. For example, people are often less willing to accept involuntary risk than voluntary risk (e.g., resistance to potential PAH exposure from eating fresh fish after DWH vs. willing consumption of smoked fish by Pacific Northwest indigenous peoples) (Yender et al., 2002). Residents of the Gulf Coast have long been familiar with the oil industry for some time and hence were familiar with oil exposure through smaller spills and natural oil seeps; however they became particularly concerned with the unprecedented application of novel oil dispersants, despite the professional opinion that oil constituents remained the priority agents of concern. It should be noted however, that, industry's initial reluctance to release the specific ingredient composition of these products significantly hampered risk assessment and communication, only adding the fuel of mistrust to the fire of community unrest.

Modern approaches to risk assessment have found that for optimal effectiveness in risk communication, the process requires a bidirectional, iterative exchange of information between the technical risk assessor and any stakeholders who may be directly or indirectly affected. This relatively new approach of community-based participatory research (CBPR) has evolved as a method of choice (O'Fallon and Dearry, 2002). Early engagement of the community in the risk assessment process can help the technical risk assessors identify sources of hazards and exposure previously unknown to them. Joint formulation of

hypotheses and methods of testing can lead to greater efficiencies in data acquisition, transparency of the process, and greater trust and acceptance of the final characterization of risk. Community members also often have more insight than outsiders on how such risks can be mitigated based on intimate knowledge of community dynamics and behaviors. Ideally, a CBPR approach should begin as early as possible in the process of risk assessment so that those who may bear the risks can participate fully in the derivation of the assessment itself and trust is fostered through a mutual, transparent, consensual, and participatory process (Sandman, 1985; NAS, 1996; Slovic, 1998).

2.3.2 Treatment of Uncertainty

Common risks encountered in daily life, such as automobile accidents, risk of drowning, and falling in the elderly, are commonplace, and their incidence is fairly well documented. In many cases though, environmental health risks are not precisely known or quantified. As mentioned above, their approximation is often based on unverified assumptions and extrapolations. Various levels of uncertainty are inherent in every step of the risk assessment. These include (1) unidentified adverse reactions associated with known chemicals or novel untested chemicals themselves; (2) paucity of human dose–response data; (3) temporal, spatial, and individual heterogeneity in exposure; (4) application of hypothetical models; and (5) consideration of risk at the personal, population, and societal level. For a final risk assessment to be considered adequate, it must also include an explicit and understandable presentation of the most significant sources of uncertainty applied to the estimation and how modulation of some of the key assumptions might impact the final risk estimate (Finkel, 1990; Morgan and Hendon, 1990; NAS, 1994). Issues like sole reliance on an animal study, lack of an observable in NOAEL in a dose–response relationship, or various assumptions used in the exposure estimate need to be identified and outlined. A simple description of whether the risk characterization represents an estimation for an "average" individual in the population versus a "worst-case scenario" can go a long way toward putting risks into a proper perspective. This transparency on the deficiencies or ambiguities in risk characterization can help stakeholders, not only to weigh its importance and accept the proposed changes but also importantly to guide where more experimental work and data acquisition are needed to improve the precision of future risk assessments.

2.3.3 Placing Risks in Proper Perspective

The challenge for effective risk communication personnel and methods is to place these risks in a proper context that brings meaning to all the stakeholders who may perceive these risks through a diverse myriad of lenses, like voluntary and involuntary risks (e.g., NAS, 1989; Zeckhauser and Viscusi, 1990; Fischoff et al., 1997). Many times the parameters applied to risk assessment are expressed in quantitative terms that, by themselves, have little meaning to the nontechnical lay audience, especially considering the complexities of risk perception described above. Our scientific dependence upon the metric system can pose perception challenges for the U.S. public, especially when faced with unfamiliar quantities such as microgram, cubic meter, nanomolar, or 10^{-6}. Table 2.7 lists some familiar scenarios and situations that themselves carry risk of approximating one in a million. Understanding of concepts like parts per million or cancer risk of 1 in a million can be aided by comparison with a more familiar quantity in the United States like 1 inch in 16 miles. It can be hard for people to comprehend what an annual release of 450 tons of

TABLE 2.7 Situations and Activities Carrying an Estimated One-in-a-Million Risk of Death

Exposure or Activity	Cause of Death
Smoking 1.4 cigarettes	Cancer, heart disease
Drinking 1.5 L of wine	Cirrhosis of the liver
Spending 1 h in a coal mine	Black lung disease
Spending 3 h in a coal mine	Accident
Living 2 days in New York or Boston	Air pollution
Traveling 6 min by canoe	Accident
Traveling 10 miles by bicycle	Accident
Traveling 300 miles by car	Accident
Flying 1000 miles by jet	Accident
Flying 6000 miles by jet	Cancer caused by cosmic radiation
Living 2 months in Denver	Cancer caused by cosmic radiation
Living 2 months in an average masonry building	Cancer caused by natural radioactivity
One chest X-ray	Cancer caused by radiation
Living 2 months with a cigarette smoker	Cancer, heart disease
Eating 40 tablespoons of peanut butter	Liver cancer caused by aflatoxin B_1
Drinking Miami drinking water for 1 year	Cancer caused by chloroform
Drinking thirty 12-oz cans of diet soda	Cancer caused by saccharin
Living 5 years at the boundary of a nuclear plant	Cancer caused by radiation
Eating 100 charcoal-broiled steaks	Cancer caused by benzopyrene

Source: Wilson (1979).

VOCs from an industrial source "looks like" until a more familiar perspective is provided like being equivalent to 36,000 additional new automobiles to an area, each driving 12,000 miles a year (Fabisiak, 2016). Does describing a major ingredient of COREXIT oil dispersant by its chemical name (dioctyl sodium sulfosuccinate) bring any level of public consolation during the Gulf oil spill without pointing out that this same chemical is also the active ingredient in a widely used over-the-counter laxative product (Fabisiak and Goldstein, 2011)? Comparisons of quantitative risk estimates (e.g., Peto et al., 1984; Table 2.7) such as have often been presented for the purpose or attempt to weigh risks solely on the basis of their impacts on life expectancy (e.g., ICRP, 1991, 1997), on the quality of life (e.g., ICRP, 1991), or on their economic costs (e.g., Arrow et al., 1996) are likely to be inadequate by themselves (Slovic et al., 1981; NAS, 1989). Therefore, an effective risk communication strategy should consider the multiple factors shown in Table 2.2 like familiarity, fairness, and others that govern the overall perception of "acceptability" of any given risk, despite the fact that they may have the same quantitative impact (Sandman, 1985; Fischoff et al., 1997).

2.4 RISK REDUCTION

Two conflicting principles are frequently invoked in any discussion regarding the need, rigor, and responsibility required to limit environmental risks within the society. The precautionary principle states that for any potential "risky" activity, precautionary actions should be taken to provide protection even if some cause and effect relationships have not been fully established scientifically. Proponents cite the need for protection and

recommend cost-effective measures to prevent harm even in the face of scientific uncertainty. The precautionary principle places the burden of proof for safety on the purveyor of new technology and fosters a "better safe than sorry" attitude in a regulation-rich environment. In contrast, the proactionary principle highlights that people's freedom to innovate is necessary for technological advancement of society. Its proponents believe that restrictive limits based on perceived risks rather than actual documented risk stifle progress, and they frequently point to the cost and risks of the restrictions themselves, as well as those of missed opportunities. The proactionary principle places the burden of proof on those who propose the restrictive measures and promotes a "laissez-faire" attitude within a regulation-poor environment. Most risk reduction strategies represent some combination of these two extremes of attitude, yet many contain a predominance of one of the other. For example, the FDA approval process for new drugs typifies a precautionary approach that requires demonstration of efficacy, human toxicity studies, and detailed cost–benefit analysis at the manufacturer's expense prior to receiving FDA approval for marketing. In contrast, the Dietary Supplement Health and Education Act (DSHEA) covering nutritional supplements like vitamins and botanical health aids begins by assuming such products are generally safe and allows their marketing in the absence of any demonstrable efficacy and without FDA approval. Products can only be withdrawn from market once a safety issue has been realized and are not routinely monitored with the same quality assurance scrutiny as approved drugs. Of historical note was the extreme difficulty in removing one such dietary supplement product, ephedra, from the market despite convincing and long-standing evidence of serious life-threatening cardiac toxicity in young healthy people using this product for weight loss and athletic performance enhancement.

In spite of the fact that the importance or acceptability of risks can vary among different individuals, there is still a basic expectation for policy makers, regulatory agencies, and commercial enterprises to set and maintain certain basic standards to protect human health from undue harm (Rodricks, 1992). According to the Clean Air Act, Congress directs the U.S. EPA to set National Ambient Air Quality Standards (NAAQS) for certain common and widespread pollutants (criteria pollutants like $PM_{2.5}$) to "protect public health, including that of sensitive populations with an adequate margin of safety." While much discussion ensues among the experts regarding what those standards should be to meet the "adequate margin of safety," there is no question as to the success of this legislation in terms of improving air quality and thus reducing disease burden. Cost–benefit analysis and feasibility are always part of the risk reduction strategy. Again, toward this end, the U.S. EPA's National Emission Standards for Hazardous Air Pollutants (NESHAPS) from point sources sought to limit the permissible levels of carcinogenic pollutants to a 1-in-a-million or less lifetime risk (Travis, 1989); however it is clear that the level of risk that is judged to be acceptable varies between agents and circumstance (Fig. 2.3). Simply living with the convenience of an urban environment frequently entails cumulative risks exceeding those observed in rural locales, as well as the one-in-a-million standard (Michanowicz et al., 2013). With the discovery of the ubiquity of radon in the indoor environment, the EPA's guidance recommends remedial measures when the concentration indoors exceed 4 pCi/L, a level that poses a lifetime risk of cancer of about 7 in 1000 for nonsmokers (U.S. EPA, 2005c). Moreover, elevated cancer risks associated with occupational exposures, which reflect some matter of choice, are often deemed acceptable, in contrast to if those same levels of risk were present with ambient exposure to the general public at large (Fig. 2.3).

Failures to adequately provide protection from a variety of environmental risks may entail considerable costs, not only in human life, health, and well-being but also in economic

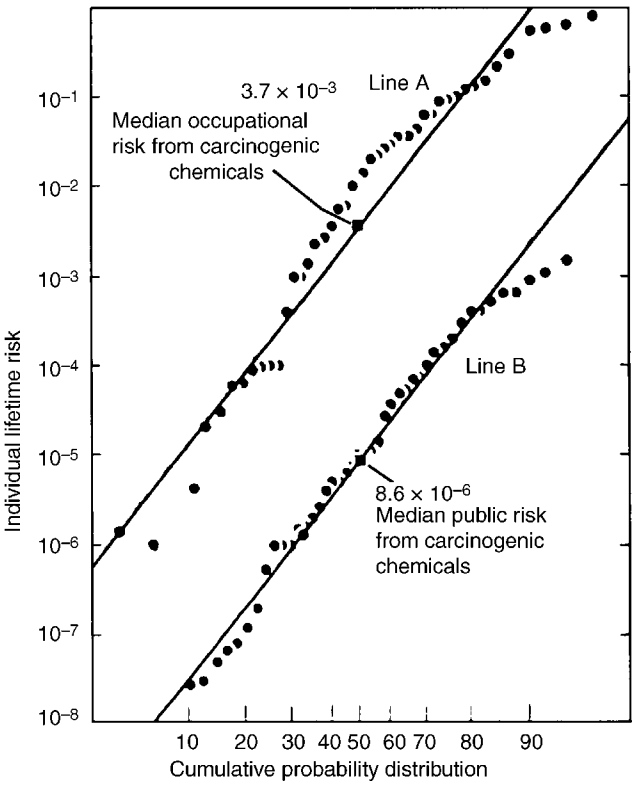

FIGURE 2.3 The level of risk judged to be acceptable varies among carcinogens. *Source*: From Travis et al. (1989).

terms (missed work or school days, premature death, health-care costs). In turn, measures to reduce environmental risk also involve costs in terms of technology investment, erosion of profitability, higher consumer and monitoring compliance costs, and perhaps, additional health risks themselves. Therefore, weighing the relative costs and benefits of all available risk management strategies is an inherent variable in deciding the most appropriate approach for reducing a given risk (Davies, 1996). Included among the criteria to be considered in such comparative risk assessments are both the risks to society and the risks to individuals (Evans and Verlander, 1997), since the acceptability of a given risk generally varies inversely with the number of individuals who may be affected (e.g., Fig. 2.4).

2.4.1 Options for Risk Reduction at the Individual and Community Levels

Environmental risks to human health can be mitigated at various points along the sequence of events that follows release into the environment, fate and transport through various media (i.e., air, water, soil), ultimate exposures, and causative health effects (Fig. 2.5). Source controls might entail setting emission limits or safer chemical substitution. Dilution, containment, and recycling strategies can impact fate and transport toward ultimate receptors. Personal protective devices or behavior guidance can be employed to limit individual exposure. All of these represent primary preventive strategies; however, in certain situations secondary measures are unavoidable such as administration of potassium iodide to a population exposed to radioactive iodine following releases of radioisotopes resulting from

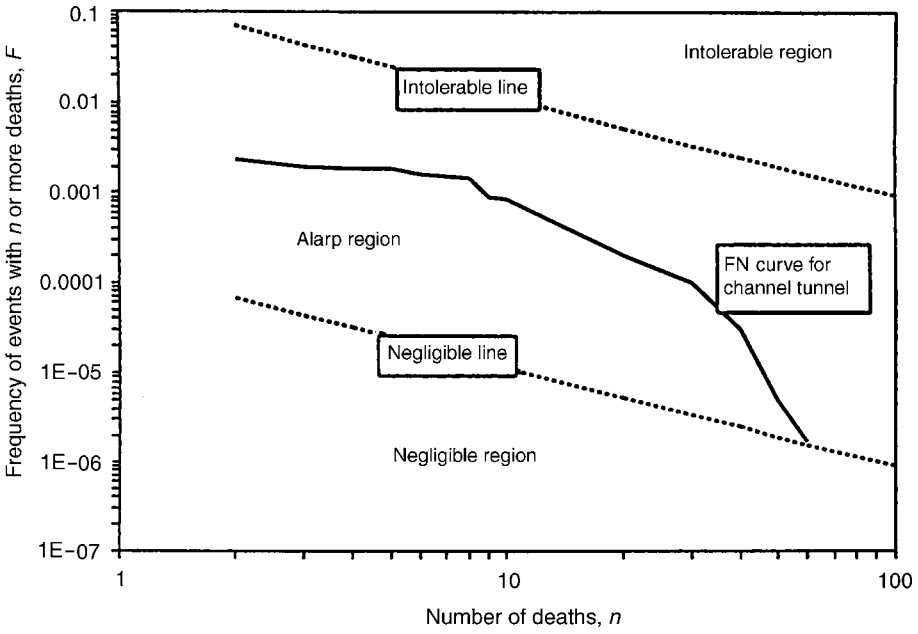

FIGURE 2.4 The level of risk judged to be acceptable varies inversely with the number of victims affected. *Source*: From Channel Tunnel Safety Study, Eurotunnel (1994).

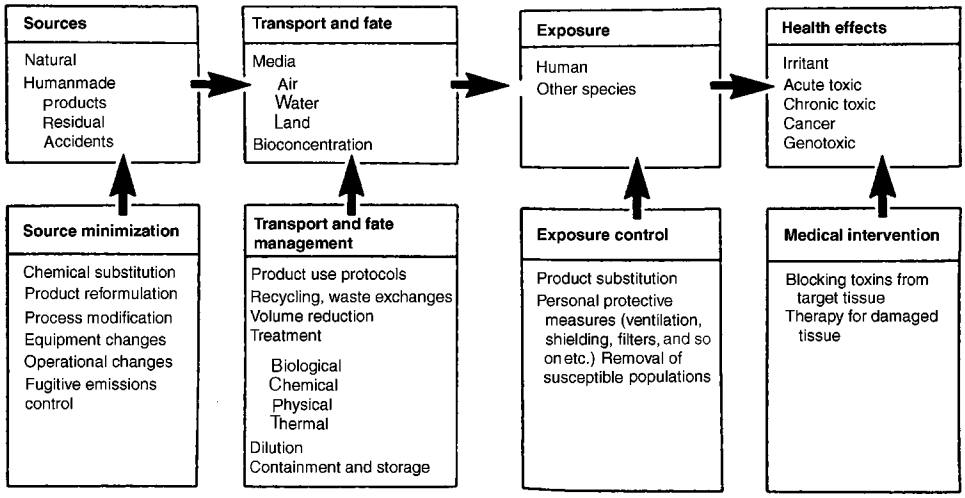

FIGURE 2.5 Options for reducing environmental risks to human health. *Source*: From Andrews and Turner (1987).

nuclear accidents. Generally speaking, the earlier in this sequence one can act, the greater the potential success in terms of risk reduction to the greatest number of people; however, each risk scenario possesses its own unique set of factors and cost–benefit equations.

Some options can be taken at the level of individual choices and behaviors with little requirement of outside intervention, such as smoking cessation, private well water testing, or employing air filtration in the home environment. Success of these actions may be

greatly facilitated by public education campaigns. Other actions, such as setting and enforcing regulatory policy, require cooperation of various stakeholders and the political and economic will at the national or community level to make them happen. All the available options, however, depend on some understanding of the risks in question and possessing the skills and resources needed to implement the strategies (Tones, 1997).

On the larger national and global level, long-term forward-looking strategies for improving risk reduction encompass a board range of activities. A few of those that should be made are listed as follows:

(1) Increased research efforts toward the rapid, efficient, and accurate identification of potentially hazardous agents, describing the mechanistic basis of their toxicity, and defining relevant dose–response relationships. The recently described "adverse outcome pathways (AOP)" framework holds promise as a way to systematically incorporate high-throughput and high-content data acquisition formats into a regulatory and policy decision-making endpoint (Wittwehr et al., 2017).

(2) Increased efforts to document the extent to which individuals and populations may be exposed to chemical hazards via multiple routes and environmental media. This can range from expanded Pb screening programs for children, improvements to measure air pollutants with better temporal and spatial resolution, and population-based biomarker or "exposome" surveys.

(3) Further refinement of identifying high risk groups because of increased levels of exposure or heightened susceptibility because of genetic and other biologic factors.

(4) A truly bipartisan political approach that rationally incorporates the precautionary principle in formulating standards and regulations, as well as technological advances that produce engineering controls that are more cost-effective in reducing chemical emissions and alternative processes that represent safer options.

(5) A rational consideration of worst-case scenarios such as accidental releases or disasters should always be discussed in order to maintain a readiness and capacity to effectively respond to environmental emergencies in the event they occur. This also includes a willingness for communities to assess their overall resilience and resources to withstand such affronts.

(6) Realization that knowledge is power and programs in public and professional education in environmental risk reduction should continue to be developed and refined as new knowledge is gained and whenever the environmental status quo of a community or region threatens to be altered (U.S. EPA, 1990; Griffith and Saunders, 1997; Omenn and Faustman, 1997; Presidential Congressional/Commission on Risk Assessment and Risk Management, 1997; Breggin and Hallman, 1999; Breggin, 2002; O'Fallon et al., 2003).

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3

REDUCING RISKS: AN ENVIRONMENTAL ENGINEERING PERSPECTIVE

MORTON LIPPMANN AND RAYMOND C. LOEHR*

3.1 INTRODUCTION

Concern about environmental risks, particularly those affecting human health, can be traced to the earliest human records (Paustenbach, 1989, 2002; Graham, 1994). However, it was not until the twentieth century that there was a concerted focus on the protection of humans from the adverse effects of chemical exposures at work sites and to human exposures to chemicals and their reaction products following their emissions into environmental media.

The purpose of this chapter is to provide a broad perspective of the use of risk assessment and risk-based decision making by industry, environmental engineers, and governmental agencies responsible for the protection of human health and the environment from effluent discharges and inadequate isolation of waste depositories. The topics covered in this chapter include:

- Risk-based decision making in the Superfund process.
- The risk-based corrective action (RBCA) framework.
- Factors that affect site-specific health and environmental risk.
- Scientific and research knowledge that can be used for site-specific risk evaluations.
- The value of site-specific parameters, rather than default assumptions, for risk-based management decisions.

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3.2 ENVIRONMENTAL RISK-BASED DECISION MAKING

3.2.1 Overview

Environmental risks have changed over time. In the early 1900s, lead arsenate was the principal insecticide sprayed on fruits and potatoes to control insects; medicinals were unreliable; industrial towns were black with soot, as were people's lungs; and workers labored at their own peril (Lowrance, 1976). The principal fatal diseases were pneumonia, influenza, and tuberculosis. More than 13% of all American children died before their first birthday. Paustenbach (2002) has provided a detailed historical timeline of events that relate to human health risk assessment issues.

Many steps have since been taken to protect public health by reducing the major earlier human health and environmental risks. As a result, other risks, which at one time were low in priority, have risen in concern. Both personal and societal risks are inherent in human activity, and it is not possible to reduce all risks to zero. Risk is an inescapable fact of life.

However, it is not risk *per se* that generally is of concern to the public. Involuntary risk is the major concern. Opinions of "acceptable" risk frequently depend on the degree of choice to decline a specific risk.

Site-specific risk assessments have been used to illuminate possible degree of impact, options, priorities, and frequently relative costs. A risk assessment provides information to policy makers, regulatory agencies, other risk managers, and the public so that the most appropriate decisions can be made. A risk assessment provides the bridge between scientific information and risk management and puts the words toxicity, hazard, and risk into perspective (Paustenbach, 1989, 2002). The real world of risk assessment is based on sound scientific and empirical knowledge.

The commonly accepted definitions of risk assessment and risk management are those adopted by the National Research Council (NRC, 1983):

Risk assessment: The characterization of potential adverse health effects of human exposures to environmental hazards; the assessment includes characterization of the uncertainties inherent in the process of inferring risk.

Risk management: The process of evaluating alternative regulatory actions and selecting among them; the selection process requires the use of value judgments on such issues as the acceptability of risk and the reasonableness of the costs of control.

Until the early 1990s, the concepts of the risk assessment paradigm, and the use of risk assessment as part of the environmental decision-making process, were viewed by environmental engineers as an esoteric practice. While possibly valuable, it was considered, at that time, to be on the fringes of environmental engineering and hazardous waste management practice, with emphasis being placed on meeting specific regulatory requirements that commonly were based on worst-case assumptions used to assure maximum protection of human health.

Today, risk assessment is an important component of many environmental engineering and waste management decisions, specific state and federal guidance is available, and it is an integral part of federal and state environmental legislation.

3.2.2 Risk Assessment Process

The risk assessment process can be divided into four major discrete steps: hazard identification, exposure assessment, dose–response assessment, and risk characterization (NAS, 1994).

Risk assessment needs to precede risk management when the estimated risk is considered to be unacceptable. Risk assessment considers how risky the situation or exposure is. Risk management considers what to do about the risk, that is, how to reduce or remove it, when it is deemed unacceptable.

The values of the risk assessment process are many. An important value is that it provides a consistent, disciplined approach to organize scientific information so that the relevant items are considered and used for subsequent risk management decisions. The risk assessment process helps identify the uncertainties inherent in the available data, as well as the assumptions that are involved. It helps indicate the time frames involved and who or what is affected. It also helps identify strategies and priorities, such as where, within the process, risk management decisions can be most effective. Overall, it helps educate all involved about the factors, exposures, effects, and relative risks that exist at a site or for a particular situation.

3.2.3 Risk Management from an Environmental Engineering Perspective

The concepts of RBCA and risk-based decision making are now being widely applied to identify appropriate risk management decisions that need consideration at specific sites to protect human health and the environment. The following example may help put the use of the risk assessment process for environmental decision making into perspective. A simplistic risk assessment paradigm is shown in Fig. 3.1.

For a chemical to pose a threat to human health and/or the environment, there must be one or more sources of contamination and releases from such sources. Once released, the chemicals must be transported to receptors of concern, such as humans, plants, animals, or fish. During the transport, there may be chemical transformations that can change the form or speciation of the chemical, which may, in turn, make the chemical more or less mobile or toxic. If the chemical contacts a sensitive area of a receptor, that is, if relevant exposure occurs, there could be negative impacts. The environmental risk associated with such negative impacts can be identified, and decisions made as to whether such effects may be considered to be acceptable or unacceptable. The risk assessment process has been formalized and has been increasingly used by the U.S. Environmental Protection Agency (EPA) and other regulatory agencies as part of environmental decision making.

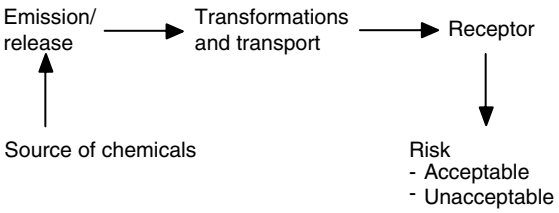

FIGURE 3.1 Conceptual risk assessment paradigm.

TABLE 3.1 Example Environmental Engineering Options to Reduce Environmental Risks

- Removing the source of contamination leading to burdens in soils and allowing natural environmental assimilative processes to control any remaining chemicals and those that have been released.
- Changing the chemical or manufacturing process that is emitting chemicals of concern, thus decreasing or eliminating the chemical causing the risk. This is the cornerstone of the pollution prevention approach now widely used by industry.
- Changing the form of the chemical, either at the source or after release. This can be done by modifying the pH of the media containing the chemical or adding other chemicals that will immobilize the chemical of concern, for example, solidification–stabilization.
- Intercepting and treating emitted and released chemicals of concern before they reach a receptor. Examples include *in situ* and *ex situ* biological and chemical treatment processes.
- Preventing released chemicals of concern from reaching a receptor. Capturing a chemical of concern at the source and *in situ* slurry walls are two such possibilities.
- Separating the receptor from the chemical of concern. Providing alternative drinking water and food supplies, purchasing property to reduce or control access, and moving residents are possibilities.

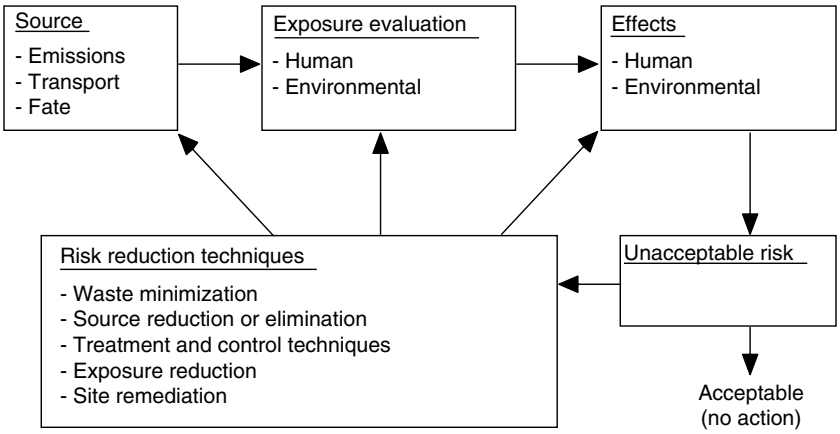

FIGURE 3.2 Risk assessment and illustrative engineering risk management approaches.

An environmental engineer uses the knowledge from the risk assessment process to identify locations and procedures by which the identified risks can be reduced. For instance, in terms of the paradigm in Fig. 3.1, possible control or remediation options are noted in Table 3.1. Schematically, these options can be included in the risk assessment paradigm, shown in Fig. 3.2. The interaction between risk assessment and risk management is shown in Fig. 3.3, which also indicates some of the nonengineering factors that are involved in a risk management decision.

For a real-world situation, there are many risk management options that can be considered. For a specific problem, the challenge for the environmental engineer is to identify, develop, and implement cost-effective technical solutions that will protect human health and the environment.

The cost implications associated with protective risk-based management decisions are not trivial. Consider the following example that resulted at an actual site where alternatives for remediation were evaluated. The detailed site remedial investigation studies indicated

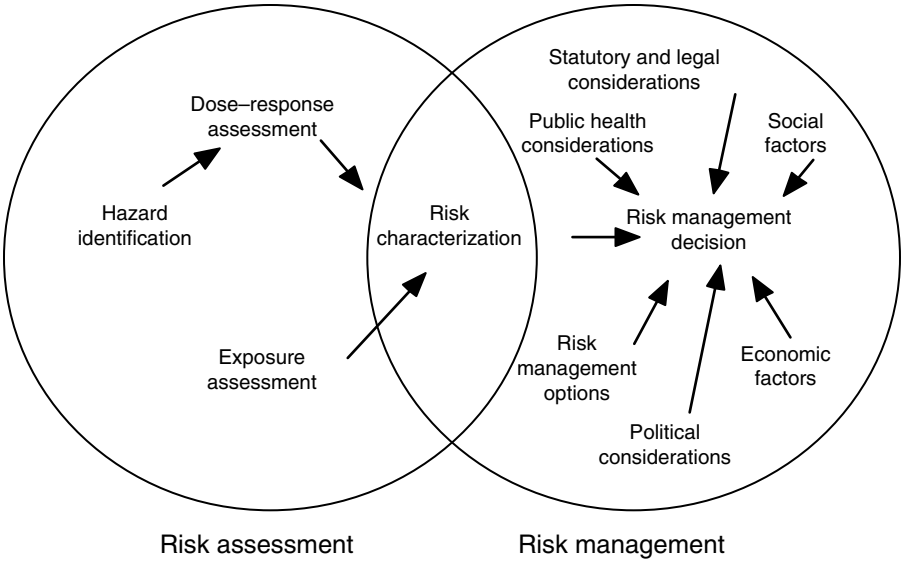

FIGURE 3.3 Conceptual interactions between risk assessment and risk management. *Source*: Adapted from NRC (1983).

TABLE 3.2 Example Situations—Relationship of Cleanup Action Levels to Remediation Life Cycle Costs

Assumed Cleanup Action Level (ppm of Chemical in Soil)	Total Estimated Remediation Site-Specific Life Cycle Costs ($ Millions)
10	1375
50	163
180	69
400	30

that the life cycle remediation costs were related to the required cleanup levels (Table 3.2). A site-specific risk-based evaluation determined that a cleanup level of 400 ppm for the chemical of concern was protective of human health. In the absence of the risk-based evaluation, a default and more restrictive cleanup level would have been required, resulting in a considerably larger site remediation cost.

Paustenbach (1989, p. 92) also provided a pertinent example of the relationship between the cost of remediation and cleanup levels. In that example, the estimated costs of soil removal and destruction for various soil cleanup levels of dioxin were shown. The cost was indicated to be about $17 million for a cleanup level of about 1 ppb dioxin to less than $1 million for a cleanup level of 100 ppb dioxin in the soil.

Evaluations of the risk associated with specific chemicals at a site can help identify the site-specific cleanup levels that are needed in order to be protective of human health and the environment. A sound site-specific risk assessment is important to assure that human health and the environment are protected and to assure that resources (time, energy, funds) are used effectively.

The resource considerations related to reducing environmental risks were stated clearly by EPA in 1984 (U.S. EPA, 1984, p. 23):

> One can argue about how much should be spent on environmental protection, but at some point the risk manager must accept that the commitment of resources for any social purpose has a finite limit. If the number of potential risk targets is very large, in comparison to the number that can realistically be pursued, which seems now to be the case, then some rational method of choosing which risks to reduce and deciding how far they should be reduced is indispensable.
>
> It is important to keep in mind that while individual risk management decisions may be seen as balancing risk reduction against resources, the system as a whole is designed to balance risk against risk. In other words, it is essential to address the worst and most controllable risks first; failure to do so means that the total amount of harm prevented is smaller than the amount prevented. Making incorrect priority choices, saving one where we might have saved two, represents a profound failure of the Agency's basic mission.

The ideas and concepts in the above statement have been recognized broadly and have stimulated many of the approaches now being used to assure that (a) the focus is on important environmental risks and (b) cost-effective environmental risk reduction strategies are utilized.

U.S. EPA continues to explore approaches to use the concept of relative risk for environmental decision making. In 1990, the EPA Administrator called for a national debate on environmental directions and policies (Reilly, 1990). What stimulated that call and the debate was the U.S. EPA Science Advisory Board Report, Reducing Risk (U.S. EPA, 1990). The 10 summary recommendations of the report are noted in Table 3.3. The recommendations emphasized targeting environmental protection efforts on the basis of environmental risk and risk reduction opportunities. They called for risk-based priorities in national planning and budgeting and for emphasis on pollution prevention rather than on end-of-pipe treatment. Efforts to better educate the public about actual risks also were recommended.

3.3 APPLICATIONS AND USE

Risk assessment evaluations have been applied in many situations for environmental management decisions, based on the following factors: (1) environmental engineers and regulators now better understand the limits of existing technologies, (2) the sciences of risk assessment and modeling now can be used to measure and compare the benefits of possible options, and (3) many studies have documented that all sites and situations do not require treatment or removal to the same generic standard.

The following sections address the use of risk-based decision making for different environmental situations and decisions. These include an overview of the risk assessment process as used to evaluate site-specific risks, experience gained in Superfund remediations, a discussion of the RBCA approach now used widely for environmental management decisions, and the type of recent information that has become available for improved site-specific risk-based management decisions.

3.3.1 Overview

There are many uses of a site-specific risk assessment (Table 3.4). From an engineering and environmental decision standpoint, an environmental risk assessment can be conducted in a forward or a reverse mode. In the forward mode, using the items in Fig. 3.1, data about

TABLE 3.3 Recommendations from the EPA Reducing Risk Report

1. EPA should target its environmental protection efforts on the basis of opportunities for the greatest risk reduction. The United States already has taken actions to address the most obvious environmental problems. EPA needs to set priorities for future actions so the nation takes advantage of the best opportunities for reducing the most serious remaining risks.
2. EPA should attach as much importance to reducing ecological risk as it does to reducing human health risk. Productive natural ecosystems are essential to human health and to sustainable, long-term economic growth.
3. EPA should improve the data and analytical methodologies that support the assessment, comparison, and reduction of different environmental risks. Setting priorities for national environmental protection efforts always will involve subjective judgments and uncertainty. The scientific data and analytical methodologies that underpin those judgments and reduce their uncertainty should be improved.
4. EPA should reflect risk-based priorities in its strategic planning processes. The Agency's long-range plans should be driven not so much by past risk reduction efforts or by existing programmatic structures, but by assessments of remaining environmental risks and the analysis of opportunities available for reducing risks.
5. EPA should reflect risk-based priorities in its budget process. Although EPA's budget priorities are determined to a large extent by the different environmental laws that the Agency implements, it should use whatever discretion it has to focus budget resources at those environmental problems that pose the most serious risks.
6. EPA—and the nation as a whole—should make greater use of all the tools available to reduce risk. The extent and complexity of future risks will necessitate the use of a much broader array of tools, including market incentives and information.
7. EPA should emphasize pollution prevention as the preferred option for reducing risk. By encouraging actions that prevent pollution from being generated in the first place, EPA will help reduce the costs, intermedia transfers of pollution, and residual risks associated with end-of-pipe controls.
8. EPA should increase its efforts to integrate environmental considerations into broader aspects of public policy in as fundamental a manner as are economic concerns. Other federal agencies often affect the quality of the environment, for example, through the implementation of tax, energy, agricultural, and international policy. EPA should work to ensure that environmental considerations are integrated, where appropriate, into the policy deliberations of such agencies.
9. EPA should work to improve public understanding of environmental risks and train a professional workforce to help reduce them. The improved environmental literacy of the general public, together with an expanded and better-trained technical workforce, will be essential to the nation's success at reducing environmental risks in the future.
10. EPA should develop improved analytical methods to value natural resources and to account for long-term environmental effects in its economic analyses. Traditional methods of economic analysis tend to undervalue ecological resources and fail to treat adequately questions of intergenerational equity. EPA should develop and implement innovative approaches to economic analysis that will address these shortcomings.

Source: U.S. EPA (1990).

TABLE 3.4 Example Uses of a Risk Assessment for Environmental Engineering Purposes

- Determines the need for remedial action at contaminated sites
- Helps set priorities and establish the urgency to remediate sites
- Determines site-specific, health-based cleanup levels
- Provides a position from which the concerned parties can negotiate cleanup levels
- Allows the use of less expensive remedial alternatives while still being protective of human health and the environment
- Helps identify suitable locations for waste management facilities, such as municipal incinerators and landfills
- Supports litigation involving chemical emissions and exposures
- Helps meet mandates of federal or state environmental regulations

TABLE 3.5 Basis Steps in a Site-Specific Risk Assessment

- Prepare a risk assessment plan for the site or situation
- Identify available data, data needs, and data gaps
- Define data quality objectives
- Prepare a sampling plan
- Identify chemicals of concern and assess toxicology
- Identify current and potential future receptor(s)
- Define exposure pathways
- Estimate exposure point concentrations
- Estimate applied or absorbed dose
- Characterize health risks and uncertainties
- Identify and state key assumptions

the chemical at the source and transformation and transport knowledge are used to assess the risk to a site-specific human or ecological receptor from a defined source, such as a leaking fuel tank, a spill, or a gaseous emission.

In the reverse mode, one works backward from the exposure to a chemical that a known receptor may receive. With that exposure information and using protective health or ecological criteria, the soil, groundwater, or emission levels that would have to exist to be protective of human health and the environment can be calculated. This reverse approach can be used to determine site remediation or chemical emission levels that would have to be achieved so that there is no adverse effect to the receptor.

Site-specific risk assessments that are part of risk-based management decisions are commonly conducted at several levels or tiers, with a screening-level assessment being done first. The basic steps of a site-specific risk assessment are indicated in Table 3.5. The detail and accuracy of the information needed, the time needed to complete the risk assessment evaluation, and the cost of the evaluation are different in each tier of the evaluation. The screening-level evaluation requires the least amount of information, since many conservative assumptions are involved. The cost of a risk assessment evaluation can increase by a factor of 5–10 when the evaluation moves from a screening level to a detailed evaluation.

The specifics of a risk assessment evaluation will be different for each site. The general characteristics of a risk assessment process for environmental engineering purposes are indicated in Table 3.6.

TABLE 3.6 Characteristics of the Risk Assessment Process for Environmental Engineering Purposes

Develop reasonable exposure scenarios
Current site and surrounding land use
Future reasonable site and surrounding land use
Real and possible hypothetical pathways from source to receptor
Evaluate multiple exposure pathways
Air
Soil
Groundwater
Surface water
Evaluate multiple exposure routes
Ingestion
Inhalation
Dermal
Involve multidisciplinary expertise
Quantify environmental multimedia fate and transport
Understand and apply toxicological principles
Understand site-specific environmental chemistry
Interpret multimedia field data
Involve statistical analysis of the data
Risk communication skills needed

3.3.2 Superfund

From an environmental engineering standpoint, the risk assessments and the site-specific risk management decisions for Superfund sites represent some of the more detailed and comprehensive evaluations. The details of a Superfund risk assessment are well developed and standardized (U.S. EPA, 1988, 1989a, 1989b, 1989c, 1991a, 1991b). The following is a summary of the Superfund site risk assessment steps and process.

The major decisions made at a Superfund site are based on answers to questions such as: (1) how serious are the problems at the site, (2) should something be done at this site, (3) what should be done, and (4) when has enough been done?

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) (also known as Superfund) was passed by the U.S. Congress in 1980 and amended in 1986. Superfund requires EPA to identify and, if needed, to reduce the risks from past inadequate waste management and disposal approaches. Different risk assessment approaches are applied in evaluating possible effects of Superfund sites on human health and the environment. Neither EPA nor the Superfund program has one standardized environmental evaluation methodology because there are so many different aspects of the environment, such as individuals, species, ecosystems, natural resources, and endangered species, which may need to be evaluated. As a result, the Superfund program has identified an orderly process for environmental risk evaluation.

Risk assessment for a Superfund site is a four-step process. The first step, data collection and evaluation, identifies contaminants present in the environmental media: soil, groundwater, surface water, air, and fish at the site. The second step, toxicity assessment, uses the results of prior research and testing to decide which of the contaminants found on site might pose a health threat.

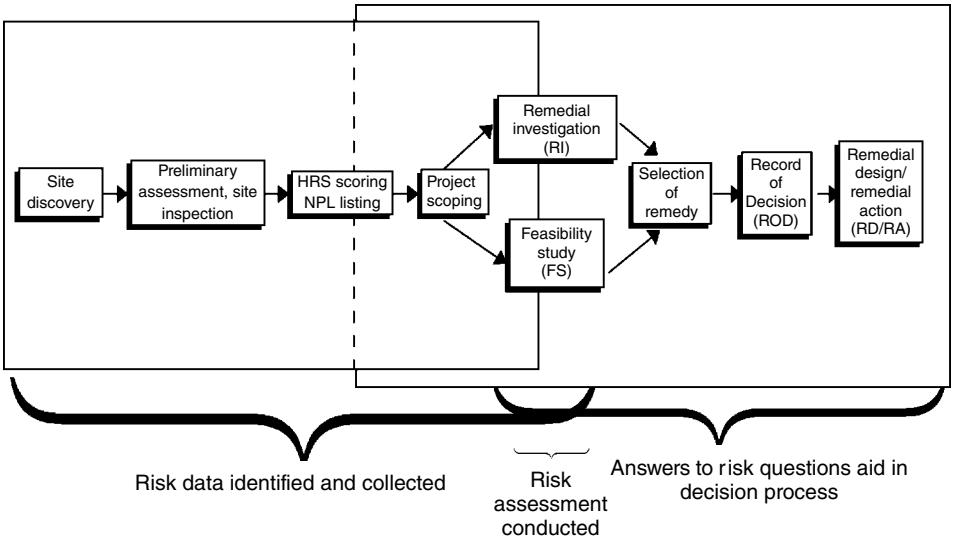

FIGURE 3.4 Major steps in the Superfund site evaluation process. *Source*: Adapted from U.S. EPA (1991b).

The third step, exposure assessment, defines which exposure pathways might bring the contaminants in contact with people. The final step, risk characterization, brings information from the first three steps together to determine the potential severity of health threats from the site.

Figure 3.4 indicates the basic steps of the Superfund site evaluation process and the role of risk assessment in that process. As indicated, the detailed risk assessment evaluation occurs at the remedial investigation/feasibility study (RI/FS) stage in the process. However, a screening-level risk assessment occurs earlier in the hazard ranking system (HRS) scoring.

The HRS analysis uses specific assessment principles to determine whether conditions at a site warrant placing the site on the National Priorities List (NPL) and using federal funds to continue with the rest of the site evaluation process.

Most of the formal risk management activities occur after a site is placed on the NPL. These activities:

- Help develop preliminary remediation goals during project scoping and their modification during the feasibility study (FS).
- Develop the baseline risk assessment during the remediation investigation (RI).
- Evaluate the effectiveness of remedial alternatives in the FS report; in the Record of Decision (ROD) to relate target cleanup concentrations to health risks; and during remedial action to monitor progress toward "acceptable risk."

The baseline risk assessment of the RI is the central risk evaluation activity in the Superfund program and is a four-step risk assessment paradigm involving data evaluation, exposure assessment, toxicity assessment, and risk characterization.

To initiate the baseline risk assessment of the RI, information on site history and data gathered during the pre-remedial program or a recent site visit are assembled and used to

guide the remedial investigation. A critical step is identifying contaminants of significant toxicity and all exposure pathways of concern. In addition to the media paths (soil, air, and drinking water), other paths such as the eating of contaminated food or recreation may be important. Knowing toxicity and exposures to be evaluated leads directly to a sampling strategy [e.g., identifying "hot spots," gathering sufficient data for reasonable maximum exposure (RME) determinations], to appropriate analytical methods (e.g., requesting special analytical services for detection at low concentration), and to related data quality objectives (DQOs) for sampling and analysis.

The exposure assessment looks for pathways of exposure to particular individuals on or near the site, since the activities of the people determine the exposure. The baseline risk assessment considers both present exposures and those that might result from current or probable future land use.

What is determined is the RME. This is to be the highest exposure that is reasonably expected to occur at a site, considering land use, intake variables, and pathway combinations. The intent is to estimate a conservative exposure case that is still within the range of possible exposures. The result is that people at or near sites will be protected, but cleanups will not be driven by assumed exposures outside the range of foreseeable possibility.

Toxicity assessments are the next step in the process and are done using available information and the expertise of toxicologists. These are weight-of-evidence classifications. EPA has standardized this approach in a five-class grouping. In general, multiple well-designed studies, studies showing adverse effects in several species of animals, and evidence of adverse effects in humans provide greater weight-of-evidence information.

Once the exposure and toxicity assessments are complete, the risk assessor must characterize the risks. This step is crucial for communicating both to individuals who must decide what actions to take at a site and to those who may live at or near the site.

Excess lifetime carcinogen risks to individuals of less than 10^{-4} (1 in 10,000) do not require remedial actions at Superfund sites. However, actions may be taken to reduce risks below 10^{-4}. If the baseline risk assessment shows risks greater than 10^{-4} to individuals, then initial cleanup target concentrations corresponding to 10^{-6} risk are chosen.

Remedial alternatives are evaluated against nine criteria (Table 3.7). The remedy selected in the ROD is the one that best satisfies the criteria. Every ROD should, at a minimum, identify the contaminants posing risks, target concentrations for cleanup, points of compliance for cleanup in each medium, and the risks that will remain after completion of the remedy if cleanup goals are achieved.

TABLE 3.7 Criteria Used to Determine Superfund Site Remediation Alternatives

- Overall protection of human health and the environment
- Compliance with other regulations
- Long-term effectiveness and permanence
- Reduction of toxicity, mobility, or volume
- Short-term effectiveness
- Implementability
- Cost
- State acceptance
- Community acceptance

Source: Adapted from U.S. EPA (1991b).

Every attempt is made to have a Superfund risk assessment and the remediation decision-making process be straightforward and progress logically. In practice, each site is unique, data are incomplete, uncertainties can be large, and professional judgment and interpretation are involved. However, the risk assessment evaluation is an extremely important part of the decision process and is the key to environmentally sound and protective site-specific decisions and subsequent management decisions.

The incorporation of risk assessment in Superfund decisions represents the most standardized environmental engineering application of the process. However, in the past decade, there have been an increasing number of real-world situations in which environmental engineers have been involved in risk-based management decisions to protect human health and the environment. In doing so, increasing use has been made of the RBCA approach and methodology.

3.3.3 Risk-Based Corrective Action

The RBCA approach takes the risk assessment methodology and applies it to situations needing evaluation. RBCA is a standardized approach for developing remediation strategies and has gained wide acceptance from regulatory agencies. It identifies the types of risks to human health and the environment at the sites and allows the types of corrective actions considered and implemented to be commensurate with those risks.

The RBCA process integrates components of site assessment, risk assessment, risk management, and remediation into a holistic site-specific approach that is consistent and technically defensible while still being practical and cost-effective. The details of the process and examples are available in several ASTM guides (ASTM, 1995, 1999).

There are some who view RBCA as an approach to avoid remediating a contaminated site. Based on experience with the use of RBCA, and with the overview provided by regulatory agencies, that is not the case. Rather, RBCA provides the following to determine site-specific effective and protective risk-based management approaches: (1) flexible framework, (2) procedural consistency, (3) a classification scheme that helps focus efforts and direct the type and urgency of response, and (4) a tiered approach that provides an increasingly site-specific assessment where site conditions warrant. It also is resource effective by focusing resources toward assessment and remedial measures on sites, exposure pathways, and substances of significant concern, with remedial goals based on reducing risk to acceptable levels.

Sites with surface and subsurface contamination vary greatly in terms of complexity and physical and chemical characteristics and in the risks that they may pose to human health and environmental resources. The RBCA process recognizes this diversity and utilizes a tiered approach involving increasingly sophisticated levels of data collection and analysis. As needed, the conservative assumptions of an earlier tier are replaced with site-specific assumptions in later tiers. Upon completion of each tier, the user reviews the results and recommendations and decides if more site-specific analysis is required.

In Tier 1, sites are classified by the urgency of need for initial corrective action, based on information collected from historical records, visual inspections, and minimal site assessment data. The user is required to identify contaminant sources, existing environmental impacts (if any), the presence of potentially impacted humans and environmental resources (e.g., workers, residents, water bodies), and potential significant transport pathways (e.g., groundwater flow, atmospheric dispersion). Associated with site classifications

are prescribed initial response actions, if such are needed, that are to be implemented prior to proceeding further with the RBCA process.

In addition, as part of Tier 1, conservative corrective action goals are based on a list of nonsite-specific risk-based screening levels (RBSLs), aesthetic criteria, and other appropriate standards such as maximum contaminant levels (MCLs) for potable groundwater use. Tier 1 RBSLs are typically derived for standard exposure scenarios using current RME and toxicological parameters as recommended by the U.S. EPA and using conservative contaminant migration models.

Tier 2 provides the user with an option for determining site-specific target levels (SSTLs) and appropriate points of compliance when it is judged that Tier 1 corrective action goals are not adequately protective of human health and the environment or if such goals appear overly conservative for the site conditions. Both Tier 1 and Tier 2 screening levels are based on achieving similar levels of human health and environmental resource protection (e.g., 10^{-4}–10^{-6} risk levels). In moving to higher tiers, the user is able to develop more cost-effective corrective action plans because the conservative assumptions of earlier tiers are replaced with more realistic site-specific assumptions.

In some cases, Tier 2 SSTLs are derived from the same equations used to calculate Tier 1 RBSLs, except that site-specific parameters are used in the calculations rather than default values. At other sites, the Tier 2 analysis may involve applying Tier 1 RBSLs at more probable points of exposure, such as property boundaries and negotiated points of compliance, and then deriving Tier 2 corrective action goals for the contaminant source areas based on demonstrated and predicted attenuation of contaminants of concern with distance.

Tier 3 provides the user with an option for determining SSTLs and appropriate points of compliance when it is judged that Tier 2 corrective action goals are not appropriate or appear overly conservative. The major distinction between Tier 2 and Tier 3 analyses is that a Tier 3 analysis is generally a substantial incremental effort relative to Tier 2, as the analysis is much more complex and may include increased site sampling and analyses, detailed site assessments, probabilistic evaluations, and more sophisticated chemical fate/transport models.

If the selected target levels are exceeded and corrective actions are necessary, the user develops a corrective action plan in order to reduce the potential for adverse impacts. One option is to utilize traditional remediation processes to reduce contaminant concentrations below the target levels. Another option is to achieve exposure reduction (or elimination) through use of institutional controls or through the use of containment measures, such as capping and hydraulic control.

In the RBCA process, remedial action is determined to be appropriate, based on comparisons of representative concentrations to the target levels determined under the tier evaluation. This allows the project to focus only on those areas or media posing a potential threat to human health or the environment. Monitoring is conducted following a remedial action to demonstrate that target levels are met and continue to be met and to verify the assumptions and predictions that were used in any Tier 2 or Tier 3 evaluations.

The original RBCA guide (ASTM, 1995) focused on sites containing chemicals at petroleum release sites. Shortly after, additional guides that addressed collecting site characterization data and considering natural attenuation as a safe remedial alternative were prepared (ASTM, 1999). Subsequently, RBCA-type efforts have been developed for exploration and production sites (McMillen et al., 2001) and for sites with residues from manufactured gas plant (MGP) operations (Linz and Nakles, 1997).

The RBCA approach to contaminated site remediation has been adopted by many states. Such rules and guidance are an improvement from the previously overly conservative approach of requiring removal of all contaminants. Such rules and guidelines also achieve protection of human health and the environment using remedies that have a high degree of long-term effectiveness.

3.4 HISTORIC BACKGROUND

In recent decades, the ability to identify site-specific human health and ecological risks has increased considerably. In the context of the concepts being discussed (Fig. 3.1), this has increased the ability to use site-specific risk-based evaluations for remediation and management decisions.

In the same period, there has been a considerable body of new knowledge developed about the factors that affect the release, emission, transformation, and transport of chemicals that may be found at a particular site. This has been the result of research using actual field soil and sediment samples and has provided specific information about actual chemical release and about the actual environmental availability and bioavailability of chemicals in a specific soil or sediment.

This increased knowledge is particularly relevant to the human and environmental risk associated with anthropogenic hydrophobic organics such as petroleum hydrocarbons, polyaromatic hydrocarbons (PAHs), and polychlorinated biphenyl compounds (PCBs).

The weight-of-evidence information that has resulted from such research and that now is in the peer-reviewed literature indicates the following:

- Results obtained from methods used for the analysis of organic compounds in a soil or sediment do not measure the chemical availability or bioavailability of chemicals such as PAH or petroleum hydrocarbons that are in such media.
- Assuming that all extractable organic chemicals in field soils and sediments are chemically available or bioavailable overestimates the risk of such chemicals.
- Desorption of sorbed hydrocarbons is not immediate. The actual desorption that occurs can be represented by a two-step pattern that consists of an initially relatively fast release followed by slow release of the remaining hydrocarbon.
- Neglect of such desorption (release) kinetics can lead to erroneous conclusions when estimating the movement and risk of a hydrophobic hydrocarbon in a soil or sediment.
- Two analytical methods, supercritical carbon dioxide extraction (SFE) and water desorption, are available to determine the fraction of a hydrophobic organic chemical that can be desorbed (released) relatively rapidly. That "fast" fraction is known as the F value for a particular organic chemical in a field soil or sediment sample.
- The rapidly released fractions (F values) of PAH and petroleum hydrocarbons in weathered field soils can be low, with many in the 0.1–0.4 range.
- The type of organic carbon in a field sample and the weathering of spilled or released hydrocarbons affects the rapidly released fraction of such chemicals.

Relevant information about these items is included in the following sections.

3.4.1 Analytical Measurements

Methods to analyze organic chemicals have been developed to extract and measure the total concentration of an organic chemical in a sample of soil or sediment. Regulatory agencies often conservatively assume that the amount of chemical measured by common and exhaustive extraction methods is 100% chemically and bioavailable. Such is not the case. Assuming that all extractable chemicals are fully available can considerably overestimate the risk of such chemicals.

3.4.2 Weathering

Unless the chemicals in a soil or sediment have resulted from a fresh spill, the chemicals at a site of concern have undergone considerable weathering. The impact of such weathering reduces the actual risk of the chemicals at a site. Weathering refers to the result of normal biological, chemical, and physical processes that can affect the chemicals that remain at a site over time.

When a spill or release of organic chemical occurs, weathering begins to alter the composition of the released material. These weathering processes include (1) biodegradation of the most readily degradable organic chemicals, (2) volatilization of the lower boiling point organics, (3) greater sequestration or sorption of residual organics to the soil particles, and (4) leaching of water-soluble low boiling point fractions.

Thus, weathering affects the presence of industrial organic chemicals that may be at an actual site over time. As a result, the chemicals that remain at a weathered spill site will have a different distribution than those that originally entered the soil. The weathering processes also will result in a lower chemical availability and bioavailability of the remaining soil-bound organic chemicals.

3.4.3 Parameter Determination

To have consistent baseline data for important chemical availability parameters (i.e., solubility, volatility, K_{ow}, and K_{oc}), such as those in references (Lyman et al., 1982; Howard, 1990; Mackay et al., 1992; Verschueren, 1996), laboratory studies have been conducted with single pure chemicals under controlled conditions. Where it was not possible to conduct controlled laboratory experiments, estimation methods have been used to determine reasonable values for organic compounds of interest.

However, such parameters are not the best measure of what happens to organic chemicals at an actual contaminated site. Current data indicate that such parameters do not represent the actual solubility and partitioning that exist at an actual site.

3.4.4 Sorption/Desorption Kinetics

Sorption and desorption of an organic chemical in a soil or sediment are fundamental to the fate of chemicals in the environment. The parameters K_{ow} and K_{oc} are measures of the partitioning (sorption) of an organic chemical to soil or sediment particles.

Numerous studies now have shown that the desorption (release) of sorbed organics in actual soils and sediments is not immediate, but is better represented by a two-stage pattern that consists of a relatively initial fast step followed by a slower one. In addition, such desorption occurs over many days. Neglect of such sorption kinetics can lead to erroneous conclusions in estimating chemical movement and risk at a site.

TABLE 3.8 Illustrative Desorption Kinetic Data (*F* Values)—Petroleum Hydrocarbons

Source	Soil	Hydrocarbon	*F* Value
Refinery site	Sandy silt	C20	0.11
Refinery site	Sandy silt	C32	0.02
Refinery site	Sandy silt	MRO	0.09
Crude oil	Clayey	MRO	0.04
Storage site			
Industrial	Sandy silt	C10	0.40
Industrial	Sandy silt	C20	0.45
Industrial	Sandy silt	C25	0.05

MRO, mineral range organic petroleum hydrocarbons.

Many studies have been undertaken to identify and quantify the rates of organic chemical desorption that do occur in actual soils. Two parameters have been determined as important. One is the fraction that is desorbed (released) rapidly—the *F* value. The other is the rate, K_2, at which the remaining organic chemical is slowly released. *F* values for an organic chemical in a soil provide an estimate of the amount of an organic chemical that is likely to have the more immediate impact on the risk of the chemical at a specific site.

The K_2 value represents the rate at which the residual organic chemicals may be released, and be chemically and biologically available, over time. The K_2 values can be important because they can be compared to biodegradation and volatilization rates for the organic chemical in the media. If the rate of volatilization, biodegradation, or subsequent sorption is faster that the initial desorption (release) rate (K_2), the slowly released chemicals may not be transported to a receptor and may not cause an adverse health or environmental impact.

As a result of extensive studies using field soils, there are now two peer-reviewed methods to determine *F* and K_2 values. These methods are SFE and water desorption (Berg et al., 1998; Cornelissen et al., 2001; Hawthorne et al., 2001). Both methods produce comparable *F* values (Hawthorne et al., 2001, 2002). In addition, a protocol based on *F* values has been developed (Loehr et al., 2003) that can be used for a relative risk evaluation of a site that has PAH or petroleum hydrocarbon impacted soils.

Extensive desorption kinetic studies indicate that the kinetics and *F* values can be different for different organic chemicals depending on the conditions at a spill or chemical release site. Table 3.8 provides illustrative *F* values for petroleum hydrocarbons at different sites.

As noted (Table 3.8), *F* values and hence rapid hydrocarbon desorption from a soil can be very low. For such situations, the data indicate that only a small fraction (some less than 10%) of a specific petroleum hydrocarbon at a site is likely to be desorbed rapidly and have an impact on human health.

An evaluation of harbor sediments (Oen et al., 2006) provided data indicating that the actual rapidly desorbing fractions (*F* values) for several PAH in these sediments can be very low. The *F* values that were determined were less than 6% for phenanthrene, 3–19% for pyrene, and 1–12% for benzo(a)pyrene (BaP).

Both laboratory and field experience indicate that the following factors influence actual F values for PAH and other hydrophobic hydrocarbons in soils and sediments:

Spill or release conditions: Hydrocarbons at a fresh spill or release site have had less opportunity to sorb to soil particles and will have higher F values.

Weathering: Residual hydrocarbons at weathered hydrocarbon spill or release sites are more tightly sorbed to site organic matter and will have lower F values.

Available carbon: Hydrocarbons sorbed to anthropogenic carbon (soot, combustion products, charcoal, coal, or coke products) have lower F values than hydrocarbons sorbed to carbon in natural organic matter.

3.4.5 Type of Carbon

The various types of carbon, particularly anthropogenic carbon, that can be in a soil or sediment at a site play an important role in determining the chemical and biological availability of hydrophobic organic compounds of concern. Such hydrocarbons are more tightly sorbed to anthropogenic carbon, such as charred wood particles and combustion products such as soot, than to natural plant-based organic carbon (Bucheli and Gustafsson, 2000; Jonker and Koelmans, 2002; Hawthorne and Miller, 2003; Hong et al., 2003). Jonker et al. (2005) studied the release of PAH from anthropogenic carbon and found very slow PAH desorption. Their results implied reduced environmental risk for PAH associated with such carbon.

These noted differences can be quite important to site-specific risk-based decisions since anthropogenic carbon can be a predominant form of carbon at industrial and other sites of concern where risk-based site management decisions may be needed. These differences also cast doubt on current risk assessment procedures and environmental quality standards for PAH and other hydrophobic hydrocarbons.

Recognition of such differences has resulted in a remedial approach that includes the possible addition of activated carbon to a site of concern (Zimmerman et al., 2005). The added anthropogenic carbon, when mixed with the soil or sediment needing attention, sorbs the hydrocarbons of concern, making them less easily released, thus reducing site risks to humans and ecosystems.

3.4.6 Partition Coefficients

Studies with field soils have developed experimentally measured K_{oc} values and soil–water partitioning coefficients, K_d ($K_d = K_{oc} \times f_{oc}$), where f_{oc} is the organic carbon fraction in the soil. Results have indicated that the experimentally measured K_{oc} and K_d values in field soil samples can be significantly greater than such values noted in reference books or estimated from chemical relationships noted in such references.

In one study, the K_{oc} values determined from analyzing field soil samples were 35–250 times higher than the respective reference predictions (Bucheli and Gustafsson, 2000). In another study (Dondelle and Loehr, 2002), K_{oc} values determined from field soil samples were from 2 to over 200 times higher than the respective reference predictions.

Such results were confirmed (Hawthorne et al., 2006) in an extensive evaluation of PAH partition coefficients in 114 contaminated sediments. The K_{oc} values for the PAH in these sediments were considerably greater than typical literature K_{oc} values. The authors concluded that using PAH K_{oc} values derived from spiked sediments and from existing predictive models can greatly overestimate the actual PAH partitioning from sediments into the overlying water.

These large differences indicate that PAH and petroleum hydrocarbons in actual field soils are more tightly sorbed than would be expected from K_{oc} and K_{ow} found in references. Thus, soil and sediment site-specific K_d or K_{oc} values should be obtained and used to identify actual human and ecological risks at a site and to identify the need for remediation actions at field sites of concern.

3.5 INTEGRATED ASSESSMENTS

Several evaluations have attempted to assess comprehensive aspects of chemical availability and bioavailability using field samples. In one study (Salanitro et al., 1997), the extent of hydrocarbon degradation, earthworm and plant toxicity, and chemical availability of soils containing crude oil was evaluated. The samples were silt loam soils containing 0.3 and 4.7% petroleum hydrocarbon (4000–27,000 mg/kg measured as total petroleum hydrocarbons (TPH). Bioremediation after 3–4 months resulted in 50–75% and 10–90% loss of the initial TPH for the two samples, respectively. Even though considerable TPH remained in the bioremediated soils, the bioremediated soils were not toxic to earthworms nor inhibited seed germination.

The results indicated that the remaining petroleum hydrocarbons, which ranged from 1000 to 8600 mg/kg as TPH, were tightly bound to the soils. In addition, these residual hydrocarbons were not able to be degraded further and were not susceptible to leaching.

A comprehensive study of the availability of PAH in soils at a site in California has occurred (Stroo et al., 2000). Site samples were subjected to a battery of physical and biological tests that focused on determining the availability of soil-bound PAH. The results demonstrated that sorption of the PAH to the soil particles, weathering, and biological treatment significantly reduced PAH chemical availability and bioavailability. When the reduced site-specific availability results were included in risk assessment calculations, the potential site risk was considerably less than that determined using California risk assessment default assumptions.

Additional evaluation of field samples from the above California site (Stroo et al., 2005b) demonstrated that the dermal and ingestion absorption factors for the PAH in those samples were far lower than factors that resulted from using default assumptions in common risk assessments. Using sample-specific absorption factors in standard risk assessment equations increased the required risk-based cleanup levels by an average factor of 72 with a range of from 23 to 142. These results indicated that, at this site, higher concentrations of the PAH could be left in the site soil. It was stated that a tiered evaluation protocol using site-specific chemical analyses, chemical release data, and *in vitro* bioassays should be used to establish realistic site-specific risk management criteria.

A more specific example of the value of site-specific data, rather than default assumptions, was obtained when the chemical bioavailability of BaP in industrial site soil was evaluated (Stroo et al., 2005a). Results indicated that the site-specific BaP dermal absorption factors were 14–107 times lower than the regulatory default assumption factors. In terms of a site-specific risk assessment, this resulted in the calculated excess cancer risk being reduced by 97% on average.

From the ecological standpoint, field data have indicated that, at sediment sites, toxicity to aquatic organisms is not related to the concentration of total measured PAH in the sediment. Instead, such toxicity is correlated to the concentration of the easily released and bioavailable PAH. It also has been noted (Cornelissen and Gustafsson, 2005) that the strong

sorption of hydrophobic organic compounds to anthropogenic black carbon is the cause of the widely different biota to sediment accumulation factors (BASFs) that occur at different sites. Such recent information reinforces the desirability of obtaining and using site-specific chemical availability data at a soil or sediment site of concern to identify human health and ecological risks.

3.6 SUMMARY

The concept of environmental risk and its use for environmental decision making and site remediation selection is now an important aspect of environmental engineering activities. The use of environmental risk for decision making and treatment or remedy selection has resulted from the recognition that (1) not all environmental problems are equally serious, (2) the resources for environmental protection are not limitless, and (3) the focus should be on sites and situations that pose the greatest risk to human health and the environment.

In the past decades, there has been considerable progress improving the knowledge related to all parts of the risk assessment process. There now is better scientific knowledge about pharmacological bioavailability that can be used for human health and ecological assessments. Equally important is the increased knowledge that has been developed about chemical release from contaminated sites, about chemical environmental availability, about chemical environmental bioavailability, and about chemical transport and transformations at real-world sites.

Such information, when used with a tiered RBCA-type framework, allows environmental engineers, regulators, and the concerned public to determine environmentally sound and protective site management and remediation decisions. In addition, scientific and field information has made it increasingly clear that site-specific data should be used for risk-based decision making, rather than depending solely on default assumptions and parameters.

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4

CLINICAL PERSPECTIVE ON RESPIRATORY TOXICOLOGY

Daniel P. Croft, Mark J. Utell, and Jonathan M. Samet

The chapters in this volume describe the adverse effects of diverse inhaled agents on the lung and, to a lesser extent, on other target organs. These effects have been characterized through multidisciplinary approaches, typically involving *in vitro* and *in vivo* laboratory studies, and often controlled human exposures and epidemiological investigations as well. The resulting evidence on adverse effects may have substantial societal impact through regulatory and nonregulatory pathways intended to reduce exposures and protect public health. However, exposed individuals sustain the associated risk and disease burden. In this chapter, we address the extension of this research to the diagnosis and management of environmentally related disease in individual patients and in exposed groups.

The care of individuals falls to the practicing physician and/or other health-care providers. Most often, the physician addresses the concerns that a patient raises about the consequences of exposure or manages the disease that may have resulted from, or been exacerbated by, exposure. The physician may also become enmeshed in legal questions as to the causation of disease in their patients and to compensation for disease that has occurred. In this chapter, we consider the clinical approach to the individual who has been exposed to a respiratory hazard. We review the tools available to the health-care provider with the purpose of describing clinical methods and their strengths and limitations in answering the frequently complex questions that arise around environmental exposures.

The evaluation of exposed populations, for example, specific worker groups, may also fall to health-care providers. However, to address the effects of exposure on populations, the approach must extend beyond simply evaluating individuals to providing measures of impact on groups. Epidemiological studies, typically cross-sectional surveys, are often conducted to evaluate potential adverse respiratory or other effects. In this chapter, we consider the tools used to conduct such surveys. Although a survey might be performed in response to recognition of a perceived hazard, persons exposed to potential or known hazards may be subject to ongoing surveillance, often required in the workplace. We also

Environmental Toxicants: Human Exposures and Their Health Effects, Fourth Edition.
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review surveillance methodology. Of necessity, our treatment of these subjects is limited. Textbooks and journals on environmental medicine are available (Rom and Markowitz, 2007; Rom and Reibman, 2015). Additionally a series of case studies has been published by The Institute of Medicine (1995).

4.1 CONCEPTS OF EXPOSURE

The concept of exposure underlies consideration of the risks associated with environmental toxicants and their management. Figure 4.1 presents a general schema of exposure that is integrative in setting out sources and their drivers, time–activity patterns of people, and the exposures that result. Moving through the diagram, the leftmost side sets out sources of exposures, including the upstream drivers of exposures, that is, factors determining the types of sources, their locations, the intensity of exposures, and the control measures used. Construed broadly, the upstream drivers reach across the range of social determinants of health; for example, in many urban locales, industrial sources are located more densely in poorer neighborhoods as are heavily trafficked roadways, and older inner-city housing often has unmitigated lead paint.

Moving to the right, contact with exposure and the intensity of exposure is driven by what is released into the environment and how people interact with the environment, as determined by time–activity patterns, that is, the places where people spend time and the amount of time spent in these locations. The term "microenvironment" refers to locations where exposures are consistent during the time of exposure, for example, the air pollution within a home, vehicle, or office. Pollution concentrations in some locations may be temporally and spatially dynamic, such as on the sidewalks of busy streets where stalled traffic may result in "hot spots" that may be cleared by wind.

The concept of exposure (the diagram's middle) has been broadened to encompass contact of people with agents, whether external or internally incorporated. As captured by the diagram, environmental agents may enter the body, may be deposited in the lungs, may be translocated to other organs, and may interact with critical receptors that drive processes leading to injury, ill health, and disease. Here, the dose refers to the materials reaching the

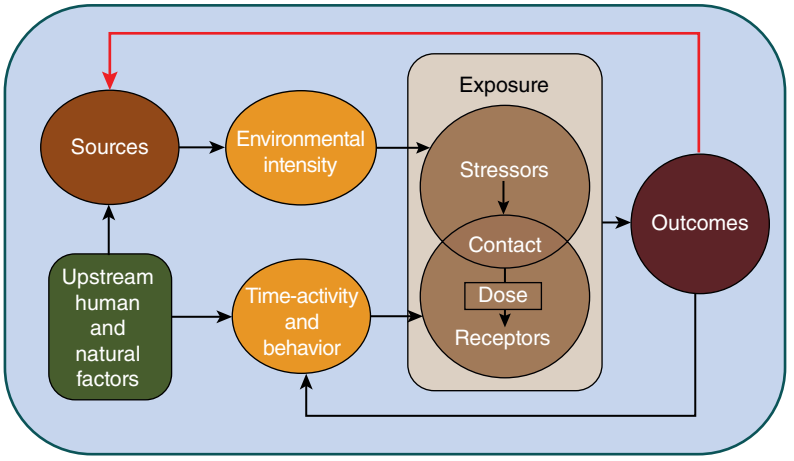

FIGURE 4.1 Drivers of exposures and outcomes.

targeted receptors. Some stressors act more generally, such as psychological stress, which may affect health through the multiple mechanisms underlying the stress response. At the far right, "outcomes" refer to the consequences of pollutant exposures, comprising a broad array of adverse effects, some transitory, for example, exacerbation of asthma or increased coughing, and some long-term and irreversible, for example, development of chronic pulmonary disease or lung cancer.

The "exposome," a broadening of the concept of exposure, is the cumulative measure of environmental influences and biological responses throughout the lifespan (Rappaport, 2011; Miller and Jones 2013; Mallon et al., 2019). The exposome encompasses all environmental, dietary, microbiome, behavioral, therapeutic, and endogenous processes experienced cumulatively throughout life.

Patterns of time use and activity place people in diverse indoor and outdoor microenvironments throughout the day. Personal exposure to air pollutants represents the time-weighted average of pollutant concentrations in microenvironments, that is, environments having relatively homogeneous air quality (Sexton and Ryan, 1988), with each microenvironment possibly having its own unique set of air contaminants. The characteristics of these microenvironments, and the time spent in them, vary with age, sex, and other sociodemographic factors. Diverse microenvironments that may be relevant for health include spaces within the home, workplace, schools, outdoors, transportation environments, and locations where recreational time is spent.

The clinician's approach to assessing exposure in these microenvironments primarily involves interviewing the patient and sometimes the family. Standardized instruments for collecting information on environmental and occupational exposures have been published, but clinicians generally take exposure histories in idiosyncratic ways, the completeness of the history reflecting the clinician's training, fund of knowledge, and familiarity with the microenvironments of concern to specific patients (Lax et al., 1998). The clinical history of exposures may touch on a few widely known hazards, for example, asbestos, but rarely inventories the duties of specific jobs, the materials handled, or the use of respiratory protection. Most physicians have limited knowledge of the exposures associated with specific occupations and, more broadly, of the different microenvironments where people spend time.

In recent decades, medical surveillance programs have been enhanced with biomonitoring applications. Since the 1990s, the U.S. Centers for Disease Control and Prevention (CDC) have measured blood biomarkers of exposure for more than 300 environmental chemicals and nutritional indicators in non-occupationally exposed populations in the United States as part of the National Health and Nutrition Examination Survey (NHANES) (CDC, 2019). The chemicals selected for measurement are found in air pollution, pesticides, plastics (such as bisphenol A), and flame retardants. Trends observed over time allow for assessment of changing patterns of exposure and differences by region and demographic factors. NHANES measured 308 chemicals in a cross section of the U.S. population, but it is estimated there are between 25,000 and 85,000 chemicals in production today (CDC, 2019; Mallon et al., 2019). Thus, the ability to assess biologically relevant dose may not be possible for most chemicals in production today. However, with newly advanced chemical profiling techniques, the technology to identify and monitor environmental exposures has dramatically improved.

Biomarkers of exposure indicate exposure to a chemical on the basis of its measurement in a biologic fluid or tissue. Biological markers of exposure are available on a routine clinical basis for only a few inhaled pollutants, including carboxyhemoglobin for carbon

monoxide, and blood or urine lead (Pb) level for inhaled lead (National Research Council, 1989, 2012). Levels of other toxicants can be measured in blood or other samples, if needed, for example, measurement of dioxins in blood or blood lipids, pesticides in blood, mercury in hair, benzene in exhaled breath, cotinine in saliva, and cadmium in urine. In itself, quantification of a specific biomarker in a biologic material establishes only that the chemical is present in the body. If the substance is not otherwise known to be endogenous, an exposure from the outside environment can be inferred. The clinical significance of the exposure depends on factors such as its metabolism and clearance, dose, individual susceptibility, and the health status of the individual (National Research Council, 2006).

Serum antibodies or skin test reactivity to intradermal antigen injection can be measured to provide an indication of past exposure and the development of sensitivity to selected antigens; tests are available for some common biological antigens, such as the house dust mite, but for only a few inhaled chemicals. Routine clinical tests for adducts, and for antibodies to adducts, are not yet available, nor are tests readily available for most intermediate endpoints that may be relevant from a toxicological perspective.

However, the routine clinical evaluation does not usually provide comprehensive or specific information concerning environmental exposures. In fact, the routine history taken by a primary care provider typically addresses only tobacco smoking and the current or usual employment; some common exposures with well-known consequences, such as asbestos, may also be covered.

Often, however, it is the identification of a disease known to be caused by a specific agent, for example, asbestosis, that prompts full questioning of the patient concerning relevant exposures, and thus, routine medical records do not usually offer any more than a superficial assessment of inhalation exposures. Alternatively, the occurrence of an uncommon syndrome or a clustering of cases may result in more complete evaluation.

A physician trained in environmental or occupational medicine routinely obtains more detailed and disease-relevant information; a physician with this training should be consulted in cases involving possible effects of complex environmental exposures. Physicians trained in pulmonary medicine may also have special expertise related to environmental lung disease, and allergists may be appropriate for addressing workplace-related allergic disorders. In evaluating the chest X-ray for the pneumoconioses, fibrotic disorders of the lung resulting from dust exposure, the chest X-ray may be interpreted according to a standard system, the International Labour Office (ILO, 1980) classification. The “B reader” certification uses this system and is given by the National Institute for Occupational Safety and Health (NIOSH) to persons who have taken a 2-day course and successfully passed an examination involving standardized interpretation of chest roentgenographs showing various occupational lung diseases. This certification reflects competency in interpreting patterns of radiographic abnormality, but does not specifically establish expertise in occupational lung disease.

4.2 TOOLS FOR STUDYING INDIVIDUALS

4.2.1 Overview

Environmental diseases frequently masquerade as common medical disorders. From a clinical perspective, primary care physicians underdiagnose environmentally and occupationally related disorders and far too infrequently enter them into their differential diagnosis.

For example, more than 80% of occupational or environmental disease diagnoses had not been correctly recognized prior to evaluation in an occupational medicine clinic, although most patients had consulted one or more physicians (Cullen and Cherniack, 1989). The inadequate knowledge base of primary care physicians in environmental medicine (Institute of Medicine, 1988) and the physician shortages in these specialized disciplines have been emphasized (Castorina and Rosenstock, 1990). It is unclear whether or not the projected increase in the training of physicians will meet future demand (USDHHS, 2006b). However, with increased state and federal support, Children's Centers of Excellence in Environmental Health and Occupational and Environmental Medicine Clinics were established in a number of U.S. communities. These facilities serve as resources both for more comprehensive investigation of potentially exposed children and for enhanced physician training resulting in earlier recognition of environmentally mediated diseases.

In considering environmentally induced lung diseases, it is important to recognize that symptoms and signs of lung damage are seldom indicative of the specific injuring agent. For the clinician, the recognition of occupational or environmental respiratory illness may be obscured by the nonspecificity of symptoms, findings on physical examination, alterations of pulmonary function, or radiographic changes. Lung biopsy is rarely indicated to confirm a diagnosis of environmentally related disease, and even when tissue is available, pathological findings may be nonspecific and not indicate a specific etiologic agent. For example, airway inflammation may reflect the consequence of a myriad of inhaled agents. Specific links between exposure and disease, such as beryllium exposure and non-caseating granulomas, are few. Rarely, specific diagnostic fibers or dusts may be identified in lung tissue. In addition, exposures are often multiple or mixed, symptoms may be minimal until disease is advanced or more severe than anticipated for the extent of physiological and radiographic abnormality, and latency periods between onset of exposure and disease development may be long. Identifying occupationally related lung disease is further complicated by the frequent overlay of cigarette smoking and the possibility of additive or even synergistic effects acting in combination with other agents (e.g., asbestos-related lung tumors); thus, the association between lung disease and occupational exposure is often neither obvious nor simple in a particular patient.

The clinician is now called on to deal not only with the more classical occupational diseases, like the pneumoconioses, but also the more problematic "environmental" illnesses of the past several decades. For example, as new construction techniques and ventilation practices were directed at conserving energy and sealed buildings began to age, outbreaks of nonspecific complaints characterized by persistent respiratory symptoms, headaches, and lassitude began to occur among office as well as industrial New York workers, who attributed their symptoms to the indoor environments where they worked (Samet et al., 1988). Now referred to as "sick building syndrome," these outbreaks continue—but seemingly in smaller numbers than previously. Even more perplexing is the appearance of illness in previously healthy individuals who, following accidental exposure to solvents, gases, or irritating fumes, develop unrelenting respiratory complaints, lethargy, and central nervous system dysfunction when subsequently exposed to trace amounts of the material or other irritants such as fumes or cigarette smoke. This symptom complex has been labeled "multiple chemical sensitivities (MCS)" (Cullen, 1987), and it poses substantial diagnostic and therapeutic difficulty. We have the general impression that this syndrome has become less common both as a research topic and as a clinical presentation. Another environmental concern is symptoms and disease resulting from exposure to mold in homes, particularly related to water damage. The flooding in New Orleans, New York

TABLE 4.1 Tools for Evaluating Individuals with Suspected Occupational or Environmental Lung Disease

General evaluation
Medical and respiratory history
Detailed occupational history
Identification of materials
Identification of toxic responses
Chest X-ray
Special studies
Immunologic and skin tests
Airway hyperreactivity testing
CT scan, PET scan
Fiber-optic bronchoscopy with bronchoalveolar lavage
Industrial hygiene information
Site or plant visit
Epidemiological investigation

City, and Houston as a result of catastrophic hurricanes has served to reinforce the potential for widespread and chronic exposure to mold. Exposure to wildfire smoke is also more common and severe than previously, particularly in the Western United States where the frequency and severity of fires is increasing. The smoke from large wildfires travels widely, leading to concern about its consequences, particularly for those with chronic respiratory diseases (Swiston et al., 2008; Gerardi and Kellerman, 2014). These environmental exposures through natural disasters will likely continue to increase in frequency and severity as global climate change worsens. In this section, we focus on the tools available to the clinician for evaluating the individual patient suspected of having an environmentally related pulmonary disorder. The emphasis on respiratory disorders is highly relevant: respiratory diseases are the most frequently diagnosed work-related conditions in industrial regions, and the best-established medical consequences of mining and farming involve the lower respiratory tract. Also, the airway is most important portal of entry for toxic agents from the environment. New technologies, such as nanotechnology, will introduce new agents into the environment and workplace with potential respiratory threats to the individual as well as larger populations. The tools commonly available for evaluating the individual are shown in Table 4.1; the general principles also apply to the evaluation of environmentally related non-respiratory disorders.

4.2.2 Clinical Approaches

For the physician, the central problem in environmental lung medicine is determining that a respiratory symptom or particular structural or functional derangement of the lung is caused by a certain inhaled agent. Table 4.2 lists examples of the pathophysiological responses of the respiratory tract to airborne particles and gases. As with all disorders associated with the environment, a careful and thorough history is mandatory. Both the work and home environments need to be considered for exposure to known allergens, irritants, chemicals, or organic dusts. Careful inquiry is necessary concerning not only the materials the individual is working with but also those being used by coworkers. The occurrence of similar problems in coworkers also should be assessed. Other clues in the history are useful. With occupational asthma, there is often a latent period between the first exposure to

TABLE 4.2 Pathophysiological Responses of Respiratory Tract to Occupational and Environmental Particles and Gases

Site Response	Agent	Potential
Nose	Pollen	Hay fever, rhinitis
	Formaldehyde	Nasal cancer
Airways	Sulfur dioxide, nitrogen dioxide	Bronchoconstriction
	High- and low-molecular-weight chemicals	Asthma
	Aeroallergens	Asthma
	Formaldehyde, wood smoke	Irritation, cough
	Radon, asbestos	Cancer
Parenchyma	Inorganic dusts	Pneumoconiosis
	Thermophilic actinomycetes, fungi	Hypersensitivity pneumonitis

the offending agent and the onset of asthma (Tarlo and Lemiere, 2014). This period may vary from a few weeks to over 20 years. Therefore, the physician attempts to correlate temporally respiratory and/or systemic symptoms with exposure in a particular environment, although varying temporal relationships between exposure and outcome may cloud interpretation of the clinical history. Thus, in some instances, the causal relationship between exposure and symptoms may not be readily recognized. Correlation of symptom occurrence with cumulative exposure may be difficult in cases of late-onset, delayed, or repetitive patterns of response or in those responses in which cough, chest tightness, or malaise predominates. This contrasts with the clinical picture of a worker who develops symptoms immediately and repeatedly when working with a particular substance. Rarely, following a catastrophic event such as occurred with the attack on the World Trade Center (WTC) on September 11, 2001, an inhalation exposure to a "pollutant mix" may cause immediate symptoms and potentially more chronic respiratory and possibly gastrointestinal diseases (Lippmann, 2015); the evaluation of both the acute and more chronic symptoms in an individual will likely be initiated by a primary care practitioner; if chronic symptoms are recognized in a significant subgroup, this subpopulation may undergo more intensive surveillance (Herbert et al., 2006). For example, the surveillance of the group of WTC workers over time revealed an increased incidence rate of sarcoidosis, an inflammatory disorder of the lung resulting in granuloma formation (Webber et al., 2017).

The initial care of individual patients exposed to potentially toxic materials usually falls to the practicing primary care physician or other health care providers. Typically, the exposure history obtained by such practitioners is limited and only characterizes the workplace qualitatively as "dusty" or "extensive smoke and fumes." Major effort should be extended to accurately identify the specific toxic agent or agents present in each work site. This may require direct contact with employers and/or assistance from governmental agencies such as NIOSH or the Occupational Safety and Health Administration (OSHA) for information. Some efforts have been focused on development of occupational/environmental history forms to assist the clinician in collecting the database (Occupational and Environmental Health Committee of the American Lung Association of San Diego and Imperial Counties, 1983; Kilbourne and Weiner, 1990; Agency for Toxic Substances and Disease Registry, 1993).

The clinically significant problems of sick building syndrome and MCS may be readily recognized if the patient presents with an unmistakable clinical picture (Brightman and Moss, 2000; Spengler et al., 2001). The clinician is faced with an individual with a myriad of

complaints, often including intractable upper respiratory symptoms, ill-defined central nervous system dysfunction, headache, fatigue, and low productivity that are attributed to odors, poor ventilation, or other aspects of a poorly ventilated building. Despite a careful physical evaluation, there may be no physical signs revealed on examination. In outbreaks with an identified etiology, a spectrum of causative agents has been identified: infectious agents, specific air contaminants, and environmental conditions such as temperature and humidity (Finnegan and Pickering, 1986; American Thoracic Society, 1997). Outbreaks without an identifiable etiology have frequently occurred in tight buildings and have given rise to the "sick building syndrome." However, these outbreaks are by no means limited to tight buildings; they may occur because of maintenance problems, changing uses, and other factors.

An even more puzzling syndrome clinically and historically is MCS, in which the affected individual becomes "sensitized" to almost all organic and synthetic chemicals (Cullen, 1987; Ashford and Miller, 1991). The history is typically that of a healthy individual who after accidental exposure to a solvent or irritant gas or fumes develops progressive symptoms with exposure to traces of the original agent or a variety of nonspecific agents. The assessment lies in the historical data, as the remainder of the evaluation is quite unremarkable. Many hypotheses have now been examined, and there are few known extrinsic causes of MCS supported by research, while diverse mechanisms have been proposed ranging from specific sensitization to broader psychogenic processes. Immunologic abnormalities now appear to be a less plausible cause (Simon et al., 1993). One early suggestion was that the clinician is dealing with a variant of a posttraumatic stress disorder (Schottenfeld and Cullen, 1986) and case reports have revealed a probable role of conditioned response, especially to odor. Although no well-controlled studies establish a clear mechanism, the MCS patient often requires comprehensive evaluation and compassionate support. Despite early calls to clarify the diagnosis of MCS by several major medical societies including the American College of Physicians (1989), the American Medical Association, Council on Scientific Affairs (1992), and the American College of Occupational and Environmental Medicine (1999), the etiology, diagnosis, and epidemiology of MCS are still debated decades later (Rossi and Pitidis, 2018).

Physical examination is often unrevealing in cases of environmental lung disease, even when the requisite full evaluation of the nose, oropharynx, and chest and lungs is completed. Abnormal lung sounds such as bibasilar rales (crackling sounds) are heard with interstitial lung disease, as in asbestosis, which results from asbestos exposure. Signs of airway disease, such as wheezing and cough (including examination upon forced exhalation), are typically associated with environmental asthma. However, neither a normal examination nor one in which wheezing persists even after avoidance of the work environment excludes environmental airway disease from the diagnostic possibilities. Furthermore, conditions that can mimic the presentation of environmental asthma such as vocal cord dysfunction can require referral to colleagues in otolaryngology and a dedicated treatment course (Dunn et al., 2015). As the diagnostic possibilities are evaluated, there can occasionally be a need to systematically correlate symptoms and lung function levels with activities and exposure in the work environment and at home. There are no pathognomonic findings with sick building syndrome or MCS.

4.2.3 Imaging Studies

Since the clinician is likely to obtain a chest X-ray in the evaluation of any individual with respiratory complaints, it is worth emphasizing that radiological examination remains a major diagnostic tool for revealing occupationally induced interstitial lung disease. The

principal disorders amenable to radiological diagnosis are the pneumoconioses, which include asbestosis, coal workers' pneumoconiosis (CWP), silicosis, and berylliosis.

The chest X-ray in asbestos exposure may serve to identify biological markers of exposure or evidence of disease providing documentation of mineral fiber exposure of the parenchyma and/or pleural reactions. Workers exposed to relatively low concentrations of asbestos fibers may develop a variety of pleural processes including effusions and calcified plaques; the pleural changes serve as markers of asbestos exposure and rarely are associated with symptoms or functional changes (Craighead and Mossman, 1982). In contrast, workers exposed to high concentrations of asbestos fibers may develop interstitial infiltrates, primarily in the lower lung fields, characterized by bilateral small irregular parenchymal opacities. Persons with this pattern also typically have reduced lung volumes.

Workers exposed to other mineral dusts, such as silica, and/or coal-mine dust typically demonstrate bilateral upper lobe interstitial infiltrates (Fraser et al., 1994). These infiltrates characteristically are nodular in appearance; the nodules range in size from 1 to 10 mm, may coalesce, and eventually distort or cause retraction in the hilar region. It should be emphasized that the diagnosis of pneumoconiosis in the absence of an abnormal chest radiograph is highly unusual. The rare patient in this category, symptomatic but with a normal chest X-ray, would require additional supportive data to substantiate the diagnosis.

For the clinician evaluating the patient with suspected environmental or occupational asthma, the conventional chest X-ray may not be of great diagnostic help. Often it is normal or reveals only minimal hyperinflation. The patient with hypersensitivity pneumonitis will often demonstrate diffuse infiltrates on the radiograph. Finally, occupationally related pulmonary malignancies present with the various X-ray patterns of lung cancers associated with smoking. The radiographic changes in various occupational lung diseases have been described in detail (Fraser et al., 1994). But even with a radiographic pattern specific for an occupational lung disease, there continues to be debate regarding the radiographic criteria for diagnosis. For example, an American Thoracic Society (ATS) committee in 1986 concluded that chest roentgenographic evidence of small irregular opacifications of a profusion of 1/1 or greater (a level of classification in the ILO system that implies certainty in the presence of pneumoconiosis) was required to diagnose asbestosis (American Thoracic Society, 1986), whereas the 2004 ATS committee concluded that profusion 1/0 (a lesser degree of certainty) was adequate (American Thoracic Society, 2004).

More sophisticated imaging techniques used in clinical practice, such as computed tomography (CT), are being used increasingly for research and surveillance, but may be useful in the evaluation of specific chest X-ray abnormalities. In environmental medicine, the CT scan is an important modality in locating and characterizing small lesions that could represent early lung tumors, and CT screening has now been shown to lower lung cancer mortality in higher risk older smokers. Due to the sensitivity of CT scans in detecting nonpathologic lesions along with cancerous lesions, the risks of false positive results from lung cancer screening are an important part of shared decision-making conversations with patients (Tanoue et al., 2015). The CT scan has been useful in assessing the location, extent, and potential for surgical resection of small lung lesions and masses. In addition, the CT scan has been useful in confirming the presence of pleural plaques noted on chest X-ray; it also raises the possibility that there are large groups of persons with pleural disease undetectable on standard radiographs (Friedman et al., 1988). High-resolution chest CT (HRCT) can detect small pneumoconiotic opacities or abnormal lung tissue associated with diffuse forms of interstitial fibrosis not visible on plain chest X-ray (Aberle et al., 1988). Schwartz

et al. (1990) observed that among asbestos-exposed patients with normal parenchyma on plain chest X-ray, patients with pleural disease are more likely than those with normal pleura to have interstitial abnormalities on the HRCT. Prospective controlled evaluations are ongoing to determine the prognostic significance of these abnormalities on HRCT among exposed persons with normal-looking parenchyma by chest X-ray.

4.2.4 Pulmonary Function Testing

Tests of pulmonary function are useful for evaluating the physiologic consequences of lung damage caused by inhaled materials. In evaluating individual patients with respiratory complaints or with potentially toxic exposures, physicians obtain pulmonary function tests. In fact, failure to obtain such studies almost invariably represents inadequate clinical evaluation. Guidelines for characteristics of pulmonary function equipment, test interpretation, and quality assurance are available (*European Respiratory Journal*, 2005).

The clinical utility of physiological function testing in individual patients lies in finding evidence of response to inhaled toxic agents. The tests can show the functional manifestations of structural changes in the respiratory system, whether the changes are reversible or transient (e.g., asthma or reflex bronchoconstriction) or permanent (e.g., fibrosis). However, these tests have little specificity for effects of specific environmental agents. The lung responds to injury in a limited number of ways, and the physiological manifestation of various types of injury is often the same, regardless of the causative agent. Thus, abnormal lung function tests are a general, rather than a specific, indication of injury. One type of testing, inhalation challenge with specific agents may lead to more specific findings.

The real workhorse in the evaluation of the individual patient is simple spirometry, a test that can be readily performed in the field or office setting. The spirometric tracing, which describes the rate of forced exhalation, distinguishes the obstructive pattern from restrictive patterns of physiologic impairment. Detailed reviews of the use of pulmonary function testing in evaluation of the patient with occupational lung disease are available (McKay and Lockey, 1991; Redlich et al., 2014). In brief, the restrictive pattern, typical of interstitial lung disease, is suggested by a forced vital capacity (FVC) <80% predicted and $FEV_1/FVC > 75\%$. In contrast, the obstructive pattern, typical of airway disease, includes an FVC > 80% predicted, $FEV_1 < 80\%$ predicted, and $FEV_1/FVC < 75\%$. In addition, the maximum voluntary ventilation (MVV) is usually reduced below 80% predicted. Predicted values take into account patient age, height, and sex. Although the MVV is a valuable test when properly performed, it requires considerable patient cooperation and effort. A reduction in the forced expiratory flow from 25 to 75% of FVC, $FEF_{25–75}$, below 70% predicted with otherwise normal flow rates is a marker of early but mild airway obstruction.

The restrictive pattern is the hallmark of interstitial lung diseases, such as asbestosis, but is not specific, and can result from such diverse processes as large pleural effusions, heart failure, muscle weakness, and even marked obesity. In the presence of significant obstructive airway disease, as in smoking-caused chronic obstructive pulmonary disease (COPD), it can be difficult to interpret the spirogram for restrictive changes unless these are extremely severe, since obstructive disease may also reduce the FVC. In this case, more sophisticated measurements of lung volumes using body plethysmography or helium dilution techniques may be necessary. Additionally, imaging can be informative. Restrictive disease is classically defined by a reduced total lung capacity below 80% predicted. A reduction in the diffusing capacity for carbon monoxide may be valuable in supporting a diagnosis of interstitial lung disease.

Although cigarette smoking is the most common cause of the obstructive pattern, a history of smoking should not preclude careful investigation into occupational exposures as contributing if not causal factors for physiological impairment. All too often, an abnormality in lung function is attributed to cigarettes to the exclusion of other potential agents. On the contrary, some of the impairment in persons with occupational lung disease may be due to smoking. Smokers are usually diagnosed with chronic obstructive respiratory diseases when the level of airflow limitation interferes with activities that they would otherwise be able to perform (Speizer and Tager, 1979). There is a continuum between health and respiratory disease. Disability typically occurs when the FEV_1 approaches 40–50% of the predicted value. Pulmonary clinicians consider the reduction of FEV_1 below 80% of the predicted value or below the 95% confidence interval as indicative of obstructive disease (Pellegrino et al., 2005); as the reduction in the FEV_1 progresses, the severity of disease increases.

In the evaluation of potential occupational or environmental asthma, it is important to objectively establish the relationship of exposure to symptom occurrence and reduction of lung function. The serial recording of peak expiratory flow rates (PEFR) with a mini peak flow meter by the patient at work or at home may be a valuable method of establishing a causal relationship. These devices are inexpensive and sufficiently accurate if used properly. The serial measurement of lung function generally provides stronger information on the effect of exposure than one or two spirometric measurements spaced over time. In following the PEFR, the individual typically measures and records the value every 2–4 h from waking to sleeping. A record is maintained for several workweeks, often followed by at least 1 week away from any work exposure. Although criteria for a positive response have not been well established, the clinician is seeking to find a pattern of exposure followed by symptoms and a change in PEFR. Improvement in lung function and reduction in symptoms during the week away from work are also helpful in confirming a diagnosis. A major concern with PEFR measurement is the effort dependence of the test, such that inadequate respiratory effort can lead to misleading data. Also, adherence in comprehensively recording data is an issue, though the advent of mobile phone applications for asthma self-management has the potential to mitigate this problem (Tinschert et al., 2017). Recognizing its limitations, the inclusion of serial home PEFR measurements in the diagnostic workup of occupational asthma has been incorporated into several guidelines (including the American College of Chest Physicians) (Moore et al., 2009).

Specialized testing, including an assessment of airway hyperreactivity, may be needed to evaluate environmental asthma. Nonspecific airway hyperreactivity is defined as an exaggerated bronchoconstrictor response to a variety of chemical, physical, and pharmacological stimuli. There is now nearly a consensus that nonspecific airway hyperreactivity is a characteristic shared by virtually all asthmatics (Boushey et al., 1980; National Institutes of Health, 1997); that is, the asthmatic develops bronchoconstriction after inhaling a lower concentration of a provoking agent than is needed to cause a similar degree of change in airway tone in a healthy subject.

In the laboratory, airway reactivity testing is divided into two general categories, depending on the choice of nonspecific versus specific agents. In both, the increased bronchoconstrictor response is assessed with pulmonary function tests. Nonspecific stimuli include pharmacological agents such as methacholine and histamine, exercise, hyperpnea with cold or dry air, and inhalation of hypertonic or hypotonic aerosols. Although pharmacological challenge is used most often in the clinical laboratory, it is less suitable for population studies, especially those involving children because of safety considerations.

Cold-air challenge with hyperventilation has been used effectively, and response to cold air is generally correlated closely with methacholine responses (O'Byrne et al., 1982). Challenge with specific agents, common antigens, chemicals such as isocyanates, and organic materials such as plicatic acid (from western red cedar) can be used to identify specific sensitizing agents. These approaches can be particularly powerful in incriminating occupational chemicals and confirming the diagnosis of occupation-related airway disease; challenge may provoke immediate and/or late pulmonary responses that do not resolve spontaneously. Even with specific agents, the interpretation of responses can be difficult and be confounded by a variety of factors, such as dose and irritant effects (McKay, 1986).

In the assessment of asthma induced by occupational agents, airway reactivity testing with nonspecific and specific agents serves a diagnostic function (Eisen et al., 1993). Tarlo and Lemiere (2014), among many others, have published comprehensive reviews on the subject of occupational asthma and the role of airway reactivity testing. Nonspecific airway hyperreactivity occurs in most workers with occupationally induced asthma despite their not having the usual factors such as atopy. Furthermore, Lam et al. (1983) found a good correlation between the degree of nonspecific bronchial hyperreactivity and the severity of response to the provoking agent, plicatic acid in workers with red cedar asthma. Measurement of hyperreactivity also assists in providing objective evidence of sensitization. The demonstration of an increase in bronchial reactivity on returning to the workplace and a decrease away from work, with appropriate changes in lung function, is evidence for a causal relationship between symptoms and the work environment. To pinpoint the etiological agent in the workplace responsible for asthma, specific challenge may be necessary. Such testing can be dangerous and should be performed only by experienced persons in a hospital setting for the following conditions: studying previously unrecognized occupational asthma, determining the precise etiological agent in a complex industrial environment, and confirming a diagnosis for medicolegal purposes. Detailed guidelines and testing procedures have been developed and published (Pepys and Hutchcroft, 1975; Salvaggio and Hendrick, 1989). In adults presenting with new-onset asthma, sensitizer- or irritant-induced asthma from an occupational source should be considered, diagnosed, and treated promptly to optimize patient outcomes (Tarlo and Lemiere, 2014).

4.2.5 Special Laboratory Investigations

In a vast majority of patients, a diagnosis of environmental or occupationally related lung disease will be made through careful history and physical exam performed in conjunction with pulmonary function tests and a chest X-ray. The clinician rarely proceeds to specialized laboratory testing. In selected cases, however, additional supportive information can be obtained from skin testing with appropriate extracts, particularly of high-molecular-weight compounds for specific agents responsible for occupational asthma. Skin tests are generally accepted for IgE-mediated protein allergens. For example, extracts from flour (Block et al., 1983) and animal products produce immediate positive reaction on skin testing in sensitized subjects. Unfortunately, these skin tests are not always available. There is no established value for skin tests against chemicals such as formaldehyde or cigarette smoke. This contrasts with more routine allergy skin tests with common inhalants and food allergens that are used to define the atopic status of the individual.

Antigen-specific IgE antibody testing can be done with RAST or by ELISA for occupational asthma. Specific IgE antibodies against low-molecular-weight compounds conjugated to a protein (e.g., isocyanate) have been demonstrated in some exposed

individuals (Butcher et al., 1980). Again, such tests are not readily available, and a positive response may occur in exposed workers without asthma. It is not appropriate to obtain antibodies to evaluate symptoms resulting from a single exposure.

Serum precipitin testing can be done for hypersensitivity pneumonitis. The precipitins can be detected in farmer's lung disease with antibodies to the thermophilic actinomyces, especially *Micropolyspora faeni*, or in pigeon breeders with antibodies to various proteins contained in pigeon serum or pigeon-dropping extracts. Office workers may develop hypersensitivity from fungal contaminants in ventilation systems; exposures have been reported to thermophilic actinomyces and penicillium species. Fungal exposure is common to other occupations and built environments, particularly those with water damage (Green, 2017).

Several studies have examined the role of bronchoalveolar lavage (BAL) in occupational lung diseases (Rom et al., 1987). Workers with farmer's lung demonstrate a predominantly suppressor T-cell lymphocytic alveolitis (Cormier et al., 1986), and workers with chronic beryllium disease show a T-helper-cell lymphocytosis (Epstein et al., 1982), whereas workers with asbestosis demonstrate an increase in alveolar macrophages with or without a modest increase in neutrophils (depending on the smoking status) (Rom et al., 1987). Not only can the cellular differentials provide a clue to the underlying disorder, but also the asbestos fibers in the lavage fluid can be quantified. Although the utility of these research techniques in patient evaluation and diagnosis remains largely investigational, BAL may have diagnostic and prognostic implications. For example, BAL fluid has been studied in chronic beryllium disease. Beryllium is a rare alkaline earth metal that is typically aerosolized during the manufacturing process of advanced technology sectors including aerospace and electronics (Balmes et al., 2014). The mechanism of chronic beryllium disease involves immune sensitization via induction of post-translational modifications in human leukocyte antigen (HLA) complexes, which then leads to a $CD4^+$ T-cell-mediated granulomatous reaction in the lung (Fontenot et al., 2016). The extent of BAL cellularity, lymphocytosis, and beryllium lymphocyte proliferation test response parallels disease severity (Newman et al., 1994), suggesting that the magnitude of the inflammatory and antigenic response in the lung may help predict disease progression or response to therapy. The depth of evidence for the toxicity of beryllium has led to support by both labor and industry groups to reduce exposure by revising the beryllium standard (Samet, 2018).

The application of molecular biology tools to examine susceptibility to toxicants is happening at an accelerated rate. Molecular approaches using arrays to analyze changes in gene expression are being applied to cells (e.g., macrophages and blood monocytes) removed from humans in exposed populations, and following controlled exposure to pollutants. Clinical studies are also incorporating analyses to identify genetic polymorphisms that make a person particularly susceptible to air pollutants. In humans, one of the candidate genes most implicated in air pollution responses is $GSTM_1$, an important enzyme in the glutathione pathway for protection against oxidant injury (Peden, 2005). $GSTM_1$ has a null allele with no protein expression, which leads to reduction in antioxidant protection and is present in 40% of the population of the United States. Children with the $GSTM_1$ null allele have reduced lung function growth and increased susceptibility to ambient ozone (Romieu et al., 2004), while in adults the $GSTM_1$ polymorphism may play a role in enhancing the nasal IgE response to diesel exhaust particle exposure (Gilliland et al., 2004). When a group convened by the International Agency for Research on Cancer (IARC) met in 2013, $GSTM_1$ among other genetic polymorphisms were highlighted due to their association with an increase in cytotoxic and DNA damage from ambient air pollution (Loomis et al., 2013). With continued advances in genomics and proteomics, the possibility of unraveling susceptibility at the population level becomes increasingly realistic.

Similarly, an increasing number of molecular methods have become available to study exposure biomarkers. The different omics techniques have been touted for their potential power in exposure assessment, epidemiology, and personalized medicine. Potentially useful applications of each method are being identified and evaluated. For example, genomics is the analysis of data derived from DNA sequencing and can be used to look for single nucleotide polymorphisms (SNP) that identify genetic predispositions to diseases caused by environmental exposures. Proteomics is the study of proteins in the host to examine molecular events after transcription has occurred. Environmental chemicals may bind to serum proteins forming protein adducts. Metabolomics is the study of the human metabolome, defined as the chemical profile of all low-molecular-weight compounds in a biological specimen, and includes endogenous metabolites, chemicals from human–environment interaction, and reactants arising from interaction of these compounds with enzymatic and bacterial processes occurring within the body. High-resolution metabolomics studies provide evidence of thousands of unidentified chemicals in human plasma. Biomarkers in human specimens can therefore provide measures of metabolites from a variety of sources including environmental chemicals. These approaches have been used, for example, to examine potential burn pit exposures sustained by the military (Mallon et al., 2016, 2019)

4.2.6 Exposure Assessments

For the clinician, insufficient information on exposure data often limits establishing a causal relationship between exposure and disease. Especially in large industries, industrial hygiene information may be available that provides quantitative estimates of dusts, vapors, or aerosols in the work environment. An understanding of the pathophysiology of environmentally induced lung disorders requires a working knowledge of the mechanisms involved in the uptake of gases, the deposition of particles, and their subsequent retention. In addition, in relating clinical disease to specific gaseous exposures, the clinician must recognize that penetration into and retention within the respiratory tract of toxic gases can vary widely depend on a number of factors: the physical properties of the gas (e.g., solubility), the concentration of the gas in the expired air, the rate of depth of ventilation, and the extent to which the material is reactive (Utell and Samet, 1990). Likewise, the aerodynamic properties of particles, airway anatomy, and breathing pattern largely determine the sites of deposition of particles and fibers in the airways. Thus, the identification and characterization of the inhaled materials are essential in linking inhaled materials with specific types of lung damage.

Patterns of time use and activity place individuals in diverse indoor and outdoor microenvironments throughout the day, each microenvironment having its own unique set of air contaminants. Perhaps because of the distinct sources contaminating outdoor air and indoor air and the separate regulatory mechanisms for outdoor air, the workplace, and the home, the health effects of inhaled toxicants have often been addressed separately for outdoor and indoor air.

However, the concept of total personal exposure is most relevant for health; personal exposure to air pollution represents the time-weighted average of pollutant concentrations in microenvironments having relatively homogeneous air quality. Thus, for an office worker, relevant microenvironments might include those at home, office, car, outdoors at work, and a movie theater. For some chemically reactive pollutants, such as ozone or acid aerosols, outdoor environments make the predominant contribution to total personal

exposure; for others, such as radon and formaldehyde, indoor locations are most important. In considering the health consequences of environmental toxicants, the physician should recognize the potential contributions of various pollution sources.

In the workplace, the safety officer or industrial hygienist can be very helpful in reviewing exposures and assisting in interpreting the results. In the investigation of building-related illnesses, indoor contaminants such as formaldehyde, environmental tobacco smoke, and microorganisms as well as the adequacy of the ventilation system need to be assessed. In special situations, a visit to the work or environmental site may be crucial in assisting the physician to better understand the potential exposure or even the adequacy of the ventilation system. Occasionally intensive investigation of the individual will lead to a recommendation for a population study as a result of introduction of new materials or issues of "safe" levels in the workplace.

4.3 TOOLS FOR STUDYING POPULATIONS

4.3.1 Overview

Persons may be exposed to inhaled pollutants in a variety of locations, including outdoors, workplaces, and other indoor microenvironments. Typically, epidemiological investigations of health effects are initiated because of concern about the consequences of exposure in a particular location, for example, outdoors or the workplace. For example, many studies of asbestos-exposed workers were undertaken after adverse consequences of exposure to this agent first became apparent. Studies of outdoor air pollution have been implemented in areas with unusual localized patterns of exposure, for example, effluents from petrochemical or other manufacturing plants, or with regional patterns of high outdoor pollution levels, for example, photochemical or acidic aerosol pollution. Similarly, groups have been targeted because of particular exposures indoors.

Epidemiological studies may also be implemented because of concern about the contribution of inhaled pollutants to particular diseases, both malignant and nonmalignant. For example, lung cancer increased in epidemic fashion among males in many countries beginning in the 1940s. Many of the initial investigations focused on exposure to outdoor air pollution in addition to cigarette smoking, which was subsequently found to be the cause of most cases. In the 1980s and 1990s, the contribution of indoor air pollutants, particularly secondhand tobacco smoke and radon, to lung cancer, was an area of active investigation. Now, secondhand smoke is established as a cause of lung cancer (USDHHS, 2006a), and the risks of radon have been well characterized (Darby et al., 2005; Krewski et al., 2006).

In most places in the developed world, although some individuals may be sufficiently impacted by inhaled pollutants to have clinically evident effects, the anticipated effects of most current exposures of concern on the population as a whole are likely to be subtler and not detectable by routine clinical assessment. However, epidemiological assessment of populations may provide evidence for adverse effects, and the finding of such effects may become a basis for regulations or legal actions. Comparisons of exposed with non-exposed populations, or of more highly exposed with less exposed populations, are made to find evidence of adverse effects that may not be manifest on evaluation of single individuals. Epidemiology comprises the methods used to study the effect of exposure on the population. Of the complementary approaches used to study inhaled pollutants, laboratory studies,

clinical studies, and epidemiological studies, only the results of epidemiological studies provide evidence of the effects of agents as exposures occur in the community. Epidemiologic data may span the full range of susceptibility and inherently incorporate the interaction among agents.

Surveillance refers to ongoing systematic collection of data on health and disease (Baker et al., 1989). Programs for health surveillance may be implemented to monitor the consequences of intervention programs or to identify "sentinel" cases signaling unacceptable exposures (Rutstein et al., 1983). Exposures to hazards can also be monitored (Froines et al., 1989). Surveillance approaches have been most widely used for monitoring the occurrence of occupational diseases; surveillance may be implemented within populations or within exposed workforces. Data sources on populations include reporting by clinicians and studies of death certificates, cancer registries, workers' compensation systems, hospital discharge records, employer reports, and special surveys (Baker et al., 1989; Freund et al., 1989; Melius et al., 1989). Large industries have implemented their own surveillance systems.

A report on silicosis illustrates the application of a surveillance system in a defined population. Valiante and Rosenman (1989) implemented a surveillance system for silicosis in the state of New Jersey; death certificates, hospital discharge data, and physicians were the principal sources for case ascertainment. For the period 1979 through 1987, 401 cases were identified, primarily by screening of hospital discharge data. Only one case was reported by a physician, and most hospitals did not voluntarily report their cases in spite of state requirements. Follow-up inspections at industries where cases had occurred identified inadequate control of exposure to crystalline silica at most. This system had the purpose of finding all cases of this fully preventable disease.

Another example emphasizing the necessity of disease surveillance is CWP (black lung disease). Although miners were long recognized as having a shorter life expectancy than other professions for several centuries, CWP became increasingly recognized as caused by inhaled coal dust particles at the turn of the twentieth century (Martin, 1954). The diagnosis and classification of CWP has evolved into two main forms based on symptoms, lung function, and imaging findings, namely, simple CWP and complicated CWP, which includes the most severe manifestation, progressive massive fibrosis (PMF) (Perret et al., 2017). Disturbingly, the United States has experienced an abruptly increased rate in the diagnosis of PMF in miners (particularly in Appalachian states) after 1996, a trend thought to be related to either changes in mining practices or possibly increased silica exposure (Almberg et al., 2018). While silicosis from silica exposure is technically a distinct entity from CWP, mixed dust exposures with high concentrations of respirable silica may be one of the drivers of the increasing prevalence of PMF (Cohen et al., 2016). Without ongoing epidemiologic surveillance of miners, this concerning trend may have been missed. As both CWP and silicosis are preventable diseases, improvement in regulations and research on regulatory impacts on health is needed.

There is also now a call for "accountability research," referring to studies that track the consequences of regulations and other measures intended to protect public health (Health Effects Institute, 2003). Such research might be considered as a form of surveillance with focus on tracking such indicators as reductions in emissions or gains in health indicators. In Southern California, the Children's Health Study has tracked community-based cohorts of children in multiple communities for more than two decades; as air pollution has declined in Southern California, those children growing up in cleaner air have greater lung growth and lower rates of respiratory symptoms (Gauderman et al., 2015; Urman et al., 2018).

4.3.2 Epidemiological Approaches

Conventional epidemiological approaches used to study the adverse effects of inhaled pollutants on the lung are the cross-sectional study, the cohort study, and the case–control study (Table 4.3). More recently, the case-crossover design has been increasingly used for assessing the short-term risk of an exposure in relation to the occurrence of discrete events. These designs have the exposed individual as the unit of observation. Ecological designs, also used to study the environment and health, have groups of individuals, for example, communities or even countries, as one unit of observation. Multilevel designs incorporate elements of both the ecologic studies and the individual-level designs. Each design has advantages and disadvantages for examining the effects of environmental exposures.

Ecological study designs have long been used to investigate the health effects of air pollution. Cross-sectional studies have compared the health characteristics or rates of disease occurrence in communities having differing levels of exposure to air pollution (Lave and Seskin, 2013). Time-series designs have also received widespread application (Bell et al., 2004). In these ecological studies, temporal associations between air pollution levels and disease measures are evaluated. For example, a series of studies have assessed daily concentrations of air pollution levels with mortality counts and morbidity measures (U.S. EPA, 2004; U.S. EPA, 2018, under review). Initially, these studies involved data from single cities, but advances in computing and statistical analysis methods now make possible the analysis of data from multiple cities. In the National Morbidity, Mortality, and Air Pollution Study (NMMAPS), the investigators used data from up to 100 cities to assess the relationship between airborne particles and mortality (Samet et al., 2000a, 2000b; Dominici et al., 2005). Even larger studies have now been carried out using administrative data bases.

Carrying out large epidemiological studies of ambient air pollution has been facilitated by the development of modeling approaches that provide estimates of air pollution concentrations at increasingly small geographic levels. Such models are most fully developed for airborne particulate matter (PM). They incorporate existing modeling data, demographic and land use information, and satellite data (Knibbs et al., 2018; Masiol et al., 2018).

A principal limitation of the ecologic design is the assumption that associations observed at the group level reflect effects at the individual level, the so-called ecologic fallacy. Nonetheless, the time-series studies have raised concern that current levels of air pollution in the United States and other developed countries are adversely affecting public health. Various approaches have been used to address potential confounding.

TABLE 4.3 Epidemiological Study Designs Used to Investigate the Effects of Inhaled Pollutants

Case–control study: An analytical design involving selection of diseased cases and non-diseased controls followed by assessment of past exposures
Case-crossover study: An analytical design involving the use of cases as their own controls by time-matching a window of exposure preceding and/or following occurrence of a health effect and comparing exposure in the case window with the control window(s)
Cohort study: An analytical design involving selection of exposed and non-exposed subjects with subsequent observation for disease occurrence. Short-term cohort studies of the health status of susceptible groups are often called “panel studies”
Cross-sectional study: Participants are identified, and exposure and disease status determined, at one point in time

The cross-sectional study is a generally economical and feasible approach, often used to investigate indoor and outdoor air pollution and occupational lung diseases. When this design is used to study indoor or outdoor air pollution, populations of children or adults are typically surveyed, and health status is assessed. Exposure to outdoor air pollution may be inferred from geographic location or the results of local area and limited personal monitoring; in studies of indoor air pollution, exposure may be categorized by the presence of sources or by monitoring the indoors. For example, in the Harvard Six Cities Study of air pollution, outdoor exposures were inferred from the city of residence and centrally sited pollutant monitors, and indoor exposures were classified by sources and monitoring for some specific pollutants (Dockery et al., 1989; Neas et al., 1991). Personal monitoring was used to assess the contributions of indoor and outdoor pollutants to total personal exposures of the subjects (Spengler et al., 1985).

The cross-sectional design is also widely used to evaluate the effects of occupational agents on the lung. In a typical cross-sectional study or survey, employed workers receive an assessment that includes a standardized respiratory symptom questionnaire, spirometry, chest radiograph, and often a limited physical examination. Exposure classification may be based on job title or duties, length of employment, reported duration of exposure to materials of interest, or a cumulative exposure measure calculated from measured or presumed concentrations of an agent and length of time at each level of exposure. For example, Samet et al. (1984) conducted a respiratory survey of long-term underground uranium miners. The study population was recruited by sampling from employed miners at two large companies. Increasing duration of underground employment, used as an index of exposure to potentially hazardous agents underground, for example, silica, radon, and blasting fumes, was associated with lower level of midmaximal expiratory flow. The prevalence of an abnormal chest radiograph compatible with silicosis increased with longer duration worked underground.

The cross-sectional study, although one of the most economical and feasible designs, is subject to potentially significant methodological limitations. Estimates of the effects of exposure may be biased by the tendency of more susceptible or more affected persons to reduce their level of exposure, for example, by leaving an industry or polluted area. For example, in the respiratory health survey of uranium miners reported by Samet et al. (1984), long-term miners who had already developed disease may have retired or even died, leaving a population less susceptible to develop silicosis or lung cancer. This type of selection bias is likely to be most prominent for agents with immediate effects on susceptible person; thus, asthmatic persons are likely to leave jobs involving exposures that worsen their disease. The temporal relationship between exposure and disease may be obscured or misrepresented in cross-sectional data because exposure and disease are assessed at only one point in time.

Cohort and case–control studies, which establish the proper sequence between exposure and disease, are also used to investigate the effects of inhaled pollutants on the lung. Cohort studies represent the optimal approach for assessing the effects of rare and special exposures, such as inhalation of toxic gases or exposure to asbestos, if a group of exposed workers can be assembled and followed. Cohort studies are termed "prospective" if the disease events will occur in the future, after the study population is established, and "retrospective" if disease events have already occurred when the cohort is assembled and follow-up is historical. The cohort design has the advantages of permitting direct estimation of disease incidence or mortality rates for exposed and non-exposed persons and of prospectively accumulating comprehensive data on exposure. The retrospective cohort design,

often applied to occupational groups, can sometimes be used to quickly assess the effects of a pollutant, since exposure and disease have already taken place when the investigation is initiated. The principal disadvantages of the cohort design include potentially high costs and losses of cohort members to follow-up.

Use of the cohort design is well illustrated by the many studies of mortality from cancer, asbestosis, and other causes of death in asbestos-exposed workers. For example, in a well-known study, Selikoff and colleagues described the mortality experience of 17,800 members of the insulation workers' union in the United States (Selikoff et al., 1979). The cohort members were active in the union on January 1, 1967; follow-up of mortality was described through 1976 in the 1979 publication. Mortality in the cohort was compared to U.S. death rates for white males. Overall, 2271 deaths were observed with 1659 expected; cancer of the lung occurred with a fivefold excess, and 49 deaths from mesothelioma were identified. In this study, comparison of the mortality of the exposed population (asbestos workers) with the unexposed population (U.S. white males) provided strong evidence for associations of asbestos exposure with excess mortality from several causes of death. Other notable cohort studies have assessed air pollution and mortality: the Harvard Six Cities Study (Dockery et al., 1993; Laden et al., 2006) and the American Cancer Society's Cancer Prevention II Study (Pope et al., 1995, 2002; Thurston and Lippmann, 2015). More recently, large national administrative databases, for example, that for Medicare, have been used to create cohorts to investigate the health consequences of air pollution; modeling techniques are used to estimate pollution concentrations at residence locations, and follow-up is accomplished through linkage approaches (Di et al., 2017).

The case–control study, like the cohort study, provides a measure of association between exposure and disease. This design has been widely used for studying lung cancer and occupational and environmental agents but infrequently for nonmalignant respiratory diseases. In comparison with the cohort study, the case–control study has the advantages of generally lower cost, greater feasibility, and usually a shorter time frame. The case–control study is the optimum approach for studying uncommon diseases. The results of this design may be limited by bias in assessing exposure or by bias in the method used to select cases and controls.

The application of the case–control approach can also be illustrated by studies of the effects of asbestos. Analyses of geographic patterns of lung cancer mortality for the United States showed the highest rates along the coastal regions, particularly in the Southeast (Blot and Fraumeni, 1976). A series of case–control studies were conducted to assess occupational and other factors potentially contributing to the excess lung cancer (Vineis et al., 1988). Cases were identified through hospitals and death certificates, and controls were sampled from persons admitted to the same hospitals with diseases other than lung cancer, from death certificate files, or from the general population.

More recently, the case–control approach has been widely used to explore the genetic basis of disease. In so-called genome-wide association studies (GWAS), SNPs are compared across the chromosomes of people with a disease of interest and appropriate controls without the disease (Bush and Moore, 2012). The case–control approach has provided a relatively rapid and informative evaluation of potential determinants of susceptibility to environmental agents.

The results of each type of study may be affected by biases, which alter the relationship between exposure to an inhaled agent and the health effect of concern; bias may increase or decrease the strength of an association. The three principal types of bias are selection bias, misclassification bias, and confounding bias.

Selection bias refers to distortion of the exposure–outcome relationship by differential patterns of subject participation depending on exposure and disease status. For example, subjects with airway hyperresponsiveness might be more likely to withdraw from occupational cohorts exposed to respiratory irritants.

Error in measuring either pollutant exposure or the health outcome results in misclassification. If the error equally affects cases and controls in a case–control study or exposed and non-exposed subjects in a cohort study, the bias reduces associations toward the null value, that is, no effect of exposure. Such non-differential or random misclassification is of concern in most studies of inhaled pollutants and the lung; pollutant exposures are generally estimated using limited measurements or surrogates, such as presence of sources or duration of employment. Statistical power, the ability of a study to detect exposure–disease associations, declines as the degree of random misclassification increases (Shy et al., 1978; Gladen and Rogan, 1979). For example, Lubin et al. (1990) estimated sample sizes needed for case–control studies of indoor radon and lung cancer. As the degree of measurement error increased from the implausible level of 0% to more plausible levels above 50%, statistical power declined well below the desired level of 80–90%.

If misclassification is differential (varying with case or control status in a case–control study or with exposure status in a cohort study), then the bias may increase or decrease the strength of association. Differential misclassification is of particular concern in case–control studies using interviews to assess exposure. Information obtained from persons with and without a disease may not have comparable validity. For example, in comparison with controls, persons with lung cancer might minimize the extent of prior smoking or better recall occupational exposures such as asbestos.

Bias from confounding results when the effect of the exposure of interest is altered by another risk factor. For example, confounding by cigarette smoking would occur in a cohort study of asbestos workers if smoking differed between exposed and non-exposed subjects. In fact, in studies of inhaled pollutants, particularly those with weak effects, confounding can be controlled through matching exposed and non-exposed subjects on potential confounding factors or through collection of data on potential confounding factors and use of appropriate data analysis methods. Specific limitations of accountability studies can include a lack of suitable comparison control populations and difficulty in controlling for time trends, among other study-specific potential biases (Rich, 2017).

4.3.3 Exposure Assessment

Diverse approaches are used in epidemiological studies to assess exposure to inhaled agents (Table 4.4). Some approaches can be used feasibly and at low cost in large populations, whereas others require intensive and costly personal or biological monitoring. The simplest approaches, such as using job title in an occupational study or residence location in a study of outdoor air pollution, are likely to be subject to the greatest degree of misclassification. Misclassification is least if personal exposures are directly monitored. However, accurately assessing an individual's exposure over time and location is quite difficult. Efforts to include personal exposure monitors in non-occupational health studies began around 1980 (Wallace and Ott, 1982). The technology of personal exposure monitoring has improved greatly over the past 30 years and is being paired with global positioning system (GPS) information to help with spatiotemporal resolution of exposure (Steinle et al., 2013). As personal monitoring improves, study methods will also need to improve to accurately account for the comprehensive burden of subjects' exposures across their everyday life

TABLE 4.4 Assessment of Inhaled Pollutant Exposure in Epidemiological Studies

Occupational Agents	Outdoor Air Pollutants	Indoor Air Pollutants
Job title and industry	Residence location	Source inventory
Length of employment	Proximity to sources	Indoor monitoring
Self-reported exposure	Self-reported exposure	Personal monitoring
Job exposure matrices	Central site monitoring	Biological monitoring
Area monitoring	Small-area monitoring	
Personal monitoring	Personal monitoring	
Biological monitoring	Biological monitoring	

environments (Dons et al., 2017). Even with improved acute exposure monitoring, the study of cumulative non-occupational exposures that contribute to chronic diseases like COPD will remain a challenge.

Biomonitoring or biologic monitoring of tissues such as blood, urine, and breast milk is an extremely valuable tool for identifying population exposures to a variety of chemicals. Biological monitoring with exposure markers that can be measured inexpensively and non-invasively is feasible in large populations for only a few pollutants, for example, carbon monoxide, lead, and nicotine. For example, the CDC has completed four reports, findings summarized in the "National Report on Human Exposure to Environmental Chemicals," giving concentrations of chemicals and metabolites in blood and urine of a representative sample of the U.S. population. The 2019 updated tables to the fourth report (CDC, 2019) include exposure information on 346 chemicals in a cohort of 2500 individuals. By highlighting the specific chemicals (and concentrations) that U.S. residents are exposed to, clinicians and scientists can improve patient care.

Tiered approaches for exposure assessment have been proposed (Leaderer et al., 1986). Nesting more intensive monitoring approaches for small numbers of subjects selected from a larger study population permits estimation of the misclassification resulting from use of less intense but feasible approaches.

For air pollution, modeling strategies may be used that incorporate information on air pollution sources and traffic-generated pollutant exposures. Models may also incorporate measured pollution data. These methods have become increasingly sophisticated, and contemporary models incorporate terms based on land use, satellite data, and actual measurements. Models have now been developed that span the globe and that are increasingly detailed spatially (Knibbs et al., 2018; Masiol et al., 2019).

4.3.4 Outcome Assessment

The range of adverse health effects caused by inhaled pollutants is wide, extending from excess mortality to subtle effects on function or symptom occurrence (Table 4.5) (American Thoracic Society, 2000; Pope and Dockery, 2006; Cohen et al., 2017). Studies of morbidity most often examine symptom and disease occurrence and level of lung function. Standardized methods have been developed for collecting data on symptoms and for assessing lung function and airway responsiveness (Ferris, 1978; Samet, 1989; Sparrow and Weiss, 1989). The ATS has published standardized respiratory symptom questionnaires, which are currently under revision, for children and for adults (Ferris, 1978). The questionnaires emphasize chronic respiratory symptoms and conditions and are not appropriate

TABLE 4.5 Health Outcomes in Epidemiological Studies of Inhaled Pollutants

Occupational agents
Mortality, specific or all causes
Occurrence of specific diseases
Respiratory symptoms
Reduced lung function
Abnormality on chest radiograph
Increased airway responsiveness
Immunologic sensitization
Outdoor and indoor air pollutants
Mortality, specific or all causes
Hospitalization
Emergency room or other outpatient visit
Absenteeism
Disease occurrence
Respiratory symptoms
Reduced lung function
Increased airway responsiveness
Immunologic sensitization

instruments for investigations of acute responses to inhaled pollutants. Questionnaires specific to the investigation of asthma are available (Burney and Chinn, 1987; Asher et al., 1995; Cloutier et al. 2012). Recommendations for spirometric testing cover the characteristics of the equipment, procedures for testing, and data acceptability and interpretation (American Thoracic Society, 1995; European Respiratory Journal, 2005).

In studying the pneumoconioses, occupational lung diseases caused by inhalation of inorganic dusts of low solubility, chest radiographs are usually classified according to the system of the ILO, most recently revised in 1980 (International Labour Office, 1980). This system classifies abnormalities of the lung parenchyma on the basis of size and extent and changes in the pleura. The system was designed to provide a uniform method for coding changes on chest radiographs and thus to facilitate comparisons across regions and over time. In the United States, training is offered in the use of this system by the American College of Radiology; those successfully passing an examination in its use are designated as "B readers" by the NIOSH. Even the use of a standardized classification and trained film readers may not eliminate strong observer bias in X-ray interpretation (Ducatman et al., 1988; Parker et al., 1989) especially if one considers the B readings utilized in asbestos litigation (Gitlin et al., 2004).

4.4 CARDIOVASCULAR RESPONSES

Although the respiratory system generally has long been considered the major target of inhaled materials, more recently it is demonstrated that for several inhaled agents, the lung serves primarily as a conduit. This has especially been the case for inhaled PM, where epidemiological studies provide convincing evidence that exposure to increasing mass-based concentrations of ambient PM triggers adverse cardiac events (Samet et al., 2000a, 2000b). Literally hundreds of epidemiological studies linking PM with adverse cardiovascular outcomes have been published over the past decade. Clinical studies on the cardiovascular

effects of air pollution and PM were the basis for an increased focus on this area (Utell et al., 2002). Brook et al. (2017) review the significant progress made in delineating the role of air pollution in cardiovascular disease, including proposed mechanistic pathways. Epidemiologic studies provide insight into where further mechanistic research should focus. For example, a study of adult cardiac patients observed an association of acute increases in $PM_{2.5}$ concentrations over the prior hour with ST elevation myocardial infarction (STEMI) but not the less severe non-ST elevation myocardial infarction (NSTEMI) (Gardner et al., 2014). The mechanism of air pollution's associations with cardiac disease, including myocardial infarction (MI), is an area of active research.

Exposure to fine PM ≤ 2.5 μm ($PM_{2.5}$) in ambient air is associated with increased mortality and morbidity related to cardiovascular disease. An increase in PM_{10} concentration of 50 $\mu g/m^3$ was associated with a 3–8% increase in relative risk of death (U.S. Environmental Protection Agency, 2006). The strongest associations were seen for respiratory and cardiac deaths, particularly among the elderly. Because deaths from cardiovascular causes outnumber those from respiratory causes, the majority of deaths are attributable to cardiovascular causes. Increased PM levels have been linked with death from MI, arrhythmia (sudden death), and congestive heart failure.

Novel noninvasive diagnostic techniques, such as electrophysiological monitoring, echocardiography, scintigraphic scanning and impedance cardiography, and implantable defibrillators with recorders, available in clinical cardiology practice have been incorporated into epidemiological, panel, and human clinical air pollution studies. For example, $PM_{2.5}$ exposure was associated with increased frequency of cardiac arrhythmias in patients with implantable defibrillators (Peters et al., 2000) and with reductions in heart rate variability (HRV) in panel studies of elderly residents (Gold et al., 2000). This observation was confirmed in a human exposure study to ozone, where adverse changes in the frequency-domain variables of HRV were observed (Arjomandi et al., 2015). These studies suggest that exposures to ambient $PM_{2.5}$ influence autonomic regulation of the heart and are associated with increased arrhythmias and MI in susceptible patients with heart disease. In addition, changes in blood viscosity, ST-segment depression, increased blood pressure, and increased circulating markers of inflammation and thrombosis have been linked with increases in $PM_{2.5}$ air pollution (Godleski, 2006).

Exposure to $PM_{2.5}$ air pollution may also have acute effects on vascular function in humans. Investigators exposed subjects to concentrated ambient $PM_{2.5}$ plus ozone and demonstrated constriction of the brachial artery of the forearm immediately after pollutant exposure (Brook et al., 2002). Inhalation of ultrafine (<50 nm) carbon particles alters blood leukocyte expression of adhesion molecules and the lung diffusing capacity in humans (Pietropaoli et al., 2004; Frampton et al., 2005). There is recent additional evidence, from both animal (Chen and Nadziejko, 2005; Sun et al., 2005) and human studies (Künzli et al., 2004), indicating potential mechanisms by which PM may worsen or even induce atherosclerosis. The Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air) study found similar evidence of accelerated atherosclerosis associated with $PM_{2.5}$ (Kaufman et al., 2016). These findings indicate that there are acute vascular effects of exposure to air pollution and provide a mechanistic link between PM exposure and cardiovascular effects in humans.

Although the cardiovascular consequences of $PM_{2.5}$ exposure have been the major focus of recent research, the effects of exposure to carbon monoxide as a result of its affinity for hemoglobin have been recognized for many decades. More recently, several reports have suggested that ozone and perhaps sulfur dioxide exposures could also be

associated with adverse cardiovascular outcomes. Future research may identify other extrapulmonary effects of inhaled toxicants as novel nontraditional approaches are applied to inhaled materials.

4.5 LIMITATIONS OF CLINICAL AND EPIDEMIOLOGICAL ASSESSMENTS OF THE EFFECTS OF INHALED AGENTS

In the twenty-first century, many respiratory hazards have been recognized and controlled in the United States and other developed countries. Concern remains, however, about the safety provided by standards for workplace and environmental exposures and the risks of new and unevaluated agents. Large segments of the population are exposed to indoor and outdoor pollutants with adverse effects, and workers expect that their jobs will not carry unacceptable risks. In response to individual and societal fears, clinicians and epidemiologists are asked to assess the effects of inhaled pollutants: the clinician to evaluate the health of exposed individuals and the epidemiologist to address the effects on exposed groups. Both types of assessments have limitations, particularly for answering concerns about safety.

Few clinicians have the proper training to evaluate patients with toxic occupational and environmental exposures. Occupational health clinicians can be broadly separated into injury care (IC), clinical specialist (CS), and management/population health (MP), though common competencies exist including understanding the U.S. Occupational Safety and Health Act (OSHA) and worker's compensation (Harber et al., 2010). While in 2006, the American College of Occupational and Environmental Medicine had roughly 6000 members (Baker et al., 2007), the aging population and longer work careers imply that a shortage of physicians remains. Most primary care providers (internists and family practitioners) lack training in toxicology and in occupational and environmental medicine. They do not have skills for characterizing exposures and making links between exposures and health outcomes; they are also unlikely to have an understanding of such key concepts as individual susceptibility, interactions among agents, and exposure–response relationships. Their skills in counseling patients concerning risks may also be limited. Furthermore, clinical methods for assessing respiratory effects may be insensitive if applied in the conventional clinical fashion. For example, pulmonary function testing may be unstandardized, and the chest radiograph may only be interpreted for gross clinical abnormalities and not for subtle changes reflective of early disease.

Epidemiology has been an extremely effective tool for investigating inhaled pollutants with either very strong (e.g., cigarette smoking and lung cancer) or very specific (e.g., asbestos and mesothelioma) effects. In investigating agents that may have effects that are not strong but of public health concern, the epidemiological studies may be limited by misclassification of exposure and disease, confounding, and other methodological problems. Large sample sizes may be needed to test for the anticipated effects, particularly if exposure estimates are subject to substantial misclassification (Lubin et al., 1990). The results of an epidemiological study cannot provide sufficient precision to exclude the possibility of some increased risk of exposure to an agent. Thus, the findings of epidemiological studies of inhaled pollutants often leave uncertainty concerning the extent of the risks posed and do not fully address the concerns and questions of exposed persons and those involved in policy and regulation.

4.6 CLIMATE CHANGE AND HEALTH

As an important driving factor to environmental illness, the negative impact of climate change on the prevalence and exacerbation of respiratory diseases is of concern (Gerardi and Kellerman, 2014). As the climate changes further, the stress on the human body and the surrounding ecosystem will continue to intensify due to increased frequency and severity of flooding, hurricanes, droughts, and other natural disasters (Hallegatte, 2014). Populations with low socioeconomic status are disproportionally impacted by the effects of climate change. One specific environmental exposure arises from forest fires, which increase the risk of pulmonary inflammation from biomass and wood smoke (Swiston et al., 2008) and can exacerbate underlying lung disease.

Aside from natural disasters, everyday exposure to biomass burning for heat and cooking is a well-studied risk to cardiopulmonary health and is particularly prevalent in developing countries (Naeher et al., 2007; Hoppin and Jacobs, 2012; Laumbach and Kipen, 2012; Sigsgaard et al., 2015). In developed countries, rather than exposure to biomass smoke, exposure to wood burning can arise from recreational exposures from home fireplaces or firepits. To improve the study of wood smoke as a specific exposure, a novel marker for wood smoke, Delta-C, has been developed (Allen et al., 2004). It is simply calculated by measuring the difference between ultraviolet BC (UVBC) measured at 370 nm and BC measured at 880 nm (Wang et al., 2012). Increased concentrations of Delta-C have been found to be associated with an increased risk of STEMI (Evans et al., 2016) and also with increased plasma concentrations of fibrinogen in cardiac patients (Croft et al., 2017). Improving the study of specific sources of air pollution can require innovative characterization of sources and monitoring. The emerging evidence on the potential dangers of wood smoke make it important to include questions on the use of wood fireplaces, firepits, and wood cookstoves when assessing patients' exposures.

4.7 NOVEL EXPOSURES

While cigarette smoking complicates the assessment of patients with occupational or environmental exposure, electronic nicotine delivery systems (ENDS) are a rapidly emerging source of inhalation exposure to toxicants for youth and adults. ENDS including e-cigarettes are battery-powered devices that vaporize a solvent (propylene glycol or a vegetable glycerin) that can contain flavors and nicotine (National Academies of Sciences, Engineering, and Medicine, 2018). ENDS are touted as an exciting new tool for cessation from combustible cigarettes and appear to produce fewer toxic chemicals than their combustible counterparts. Proponents cite the harm reduction potential of ENDS in comparison with combustible cigarettes, while other researchers and public health professionals urge caution due to human exposure studies showing adverse changes in human cells and the lack of long-term safety data and the potential for youth to become nicotine addicted from use of ENDS. The exposures from ENDS include heavy metals from the heating coil and chemicals from the solvent and flavorings (Chun et al., 2017; Muthumalage et al., 2018). The bulk of the evidence for pulmonary inflammation from ENDS exposure was performed *in vitro* and *in vivo* (cell and mouse models). Early *in vivo* human exposure studies have observed adverse changes to the lung mucosal barrier that may increase susceptibility to infection and chronic lung disease (Ghosh et al., 2018; Reidel et al., 2018). The liquid

nicotine used in ENDS can result in dermal exposure to nicotine and even potentially lethal doses of nicotine to children. The batteries in ENDS can explode, causing severe facial injuries and even death when mishandled or used in unstable conditions in modified ENDS. The current lack of regulation of ENDS has led to incredible heterogeneity in both the liquids and devices, increasing the difficulty in accurately assessing the risk to individual users.

The National Academies of Sciences, Engineering, and Medicine produced an extensive review of the current controversies surrounding ENDS including defining chemical exposure profiles, health effects, and public health issues including its potential efficacy in smoking cessation and its potential to increase youth nicotine addiction (National Academies of Sciences, Engineering, and Medicine, 2018). In the absence of conclusive data on the full consequences of ENDS, public health professionals disagree on the overall societal risks versus benefit of ENDS. Public Health England in the United Kingdom has embraced the potential for harm reduction and encouraged the use of ENDS for smoking cessation (McNeill et al., 2018). The United States has overall taken a more conservative approach, with the surgeon general report focusing on the risk of ENDS to youth and then need for ongoing study of potential health effects (USDHHS, 2016).

Irrespective of the clinician's country of practice, each clinician should have a basic understanding of ENDS to effectively screen and advise patients. While the literature base develops, a pragmatic approach proposed by the American Cancer Society (2018) is for patients to first try FDA (or equivalent governing body) approved tobacco cessation methods (nicotine replacement and pharmacologic treatments), reserving the unproven ENDS for patients unable to quit smoking with proven approached (ACS, 2018). If ENDS are employed, clinicians must counsel patients to switch completely from combustible cigarettes to ENDS (no dual use) and that a plan to stop using ENDS should also be made.

4.8 ADVICE AND COUNSELING OF PATIENTS

4.8.1 Patient Oriented

Although often called on to advise on control strategies for minimizing exposure to occupational and environmental exposure, the primary care physician may have little training or experience in this arena. Approaches for limiting the health risks of breathing polluted ambient air have received little investigation and dissemination to primary care practitioners. Present understanding of the determinants of exposure suggests that modifying time–activity patterns to limit time outside during episodes of pollution represents the most effective strategy for dealing with acute effects. The levels of some reactive pollutants tend to be lower indoors than outdoors. Ozone levels in buildings are lower than outdoor levels but can be driven upward by increasing the rate of exchange of indoor with outdoor air. Fine acid aerosols can penetrate indoors, but neutralization by ammonia produced by occupants, household products, and pets may reduce concentrations. Other types of particles in outdoor air may also enter indoor air. Nevertheless, health care providers can reasonably advise patients to stay indoors during pollution episodes. Vigorous exercise outdoors, which increases the dose of pollution delivered to the respiratory tract, should also be avoided at such times.

Defining what represents an adverse health effect is an ongoing challenge, and most recently, has been addressed by a joint ATS/ERS committee. The committee proposed a set

of considerations to help the clinician understand the adverse health effects related to air pollution rather than providing an exhaustive catalog of what should or should not be considered an adverse effect (Thurston et al., 2017).

Susceptible patients should be counseled concerning the nature and degree of their susceptibility. The use of medications should follow the usual clinical indications, and therapeutic regimens should not be adjusted because of the occurrence of a pollution episode without evidence of an adverse effect on symptoms or function. In the laboratory, inhalation of cromolyn sodium and bronchodilating agents may block the response to some pollutants, but use of these drugs solely because of exposure to air pollution cannot be advised.

Respiratory protective equipment has been developed for use in the workplace in order to minimize exposure to toxic gases and particles. Many of these devices, particularly those likely to be most effective, add to the work of breathing and cannot be tolerated by persons with respiratory disease. Respirators can provide effective personal protection only when they are properly selected and when they are used in the context of a comprehensive respiratory protection program. OSHA has specified the minimum requirements for an acceptable program. In order for respirators to provide adequate worker protection, the proper selection, facial fit, and the correct use of respirators are essential. Respirators are the least preferred method of protection from respiratory hazards, and they should be used only when engineering controls are not technically feasible, while controls are being installed or repaired, or in emergency or other temporary situations. Under most circumstances, health care providers should not suggest respiratory protection as a method of reducing the risks of ambient air pollution. Similarly, aside from certain populations (asthmatics), household air cleaners have not been shown to have health benefits, whether directed at pollutants generated by indoor sources or those brought in with outside air.

While nutritional supplementation with omega-3 fatty acids has been explored as a potential intervention to attenuate the harmful health effects of air pollution, this approach remains an area of active research (Tong et al., 2015; Croft et al., 2018).

A variety of federal agencies are active in the area of environmental health and have services of some degree of value to practicing physicians. Unfortunately, the responsibilities among the agencies are often fragmented, and no one source is targeted for providing assistance to the practicing physician. If the clinician wishes further information or consultation, the following agencies may be of value:

1. *Agency for Toxic Substances and Disease Registry (ATSDR):* Funded via Superfund legislation, this agency is part of the U.S. Public Health Service and affiliated with the CDC. Among its mandates are the educations of physicians concerning toxic hazards associated with hazardous wastes. Its Toxicological Profiles are a useful source.
2. *Centers for Disease Control and Prevention:* This agency is available to investigate illnesses that may be linked to environmental hazards.
3. *Environmental Protection Agency (EPA):* This federal agency has the responsibility of protecting humans and the environment from the unwanted effects of chemicals and physical agents and sets for pollutant concentration limits for ambient air and drinking water. It has focused primarily on providing the public with information on environmental risks and has not been a major resource for the practicing physician.

4. *National Institute for Occupational Safety and Health (NIOSH):* This agency, a component of CDC, has responsibilities for research and professional training issues related to worker health. It can provide information about specific workplace hazards and has the authority to enter workplaces to evaluate health and safety problems.
5. *National Institute of Environmental Health Sciences (NIEHS):* This federal agency is a component of the National Institutes of Health (NIH) and has responsibility for research related for understanding the health hazards related to environmental chemicals. It can provide information about the known toxicity, mechanisms of toxicity, and potential carcinogenicity of specific environmental chemicals
6. Other important resources may include professional organizations, medical school faculty with expertise in areas of environmental and occupational medicine, poison control centers, and occupational and environmental medicine clinics. The ATS has a number of relevant statements.

As a result of the OSHA Hazard Communication Standard, industries that use chemicals in the workplace have a material safety data sheet (MSDS) for each hazardous chemical. The companies should have these available on request of the physician or the worker. Goldstein and Gochfeld (1990) present additional discussion of resource agencies for clinicians and mechanisms for making professional contacts.

4.8.2 Community Oriented

Communities frequently become concerned about the impact of particular local sources, perhaps a power plant or manufacturing facility. Concern about the health risks may quickly lead to controversy and litigation. Thus, understanding the health risks posed by local sources may be difficult and require skills in community health as well as in epidemiology and toxicology. Local physicians may become involved through concerns about the health of their patients or as advocates for the community's environment or for the polluting facility. Most often the dimensions of such complex problems exceed the skills of local physicians. This competence gap has driven calls for formal advocacy training for physicians (Croft et al., 2012) specifically including advocacy to address climate change (Macpherson and Wynia, 2017). Evidence-based advocacy surrounding other inhaled exposures such as ENDS is also needed.

In the United States, no regulatory agency has authority over indoor air quality. Workplaces with more than 25 people are regulated by OSHA, although standards do not directly address the new problem of building-related illnesses. Good practice guidelines that are often adopted by states and localities as standards (actually guidelines) for ventilation in nonresidential buildings are set by the American Society of Heating, Refrigerating and Air Conditioning Engineers (ASHRAE).

The interplay of factors that must be manipulated to prevent environmentally induced disease is complex, but standards are considered to be objective indicators of success preventive measures or strategies. In 1976, the EPA proposed cautionary statements for public reporting of outdoor air quality, the Pollutant Standards Index (PSI) for criteria pollutants; in 1999, the PSI was replaced with the Air Quality Index (AQI) to incorporate new $PM_{2.5}$ and ozone national ambient air quality standards (NAAQS). The actions taken when "alert levels" are reached or expected to be reached include the issuance of health advisories (or

cautionary statements) to the public. The EPA's advice is intended to be applied by local air pollution agencies in preparing daily air quality summaries, which are disseminated to the media. Although the cautionary statements require some revisions, especially as related to ozone exposures, useful guidelines are offered for physicians and public health officials.

Finally there are important impacts to the community and workplace resulting from changes in health care insurance and the delivery of health care. A large proportion of employees in the United States are enrolled in health plans through health maintenance organizations (HMOs) and their provider networks. Both physicians and employees are subject to managed care's efforts to hold health care professionals more accountable for the medical and financial aspects of care. This often results in managing occupational health care demands and workers' compensation according to established guidelines. Although the original goal of all of these programs was to provide high-quality care, the focus of many stakeholders has been cost containment. With the introduction of workers' compensation managed care, many businesses have developed and implemented transitional work programs, developed alliances with occupational medicine clinics, retained the services of case managers, requested more independent medical examinations, and contested more claims, all in an effort to contain costs.

Although it is premature to assess the impact of managed care, it certainly has the potential to negatively impact health care of the injured worker. However, the Managed Care Pilot Project in Washington State (Sparks and Feldstein, 1997) studied the effect of experience-based capitation on medical and disability costs, quality of care, worker satisfaction with medical care, and employer satisfaction. Much of the care, and all of the treatment coordination, was provided by physicians specialized in occupational medicine and oriented toward timely return to work. The study revealed that "medical costs were reduced by approximately 27%, functional outcomes remained the same, workers were less satisfied with their treatment and access to care initially, and employers were much more satisfied with the quality and speed of the information received from the providers." As the effort to contain costs extend into occupational health, it will be important to carefully track injury outcomes and worker satisfaction.

4.9 SUMMARY

Environmental medicine requires a highly interdisciplinary approach. For the primary care physician tackling an occupational or environmental medicine problem, there is a necessity not only to work closely with nonmedical personnel such as industrial hygienists, ergonomists, toxicologists, epidemiologists, lawyers, regulators, and union representatives but also to become knowledgeable about these various disciplines. The diseases of individuals may provide indications of unacceptable exposures in the environment or workplace; health care providers should be able to recognize such "sentinel" disease and respond appropriately by contacting employers and regulatory or public health agencies. Ultimately, it may be necessary to visit a workplace or environmental site, request industrial hygiene data, and consider screening the exposed population of individuals in order to determine whether an environmental pulmonary hazard exists. In the final analysis, an inquisitive mind and a bit of detective work are often prerequisites for establishing causation between an environmental exposure and a pulmonary or other health-related disorder.

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5

INDUSTRIAL PERSPECTIVES: TRANSLATING THE KNOWLEDGE BASE INTO CORPORATE POLICIES, PROGRAMS, AND PRACTICES FOR HEALTH PROTECTION

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5.1 INTRODUCTION

The purpose of this chapter is to present an overview of the important considerations involved in establishing policies, programs, and practices for manufacturing, use, and disposal of commercial chemicals in ways that are reflective of the available knowledge of potential hazards and human health risks. Manufacturers of broad and diversified product lines of basic chemicals, plastics, and specialty products maintain extensive industrial hygiene, occupational health, toxicology, epidemiology, and other supporting product stewardship programs that are essential to assuring the safety of its products, importantly including associated manufacturing, distribution and use, and waste disposal operations. Over the last several decades, the chemical industry has directed increasing international attention and resources to a program of Responsible Care®, a set of voluntary codes addressing such topics as process safety, emissions reduction, and product stewardship.

Knowledge of the potential adverse health effects of environmental toxicants is an essential component for establishing corporate or industry-wide programs supporting adequate protection for workers, consumers of products, and the communities at large. A key component of understanding the overall health hazards and risks of chemical product

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operations is its intimate integration with the overall health information generated in toxicology, industrial hygiene, occupational medicine, and epidemiology data, examples of which are amply described in this volume.

Consistent with the scope of this volume, this chapter focuses on industry practices designed to assure adequate protection of human health over a broad spectrum of human contact scenarios. However, it is important to note that the policies and programs are not limited just to human health protection, but also embody stewardship for environmental concerns and for other safety concerns such as process safety and chemical reactivity.

5.2 THE LIFE CYCLE OF A CHEMICAL: MANY POINTS FOR POSSIBLE INTERVENTION

Design of appropriate health protection policies, programs, and practices for environmental toxicants requires consideration of the entire life cycle of the substance. A generalized description of a typical life cycle for a commercial chemical is shown in Fig. 5.1 and involves the sequence of manufacture, transportation, use, and ultimate disposition. The life cycle for a specific chemical may take divergent patterns dependent on its use scenarios. For example, benzene as a component for gasoline will become widely dispersed in the environment, whereas when benzene is used as a chemical intermediate in the manufacture of styrene, the benzene is consumed and only relatively small quantities are released to the environment. As another example, formaldehyde has many uses; some uses may involve the potential for exposure at low concentration levels to large numbers of people in the population, while some chemical intermediate uses may involve only limited potential for exposure and only to the workforce involved in the processing.

Many of the environmental toxicants reviewed in this book are exclusively or largely unintentional by-products of manufacturing, processing, or energy production, for example, carbon monoxide, diesel engine exhaust, nitrogen oxides, ozone, and sulfur oxides. However, even for such unintentional by-products, the life cycle sequence shown in Fig. 5.1 is essential to the design of control and stewardship practices.

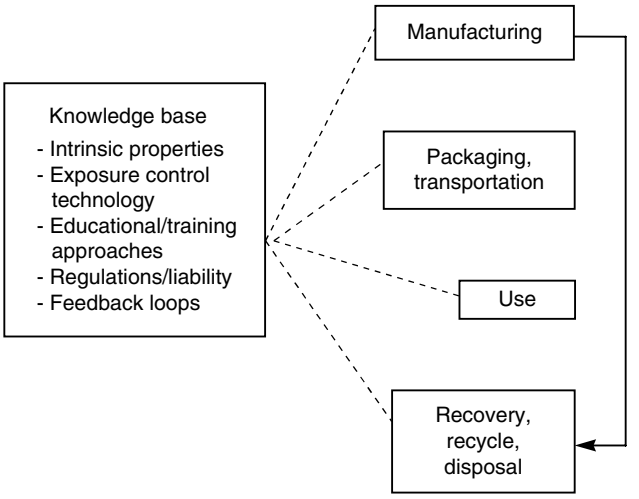

FIGURE 5.1 The knowledge base is relevant to all phases of the life cycle of a chemical.

5.3 THE KNOWLEDGE BASE FOR THE IDENTIFICATION OF HAZARD AND HEALTH PROTECTION CONTROL STRATEGIES

Most of the other chapters of this volume describe the complexity and breadth of the health effects database available for many specific environmental toxicants. These chapters illustrate the extensive science and policy considerations involved in interpreting the available peer-reviewed information to determine the appropriate toxicological endpoint(s) and associated risk assessments that are used in establishing regulatory and/or product stewardship risk reduction controls. For these specific chemicals or chemical classes, strategies for protection of human health are specific because of the multiplicity of types of health effects that might be of concern from one substance to another, such as pulmonary effects from asbestos, neurotoxicity from lead, and leukemia from benzene. Added to these complexities in designing appropriate controls, environmental and process (production) safety considerations need to be integrated into the overall risk control strategies. Thus, it is important to highlight the additional features of the knowledge base that are essential parts of the designing and planning of protective measures during the life cycle of a specific chemical or chemical class.

Important components of the overall knowledge base are shown in Table 5.1 and include the intrinsic physicochemical and toxicological properties of the substance, the current knowledge of options for exposure control technology, the educational and training approaches that are available, and information feedback loops. The general knowledge of options for exposure control and education/training can be tailored to the specific substance and to specific points in the life cycle.

TABLE 5.1 Components of the Knowledge Base for Establishing Hazard Control Strategies

Intrinsic Properties
Toxicological and clinical information
Flammability and chemical reactivity measurements
Other physical and chemical property information
Ecological and environmental fate characteristics
Product use characteristics
Exposure control technology
Restriction of use
Engineering controls
Process efficiency, waste reduction
Emission/discharge treatment or destruction
Educational and training approaches
Labels
Material safety data sheets
Technical brochures
Workforce training
Public availability
Regulatory requirements' liability potential
Feedback loops
Industrial hygiene and environmental monitoring
Health surveillance programs

Several intrinsic properties of a substance can be important in considering hazard control strategies. Certain flammability and chemical reactivity properties may be critical for process safety and selection of containers and packaging. The toxicological properties are a key component to hazard and risk evaluation in that they provide foundational hazard identification and dose–response information addressing the potential of chemicals to adversely impact health in the context of real-world and/or anticipated external exposures. Knowledge of downstream product use and disposal practices is essential for evaluating points of environmental release and their magnitude. Basic ecological properties, such as phytotoxicity and information on toxicity for typical aquatic and mammalian species, and environmental bioconcentration potential, fate, and transport also are essential to evaluate potential ecological hazards.

The intrinsic properties of potential concern at various stages in the life cycle of a chemical are key drivers of options to be considered from the array of possible exposure control and monitoring technologies. Depending on the outcomes of integrated assessments of the overall components of the knowledge base, decisions may be made to restrict or tightly control uses of a substance, such as has been done by manufacturers and/or regulatory agencies in the cases of asbestos, PCBs, DDT, and flame retardants in children's sleepwear. More routinely, however, a wide and technologically expanding array of engineering controls and operating practices have evolved over the years to minimize environmental exposures—a few examples include vapor recovery systems for tanks and vents, enclosed systems, designs to improve processing efficiency, waste reduction programs, product formulation improvements minimizing volatilization, and biological or chemical treatment or incineration of emissions and discharges. In addition, product substitution of agents with improved hazard and risk properties is a possibility with appropriate maintenance of desired efficacy.

Education and training, that is, approaches to information transfer, are important aspects of control strategies. Such efforts apply not only to the production workforce but also to downstream commercial and general public users. Over the years, there has been an evolution of information transfer approaches, including product labeling, the use of material safety data sheets (MSDSs), product brochures containing summaries of hazard information, and frequent recommendations for precautionary design of aspects of storage or operation. Increasingly, there has been attention to the implementation of training programs for the workforce; these have evolved to include emphasis on leak control and cleanup of spills and on operating increasingly sophisticated technology to minimize process perturbations, leading to unanticipated releases. As a part of the industry Responsible Care program detailed below, many chemical companies now include product safety summaries describing key health and environment properties of marketed products on their company-specific websites. In addition, more than 4500 fully searchable product summaries from across the chemical industry are available on the International Council of Chemical Associations (ICCA) Global Product Strategies (GPS) Chemicals Portal website (ICCA, 2018).

The fields of industrial hygiene and environmental monitoring have evolved many analytical methods and techniques for detecting and quantitating exposures to environmental toxicants. In recent years, monitoring programs have been augmented by substantial advancements in mass spectrometry and liquid chromatography analytical technologies, allowing for greatly increased analytical sensitivities, operational throughput, and ability to perform complex matrices analyses. These approaches provide valuable feedback loops for evaluating the effectiveness of planned exposure control strategies. Another feedback loop contributing to effective exposure controls is industry occupational health surveillance

programs. If worker health surveillance or epidemiological programs identify or suggest a cause–effect relationship between an adverse health effect and an exposure, adjustments in exposure and health protection strategies can be made as the data dictate. Of course, knowledge of animal toxicological information is also a key component that is integrated into health assessments.

Superimposed on the pragmatic considerations outlined above is a fabric of regulations, public perceptions, and potential for liability that influence corporate policies and programs. Many government regulations mandate health and/or environmental testing to identify toxicological properties of chemicals, and regulatory agency risk evaluations (including exposure assessments) can likewise require proscribed occupational and/or user exposure controls. In addition, the risk of litigation and public perceptions of hazard and excess risk also influence company-initiated exposure control options.

The need for feedback loops from manufacturing and commercial operations to the knowledge base and the need to assure compliance with company or regulatory emission or ambient standards have prompted the continued evolution of industrial hygiene and occupational health programs in many companies. An overview of the specific industrial practices underpinning the considerations for establishing these programs is described in the following sections.

5.4 INDUSTRIAL HYGIENE AND OCCUPATIONAL HEALTH PROGRAMS: IMPLEMENTING THE KNOWLEDGE BASE

5.4.1 Exposure Estimation

Much attention has been directed to measuring exposure to chemicals and other stresses in occupational settings. The broad subject of how to do this from a sampling and analytical standpoint is beyond the scope of this chapter; however a few general observations are offered regarding strategies for generating reliable estimates of actual exposures. That knowledge can then be applied to test the efficacy of exposure controls intended to protect worker health.

There are three main objectives of monitoring:

1. To assure that untoward health effects are not likely to be encountered.
2. To describe what the exposure actually was for future use in exposure (dose)-response assessment of findings from toxicology and epidemiology studies.
3. To assure that exposures are within legal requirements, for example, U.S. Occupational Safety and Health Agency (OSHA) permissible exposure limits (PELs; OSHA, 2018) or consistent with other worker exposure guidelines, for example, American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs®; ACGIH, 2018).

These three objectives are not mutually exclusive, but may require somewhat different approaches.

Some knowledge of the type of effect a chemical may exhibit and an estimate of its toxicity dose–response and toxicokinetic properties are important to meet the first two monitoring objectives. For example, for gases or vapors that are believed to cause only immediate respiratory irritation, a method should be chosen that measures peak exposures

in that the toxic effect of such materials is likely to be linked more closely to the highest concentration reached than to the total dose received. Hydrogen chloride and other acid gases are examples of chemicals in this category. In contrast, the toxic effects of some materials are more closely associated to the total dose received than to the concentration seen over any short time period. Vinyl chloride is a good example of this type. Vinyl chloride induces hemangiosarcomas of the liver, and it appears that high exposures over relatively long periods (years) are required to produce this carcinogenic effect. This suggests that exposure over a short period is relatively unimportant compared with cumulative exposures over longer times. Thus, an exposure measurement strategy that gives an estimate of the time-weighted average over lengthy periods is most informative of health risk.

In order to address these varied exposure–outcome relationships, occupational exposure limit (OEL) setting bodies such as ACGIH (2018) describe chemical specific and health-protective exposure control strategies for the workplace (point 3, above). Thus, an OEL may be set only as a single time-weighted value (TLV-TWA) reflecting an 8 h/day, 5 days/week exposure that is not expected to cause adverse health effects in workers. However, dependent on toxicological properties, the OEL may include exposure control notations used in concert with the TLV-TWA, such as a short-term exposure limit (TLV-STEL). The TLV-STEL is an exposure not be exceeded for more than 15 min at any time during a single 8-h work shift. For highly active respiratory irritants, ACGIH can assign a ceiling value (TLV-C), which is concentration that should not be exceeded at any time during the work shift.

For the second objective, measurements useful for informing interpretation of toxicology and epidemiology studies, another key need is to know as much as possible about historical exposures, primarily to better assess whether the health effect of concern exhibits one or more dose–response relationships. The dose–response relationship is a key element to establishing Bradford Hill's causality for adverse health outcomes (Hill, 1965).

When much is known about the biological properties of a chemical (i.e., an extensive toxicology database is available), it is easier to design an appropriate OEL approach and associated sampling strategy. However, it should be borne in mind that new effects may be discovered in the future that may require alterations to existing OELs or the need for new OELs (i.e., feedback loops between toxicology, occupational medicine, and industrial hygiene practices). To facilitate development of health-protective OEL assessments as complete a description of exposure as feasible should be obtained. For example, even though it may be thought that exposure over relatively long periods of time is the most important concern for a material, it is best to estimate both long-term (full-shift) and excursion (short-term peak) exposures.

How the chemical is used should also be a factor in deciding when, where, and how long to take samples or to record the time-varying measurements of direct-reading instruments. It is important to know what the probability will be of a person actually encountering a given concentration before deciding on a measurement plan. For example, if an in-line filter in a chemical process is periodically changed, it would be most sensible to measure the level of chemical encountered over the range(s) of time it takes to change the filter and include measurements at the immediate location of the filter change and the larger surrounding area. On the other hand, if a chemical is likely to be present in the general work area either continually or for major portions of a work shift, a full-shift-length sample would probably be the best type of sample to take for the estimation of cumulative exposure.

In designing sampling approaches, it is necessary to consider each situation in the light of how the chemical(s) are encountered, as well as their toxic properties, and how they are used. In judging the seriousness or acceptability of an exposure (e.g., rapid death; fetal toxicity), adjustment to permissible exposure limits should be taken into account. In addition, the toxicokinetic properties (how a chemical is absorbed, distributed, metabolized, and excreted) also inform the internal systemic (body or organ) dose resulting from an external chemical exposure. For example, some chemicals are poorly absorbed orally or through the skin or if orally absorbed are rapidly metabolized by the liver, resulting in limited distribution of the parent compound to other systemic organs. Such considerations aid in the interpretation of toxicological and epidemiological findings that may be dependent on the nature of the occupational (or general population) exposures most commonly/realistically encountered and/or the dose/exposure conditions under which the toxicity test was conducted. For example, toxicity data collected from an oral dosing study may not inform the health risks of substance for which human exposures are predominately dermal or inhaled; further information on dermal absorption and respiratory tract deposition are necessary to make appropriate use of the oral toxicity data.

5.4.2 Biomonitoring

Biomonitoring is defined, for the purposes of this discussion, as the evaluation of the internal exposure of a human to a chemical agent as determined by the analysis of biological specimens (e.g., urine, feces, blood/serum, exhaled air). Depending on the chemical agent and biological specimen collected, the internal exposure reflects the amount of the chemical recently absorbed (except for bioaccumulative compounds), the amount (dose) of chemical in the body, or, less frequently, the amount of the chemical at its action site(s). Biomonitoring approaches are widely applied to occupational and nonoccupational exposures.

For workers, biological monitoring techniques potentially can offer better estimates of internal exposure than environmental monitoring because biological parameters related to internal exposure are more directly associated with potential systemic adverse health effects than environmental measurements. Biological monitoring takes into consideration total absorption by all routes of exposure. Biological monitoring also provides a better estimate of an individual worker's actual systemic exposure than environmental measurements because of individual differences in work habits, for example, use and type of personal protection equipment such as gloves and/or respirators. Biomonitoring also captures individual differences in the way chemicals are handled in the body due to age, gender, disease status, genetics, co-exposures to other chemicals, other lifestyle factors, and so on. The ACGIH (2018) has developed Biological Exposure Indices (BEIs®) that offer quantitative or qualitative estimates of workplace exposures to specific chemical agents, further refining potential assessments of compliance with workplace OELs or confirming whether exposures have even occurred. It is important to note that biomonitoring is not restricted just to analytical measurements of chemicals and their metabolites, but may include measurement of biological biomarkers such as inhibition of acetylcholinesterase, as is the case with occupational exposures to organophosphate pesticides. For example, ACGIH has set a BEI for exposure to cholinesterase-inhibiting pesticides as an end of shift red blood cell acetylcholinesterase activity of 70% of a worker's baseline activity (ACGIH, 2018).

It is important to distinguish, however, between measuring just the amount of material in the bio-sample versus understanding the relationship between that amount to an actual

external environmental exposure and the corresponding health risk. It is often possible to quantify the amount of a chemical and/or its metabolite(s) present in a urine sample. However, simple detection does not equate to health risks, and further information correlating relationships of analyte(s) to external exposures, including the time(s) at which they are sampled, are necessary elements to establishing the viability of a meaningful biomarker. Such circumstances are particularly of concern, for example, when urinary metabolite(s) is common to differing chemicals, thus limiting attribution of the measured urine analyte(s) to a specific chemical exposure.

Given the preceding considerations, establishment of a risk-informative biomarker ideally is supported by controlled experiments in humans that determine the quantitative relationships between exposures and concentrations of the chemicals or metabolites in the medium to be measured. When this is not possible, it may still be possible to arrive at concentration ranges of acceptability by referring to animal studies when toxicokinetic models can be constructed with confidence that relate how chemicals are handled in the animal relative to humans. Biological monitoring techniques may not be appropriate, however, if the primary adverse response involves acute site-of-contact injury to the respiratory tract, skin, or eyes. Using biological monitoring to detect peak chemical exposure levels is also challenging, since it requires knowledge of the time interval between the onset of exposure and appropriate collection time as influenced by the overall toxicokinetic behavior.

Over the last couple of decades, the value of biomonitoring efforts for informing potential human health risks has been widely extended beyond occupational evaluations to assessing environmental nonoccupational exposures to the general population. One of the largest of such biomonitoring programs is conducted by the U.S. Centers for Disease Control and Prevention (CDC) and is subcomponent of the U.S.-wide CDC National Health and Nutrition Examination Survey (NHANES). Beginning is 1999, and conducted in 2-year analysis cycles since, the CDC surveys blood and urine concentrations of a wide range of environmental chemicals of interest across a representative spectrum of the general population (geography, gender, young to old, racial). The biomonitoring reports are released regularly as updated two volume publications (CDC, 2018). As with occupational monitoring, the CDC importantly notes that simple detection of blood or urine concentrations, as reported by the program, does not infer potential adverse health risks. Rather, the primary purpose of the program is to identify trends of increasing or decreasing exposures to key environmental agents of interest, both of which are useful for informing priorities for future risk management options and population-based epidemiological studies.

Recently, the concept of "Biomonitoring Equivalents" (BEs) has been introduced in which concentrations of human biomonitored substances can be interpreted in context to human risk-based health safety standards, such as reference doses or concentrations (RfD; RfC). The BE concept uses toxicokinetic data to predict human blood and/or urine concentrations resulting from exposures to environmental chemicals at the RfD or RfC, that is, "Biomonitoring Equivalents" to theoretical human exposures at an RfD or RfC. Since RfD and RfC values are conservatively set to be protective of human health, if the actual biomonitored concentrations are less than the BE-predicted blood/urine concentrations, the assumption is that such exposures are likely in compliance with health-protective regulatory standards and thus to have a low priority for additional risk management attention (Hays et al., 2008).

Since the introduction of the BE approach, this useful screening-level approach has been applied to a large and growing number of biomonitored environmental substances, including those from the CDC NHANES program (Aylward et al., 2015; Aylward, 2018).

An example of how the BE approach informs potential health risks resulting from real-world exposures to an environmental agent is the BE derived for the widely used herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) (Aylward and Hays, 2015). The current U.S. EPA-derived RfD for 2,4-D of 0.21 mg/kg/day was based on a no observed adverse effect level (NOAEL) dose of 21 mg/kg/day for mild kidney toxicity in adult rats treated in an extended one-generation reproduction toxicity study (i.e., the RfD is 100-fold less than the rat NOAEL). Using human 2,4-D toxicokinetic data, urinary BEs of 10,500 and 7000 μg/L for parent compound 2,4-D were calculated for adults and children, respectively (2,4-D is rapidly and completed excreted into urine following systemic absorption). Upper-bound estimates of actual 2,4-D urine concentrations from multiple biomonitoring studies were more than 5000-fold lower than the calculated 2,4-D BEs, indicating exposure controls for this important herbicide are considered fully adequate and current use patterns of 2,4-D present a low-priority health concern.

5.4.3 Toxicity Testing and Data Evaluation

In addition to exposure analyses, toxicity testing in whole animal (*in vivo*) and cell-based (*in vitro*) systems is an essential component of industry-conducted evaluations of chemical-induced health hazard and risks. Because epidemiological studies generally are not sufficiently powered to fully characterize human health risks associated with chemical exposures and can only be ethically conducted after exposures and potential health effects have already occurred, proactive animal toxicity testing has long been the hallmark approach to health evaluation of environmental chemical exposures. The objectives of animal toxicity testing are to identify potential toxicity hazards (i.e., identification of intrinsic biological responses of a given chemical) and to conduct dose–response analyses allowing for characterization of human risk potential (i.e., identification of doses that produce both no effects and effects and relationships of those responses to human exposures).

Toxicity testing in industry goes back many decades and foreshadowed the advent of government regulatory mandates that are now in place for testing of most drugs, pesticides, and other workplace and general environmental chemicals. At present, industry-based toxicity testing largely relies on a series of globally accepted test protocols developed by regulatory agencies to evaluate toxicity potential associated with a range of specific health endpoints of concern. The test protocols, and associated guidance for their conduct, are detailed by U.S. Environmental Protection Agency (EPA) and Organization of Economic Cooperation and Development (OECD) (EPA, 2019; OECD, 2019). These protocols are generally recognized across multiple countries and geographies in order to reduce and harmonize country- and area-specific testing requirements. The toxicity endpoints addressed by these protocols are acute oral/dermal/inhalation (lethality and general toxicity), primary eye/skin irritation, acute neurotoxicity, dermal sensitization, *in vitro* and *in vivo* bacterial and/or mammalian genotoxicity, repeat dose (21–90 days) subchronic general toxicity (oral/dermal/inhalation), subchronic neurotoxicity, prenatal developmental toxicity, two-generation or extended one-generation reproduction toxicity, immunotoxicity, chronic plus lifetime carcinogenicity, and metabolism and toxicokinetics.

More recently, EPA has issued guidance for conduct of *in vitro* and *in vivo* screening-level testing for endocrine activity targeted to the estrogen, androgen, and thyroid hormone pathways (Borgert et al., 2011). In order to reduce the use of animals in toxicity testing, regulatory agencies have also developed protocols that combine multiple endpoints into a single screening-level protocol, for example, the OECD 421/422 protocols (OECD, 2016)

combine an assessment of adult subchronic toxicity and screening-level assessments of reproductive and developmental toxicity and adult/developmental neurotoxicity. In addition, the extended one-generation reproduction protocol allows for combined high-tier evaluations of several apical toxicological endpoints that would otherwise be typically assessed in stand-alone toxicity test protocols consuming far larger numbers of animals and other resources (Cooper et al., 2006; OECD, 2011; Rorije et al., 2011; Marty et al., 2013). The combined apical endpoints include chronic toxicity and reproductive toxicity in adults; developmental neurotoxicity; estrogen, androgen, and thyroid endocrine responses; and adult and developmental immunotoxicity. Such screening-level and apical protocols are a key component to tier-based testing strategies implemented by industry (detailed below).

Rats are the primary test species common to all studies, with other species used for selected endpoints (e.g., mice: carcinogenicity, immunotoxicity; dogs: subchronic/chronic toxicity; rabbits: acute dermal, eye/skin irritation, developmental toxicity). Although these protocols are generally used/required for industry-conducted/sponsored toxicity studies, the protocols are designed by government regulatory agencies, with inputs from academic and industry toxicologists. The protocols are regularly updated to reflect advancements in knowledge of toxicological science but are constrained to methods that have been appropriately validated for confidence in the relationship of the test endpoints of concern to clear adverse health outcomes, cross-laboratory reproducibility, and consistent and transparent data interpretation.

A common design element of all the protocols is use of the maximum tolerated dose (MTD) as the recommended means for selection of the study top test dose. The MTD concept was implemented in the early stages of modern toxicity testing design in the 1960s and is defined as a dose sufficient to cause no more than a 10% loss of animal body weight over the course of repeated dose studies and/or with other evidence of organ system injury (Carr and Kolbye, 1991). The MTD concept was adapted as a means to assure adequate identification of potential health hazards, because animal toxicity studies generally composed of 50 animals or less per dose group were intended to represent very much larger and diverse human populations. To be valid for risk assessment extrapolations, however, the MTD approach intrinsically assumes that the animals' biology responds linearly to dose over the complete range of doses up to and including the MTD. This fundamental assumption is now recognized to no longer holding true for many chemicals, with many studies having shown that the toxicokinetic processes of absorption, distribution, metabolism, and clearance can all be subject to metabolic saturation with increasing doses (Bus, 2017). Toxicity occurring at doses saturating toxicokinetics is generally regarded as not relevant to informing health risks of human exposures occurring at sub-saturating exposures (Bus, 2017). Thus recent OECD (2012) dose selection guidance recommends that the top dose used in toxicity testing not exceed the onset of nonlinear toxicokinetics as long as the inflection point is well above expected or known real-world human exposures. This dose selection strategy has been termed the "kinetically derived maximum dose," or KMD (Saghir et al., 2012), and is important for improving the overall human relevance of animal toxicity studies, particularly in the face quantitatively declining human exposures due to decades of industry product stewardship and regulatory exposure-reduction practices.

It is important to note that doses, that is, concentrations, of test chemicals used in *in vitro* toxicity testing systems must also be considered in the context of blood and tissue concentrations resulting from realistic human exposures in order to be of value for informing human risk assessments (Bus, 2017). Such considerations are of increasing importance, given encouragement for use of such methods by the NAS (2007), with the intent that such

approaches allow for higher-throughput toxicity evaluations relative to the higher time and animal resource consuming conventional toxicity testing. The combined government, industry, and academic toxicology research communities are aggressively developing how these higher-throughput methods can be best used to advance and inform human health risks (Thomas et al., 2013; Wetmore et al., 2015).

Because of the ubiquitous nature of potential pesticide exposures, regulatory agencies generally mandate conduct of the complete battery of toxicity tests, described above, in order to establish a pesticide registration. For non-pesticide agents, however, toxicity testing is generally designed using "fit-for-purpose" approach, that is, individual toxicity tests are selected based on anticipated/known human exposures using "tier-based" testing assessments (Becker et al., 2007; Plunkett et al., 2010). Tier-based toxicity testing allows for certain test result "triggers" to drive a progression of increasing detailed higher-tier (i.e., more definitive) toxicity testing. For example, a higher-tier two-generation toxicity test may be triggered if lower-tier shorter-term subchronic studies indicate toxicity to male or female sex organs, but no such trigger if no such evidence is present and human exposures are well below the doses producing no effects in the shorter-term studies. Carcinogenicity studies may not be necessary if the compound is not genotoxic and subchronic studies do not indicate histopathological changes consistent with potential pre-neoplastic changes and if human occupational and/or public use conditions are infrequent and/or exposures are well controlled below doses producing no effects (NOAELs) in the lower-tier studies. Complex developmental neurotoxicity studies may not be required if lower-tier findings in adult or developmental screening studies indicate no evidence of functional (clinical signs) and/or pathological evidence of neurotoxicity.

Another key characteristic of industry-conducted and regulatory guideline-driven toxicity testing is its compliance with Good Laboratory Practices (GLP). Introduced by regulatory agencies in the late 1970s to minimize potential concerns of fraud in industry toxicity testing, GLP is a quality management system focused on transparently recording the details of the planning, conduct, monitoring, data recording, and archival of toxicity study data (Jena and Chavan, 2017). Although GLP is not intended to replace the use of sound scientific knowledge for study interpretation, it is intended to increase the confidence that studies follow validated scientific methods and that the experimental details of the study are implemented in accord with pre-study specified test protocols (and any deviations from such are transparently documented). GLP requires the use of a spectrum of quality control procedures such as analytical confirmation of doses and demonstrated operational verification of all instrumentation associated with data collection and analysis. Importantly, GLP requires that all aspects of study reporting be transparently and fully described and retained for external third-party audits by competent regulatory agency scientists. Failure to adequately comply with GLP can result in invalidation of a study for use as a regulatory submission (Becker et al., 2009) and, if the deviations from GLP are particularly egregious, can result in suspension of the testing laboratory from offering test results to regulatory agencies. Because proprietary and other reasons may limit whether industry-conducted toxicity tests are reported in the peer-reviewed scientific literature, the GLP process provides an important level of confidence that such studies are competently conducted and follow validated toxicity testing protocols designed to be highly reproducible across testing laboratories. It is important to note, however, that GLP studies submitted just to the agencies are subjected to independent agency scientist expert reviews and produce detailed, publicly available, summary reports.

An important consideration with all animal toxicity testing is whether the animal models adequately reflect human biological responses, whether that be from intrinsic qualitative differences between the biology of the animal test models and humans or quantitatively different due to alterations of metabolism of chemicals and/or interactions with target receptors. In order to better inform the human relevance of animal toxicity study findings to human hazards and risk, industry has long worked in collaboration with academic and regulatory scientists in the development and promotion of mode of action (MOA) investigations. These specialized studies are designed to identify and evaluate the contributions of a series of biologically plausible events that are key to the ultimate expression of the toxicity(s) of concern. Because of the importance of MOA investigations to informing human risk assessment decision making, robust globally accepted frameworks have been developed to promote transparent and reproducible MOA assessments (Meek et al., 2014a, 2014b). Understanding of MOA becomes particularly important when animal toxicity is observed at doses that are close, or even common, to humans in real-world (most often occupational) exposure scenarios. An example of such a concern is with the high volume commodity chemical styrene, which produces lung tumors in mice at inhalation exposure concentrations commonly encountered by workers in the reinforced plastics (boatbuilding) industries. Importantly, no cancer was observed in rats exposed to concentrations (1000 ppm) far higher than those encountered in the workplace, raising the question of whether humans respond to styrene like the sensitive mice or insensitive rats. A decades-long series of MOA investigations sponsored by the Styrene Information and Research Center, an industry consortium supporting styrene product stewardship, has demonstrated that the MOA of styrene mouse lung carcinogenicity is likely not qualitatively or possibly quantitatively relevant to humans (Cruzan et al., 2018). The styrene MOA indicates that mouse lungs preferentially metabolize styrene to ring-oxidized metabolite(s) through a metabolic enzyme, cytochrome P450-2F2 (CYP2F2) that is not present in rats or humans, and that variants of this enzyme in rats (CYP2F4) and humans (CYP2F1) are substantially less able to ring-metabolize styrene. The styrene MOA investigations were substantially facilitated by use of new genetically modified mouse models in which CYP2F2 was genetically "knocked out" or in which the human CYP2F1 enzyme variant was inserted as a replacement for normal mouse CYP2F2. These new and advanced toxicological whole animal test models revealed the critical role of CYP2F2 as a key metabolic gateway for mouse lung-specific toxicity and tumors, that is, a complete absence of toxicity in CYP2F2 knockout mice and CYP2F1 humanized mice relative to extensive toxicity in the wild-type mice. Importantly, this MOA conclusion also is consistent finding that styrene was not a carcinogen in the most highly exposed styrene workers (Collins et al., 2013; Collins and Delzell, 2018).

5.5 PRODUCT STEWARDSHIP

Knowledge of the potential health and environmental effects of chemicals has been evolutionary, whether indicated by increases in the amount and sophistication of toxicological and epidemiological literature, the number of professionals in the field, or the proliferation of industrial hygiene, environmental health, and environmental medicine curricula and postgraduate research programs. Approaches to expanding the scientific knowledge base, and their applications to product stewardship within the chemical industry, have also been evolutionary. For example, The Dow Chemical Company, a pioneer and leader in many

aspects of environmental health and product safety, established one of the first toxicology research laboratories for the study of industrial chemicals in the early 1930s. Industrial hygiene and occupational medicine departments were established in the 1940s, and epidemiology and environmental science groups in the early 1970s.

The evolutionary industry progress in efforts to protect human health increasingly recognized that the knowledge base had to be transferred to customers, many of whom further processed or formulated the chemicals into other products. This recognition led to the development of what is now termed product stewardship—a philosophy or ethic, with associated practices, to promote a synergistic knowledge partnership between manufacturer and customer. The philosophy of product stewardship, or product safety as it is referred to in some companies, is now practiced across a large number of chemical companies. In short, product stewardship is a commitment to action—to "do the right things." This commitment is intended to permeate all levels and functions of the organization, from top management policy to planning to day-to-day decisions and practices.

The purpose of product stewardship is fivefold:

1. Protect employees, public health, and the environment.
2. Protect the environment from harmful products.
3. Reduce liability.
4. Help prevent adverse publicity.
5. Build trust with employees, customers, and ultimately the public.

Each of these purposes is self-explanatory. Although protection of people is extremely important, it is also important that the environment likewise be protected from misuse, abuse, and improper disposal of chemical products. Obviously, failure to adequately attend to the objectives of product stewardship can lead to calls for product bans, product restrictions, and tighter regulations.

Effective product stewardship must be the responsibility of all employees. It cannot just be the responsibility of a safety coordinator or a single department. It ranges from the chemist experimenting on a product for the future to the sales organizations taking orders for large quantities of an established commodity such as caustic soda. Industrial health and environmental scientists have the responsibility for generating safety data evaluating its impact on potential or existing products applications and ultimately communicating this information in appropriate terms to all concerned. Manufacturing employees must be informed of potential health effects and have necessary training to assure operational safety. It is also the responsibility of effective product stewardship to insure adherence to pollution control and industrial hygiene standards and practices and compliance with all regulatory requirements.

As stated above, stewardship does not end when a product leaves the plant. Distribution personnel must select carriers, warehouses, and terminals that will perform consistently within guidelines and assure that products reach the customer in a safe manner. Marketing personnel must furnish customers and distributors with appropriate handling and application information and be on the lookout for potential misuse, mishandling, or improper disposal of products. They should form supplier–customer partnerships that promote safe uses and applications of chemicals.

Another key person to a robust product safety program is the product steward—the person responsible for advocacy to the businesses for necessary support of health and

environmental studies and safety assessments for each product. In addition, these stewards help evaluate the appropriateness of a customer's use and disposal of a product and facilitate preparation of safety training tools. The objective is to have a steward assigned for each product and/or related product portfolios, with responsibilities of maintaining contact with sales representatives, customers, health and environmental scientists, and government regulators in order to assure that appropriate health and environmental information and concerns are considered in all aspects of product development and use.

A number of considerations are central to a product evaluation. How is the product manufactured? What are its raw materials? What is the manufacturing process? What impurities does a product contain, and what problems can be expected if changes are made in the process? In addition, product distribution networks must be evaluated; is the product transported by road, rail, or water? Does it go through terminals? Is it handled by distributors? How is it packaged?

Other considerations include how the product will be used and how, potentially, it can be misused. Can it be ingested, inhaled, or touched? What are recommended disposal practices? Will it be burned, be landfilled, or be part of treated waste water? What would happen if the product is accidentally spilled or otherwise released?

Additional considerations include evaluation of the probability and extent of human exposure. In the early years of industrial hygiene practice, only worker exposures of healthy males between the ages of 18 and 65 were considered. Today the health of women and their unborn fetuses also must be considered. If the product leaves a plant site, the potential for adverse effects on sick people, children, the elderly—every demographic group—must be considered. Furthermore, the probability and amount of release to the air, water, and land must be evaluated along with the toxicity and persistence of the product in the environment.

In more recent years, health and environmental concerns have often dominated product stewardship evaluations. However, safety concerns involving fire, explosion, and reactivity cannot be underestimated or overlooked. The Bhopal, India, incident in 1984 resulted in thousands of deaths and injuries and was caused by a runaway exothermic reaction of methyl isocyanate with water, resulting in a high-pressure leak of this highly toxic gas. The Bhopal incident catalyzed a series of intensive follow-up industry and governmental regulatory assessments of needs and mandates for improvements in process safety [reviewed in National Research Council of the U.S. National Academy of Sciences (NAS), 2012].

How much health and environmental data are needed on a new product is a question always asked during the developmental cycle. For early stage research work, range-finding toxicological data are obtained. These studies include acute oral, eye irritation, and skin irritation studies. In addition, it is at this point that reactive chemical data should be obtained to determine if a product is shock sensitive, is flammable, undergoes exothermic decomposition, and so on. Its reactivity with other chemicals and construction materials should also be determined or anticipated. When a potential new product reaches pilot plant stage, range-finding studies may still suffice, depending on the results and the applications considered for the material. Finally, when a material is supplied to potential customers for applications development, a review must be conducted to determine the need for additional toxicological and environmental testing, depending on the intended use(s) of the product (Section 5.4.3, tier-based toxicity testing).

An important link in the product stewardship philosophy is to establish supplier–customer partnerships in health, safety, and environmental matters. Experience has shown that in addition to the commercially important consideration of having high-quality products at

competitive prices, the supplier must be the buyers' expert in avoiding injury or environmental damage from downstream use of its products—a trend that is in the suppliers' own interests. The time when companies could sell products merely on the bases of competitive pricing is long past. Today, the key to successful marketing is to reduce the knowledge gap between seller and buyer as a value-added contribution in the overall value chain of a chemical.

The supplier has to focus attention on the buyer's needs and experience in handling chemicals. Regulation is only one factor influencing these needs; the most essential factors are product knowledge and an attitude of commitment to developing and carrying out sound practices. Knowledge about health, safety, and environment is a specialty that the supplier must offer with its products, and such knowledge is essential for customer success. Only if customers are successful in avoiding adverse health and environmental impacts will the supplier likewise be successful. The amount of time and effort required for product stewardship attention depends on the properties of the product and its intended uses and the resources and expertise of the customer. Some of the approaches to supplier–customer interchanges are shown in Table 5.2. Obviously, more supplier resources are required for a highly toxic, high volume product such as chlorine being used by a customer with limited resources than for a polystyrene resin sold to another large and well-resourced manufacturing company. In both cases, however, it is important that a safety partnership be developed between the supplier and the customer.

In addition to industry-specific product stewardship initiatives, it is important to note that there has been an extensive parallel development and implementation of comprehensive global government regulatory frameworks providing another important layer to overall chemical safety. While beyond the scope of this chapter, examples of regulatory oversight include the U.S. Toxic Substances Control Act (TSCA) and Federal Insecticides Fungicides and Rodenticides Act (FIFRA), Canadian Environmental Protection Act (CEPA), and the European Union's Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) regulation.

A challenge for the future is to build supplier–customer relationships on an industry-wide basis, with emphases on all areas of stewardship—manufacturing, transportation, storage, use, and disposal. This challenge is addressed and further catalyzed by the Responsible Care initiative of American Chemistry Council (ACC) [renamed from

TABLE 5.2 Product Stewardship: Types of Information and Consultation Provided to Customers

Material safety data sheets
Technical literature and summary brochures
Label instructions
Posters and video or audio tapes for training
[a]Phone consultation
[a]Presentations at safety meetings by experts
[a]Seminars on regulatory compliance
[a]Plant visitations and information exchange
[a]Industrial hygiene surveys at customer's site
[a]Vent stack monitoring
[a]"How-to" presentations at professional and trade association meetings

[a]These depend on the nature or the product, its intended use, and the resources of the customer.

the Chemical Manufacturers Association (CMA) in 2000], a trade association representing companies that produce approximately 90% of U.S. industrial chemicals (ACC, 2019). Beginning in 1988, adherence to Responsible Care was a condition for membership in the ACC. Through the efforts of the International Council of Chemical Associations (ICCA), the principles and operational commitments of Responsible Care have been extended to the broader global chemical industry members representing over 90% of global chemical sales (ICCA, 2018) and covering 68 economies around the world (ACC, 2019).

5.6 RESPONSIBLE CARE

Responsible Care has two critical program elements. The first is intended to guide companies toward continually improving their health, safety, and environmental performance; the second is designed to assist companies to do a better job of understanding and responding to public concerns about safely managing the use of chemicals. The framework to accomplish these objectives consists of 12 Guiding Principles, which serve as the operational philosophy of the program, and 10 Product Safety Codes of Management Practices, which describe the key activities companies must undertake to manage chemicals as safely as possible while constantly improving performance (ACC, 2019).

The purpose of the Guiding Principles is to provide the foundation for instituting cultural change within the chemical industry, change resulting in improved openness with the public accompanied by a commitment to continued improvement in performance. The Guiding Principles are described as follows:

1. "To lead our companies in ethical ways that increasingly benefit society, the economy and the environment.
2. To design and develop products that can be manufactured, transported, used and disposed of or recycled safely.
3. To work with customers, carriers, suppliers, distributors and contractors to foster the safe and secure use, transport and disposal of chemicals and provide hazard and risk information that can be accessed and applied in their operations and products.
4. To design and operate our facilities in a safe, secure and environmentally sound manner.
5. To instill a culture throughout all levels of our organizations to continually identify, reduce and manage process safety risks.
6. To promote pollution prevention, minimization of waste and conservation of energy and other critical resources at every stage of the life cycle of our products.
7. To cooperate with governments at all levels and organizations in the development of effective and efficient safety, health, environmental and security laws, regulations and standards.
8. To support education and research on the health, safety, environmental effects and security of our products and processes.
9. To communicate product, service and process risks to our stakeholders and listen to and consider their perspectives.

10. To make continual progress towards our goal of no accidents, injuries or harm to human health and the environment from our products and operations and openly report our health, safety, environmental and security performance.
11. To seek continual improvement in our integrated Responsible Care Management System® to address environmental, health, safety and security performance.
12. To promote Responsible Care by encouraging and assisting others to adhere to these Guiding Principles."

Prior to the advent of Responsible Care, the first code of management or practice, community awareness and emergency response (CAER), was approved by CMA in 1989. This program element was implemented in part as a response to the 1984 chemical release incident in Bhopal. At that time, few of its members had emergency plans that addressed what responses needed to be implemented and coordinated with local emergency services should an accidental release occur. The CAER program action steps, many of which were incorporated into elements of the 1986 Superfund Amendment Reauthorization Act (SARA), required formation of State Emergency Response Commissions and Local Emergency Planning Committees (LEPCs). Importantly, this early code element also mandated companies to implement community outreach through formation of Community Advisory Panels (CAPs). CAP members are citizens who provide perspectives of issues and questions arising from chemical manufacturing facilities located within their communities. As of 1998, over 300 CAPs were established in communities where chemicals are manufactured across the United States, and these advisory panels have been carried over into the Responsible Care program (ACC, 2019). The CAER program also provided an important mechanism for the chemical industry to facilitate the development of community communication plans consistent with the EPA-mandated Risk Management Planning (RMP) rule, which requires disclosure of worst-case release scenarios to public emergency response teams.

The Responsible Care Product Safety Code of Management Practices has continued to evolve since its institution and is currently characterized by 10 Codes of Management Practices (ACC, 2019). As an extension of company-specific product stewardship programs, the Code goes beyond existing regulatory requirements and consists of the following elements:

1. Leadership commitment—engagement of senior company leadership.
2. Accountability and management—product safety/stewardship are integral to business processes and employee expectations.
3. Prioritization of products—having a risk-based process to prioritize products for further evaluation.
4. Product information—maintain information on safety, health, and environmental hazards and risks.
5. Risk characterization—assess risks based on product hazard, uses, and exposures over their complete lifecycle.
6. Product safety management—processes to identify, implement, document, and communicate product information important to assuring products are safe for their intended uses.
7. Management of new information—process to identify and evaluate new information that many trigger refinement to existing risk assessments or other product safety information.

8. Product design and improvement—consideration of health, safety, and environmental impacts in product manufacture, uses, innovation, design, development, and improvements.
9. Value chain communication, cooperation, and outreach—foster product safety management and information exchange across suppliers, customers, and value chain participants.
10. Information sharing—publicly available product safety and stewardship information to enhance public knowledge and confidence in safety of chemical products.

In 1999, the ACC also initiated a chemical public health research program, the Long-Range Research Initiative (LRI) (https://lri.americanchemistry.com/; ACC, 2019), focused on fostering improvement to the science-based understanding of potential chemical risks to humans and the environment. This program included assuming governance and partial financial support for the Chemical Industry Institute of Toxicology (CIIT), a not-for-profit research institute that was committed to independent research into mechanisms of chemical toxicity. In 2007, the CIIT became a standing institute within the Hamner Institutes. Although the Hamner Institutes ceased operations in 2016, the LRI program is maintained as a joint research commitment of the ACC, CEFIC (European Chemical Industry Council), and JSIA (Japanese Chemical Industry Association) (ICCA, 2018). The research objectives of the LRI are to develop new tools to improve safety assessment, with particular emphasis on (1) high-throughput *in vitro* assays and genomics as refinements or replacements to conventional whole animal testing, (2) better translation of everyday chemical exposures to science-based risk assessment methods, and (3) augmenting translation of research outcomes to improved public/consumer confidence in product safety.

A key issue fundamental to the public credibility of Responsible Care is external verification of performance. To facilitate this objective, ACC has implemented a management systems verification (MSV) program (ACC, 2019). This process is based on the framework of "plan-do-check-act" in which companies must:

1. Plan—identify potential hazard and risk of their products and establish goals to address them
2. Do—do what is planned, document, and communicate to vested partners including employees;
3. Check—conduct performance measurement and corrective actions through self-assessments;
4. Act—senior management reviews adequacy of the management system and makes changes supporting enhancing performance and share actions with company stakeholders.

An important element of the verification program is mandatory certification by an independent accredited auditor that verifies companies have the structures and systems in place to verify performance. Certification is required every 3 years using either the Responsible Care Management System (RCMS®) or RC14001® (combination of Responsible Care and ISO 14001).

5.7 CONCLUDING PERSPECTIVE

In order to assure industry sustainability, meeting health, safety, and environmental concerns is and will remain a top priority. The sections of this chapter outline the substantial commitments of the chemical industry to protect the human health of its workers and downstream users. Despite these long-standing efforts and paralleling activities of global regulatory agencies, there is a continuing gap between public perceptions of chemical product risks versus the realities of risk as evaluated by scientific methods. Resolution of such gaps remains challenging in an era of social media, but product stewardship activities represent a publicly transparent commitment to the chemical industry's responsibilities for continual improvement of its investments in product safety. In the end, the objective of product stewardship programs is to keep a sharp focus on identifying meaningful gaps in the overall health knowledge, assuring that the finite resources of the industry as well as government research continue to identify and address the highest priority health and environmental concerns.

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6

FOOD CONSTITUENTS AND CONTAMINANTS

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6.1 INTRODUCTION

6.1.1 Composition of Food

Food is by far the most chemically complex part of the human environment. We have no reliable estimate of the number of distinct chemical compounds in the different items of food and drink we select for nourishment and pleasure, but it is surely in the hundreds of thousands. The chemical structures of many of these are unknown, but the known constituents display immense variety. To make matters more complex, the chemical composition of the human diet varies from culture to culture and over time within cultures. We will probably never identify more than a fraction of the chemicals we ingest every day of our lives (NRC, 1996).

The natural constituents of foods and beverages represent the major share of dietary chemicals. In addition to the hundreds of distinct compounds that supply nutritional requirements, there are thousands more that impart flavor and color. Food plants also contain large numbers of natural constituents that contribute neither nutritional nor esthetic properties, but are present because they play some role in the lives of these plants. It has been estimated, for one small example, that a freshly brewed cup of coffee contains more than 600 distinct compounds (Smith, 1991). We also need to note the additional burden of natural products from the hundreds of herbs and spices used in food preparation.

Also among the constituents of the human diet are substances that arise during food and beverage preparation. Fermentation, for example, produces numerous chemical alterations of organic compounds, yielding products bearing little chemical similarity to the starting materials. Little scientific skill, and not much gastronomic skill, is needed to recognize that each variety of wine and cheese possesses a unique chemical composition and

Environmental Toxicants: Human Exposures and Their Health Effects, Fourth Edition.
Edited by Morton Lippmann and George D. Leikauf.

that none of these bears much resemblance to grape juice and milk. Roasting, broiling, baking, smoking, and other means of preparing and processing foods each set off many chemical reactions. Because most methods of food preparation have been in use for centuries, people think of the products of preparation as "natural," perhaps appropriately. Strictly speaking, they actually result from human manipulation of raw food products.

Human beings have never been satisfied to leave nature as it is and have added substances to food to achieve any number of desirable technical effects. Food preservation using various inorganic salts was one of the earliest examples of this practice, but adding substances to color, sweeten, emulsify, flavor, and alter taste perception is also an ancient practice that continues to this day.

Many chemicals that are intentionally used in food production, processing, and storage end up in the diet, although usually in very small concentrations. Among these indirectly added substances are (1) residues of drugs and feed additives used in animal production, (2) crop-use pesticides and their metabolites, (3) degradation products, (4) migrants from materials coming into contact with food during processing, and (5) packaging. Several thousand direct and indirect additives that may be present in foods add a significant increment to the large numbers of natural substances and those resulting from food preparation.

Foods may also contain environmental contaminants—unwanted by-products of nature or human industry that come to be present in food. These include bacterial and fungal metabolites resulting from their growth on food, organic chemicals of industrial origin, and various metals and other inorganic species that arise either because of their natural presence in soils and water used for food production or because they have accumulated to unusually high environmental levels as a result of mining, industrial, or other human activities. For completeness, we need to include bacterial metabolites that are not produced directly in food but rather in the intestines following ingestion of foods contaminated with the offending organisms. Some contaminants are fairly regularly occurring constituents of certain foods, whereas others arise only occasionally (and unpredictably) because of a human or natural mishap. As with the other dietary constituents, the total number of possible dietary contaminants is unknown, although those most important to human health are fairly well documented (IOM, 1998). The major categories of the constituents of food are summarized in Table 6.1.

6.1.2 The Problem of Understanding Food-Related Health Risks

Given the complexity of food, we find little uniformity in the study of the health risks associated with its constituents. One approach is that of clinicians and epidemiologists interested in diet and health. They produce studies of health trends in populations with high caloric and animal fat intake and low intakes of fiber on the one hand, and cardiovascular disease and certain forms of cancer on the other. Epidemiologists have also uncovered associations between excessive intakes of specific dietary constituents, such as salt, nitrates, methylmercury, and aflatoxins, and specific human diseases, although epidemiological science is usually working close to its statistical limits in many such situations. Nutritionists rely on the tools of epidemiology but also turn to clinical studies and studies in laboratory animals to learn about the risks and benefits of nutrients. Most of their efforts have focused on the major constituents and essential nutrients (Reddy and Cohen, 1986; NRC, 1996; IOM, 1997–2002; NASEM, 2017).

The contributions of toxicologists, whose main tools are experimental investigations, have generally been limited to the study of individual constituents. The efforts of toxicologists are primarily driven by regulatory requirements to establish limits on human exposure

TABLE 6.1 Classes of Food Constituents and Contaminants and Their Known and Potential Health Impacts

Class	Description	Methods Used to Study Risks and Benefits	Importance and Source of Health Risks[a]	Known and Possible Health Benefits[a]
Nutrients	Macronutrients (supplying energy) and micronutrients	Clinical/epidemiological	Major Excessive energy, saturated fat intake; abusive intakes. Inadequate or excessive intakes may be significant for some micronutrients	Major Essential for life. Levels in excess of recommended intake levels may prove to be beneficial
Natural, nonnutritive constituents	Largest and chemically most diverse class	Little systematic study, mostly experimental	Insufficient knowledge A reasonable conjecture is that they are at least moderately important	Insufficient knowledge Some appear to offer benefits, and many may prove to be beneficial
Intentionally introduced substances, direct and indirect	Food additives, GRAS substances Pesticides, veterinary drugs, feed additives Indirect additives	Experimental studies well developed. Little epidemiological study	Minor/moderate Many substances introduced decades ago have not been studied using current experimental methods. Cumulative effects unknown	Minor Some may provide benefits through pathogen reduction and preservation of foods
Chemicals produced during processing, preparation	Reaction products from heating, irradiation, etc. Chemically very diverse	Little systematic study, mostly experimental	Insufficient knowledge A reasonable conjecture is that they are of minor but not negligible importance	Insufficient knowledge Not expected to confer significant benefits
Contaminants	Substances not expected to be present. Industrial and natural chemicals, biological organisms	Several contaminants very well studied, many not Epidemiological and experimental	Moderate Pathogens produce largest number of "countable" cases of food-related illnesses; most not serious or irreversible. Persistent, bioaccumulative chemicals are of concern	Insufficient knowledge Not expected to confer benefits
Dietary supplements	Substances permitted under law to be sold as supplements	Little systematic study Epidemiological/clinical, experimental all applicable	Insufficient knowledge Reasonable conjecture not possible Little systematic study	Insufficient knowledge Many perceived as beneficial. Little systematic study, but increasing
Alcoholic beverages	Ethanol plus many fermentation products	Epidemiological studies extensive	Major Abusive intakes associated with much mortality and morbidity	Moderate Range of low intakes almost certain to decrease risk of CHD

[a] Some conclusions offered here should be taken only as conjectures of the authors, not as well-documented facts.

to additives and certain well-recognized contaminants. Up to the present, relatively little systematic study of the thousands of natural constituents of food, except for those that have made themselves known by high and easily detectable (usually acute) toxicity, has been carried out by epidemiologists, nutritionists, and toxicologists (Doull, 1981; Kotsonis and Burdock, 2013; NASEM, 2017).

It is almost certain that the collection of toxicology data on each of the individual constituents and contaminants of food (a clearly impossible task) would still not provide a thorough picture of food-related risks. The total health risk associated with food is surely not simply the sum of the risks associated with each of its individual constituents and contaminants. Moreover, the picture is becoming increasingly complicated by the fact that many constituents of food, in addition to the nutrients, may confer health benefits (NRC, 1996; Brock et al., 2003; NASEM, 2017). Indeed, most of the major influences of food on health, both positive and negative, have more to do with overall dietary patterns than with the effects of individual constituents. Table 6.1 provides a glimpse of what is now understood about the health impacts of food and its many constituents and contaminants and of the significant gaps in understanding that remain.

6.1.3 Scope and Limitations of This Chapter

Rather than attempting to provide a complete evaluation of the role of chemicals in food in human health, we focus on individual constituents, additives, and contaminants and on the methods by which they are evaluated. Unlike most of the chapters in this volume, which deal with one or a few classes of chemicals, we are forced somehow to consider thousands of individual substances. It makes little sense to provide detailed exposure and toxicology reviews for a few important food substances, because little of general value can be learned by such an approach. Instead our choice was to emphasize the principles and methods for evaluating individual constituents, for assessing their health risks, and for establishing limits on human exposure to them. Broad surveys of the major categories of food constituents and contaminants are presented, and examples are drawn from several of these categories to illustrate certain principles and methods. The contaminants reviewed are those commonly found and not those resulting from accidental releases or acts of deliberate adulteration. Because it is the subject of Chapter 22, the matter of pesticide residues in food is omitted here.

Because much of what has been learned about food constituents and contaminants resulted from the scientific investigations that have been conducted because of legal requirements, we begin the discussion with the regulatory framework under which these substances are treated and the methods used to assess risk and safety. We then proceed to discuss (1) nutrients and substances directly and indirectly added to food, (2) contaminants of industrial origin, (3) constituents and contaminants of natural origin, (4) substances produced during food processing and preparation, and (5) the special problem of dietary supplements. A review of important food safety institutions, both national and international, is then offered. The closing section deals with gaps in understanding and some suggestions regarding possible avenues toward improvements in knowledge.

6.2 LEGAL AND REGULATORY FRAMEWORK IN THE UNITED STATES

Although this chapter emphasizes the scientific evaluation of risks from food constituents and contaminants, we also include some background on the national and international legal and regulatory contexts. We do not discuss the intricacies of the U.S. Federal Food, Drug,

and Cosmetic Act (FDCA) and the recent Food Safety Modernization Act (FSMA), the laws governing food safety in the United States, but only to summarize certain broad features of them. Legal experts may consider this summary to be inadequate (but, we hope, not misleading). It is intended to provide scientists with some understanding of why certain categories of food constituents have received more extensive study than others and about the role of risk information in decision making.

In connection with the risks of food constituents and contaminants, the FDCA recognizes and distinguishes among at least three categories (IOM, 1998): (1) substances that are intentionally added to food, both directly and indirectly; (2) substances considered to be unavoidable contaminants of food; and (3) substances that are natural components of food, including nutrients. Dietary supplements are subject to legal requirements that were enacted in 1994 (Table 6.1).

The major U.S. federal agency responsible for enforcing FDCA and FSMA is the Food and Drug Administration (FDA). The U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) has enforcement responsibility for the Federal Meat and Poultry Inspection Act, and most of its provisions dealing with potential food risks match those of FDCA and FSMA. Although the law provides these agencies with broad authority to act to ensure the safety of the U.S. food supply, it places different burdens on the agency and on the regulated industries for the different categories of food constituents and contaminants. For the first group listed above (substances intentionally added to food),[1] the FDA has power to prevent their addition unless certain safety criteria are met. The FDA has responded to this legal mandate by specifying the types of toxicity and human intake studies that must be undertaken prior to the introduction of the added substance and the criteria by which safety is to be judged. In essence, such substances can be introduced only if they are "shown to be safe"; the sections of food law governing intentionally introduced substances do not permit other considerations (e.g., any possible benefits conferred) to influence the decision about the acceptability of these substances. "Safe" is defined as the "practical certainty of no harm" under proposed conditions of use. The Delaney Amendment, introduced in 1958, further specified that no additive found to induce cancer can be judged safe at any level of addition (clarification of the limits of applicability of the Delaney clause is offered later in this chapter). The burden of demonstrating safety falls primarily on those seeking to add substances to food (the "petitioner"); FDA can prevent such actions simply by showing that the burden has not been met. Except for substances generally recognized as safe (GRAS), substances can be intentionally introduced into food only in conformance with written regulations, whose content depends, in large part, on the toxicology and human intake data supplied to FDA by the petitioner (Merrill, 1996; Frankos and Rodricks, 2001).

Somewhat different burdens and criteria apply to contaminants. Clearly, if certain lots of food are deliberately or accidentally contaminated, or if the contamination can be readily avoided by good manufacturing practice (e.g., botulinum toxins), FDA and FSIS have substantial authority to ban or otherwise limit human exposure to the contaminated food and can take powerful emergency actions if the risks are judged substantial or imminent. The more difficult problem concerns contaminants the agency considers unavoidable, but that may be present at levels that pose significant (though perhaps not imminent) health risks. There are, for example, certain substances that may enter the food chain because of their widespread presence in the environment. Chemical pollutants such as arsenic (As),

[1] This group consists of several subgroups, and distinctions are made among these; they are discussed later in this chapter.

mercury (Hg), lead (Pb), polychlorinated biphenyls (PCBs), and several chlorinated hydrocarbon pesticides can be found in certain foods on a fairly regular basis, mostly at low (but not always insignificant) levels. These substances are not present because of any deliberate act of food adulteration. They are present because of industrial practices that led to widespread environmental contamination and, with the metals at least, because there are also certain levels of spatially variable natural occurrence. Obviously, with enough foresight, significant PCB and pesticide contamination of the environment might have been prevented by the institution of strict controls from the first days of their commercial extraction and/or production. While this was not done in ways that would be considered appropriate by today's standards, regulators are now faced with problems of food-chain contamination that can only be controlled in the short term by banning or limiting consumption of the affected food itself. This situation is clearly different from that of the deliberately added substances, and under the law the FDA has authority to balance health risks from the contaminant against certain costs—notably the loss of portions of the food supply—in setting limits on human exposure. The FDA has also applied these criteria to certain contaminants of natural origin, such as the aflatoxins (IOM, 1996; Merrill, 1996).

Contaminants differ from intentionally added substances in another significant respect: no specific responsibility for developing the data necessary to characterize health effects, human exposures, and risks is assigned under the law. Generally, the FDA, relying on its own, or on other governmental testing programs, or on data appearing in the scientific literature, has the burden of demonstrating contaminant risks prior to devising programs to control them. Thus, although some contaminants, such as Pb and Hg, have received considerable scrutiny from toxicologists and epidemiologists because of their widespread environmental occurrence, many have been only poorly characterized as to the risks they pose, certainly less thoroughly than intentional additives.

Nonnutritive, naturally occurring constituents (not contaminants) of food have received relatively little attention, in part because the law prefers not to tamper with food itself unless the risks are clearly substantial. Thus, for example, certain plants that might otherwise be considered suitable for food historically have been excluded, not by the FDA, but because they contain levels of toxicants sufficiently high to cause immediate adverse or even deadly affects. However, we readily accept many natural substances that produce subclinical effects under normal rates of intake, or substances that produce serious, chronic toxicity at high doses in animal tests, when it is clear their intentional addition to the diet would be prohibited because they fail to meet the safety criteria applied to additives. And, as with contaminants, no special responsibility is assigned under the law for the development of risk-related data on these substances. Not surprisingly, most investigations into the toxicity and risks of natural constituents of the diet (and they are very few in number relative to the size of this class of substances) tend to be of limited scope.

It is well recognized that nutrients may pose risks when intakes are less than those necessary to prevent deficiency diseases and when intakes are excessive and may cause toxicity. There is also now much interest in the roles of nutrients in reducing or increasing risks of certain chronic diseases (NASEM, 2017). Regulation related to nutrients takes on many different forms, with a heavy emphasis on providing consumers and public health professionals adequate information on desirable levels of intake.

FSMA, signed into law on January 4, 2011, represents the most significant expansion, to date, of FDA's food-related regulatory authority since the passage of FDCA. While the safety-minded principles of FDCA remain intact under FSMA, FSMA specifically outlines the responsibilities and procedures that food manufacturers, processors, handlers, and distributors must follow in order to produce food that is not adulterated or misbranded.

Broadly, FSMA requires food entities to identify potential hazards and implement risk-based preventive controls in order to prevent both unintentional *and* intentional food contamination or misbranding. For example, a food industry considered "higher risk" for contamination or adulteration (e.g., fresh or frozen food manufacturers) is required to implement more substantial preventive controls than a "lower risk" food industry (e.g., certain food contact substance manufacturers). Under FSMA, FDA maintains the authority to determine the "risk level" associated with specific food industries and released draft guidance summarizing its approach (FDA, 2014a). Among other requirements, FSMA requires food manufacturers to have in place foreign supplier verification programs, adequate quality management systems (including effective systems for conducting recalls), programs against intentional food adulteration, and sanitary storage and transportation practices. The law was designed in response to a number of high-profile and deadly food-borne illness outbreaks in the early 2000s and represents FDA's increasing utilization of risk-based approaches in regulation (UMN, 2014).

These various legal criteria (and others not mentioned here) help to explain why the extent of our knowledge of the classes of substances to be discussed later varies so greatly among them. They also reveal why different approaches to risk management have been taken for different constituents of the diet.

6.3 SAFETY CRITERIA AND THEIR SCIENTIFIC BASES

6.3.1 The Acceptable Daily Intake and Other Safety Criteria

An important risk criterion for judging the acceptability of substances intentionally added to food is the acceptable daily intake (ADI). The ADI is a level of daily intake that is not expected to cause adverse health effects when maintained over a full lifetime (Food Protection Committee, 1970; Joint Codex Alimentarius Commission, 1979; Dourson and Stara, 1983; Tennant, 1997).

Arnold Lehman and O. Garth Fitzhugh of the FDA introduced the ADI approach in the early 1950s to assist regulation of pesticides and other substances added to food. The ADI has been widely used in all areas of regulation; the FAO/WHO Joint Expert Committee on Food Additives (JECFA) has published ADIs for many food additives (some conditionally because of data gaps or uncertainties), and they are generally recognized as authoritative in member countries.

The theoretical basis for the ADI is that, for all toxic effects, with the possible exception of carcinogenicity (see later), a threshold dose must be exceeded before any toxic response is produced. Experimentally measured thresholds, or no observed adverse effect levels (NOAELs), are divided by various "uncertainty factors" (UFs) to estimate the corresponding threshold dose for the general human population. The U.S. Environmental Protection Agency (EPA) now uses the term toxicity reference dose (RfD) for what is the practical equivalent of the ADI, while the World Health Organization (WHO) International Programme on Chemical Safety (IPCS, 2004) has adopted the term tolerable daily intake (TDI), typically for application to contaminants. UFs are used to account for uncertainties regarding variability in susceptibility between animals and humans and among members of the human population (see Table 6.2; Dourson et al., 1996).

In recent years EPA and other agencies have increasingly turned to the lower confidence bound on the benchmark dose (BMDL) rather than the NOAEL as the starting point for deriving ADIs and RfDs (EPA, 2012).

TABLE 6.2 Typical Uncertainty Factors Used to Derive ADIs from Animal Toxicity Data

Source of Uncertainty or Variability	Factors Typically Applied
Extrapolation from animal NOAEL to estimate NOAEL for "average" human[a]	10
Variability within human population; "average" to most susceptible	10
Chronic NOAEL from subchronic NOAEL	10
Chronic LOAEL to chronic NOAEL	2–10
Limited database (e.g., data available from a single species only)	2–10

NOAEL, no observed adverse effect level; LOAEL: lowest observed adverse effect Level.
[a]Most sensitive species. Data available on specific compounds may allow departures from these standard "default" factors.

Typically, a 100-fold UF is applied to NOAELs or BMDLs from chronic studies to derive a chronically applicable ADI. Exceptions to the use of a 100-fold factor are made when data are available to reduce uncertainties regarding inter- or intraspecies extrapolation or when certain data are lacking or are inadequate in some way, in which case larger factors are used (Table 6.2). A substance is eligible for addition to foods as long as the total daily intake from all sources does not exceed its ADI (Codex Alimentarius, 2016).

An ADI is generally derived only when sufficient toxicity data have been developed, according to regulatory testing requirements. An RfD may be developed based on whatever data happen to be available; for many substances that do not require premarket testing, data gaps may be substantial.

The ADI is not a sharp dividing line between "safe" and "unsafe" intakes. It is by no means certain that intakes at or below the ADI are "risk-free" or that intakes above it pose significant risks. The ADI provides no insight into the question of risk because the generic UFs used, and even those that are in part based on chemical-specific data, provide no information on the fraction of the population whose thresholds are below or above the ADI. Risk might be expressed in terms of those fractions (on the theory that thresholds are distributed in some regular fashion among members of the population), and policy decisions could then be made to ensure that no more than the tiniest fraction would, in theory, be exposed at intakes exceeding their thresholds; this is similar, conceptually, to the approach taken for carcinogens (see later). No standardized methodology is available to estimate risks for threshold agents in this fashion; indeed, toxicology data are typically reported in insufficiently quantitative terms to permit risk assessors to move toward more quantitative evaluations of risk for these classes of agents. The WHO has recently published guidelines that are useful for treating uncertainties quantitatively and deriving quantitative risk estimates for toxic substances acting through threshold mechanisms (IPCS, 2014).

6.3.2 Toxicity Testing for Additives

The types and numbers of toxicity tests specified by the FDA were first described in detail in a publication entitled *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food*. This document is commonly known as the "Red Book" because of the color of its cover when it was originally published in 1982 (FDA, 1982a).[2]

[2]The FDA Red Book is not the more famous National Academy's Red Book of 1983 that set forth important risk assessment principles.

The FDA Red Book sets forth practices that have evolved over the years based on knowledge of toxicological properties associated with certain types of chemical compounds. It reflects an awareness of the need for toxicological information commensurate with the potential of an additive to cause safety concerns. Thus, the extent of toxicological testing required for a food additive, or a color additive used in food, is determined on a chemical-specific basis and relates to its chemical structure (insofar as chemical structure reflects toxicological potential) and the extent of expected human exposure. The FDA Red Book also provides guidance regarding the criteria used for food and color additive safety evaluations.

The FDA Red Book invokes the term "level of concern" to establish a system for gathering necessary safety information.

Table 6.3 presents the levels of concern for various anticipated exposure levels for direct additives classified according to their structural similarity to compounds of known biological activity. Compounds in category C represent those potential additives that may exhibit significant toxicological activity. For example, organic halides, compounds with highly reactive heterocyclic ring systems, such as epoxides or unsaturated lactones, are structure C compounds. At the other extreme, substances such as simple aliphatic and noncyclic hydrocarbons, saturated noncyclic straight-chain alcohols and carboxylic acids, and compounds identical to normal human metabolites are placed in the structure A category. Based on anticipated human exposure level, the FDA derives a level of concern for a potential additive once it is classified according to chemical structure.

Once a level of concern is determined, it then becomes possible to identify the studies required by the FDA to support safety. Table 6.4 lists the studies required for a direct food additive with the highest level of concern. The list of studies does not include acute or subacute toxicity tests, but it is hard to imagine appropriate design and conduct of the studies listed here without this information. The example is, of course, illustrative and requirements for specific additives may vary significantly. Moreover, the need for additional information may arise when the results of testing become known.

TABLE 6.3 Levels of Concern for Direct Food Additives at Different Concentrations in the Diet[a] and Expected Toxicity Categories Based on Chemical Structures

Expected Toxicity (Structure) III	Anticipated Human Exposure (ppm in Total Diet)	Concern Level[b]		
		Level I (Least)	Level II (Moderate)	Level III (Most)
A. Low	>1.0			+
	0.05–1.0		+	
	<0.05	+		
B. Moderate	>0.50			+
	0.025–0.50		+	
	<0.025	+		
C. High	>0.25			+
	0.0125–0.25		+	
	<0.0125	+		

[a]Adapted from the Red Book (FDA, 1982a). See revisions in the 1993 Red Book and 2000 Red Book (https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm2006826.htm).

[b]Extent of toxicity testing needed increases with increasing concern level.

TABLE 6.4 Recommended Toxicological Tests for Direct Food Additives of Level III (Highest Level of Concern)

Genetic toxicity tests
Short-term toxicity tests with rodents[a,c]
Subchronic toxicity studies with rodents[a,c]
Subchronic toxicity studies with non-rodents[a,c]
One-year toxicity studies with non-rodents[c]
Chronic toxicity or combined chronic toxicity/carcinogenicity studies with rodents[c]
Carcinogenicity studies with rodents
Reproduction studies[c]
Developmental toxicity studies[b,c]
Metabolism and pharmacokinetic studies[b]
Human studies[b]

Source: From https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm054658.htm.

[a]If needed as preliminary to further study.

[b]If indicated by available data or information.

[c]Including screens for neurotoxicity and immunotoxicity.

6.3.3 Information from Clinical Studies in Humans

Under the FDCA, substantial clinical data are necessary to gain approval for a human drug. No such requirement exists for food additives; the basis for introducing such materials into the human diet can rest entirely on the results of animal studies. This may seem odd, in that human exposure to some additives will ordinarily be much more widespread than drug exposure, and, moreover, additives are not introduced with the expectation that they directly confer health benefits. Nor is there a general requirement for post-approval monitoring of health effects, as there is for some drugs.

Clinical studies are widely used for nutrients and for some natural components of food when it is clear they will not be harmful to the study participants. But these studies are primarily focused on health benefits, not risks (NASEM, 2017). Clinical studies may also be used to evaluate "tolerance," particularly for substances added at high levels to the diet, such as low-calorie sweeteners, and such studies can detect signs of adverse responses.

6.3.4 Carcinogens and the Use of Risk Assessment

A demonstration of carcinogenicity by "appropriate" tests (interpreted by the FDA to mean tests by the oral route) invokes the Delaney Amendment; no "safe" intake level (no ADI) can be legally assigned to a substance having this property (Merrill, 1996). The FDA may discount any positive finding because of demonstrated irrelevance to humans or because the carcinogenic effect is secondary to toxic phenomena for which a threshold of action might be applicable and there are cases (sodium saccharin, melamine) in which the FDA has found reasons to discount such data. The FDA generally requires the development of a firm understanding of a chemical's mode of action (MOA) (see below) before taking such an action.

In 1973, the FDA proposed the use of risk assessment coupled with the notion of insignificant risk to deal with a certain class of intentionally added substances. The Delaney Amendment, as we have noted, applies to intentionally introduced substances, but in 1968,

Congress modified the law to deal with carcinogenic animal drugs that were used in food-producing animals. Such drugs, Congress allowed, could be used as long as "no residue" of the drug could be found in food (meat, milk, eggs) from treated animals. One supposes that, in the Congressional mind, "no residue" was not different from "no addition," as applied to directly introduced additives, but Congress later modified "no residue" by adding "by a method of analysis approved by the Secretary" (read FDA). The FDA was then charged with the decision of whether any given "method of analysis" was adequate to show the absence of residues (Rodricks, 2007).

The critical question facing the FDA was the detection limit such methods should possess. In scientific terms, "no residue" meant only that the carcinogenic drug was not present above the detection limit of the analytical method used. In fact, it was likely to be present in food at some nonzero level once an animal was treated and could be present at any level up to the detection limit.

The FDA introduced risk assessment as a regulatory tool to deal with this class of agents in the following way (FDA, 1979a):

1. The risks (or upper bounds thereon) of the particular animal drug would be quantified based on rodent bioassay data and the application of a linear no-threshold model of the dose–response curve.
2. The level of daily intake of drug residue corresponding to a (upper bound) lifetime cancer risk of 10^{-6} would be estimated.
3. An estimate would be made of the concentration of the drug in food (meat, milk, or eggs) that would yield, when the food is consumed, the level of daily intake estimated in step 2.
4. The petitioner would be required to demonstrate that a reliable analytical method is available that has a detection limit at least as low as the concentration estimated in step 3.
5. The petitioner would be required to show that, under the proposed conditions of drug use, "no residue" remains in food when the analytical method of step 4 is applied.[3]

This "sensitivity of the method" approach, as FDA has dubbed it, constituted the first regulatory use of quantitative risk assessment for carcinogens, as well as the first time that regulations defined safety explicitly in terms of risk.

The presence in food of carcinogenic animal drugs can be detected only by the application of an analytical method. In this respect, they are different from directly added substances, but similar to migrants from packaging and other food contact materials (typically monomeric precursors of polymers). Migrants become "additives" only if they can be detected in food. How far should we search? What analytical method, with what detection limit, should be used? The FDA seems satisfied if the methods that are used are shown capable of detecting residues of carcinogens at concentrations that create daily intakes corresponding to lifetime risks no greater than 10^{-6}. In the absence of a finding that a carcinogen has migrated from food contact materials using an analytical method of sufficient detection power, there is no need to apply the Delaney restriction, because no "additive" is introduced (Rodricks, 2007).

[3]The full basis for the approach, and for the 10^{-6} lifetime risk target, is described in Rodricks (2007), chapter 11.

Logic would have it that if a lifetime risk level of 10^{-6} is of insignificant public health concern for indirectly introduced food additives, it ought to hold that directly introduced substances that are carcinogenic but pose similarly small risks would be of no concern. Indeed, the FDA attempted to apply this logic to some color additives (some of which showed lifetime risks of the order of 10^{-8} or less), but its efforts were thwarted by the U.S. Court of Appeals on the ground that the Delaney Amendment does not exempt substances because they cause negligible health risks: it is clearly a zero-risk (no addition) law (Merrill, 1979, 1996). So, although risk assessment has utility in helping to define the required characteristics of analytical methods used to search for indirectly introduced substances, the "no-risk" requirement of Delaney holds for directly introduced food and color additives, the presence of which in food does not depend on a search with analytical methods. As we see later, the FDA has also used risk assessment as a tool in the regulation of certain carcinogenic food contaminants.

6.3.5 Threshold of Regulation

For substances used in food contact articles (e.g., food packaging or food processing equipment) that migrate, or that may be expected to migrate, into food, FDA created an exception from the regulatory requirements of a food additive for substances (except carcinogens or substances reasonably likely to be carcinogens) that enter the diet only at very low levels—dietary concentrations of less than 0.5 parts per billion (ppb) or dietary exposure levels below 1.5 μg/person/day (21 CFR 170.39). This exposure level was derived by FDA, based on an analysis of 477 animal carcinogens. Based on the range of potencies exhibited by these 477 animal carcinogens, FDA determined that most known carcinogens pose less than one in a million lifetime risk if present in the daily diet at 0.5 ppb (58 FR 52719). Someone who proposes to use such a substance must submit an application to FDA for an exemption from the food additive regulations, documenting the low level of dietary intake that would result from the proposed use, but if the usage qualifies, no safety testing is needed.

6.3.6 Mode-of-Action Considerations

As noted in Section 6.3.4, for chemicals that have been shown to be carcinogenic in animals, FDA may discount that finding if it can be shown that the MOA of the chemical in causing cancer in animals is one that is not relevant to humans at the anticipated level of human intake. Examples include D-limonene, which causes kidney cancer in male rats by an MOA (alpha 2u-globulin accumulation in the kidney) that is unique to male rats and could not occur in humans (EPA, 1991). Melamine is a little different. It causes bladder tumors in rats, but only at dose levels so high as to cause formation of calculi in the urine, which produce urothelial damage, consequent regeneration, and bladder tumor formation. While this MOA is theoretically possible in humans, the level of intake needed to cause formation of urinary calculi would be so high, and exposure would need to be so prolonged as to be implausible, or at minimum would permit the identification of a threshold exposure level below which calculi would not be formed and, therefore, tumors would not develop (Cohen et al., 2004).

These examples illustrate the importance of MOA information for evaluating possible cancer risk, but the importance of MOA for assessing noncancer toxic effects has also been the subject of discussion. The IPCS (2004) of WHO has published a two-part document

discussing the importance of MOA for both cancer and noncancer effects. They call this a human relevance framework (HRF). The purpose of the HRF for cancer is stated to be:

- To provide a generic approach to the analysis of data to contribute to harmonization.
- To encourage transparency of the consideration and use of available data and reasons for the conclusions drawn.
- To provide guidance in the presentation of data.
- To identify critical data deficiencies and needs.
- To inform the quantitative assessment of carcinogenic risk to humans.

Similarly, the IPCS meeting concluded that the noncancer HRF would have multiple uses in chemical risk assessment:

- It would provide an internationally harmonized approach to the establishment of an MOA in experimental animals and its relevance to humans.
- It would generate criteria for the MOA against which subsequent cases could be considered—that is, to show whether a compound shares an established MOA.
- It would enable clarification of key information relating to the human relevance of the MOA, and this would inform the assessment of other chemicals that share the MOA.
- In general, application of the framework would enable critical data deficiencies and research needs to be identified and inform qualitative and quantitative assessment.

6.3.7 Human Exposure Assessment

The evaluation of carcinogenic and noncarcinogenic risks for food constituents depends not only upon toxicity criteria (ADIs or carcinogenicity potencies) but also upon estimates of human exposures to those constituents. The typical term used in food constituent risk assessment is the estimated daily intake (EDI). Generally, for intentionally introduced food constituents that are not carcinogenic, FDA's safety criteria (risk management goals) are satisfied if the constituent's EDI is less than its ADI. Carcinogenic risks are estimated by multiplying potencies (upper bounds on estimated lifetime risks per unit of daily intake) by EDIs.

EDIs are estimated for directly introduced constituents by multiplying the concentration of the constituent in the food by the weight of that item of food consumed per day (Pao et al., 1982; Brock et al., 2003). High-end consumers, at the 90th or 95th percentile of food consumption rates, are the usual targets for the risk assessment; if such users are protected, then it can be concluded that the safety standards set forth in law are satisfied (FDA, 1988). Estimating intakes for indirect additives (e.g., substances migrating from food contact surfaces and packaging) is more complex, because it depends upon analytical data regarding the amount of migration into food and the chemical identities of migrants. FDA's Recommendations for Chemistry Data for Indirect Food Additive Petitions (FDA, 1988) sets forth procedures to be followed to collect relevant data. Various food-simulating solvents are used, and extraction studies performed to estimate food concentrations; these data are combined with information on expected food contact surface areas and the daily intake of food to estimate EDIs. The vinyl chloride example, presented below, illustrates the evaluation procedure (Frankos and Rodricks, 2001).

Similar to FDA's requirements in the United States, the European Food Safety Authority (EFSA) requires manufacturers to evaluate the safety of new food additives, enzymes, and flavorings prior to introduction into the European Union (EU) market. To help facilitate the exposure assessments required of petitioners, EFSA has developed the "Food Additives Intake Model" in order to help petitioners estimate exposures to food additives across various use conditions. Specifically, the Food Additives Intake Model utilizes the data of the EFSA Comprehensive European Food Consumption Database for five population groups across 17 EU member countries in order to help generate mean- and high-level exposures to the proposed additives. Population groups include toddlers, children, adolescents, adults, and "the elderly" (i.e., those over 60 years of age). Although the tool exhibits limitations common to any exposure model (e.g., under- and over-reporting of food consumption, uncertainty with usual intake estimates), the model represents a modern and accessible tool aiming to harmonize exposure assessment (EFSA, 2014).

6.3.8 Risk-Based Decision Models

Risk management decisions for substances intentionally introduced into food are relatively straightforward. Such substances can be introduced only if safety criteria are met, and those criteria are met when EDIs resulting from their presence in food are less than the ADI's established for those substances. The ADI can be described as a "bright line" separating safe from unsafe exposures.

Substances that come to be present in food, but are not intentionally introduced, present greater challenges for decision making. Thus, food contaminants of both industrial and natural origin, natural constituents of food, and substances created during food preparation and processing cannot be readily avoided—their introduction into food cannot simply be halted or, in the case of natural constituents, cannot be avoided at all. In some cases a decision model that is based on application of a TDI may be used, but use of such a "bright-line" standard will require that foods having levels of contaminants or other substances that are not readily avoidable be prohibited. Because the risk associated with the TDI is not quantified, and because the effect on food supply (amounts of food not permitted into commerce by enforcement of a TDI-based standard) is not explicitly considered in establishing the standard, there is no basis in this decision model for understanding whether the risk reduction achieved is commensurate with the costs incurred. To seek a decision model that explicitly and quantitatively considers whether the costs incurred (measured as amounts of food removed from commerce) are commensurate with the health benefits (risk reductions) achieved is not to equate health protection and costs. It is rather to seek a decision model that allows understanding of how risk declines as exposure is reduced and further to understand the costs of achieving those exposure reductions. At some point the technical ability and costs of achieving exposure reductions might be judged (as a policy matter) excessive relative to relatively minor gains in public health protection. A decision model of this nature is promoted in the 2009 National Research Council report "Science and Decisions: Advancing Risk Assessment" (NRC, 2009). The use of such a model requires quantitative risk assessments. The cancer risk assessment model described earlier is suitable for this purpose. Quantitative risk models have been advocated for noncancer endpoints, and some guidance for achieving this goal is available (IPCS, 2014). Until quantitative risk models are developed for noncancer endpoints, margin-of-exposure (MOE) approaches will prove useful. Increases in MOEs achieved with different approaches to exposure reduction can provide guidance to decision makers. An ILSI working group

has demonstrated how such a decision model might be applied to evaluate to process-formed chemicals such as acrylamide (Benford et al., 2010).

The various groups of food chemicals that are not readily avoidable are discussed in Sections 6.6–6.8. It cannot be said that current approaches to their control have been evaluated under the decision model described here, but exploration of its utility for these substances should perhaps be encouraged.

6.4 NUTRIENTS

Nutrients present risks to health (1) when intakes are less than required and (2) when intakes are sufficiently high to produce toxicity. Dietary Reference Intakes (DRIs) have been in development since the 1990's by committees of the Institute of Medicine (now the Health and Medicine Division of NASEM); these efforts have been sponsored jointly by the U.S. and Canadian governments, and the recommended DRIs arising from them are used to guide decisions regarding labeling, diet planning, and other nutrition-related activities.

DRIs include Recommended Dietary Allowances (RDAs) and other measures of adequacy for both micronutrients (vitamins and minerals) and macronutrients. A framework for inclusion of what are termed Tolerable Upper Intake Levels (ULs) was developed in the 1990s and continues to be used.

The UL is defined as the highest average daily intake that is likely to produce no risk of toxicity. The need for ULs was, in part, prompted by concerns regarding increasing uses of dietary supplements and fortified foods and also because of evidence that excessive intakes of some nutrients could be harmful. Excessive intakes of vitamin A, for examples, have been associated with liver toxicity, bone mineral density declines, and teratogenicity. Excessive calcium intakes can lead to nephrolithiasis (stones), renal insufficiency with and without metabolic alkalosis, progressive renal failure, and interactions effecting absorption of other nutrients. Interactions among nutrients are common (those between folate and vitamin 12 are especially complex) and need to be considered in deriving ULs. Some critical endpoints used in the derivation of ULs are shown in Table 6.5 (IOM, 1998).

The method used to derive ULs is conceptually similar to that used for developing ADIs and RfDs (IOM, 2008). While UFs were applied, there is apparently, in the published

TABLE 6.5 Critical Adverse Effects Used to Derive ULs for Some Nutrients

Nutrient	Adverse Effect
Calcium	Milk-alkali syndrome
Phosphorus	Elevated serum P_i
Magnesium	Osmotic diarrhea
Vitamin D	Serum calcium >11 mg/dl
Fluoride	Children: moderate dental fluorosis Adults: moderate skeletal fluorosis
Niacin	Flushing
Vitamin B_4	Sensory neuropathy
Folate	Neuropathy in B_{12}-deficient individuals
Choline	Hypotension, fishy body odor

reviews, much more professional judgment involved in that selection than is common in deriving ADIs and RfDs. In some cases, application of standard UFs used to derive ADIs would lead to ULs less than the RDAs established for those nutrients. Such an outcome is not surprising, because the standard UFs do not account for the many mechanisms at work for the body's controls over exposures resulting from oral ingestion of nutrients. UL development was also examined by committees of the WHO, and their findings are generally consistent with those of the IOM committees (NASEM, 2017).

In 2016 an expert panel established by the governments of Canada and the United States published a review of scientific approaches (options) that might be used to incorporate chronic disease endpoints into the development of DRIs (Yetley et al., 2017). Although some efforts had been made to incorporate such endpoints into the establishment of DRIs, most DRIs had been based on traditional nutrient deficiency diseases. The Health and Medicine Division of NASEM was asked to evaluate the published options and recommend specific approaches; the report on this topic was recently published (NASEM, 2017). Its first application to nutrients has started, with the focus on sodium (Na) and potassium (K). The DRIs based on chronic disease endpoints are to be established to reduce risk by identifying intakes likely to minimize risks of diseases whose risks are increased by nutrient exposure or identifying intakes likely to maximize benefits from nutrients that decrease chronic disease risks.

Interestingly the NASEM report focused not only on traditional nutrients but also considered risks and benefits that might be associated with other natural constituents of food. As noted in the Introduction to this chapter, these natural substances comprise the single largest group of food chemicals, and some appear to have the capacity to affect disease risks.

The dose–response relationships for chronic disease endpoints are quite different from those relating to deficiency diseases. In the case of deficiency diseases, adequate intakes will reduce risks to near zero for everyone, and the absence of any intake will cause disease in everyone. In the case of multifactorial chronic diseases, no single factor will have anything greater than a partial effect on risk (NASEM, 2017). This entire area of research and its applications is just beginning to establish a foothold.

6.5 SUBSTANCES INTENTIONALLY INTRODUCED INTO FOOD

Under the law, the term "food additive" has a specific meaning (FDCA):

> …any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component of or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food; and including any source of radiation intended for any such use).

The statute goes on to exclude the following:

- GRAS substances.
- Pesticide chemicals in or on raw agricultural commodities.
- Color additives.
- New animal drugs.

These four categories of excluded substances (and some others as well) are certainly "intentionally introduced additives" in the popular and even technical use of the term, but legally they are not "food additives." For our purposes, many of the legal distinctions among these groups of additives are not important: safety must be demonstrated for all of these various groups of additives, although data requirements and criteria for acceptability vary among them (Merrill, 1996; IOM, 1998; Kotsonis and Burdock, 2013).

6.5.1 GRAS Substances

When the food additive amendments to the federal FDCA were enacted in 1958, certain food ingredients that had long been in use were exempted from the premarket testing and approval process required for food additives. Ingredients in use prior to January 1, 1958, could be considered GRAS based on a common use in food or through scientific evaluation procedures. Any food ingredient can be classified as GRAS if it is "generally recognized, among scientific experts qualified by scientific training and experience to evaluate its safety…to be safe under the conditions of its intended use." These criteria are quite general and essentially leave the decision about GRAS status to scientific experts.

After public review of its proposals, the FDA published a GRAS list with the following commentary (Frankos and Rodricks, 2001):

> It is impracticable to list all substances that are GRAS for their intended use. However, by way of illustration, the Commissioner regards such common food ingredients as salt, pepper, sugar, vinegar, baking powder, and monosodium glutamate as safe for their intended use.

The FDA's published GRAS list includes more than 600 substances, but this list by no means included every substance that was, or could be, considered GRAS. Indeed, classification of substances as GRAS can be made by any group of qualified experts. The Flavor and Extract Manufacturers Association (FEMA), for example, convened such a group in 1960, and the group listed more than 1000 flavoring ingredients and their levels of addition to food that could be considered GRAS; FDA has generally considered the FEMA process and lists as meeting the criteria set forth in the Act (Oser and Hall, 1977).

A selected list of GRAS substances is presented in Table 6.6. The levels of addition of these substances to food are specified for some, but for most usage levels are simply defined according to the amounts consistent with "good manufacturing practice." For those having specified limits, new uses that would result in increased exposures have to be justified based on "scientific procedures" recognized by experts. The FDA may also "affirm" the GRAS status of substances by regulation (Merrill, 1979). The required "scientific procedures" are similar to those described below for food additives.

Most chemicals directly added to food are GRAS. Scrutiny of the highly abbreviated list presented in Table 6.6 reveals the presence of agents having diverse toxicological characteristics. Most are present because they have had a long history of use in food with no significant reports of adverse health effects (FDA cannot remove a substance from GRAS status unless evidence appears showing that it can no longer be considered safe for its intended use). The quantity and quality of available toxicology data vary greatly among these substances, and decisions about the adequacy of these databases to judge risk and safety have been in the hands of experts, both within and outside of FDA (Select Committee on GRAS Substances, 1981).

TABLE 6.6 Selected GRAS Substances Listed by the FDA

Spices and other natural flavors		
Anise	Geranium	Parsley
Basil	Ginger	Spearmint
Capsicum	Glycyrrhiza	Vanilla
Elder flowers	Licorice	
Multipurpose substances		
Acetic acid	Hydrogen peroxide	
Aluminum sulfate	Lecithin	
Caffeine	Methylcellulose	
Calcium carbonate	Papain	
Caramel	Propane	
Carbon dioxide	Rennet	
Affirmed as GRAS by FDA regulations		
Benzoic acid	Potassium iodide	
Clove	Propyl gallate	
Ethyl alcohol	Sorbitol	
Garlic	Dextrans	
Guar gum	Gum tragacanth	

The Center for Science in the Public Interest, Consumers Union, Environmental Working Group, and Natural Resources Defense Council (NRDC) have criticized GRAS process as not providing adequate FDA oversight of the safety of substances before they are added to the food supply. In part to address criticisms such as these, FDA has introduced a voluntary notification procedure under which individuals who consider a substance to be GRAS can notify the FDA of their opinion by submitting a document summarizing the basis for this opinion, and FDA has the option of declaring that it has "no objection" to the conclusion of GRAS status, or declaring that it does not believe there is a basis for GRAS status (81 FR 54960). To avoid the latter, the submitter may withdraw the notice. Conclusions regarding GRAS are often reached by panels of food safety experts convened by proponents of the substance under consideration, and FDA has also recently issued draft guidance on "Best Practices for Convening a GRAS Panel" (82 FR 53433). In this document FDA provides guidance on identifying GRAS panel members who have appropriate and balanced expertise, reducing the risk that bias (or the appearance of bias) will affect the credibility of the GRAS panel's output, and limiting the data and information provided to a GRAS panel to public information (since in order to be "generally recognized" the information supporting safety has to be publicly available).

Except for substances that have a history of safe use in food dating from before 1958, GRAS status must be based upon the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation (as described in Section 6.3.2) (21 CFR 170.30).

6.5.2 Direct and Indirect Food Additives

As noted, there are hundreds of direct and indirect food ingredients listed as GRAS. There are also more than 100 direct and indirect food additives regulated as "food additives" in the legal sense of the term; that is, they have been the subject of a petition submitted to FDA since 1958, containing toxicity and human intake data adequate to meet FDA's safety

TABLE 6.7 Selected Examples of Direct and Indirect Food Additives Regulated by the FDA

Major Categories of Direct Additives	Example	Notes
Primary direct additives		
Food preservatives	BHA, BHT	GRAS for some uses
	Nitrites, nitrates	Antimicrobial actions, color fixation in meat, poultry, smoked fish: up to 2% in various foods
Anticaking agents	Silicon dioxide	Amount not to exceed 2% by weight of food
Flavoring agents	Elder tree leaves	Alcoholic beverages, 25 ppm limit on HCN
Coating agents, films	Coumarone-indene resin	Citrus fruits
Gums, chewing gum bases	Arabinogalactan	Used in minimum quantity required to produce intended effect as an emulsifier, stabilizer, binder, or bodying agent
Special dietary, nutritional agents	Nicotinamide–ascorbic acid complex	Source of ascorbic acid and nicotinamide in multivitamin preparations
Multipurpose agents		
Emulsifiers	Sodium lauryl sulfate	Egg white solids
Dough conditions	Azodicarbonamide	Also used for aging, bleaching of flour
Dispersing agent	Polysorbate 60	Also used as emulsifier, dough conditioner
Stabilizing agents	Propylene glycol alginate	Emulsifier, baked goods, cheeses, etc.
Nonnutritive sweeteners	Aspartame	FDA (1983)
Secondary direct additives: processing aids		
Polymers, resins	Acrylate–acrylamide resins	Clarification of juices
Enzyme preparations	Catalase derived from *Micrococcus lysodeikticus*	Cheese production
Microbial agents	*Candida lipolytica*	Foods resulting from fermentation
Solvents, related agents	1,3-Butylene glycol	Extraction of flavors from spices
Indirect food additives		
Adhesives and components of coatings	Acrylate ester copolymer coating	Approved for food contact
Paper and paperboard components	Acrylamide–acrylic acid resins	Specifications for monomer
Polymers	*n*-Butyl methacrylate	Approved for food contact
Adjuvants, production aids, and sanitizers	Hydrogen peroxide	For sterilizing food contact surfaces

criteria for food additives (as discussed earlier). Some major use categories for direct and indirect additives, along with specific examples, are given in Table 6.7.

6.5.3 Food Contact Materials

In 1975 FDA proposed to prohibit some uses of vinyl chloride polymers (homo- and copolymers), including their use in semi rigid and rigid food contact articles such as bottles and sheet. The FDA withdrew this proposal in 1986 and, in its place, proposed to amend its

regulations to provide for the use of vinyl chloride polymers. This change of position resulted from (1) improved production technology that reduced the level of residual vinyl chloride monomer by a large factor and (2) new agency policy, as described earlier, concerning the regulation of food and color additives that may contain carcinogenic impurities (FDA, 1986).

The FDA decided to regulate the use of vinyl chloride polymers to ensure that the polymer that is marketed does not contain unsafe levels of the monomer. The agency proposed that the Delaney Amendment not be triggered unless the additive as a whole (polymer) is found to induce cancer. An additive that has not been shown to induce cancer, but that contains a carcinogenic impurity (vinyl chloride), is evaluated under the general safety clause of the statute using risk assessment procedures to determine whether there is a reasonable certainty that no harm will result from the proposed use of the additive. The FDA has evaluated the safety of this additive under the general safety clause using risk assessment procedures to estimate the upper bound limit of risk presented by the carcinogenic chemical present as an impurity in the additive (as a by-product of polymer synthesis).

The FDA conservatively estimated that the lifetime average individual exposure to vinyl chloride monomer from the probable food contact uses of vinyl chloride polymers [e.g., liquor bottles, wine bottles, oil bottles, vinyl chloride homopolymer film, vinyl chloride–vinylidene chloride copolymers (films and fresh citrus fruit coatings), and miscellaneous uses] will not exceed 25 ng/day.

The agency used a quantitative risk assessment procedure (linear no-threshold model) to extrapolate from the doses in animal carcinogenicity studies of vinyl chloride to the very low doses of possible human exposure. The FDA estimated that the individual lifetime risk of cancer from exposure to vinyl chloride monomer at 25 ng/day is less than 1 in 10 million. The agency concluded that there is a reasonable certainty of no harm from these exposures. The monomer, though carcinogenic, need not be treated as an additive subject to the Delaney clause (FDA, 1986).

Other monomeric residuals in food contact materials have been subjected to similar scrutiny. Of particular current interest is bisphenol A, which is present in certain plastics used as food and beverage containers. The EPA established an RfD for this important product at 50 mg/kg/day, based on data from toxicity studies published in the 1980s.

Current human exposures through food seem to be well below the RfD. But in the time since the RfD was derived, many investigators have pursued research into the compound's reproductive and developmental effects, and some report biological activities said to be related to endocrine system disruption at doses below the RfD (which was derived by the application of large UFs to the earlier toxicity studies). Either the newer test systems are yielding irrelevant toxicological findings, or they are telling us that traditional study protocols fail to uncover many important endocrine system effects. Many investigators are in pursuit of answers (see Chapter 15).

In addition to a variety of industrial uses, including use in firefighting foams, per- and polyfluoroalkyl substances (PFAS) have been widely used in nonstick, stain-resistant, waterproof, and oil-resistant consumer products because of the hydrophobic and lipophobic properties of the PFAS molecules. These uses have included uses in food contact paper and paperboard (e.g., popcorn bags and pizza boxes) (Schaider et al., 2017). Because of the long residence times in the human body of the longer-chain PFAS, resistance to degradation in the environment, extensive worldwide dispersion, and concern for possible toxicity, major U.S. manufacturers in cooperation with the U.S. EPA have phased out production of

the longer-chain PFAS, and EPA has taken action under the Toxic Substances Control Act (TSCA) to restrict manufacture or importation of these substances (EPA, 2017). Shorter-chain PFAS, which show lower toxicity and shorter residence times in the body, but share a resistant to chemical degradation like their longer-chain analogs, have replaced the longer-chain forms in many industrial and consumer product uses.

Because of these concerns, some manufacturers have also withdrawn use of some of these materials for food contact purposes, and FDA has withdrawn approval for some indirect food additive uses in paper and paperboard (81 FR 83672), but food contact uses of other PFAS continue (FDA, 2018a). As a result, some of these substances are likely to continue to appear in food packaging and in food (Schaider et al., 2017). Whether coming from food packaging materials or other sources, exposure to PFAS via the diet has been well documented in various countries (Domingo, 2012; Domingo and Nadal, 2017; Fujii et al., 2017; Glynn et al., 2017; Papadopoulou et al., 2017).

Health effects associated with some members of this class of compounds include effects on fetal/infant development, the immune system, the liver, and cancer. Detailed information on the toxicology of PFAS has been reviewed by DeWitt (2015), and the toxicology of two of the best-studied PFAS, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), have been reviewed by EPA (2016a, 2016b). PFAS are also contaminants of food and are further discussed in Section 6.6.3.2.

6.5.4 Color Additives

Color additives have wide use in foods, drugs, cosmetics, other consumer products, and medical devices. Specific colors are listed or approved for food uses under FDA color additive regulations. Synthetic colors have to be certified by FDA on a batch-by-batch basis to assure that each new batch of the color additive meets certain standards of purity representative of the material tested for safety. Colors exempt from certification mainly include those of natural origin, such as beet powder, grape skin extract, titanium dioxide, and various fruit and vegetable juices. Of particular concern among the certified colors are trace constituents, typically starting materials used in color synthesis that are carcinogenic, including certain azo compounds and aromatic amines.

Although there are some legal distinctions that result in different scientific requirements, safety criteria for color additives to be added to food are generally similar to those applicable to food additives. The Delaney Amendment also applies. An issue of some importance, similar to the vinyl chloride polymer matter, concerns the presence of trace amounts of known carcinogens in some colors that, when tested in cancer bioassays, do not themselves provoke a detectable carcinogenic response. The color additive, including its trace constituents, is thus not carcinogenic, but it is known from other data that the trace constituent(s) is carcinogenic. Such outcomes are not surprising, given the limited detection power of cancer tests and the typically low level of the contaminant in the color additive. FDA's "constituents" policy allows the use of such colors as long as application of risk assessment to the trace constituent reveals that carcinogenic risks associated with it are negligible. Thus, for example, FDA found that the excess risks from *p*-toluidine, a trace contaminant of FD&C Green No. 6, a well-tested and noncarcinogenic color, did not exceed 10^{-8} and permitted use of the color to continue (FDA, 1982b).

Another example that has received more recent publicity concerns some forms of caramel color that are manufactured using ammonia or ammonium salts (caramel color III and IV) and used in some cola-type beverages and other foods. These can contain low levels of

4-methylimidazole (4-MEI), which showed evidence of carcinogenicity in mice (but not rats) when tested by the National Toxicology Program (NTP, 2007). 4-MEI is also formed when coffee is roasted and in some other foods and beverages during the normal cooking process (FDA, 2014b). FDA (2014b) concluded that it "has no reason to believe that there is any immediate or short-term danger presented by 4-MEI at the levels expected in food from the use of caramel coloring." The EFSA (2011) reached a similar conclusion.

6.5.5 Animal Drug Residues

Drugs are permitted for use in animals used for food as long as residues of those drugs found in food (meat, milk, eggs) are established as safe. Manufacturers seeking approvals must submit to the FDA studies relevant to safety assessment; FDA then reviews the submitted information and makes a determination regarding approval. As with other classes of substances indirectly added to foods, the established conditions of safe use are described in regulations and on product labels.

Evidence must be developed on concentrations in foods of drug residues (including metabolites of those drugs) after administration of the drugs to the target animals. Separate toxicology studies on the drug are undertaken to establish ADIs, and safety criteria are satisfied if the EDI of the residues is less than the ADI. In many cases, it is necessary to evaluate the pharmacokinetic behavior of the drug in target animals to establish the point in time after drug administration when food concentrations decline to safe levels. A so-called withdrawal time may be required to ensure safety and, if so, that withdrawal time is specified in regulation and on drug label. Monitoring of meat, milk, and eggs to ensure compliance with regulations is a function of USDA's Food Safety and Inspection Service.

Most attention is currently focused in antimicrobial drugs. The FDA is attempting to alter some practices that increase the risk of the development of antimicrobial resistance and is providing guidance on phasing out the use of medically important antimicrobial drugs (those used in human medicines) for production purposes. The latter involves long-term administration of antimicrobials to enhance growth and to improve food efficiency and appears to carry a greater risk of resistance development than the strictly therapeutic uses of these drugs. FDA seeks greater control over antimicrobial usage by requiring greater oversight of their use by licensed veterinarians. While the development of antimicrobial resistance is not a toxicology issue, concern with its development has become a worldwide public health issue and is perhaps the most significant animal drug use issue now under scrutiny.

A second group of animal drugs of significant importance includes hormone implants used to enhance growth. The use of DES for this purpose ended in the United States in 1980, but a number of natural hormones, including estrogen, progesterone, and testosterone, are currently in use, as are a number of their synthetic versions. FDA approval of these hormones rests upon the type of safety data and residue information described above, and the FDA has determined that "zero-day" withdrawal is appropriate for these drugs—food is safe to consume at any time after animals are treated. No hormone implants are approved for use in dairy cows, veal calves, pigs, or poultry. Hormone use in this fashion is somewhat controversial, but most safety reviews rely heavily on the fact that the use of these implants has an extremely small effect on normal background levels of hormones.

Table 6.8 lists some once widely used drugs no longer permitted for use in the United States, the EU, and Canada (at the least) in food-producing animals for purposes not listed in labels. Prohibitions such as these are not uniform throughout the world, and this leads to some difficulties in international trade in animal products.

TABLE 6.8 Some Drugs Prohibited from Extra-label Uses in Food-Producing Animals

Drug	Class
Chloramphenicol	Antibiotic
Clenbuterol	Sympathomimetic growth-promoting beta-agonist
Diethylstilbestrol	Synthetic estrogen, growth promotion
Dimetridazole	Antiprotozoan, once used widely in chickens

6.6 FOOD CONTAMINANTS OF INDUSTRIAL ORIGIN

6.6.1 Classes of Contaminants

Contaminants are substances present in environmental media in which they are not expected to be present or present at levels in excess of expected (i.e., background) levels. Thus, substances naturally present in foods, and substances approved for intentional addition to foods, directly or indirectly, are not classified as contaminants. Chemical contamination of foods can sometimes occur because of an industrial or other type of accident and even through the intentional adulteration of food. But many contaminants are found in foods on a regular basis, and human exposures to them are widespread and typically chronic in nature. This section reviews the important classes of chemically persisting food contaminants.

Metals are the oldest known class of food contaminants. All metals occur naturally, and it is not unexpected that some levels of virtually all of them can be detected in foods. Several metals are essential mammalian nutrients, and their presence in food is necessary for health. But because many metals have found important industrial and product applications, beginning centuries ago, some have found their way into the food chain, through water, soil, and air pollution, at levels in excess of normal background. Some members of this important class of food contaminants are discussed in Sections 6.6.2.1–6.6.2.4.

As is well known, large numbers of industrial chemicals and by-products of industrial activity are common environmental pollutants, and some have entered the food chain. Of particular interest and concern are organic compounds resistant to environmental degradation and, in some cases, having significant capacity for bioaccumulation. The first group of such chemicals coming under scrutiny included chlorinated pesticides introduced in the 1930s. Although uses of most of these pesticides ceased by the 1980s, residues of biopersistent varieties can still be detected in certain foods, arising from the environmental contamination that resulted from once approved uses. As will be seen in Section 6.6.3, halogenated organic chemicals of several types are now recognized as important food contaminants.

The next section contains brief reviews of well-established and emerging food contaminants of industrial origin. Many of the chemicals discussed are subjects of detailed toxicology reviews in other chapters of this book. The challenges associated with understanding and managing the health risks associated with these contaminants are elaborated in Section 6.6.4.

6.6.2 Inorganic Contaminants

6.6.2.1 Lead The concentrations of lead (Pb) in foods have declined significantly since the 1970s, when the use of Pb solder in sealing food cans began to be phased out, and efforts were made to eliminate ceramic ware Pb-containing glasses. The many new environmental

regulations aimed at reducing Pb exposures that began in the 1980s have done much to reduce Pb exposures through air and, albeit to a lesser extent, through water, soils, and dusts. Because all of these environmental media are pathways through which Pb enters the food chain, these regulations have also resulted in Pb declines in food. In a recent FDA survey of more than 8800 foods, less than 12% had detectable levels of Pb (FDA, 2016a). This effort was part of FDA's decades-long Total Diet Study, a market basket survey of 280 core foods collected, prepared as for consumption, and analyzed for contaminants and nutrients. The recent lead findings indicate significant declines in Pb-contamination since program inception.

But as human exposures to Pb have declined significantly over the past 30 years, continuing research has uncovered new health concerns, many related to behavioral and cognitive development in children, at significantly lower exposure levels (measured as blood Pb levels) than had previously been recognized. The JECFA determined in 2010 that it was not possible to establish a level of Pb exposure that would be fully protective (JECFA, 2010). This decision was consistent with recommendations from the CDC and EPA. Although the problem of Pb contamination has no doubt lessened, vigilance is still necessary. Indeed, the Environmental Defense Fund (EDF) has reported on its evaluation of data collected by the FDA from 2003 to 2013 and concludes that 20% of baby food samples had detectable levels of Pb, a higher proportion than was found in other food samples. Moreover, the findings from the United States and other more developed countries almost certainly do not apply in many lesser developed countries.

6.6.2.2 Cadmium The natural occurrence of cadmium (Cd) is relatively rare; its industrial uses are both broad and common. It is found in nickel–cadmium (Ni–Cd) batteries, pigments for ceramics, glasses, and plastics, and coatings for many types of metals and in numerous other products. Atmospheric emissions from mine tailings, smelting, and coal combustion result in soil deposition. Zinc (Zn) mining and smelting also releases Cd. Although the United States was, as recently as 2004, the world's major producer, production and applications have moved in large part to China, Korea, and Japan.

Foods relatively high in Cd include rice and other grains, shellfish and seafood, meat, and certain leafy vegetables. Cd uptake from soil into plants is relatively high; tobacco, in particular, accumulates high levels from soil. Kidney toxicity, osteoporosis, and certain metabolic disease are associated with excessive Cd intake (ATSDR, 2012). In 2010, JECFA established a provisional tolerable monthly intake (PTMI) of 25 μg/kg bw (JECFA, 2011c). Absorption of Cd following dietary exposure is relatively low (~5%) compared with exposures occurring by inhalation.

Several agencies have reviewed Cd exposures and risks because of its presence in food, but none has identified a significant health risk (FDA, EFSA, JECFA). The basis for this opinion is not clear.

6.6.2.3 Methylmercury This organic form of mercury (Hg) occurs in fish and shellfish found in both the ocean and freshwater systems. The inorganic Hg that is the source of methylmercury arises from power plant emissions and industrial processes. Some even comes from dental amalgam wastes and from natural sources in the ocean bed. Methylmercury is a developmental toxicant and causes behavioral and learning impairments in the offspring of women exposed during pregnancy (Chapter 18).

Several epidemiological studies have involved examination of populations that consume unusually high quantities of fish. One of these, conducted in the Seychelles Islands,

has so far not revealed these behavioral and learning impairments in children whose mothers exhibited Hg levels (measured in hair) higher than those typically seen in the United States and European countries. But another study, conducted in Faroes Islands, turned up evidence of cognitive and behavioral impairments in children. Scientists have struggled to understand why two well-done studies have turned up with such different outcomes, and some possible reasons have been suggested. The EPA and public health officials have acted on the basis of the Faroes data, out of both caution and also because they seem to be supported by other, more limited data and by experimental studies. The debate is not so much over whether methylmercury is a developmental toxicant, but rather over the dose required (NRC, 2002).

This particular issue of developmental toxicity is complicated in a most interesting way. Fish are a critical source of certain fatty acids that are important contributors to normal neurological development. So, should we ask women who are pregnant to reduce their intakes of chemicals that may adversely affect those same developmental processes? This is a risk management problem with no easy solution. The FDA and EPA have jointly issued guidelines on fish consumption during pregnancy, and they seem to reflect this concern for adequate nutrition during this critical period (82 FR 6571).

6.6.2.4 Arsenic A naturally occurring metalloid, arsenic (As) represents the 20th most abundant element in the Earth's crust (Valberg et al., 1997; Nachman et al., 2017). Within the environment, As exists as "inorganic arsenic" (iA) when bound to oxygen, chlorine, or sulfur, while "organic arsenic" refers to As bound to carbon compounds. Of the two forms, iA is much more toxic than organic As and is responsible for an array of health effects related to chronic consumption. Chronic health effects related to inorganic As ingestion range from skin pigment changes and hyperkeratosis (i.e., the thickening of the skin) to liver, bladder, and lung cancers and reproductive and developmental effects. The adverse health impacts of iA consumption are as widespread as its presence in the environment; the WHO estimates that at least 140 million people in 50 countries consume As-contaminated water exceeding its guideline value, described further within this section (WHO, 2017a).

Excluding occupational exposures, recent evidence suggests that drinking water (including beverages utilizing drinking water) represents the most critical source for iA intake, followed by food (WHO, 2017b). Typical As concentrations in waters range from <1 to 2 μg/L, but may exist in elevated concentrations in areas near As-containing rocks or sediments (e.g., areas proximal to sulfide mineral deposits) or near anthropogenic sources (ATSDR, 2007; WHO, 2017b). Anthropogenic activities—namely, nonferrous metal mining, smelting, pesticide applications, coal combustion, wood combustion, and waste incineration—release the greatest concentrations of iA into the environment (ATSDR, 2007). Behind drinking water, diet is increasingly considered an important contributor to iA intake, particularly in areas where iA drinking water contamination is low (NRC, 2013). The 2003–2004 National Health and Nutrition Examination Survey (NHANES) estimated the major contributors to iA intake in the United States via diet to include vegetables (contributing to 24% of intake), fruit juices and fruits (18%), and rice (17%) (Xue et al., 2010; NRC, 2013). Fish and shellfish also contain relatively high concentrations of As, though the less toxic organic As comprises 90% of the total As in these foods (ATSDR, 2007).

The health effects of iA are well established and well investigated through epidemiological studies, although the exact mechanism(s) by which iA exposure causes cancers remains unclear, particularly at low-dose exposures (Schmidt, 2014). Nevertheless, regulatory agencies around the world have taken steps to apply risk-based approaches to

determine "acceptable" levels of iA (or general As) intake. In 2001, the EPA established an As drinking water standard of 0.01 mg/L (10 ppb) based on the input and substantiation of the National Academy of Sciences (NRC, 2001) and other advisory groups (EPA, 2001). The drinking water standard was largely driven by results of epidemiological studies conducted in southwestern Taiwan (Chen et al., 1985, 1988, 1992; Wu et al., 1989), which are considered the best-quality studies for establishing dose–response relationships. Despite the relative strengths of these studies, however, uncertainties related to lifestyle and diet represented a challenge to extrapolating the findings to U.S. populations. In 2011, the WHO established a provisional guideline value[4] of 10 µg/L. The WHO principally relied upon documents generated by scientific advisory councils (e.g., International Agency for Research on Cancer [IARC], the Food and Agriculture Organization of the United Nations, and WHO), which also consider the Taiwanese studies relied upon by EPA.

More recently, the FDA has started to investigate the impacts of dietary As on human health and has accordingly begun to propose "action levels," or legal and enforceable upper limits, in food and drinks. In 2013, FDA proposed a 10 ppb As action level in apple juice, while FDA most recently proposed a 100 ppb As action level in infant rice cereal (FDA, 2013, 2016b). In addition to relying upon a variety of primary studies and authoritative reports, FDA modeled iA intake using NHANES consumption data. Interestingly, in the case of the apple juice evaluation, FDA makes a point to clarify how its evaluation differs from that of EPA. Particularly, FDA identifies differences in cohort studies considered, study dose estimates, dose–response models, lifetime disease rate estimation, and uncertainty analyses (FDA, 2013). Such differences in approaches, even between agencies within the same country, demonstrate the complexities in utilizing the "best available data" to make policy decisions, particularly as new information continues to emerge.

The decision model outlined in Section 6.3.8 would seem to have much value for the type of complex problem represented by As in food, but no attempt has been made to apply it.

6.6.2.5 Emerging Inorganic Contaminants Perchlorate is an oxidizing agent used in solid rocket fuels and propellants and in various explosives. It is fairly widespread in drinking water supplies and in the past decade has turned up in milk and some vegetables (NRC, 2005). Levels have typically been less than 10 ppb. Based on results from its Total Diet Study, the FDA has estimated average intakes in the United States to about half the EPA's reference dose of 0.7 µg/kg/day, based on protection against perchlorate-induced hypothyroidism.

In a study published in 2016, data suggested that although perchlorate levels increase in some foods, the average levels across all foods surveyed did not change (FDA, 2017). The study authors suggested that the increases might be explained by the FDA's approval in 2005 to allow perchlorate to be used in food packaging as an antistatic agent or sealant. A citizen' petition calling for revoking the approval was submitted to FDA in 2016, but the agency has, to date, made no decision.

Ni-induced allergies, due to its presence in certain foods, have attracted much attention. The EFSA has also raised concerns based upon findings of reproductive and developmental

[4]The provisional guideline value represents a drinking water constituent concentration "that does not result in any significant risk to health over a lifetime of consumption." The WHO provisional guideline values for drinking water constituents are established based "on the practical level of treatment performance or analytical achievability" and are higher than calculated health-based values (WHO, 2017b).

effects in animals. The EFSA has suggested that current food exposures exceed the agency's TDI of 2.8 μg/kg/day (EFSA, 2015).

Ni is an essential nutrient and, as discussed in Section 6.4, all essential elements may cause some form of toxicity if intakes exceed ULs. There is as yet no comprehensive examination of intakes from foods possibly contaminated with these elements and of their relationships to ULs.

6.6.3 Organic Contaminants of Industrial Origin

The carbon–halogen bond is the strongest to be found in organic molecules and is resistant to both chemical and enzymatic cleavage. The order of stability is C–F > C–Cl > C–Br > C–I. Generally, when the carbon is part of an aromatic ring, the bond is stronger than when it is aliphatic. It follows that compounds in these classes are, to varying degrees, resistant to environmental degradation and to metabolism. They also display, again to varying degrees, high lipophilicity and thus many not only persist but also bioaccumulate and therefore enter the food chain.

Many halogenated aliphatic and aromatic compounds proved to possess properties useful for many commercial applications—as pesticides, solvents, flame retardants, and, in the case of PCBs, dielectric fluids for capacitors and transformers. During the 1950s it became clear that DDT and several other halogenated compounds used as pesticides persisted in the environment long after applications. And, in the late 1960s, it was revealed that PCBs could be detected in wildlife, in some cases at great distances from their sources. Evidence that PCBs and halogenated pesticides had become significant environmental contaminants continued to accumulate during the 1970s, and by the end of that decade, the manufacture and uses of these substances had largely ended. During this same period, polychlorinated dioxins became recognized as similarly persistent and bioaccumulative environmental contaminants. These substances and related compounds were not intentionally produced for commercial purposes, but rather entered the environment as by-products of certain industrial processes (including those used to produce Agent Orange), waste incineration, and even forest fires. Other classes of halogenated hydrocarbons, including PCBs and biphenyl ethers used as fire retardants, and several classes of polyfluorinated compounds were introduced into commerce in the mid- to late twentieth century for a wide range of uses, and evidence that some of these substances have entered the food chain had begun to accumulate.

Immense amounts of data on the adverse health effects of these many halogenated compounds, including the many halogenated aliphatic used as solvents (chloroform, chlorinated ethylene, etc.), have accumulated, sufficient in many cases to support listings under the treaty issuing from the 2004 Stockholm Convention on Persistent Organic Pollutants (POPs). PCBs, dioxins, dibenzofurans, and 10 chlorinated pesticides were the first compounds to be listed as POPs, and 14 more have been added since then. Note also that POPs have been found to migrate through long distances in air and water and are found throughout the world. The Stockholm Convention treaty calls for the 90 countries that are signatories to eliminate most from production or use, to restrict the production and use of some, and to reduce releases of some that are not intentionally produced (e.g., dioxins).

6.6.3.1 Polychlorinated Biphenyls (PCBs) and Dioxins Food concentrations of dioxins and dioxin-like compounds, including PCBs, have been monitored by government agencies in the United States, Europe, and several other countries for several decades. The major

dietary sources of these compounds are animal products (meat, poultry, eggs, fish, milk, and other dairy products). Breast milk has also been identified as a source of exposures for infants (WHO, 2002). Where sufficient data are available to study trends, there is evidence that dietary exposures have declined since the early 1990s. Whether this is true for breast milk has not been evaluated. Most of the monitoring effort has been devoted to identifying sources of exposures and finding ways to reduce them. The WHO has estimated that greater than 90% of exposure to dioxin and dioxin-like compounds arises from the diet and has devoted significant sources to evaluating those exposures on a global basis and to providing guidance on reducing exposures (WHO, 2002).

The PCBs represent a particularly difficult problem for the risk assessor. Most pertinent toxicology data have been collected on the commercial products: Aroclors in the United States, Clophens in West Germany, and Kaneclors in Japan. The difficulty for the risk assessor increases when it is realized that PCB mixtures are modified as they move through the environment. The mixtures of chlorinated biphenyl found in fish, for example, vary widely in composition according to source of PCB, length of time the chemicals have been in the environment, and the species and age of the fish that has accumulated the compounds. And none of these matches in composition the commercial products for which cancer bioassay are available. The risk assessor can do no better than to use dose–response data from the commercial product that most closely matches in composition the mixture found in fish. Of increasing interest is the potential for certain PCBs to display estrogen-like properties and to affect reproductive functions in animals. Certain isomers of PCBs bear structural similarities to certain chlorinated dibenzodioxins and may act through common, and apparently receptor-mediated, mechanisms. The EPA has suggested, in its current and continuing review of chlorinated dioxins, that normal human background levels of the combination of structurally related chlorinated biphenyls, dibenzo-*p*-dioxins, and dibenzofurans are within an order of magnitude of the level at which they may cause adverse effects on endocrine systems (EPA, 1997). Fatty foods are thought to constitute the major media through which human exposures are created. This issue remains one of intense debate.

The European Commission has recently adopted new legislation setting maximum levels for dioxins and dioxin-like PCBs in food and feed. Maximum levels for dioxins in food of animal origin and all animal feed have been applicable since July 2002, but as of November 2006, any food or feed in which the sum of dioxins and dioxin-like PCBs exceeds these maximum levels could not be marketed in the EU. The effects upon commerce of this type of restriction are unpredictable.

In the past several years, much international attention has been directed to the family of compounds known as polybrominated diphenyl ethers (PBDEs). Some members of the family have been used widely as flame retardants in computers and other electronic devices. The substances are persistent in the environment, and much evidence regarding their presence in food fats has accumulated in the worldwide literature. Regulations on their presence in food have not yet been issued.

6.6.3.2 Perfluorinated Compounds Considerable attention is now focused on the large class of perfluorinated chemicals, compounds in which very hydrogen atom bonded with a carbon atom has been replaced by fluorine. The important members of this class of chemicals have carbon atoms as part of an aliphatic, not aromatic, moiety. The most commercially important and widely studied of these compounds are two perfluoroalkyl acids (PFAAs): perfluorooctonate (https://pubchem.ncbi.nlm.nih.gov/compound/9554 (PFOA)) and perfluorooctane sulfonate (https://pubchem.ncbi.nlm.nih.gov/compound/74483)

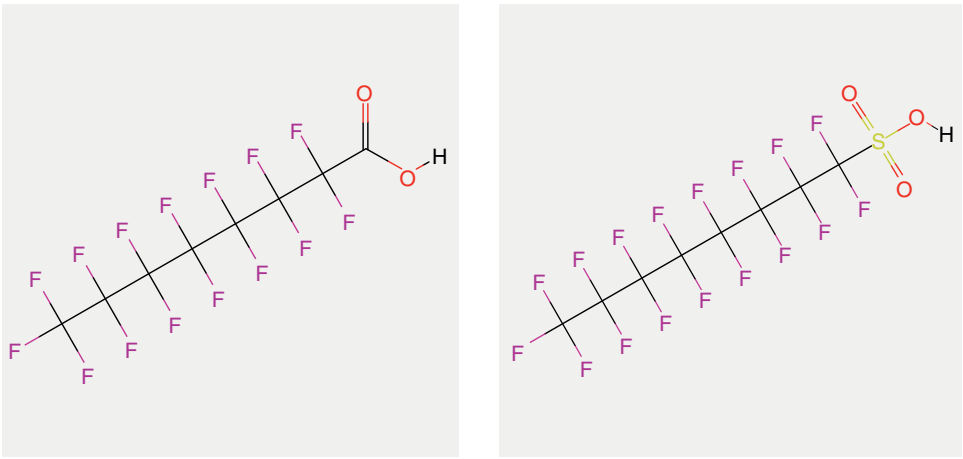

FIGURE 6.1 Chemical structures of perfluorooctanoic acid (PFOA, left) and perfluorooctanesulfonic acid (PFOS, right). *Source*: Images courtesy of PubChem (Kim et al., 2016).

(PFOS) (Fig. 6.1). Analogous compounds having shorter and longer carbon chain lengths are in production, and some of the shorter-chain compounds have replaced the C8 compounds for some applications. The PFAAs have more than 200 industrial and consumer product uses, including water- and stain-resistant coatings for textiles, greaseproof paper products for food packaging (see Section 6.5.3), waxes and polishes, and firefighting foams. A range of PFAA derivatives are also in commercial production, and some can degrade to PFAAs, which are thus highly persistent.

The fluorinated carbon chains in the PFAAs have the lipophilic characteristics found in chlorinated hydrocarbons but also have the carboxylate or sulfonate functional groups, which are highly lipophobic. PFOA and PFOS are thus highly stable surfactants, which make them very useful chemicals but highly persistent environmental contaminants (Hu et al., 2016).

PFAAs are important drinking water contaminants, and investigations into methods for removal are underway. The EPA has advised that PFOA and PFOS drinking water levels should not exceed 20 ng/L (parts per trillion). Although both compounds have been shown to produce a variety of toxic effects, including carcinogenicity in rodents, the EPA based its reference dose on developmental toxicity, which it considered the most sensitive endpoint. The EPA also introduced a factor of greater than 100 for cross-species extrapolation, based on the exceptionally long elimination half-lives of these compound in humans (2.3–3.8 years for PFOA and 5.4 years for PFOS) relative to rodents (half-lives measured in days). Shorter-chain PFAS have much reduced elimination half-lives, and this is one of the reasons major manufacturers have phased out production of the C8 compounds and switched to shorter-chain PFAAs (EPA, 2016a, 2016b).

Because PFAAs are amphoteric and have surfactant properties, lipid uptake and storage is not as significant as it is for the highly lipid-soluble hydrocarbons. Several investigations have pointed to dietary intake through fish and shellfish as a major pathway of human exposure. Thus far, most studies of dietary exposure have been conducted in Europe and Canada. The overall database on this subject is relatively small.

The PFAA's contamination issue is emerging as a possibly significant food safety issue, but much remains to be done to determine where there is a significant public health related to this class of compounds.

Several other classes of unapproved compounds can be found in the diet, including certain N-nitroso derivatives of amines, amides, ureas, and carbamates (Al Kaseem et al., 2014; Herrmann et al., 2015; Tricker and Preussmann 1991). Some brominated flame retardants have been identified by the EFSA as possible food contaminants needing attention (EFSA, 2012). Certain polycyclic aromatic hydrocarbons have for many years been recognized as food contaminants arising from contaminated environments, including those in which foods are cooked over fire. Chemical contamination of the diet is a continuing issue, requiring vigilance throughout the world.

6.6.4 Risk Assessment and Management

TDIs or RfDs have been developed for some of the important food contaminants (Table 6.9). If adequate data are available to assess human exposure to food contaminants, those exposures can be compared with the TDIs or RfDs to determine whether a safety problem exists. This approach has generally been taken by regulating agencies (Brock et al., 2003).

This approach may, in some cases, be limited or even misleading if efforts are not made to examine total exposures resulting from all sources of the contaminant. Isolating and examining only one source (e.g., diet) among many may significantly underestimate human risk. This problem presents significant difficulties, because it requires decisions about fractions of a TDI or RfD that should be allocated to each source. This problem has been dealt with in some circumstances (e.g., in the United States EPA typically allocates 20% of an RfD to establish limits for drinking water exposure), but no general solution has emerged.

If it is determined that a possible health risk exists because exposures exceed an established RfD or TDI, it is not possible to estimate the magnitude of that risk. These measures of safety are not developed to meet some specified, quantified risk target, and the risks associated with exposures greater than, equal to, or less than these measures carry risks of unknown magnitude. The "bright-line" approach to risk assessment represented by the use of TDIs of RfDs is not an ideal decision-making model for substances that cannot simply be "removed" from foods in the way that is possible for intentionally introduced chemicals.

TABLE 6.9 Tolerable Daily Intakes or Toxicity Reference Doses for Some Important Food Contaminants

Contaminant	ADI/EDI/RfD (mg/kg bw/day)	Source
Arsenic	0.0003	EPA IRIS (https://www.epa.gov/iris)
Cadmium	0.001	EPA IRIS (https://www.epa.gov/iris)
Methylmercury	0.0001	EPA IRIS (https://www.epa.gov/iris)
Nickel	0.0028 (EFSA TDI)	EFSA (2015)
	0.02 (EPA RfD)	EPA IRIS (https://www.epa.gov/iris)
Perchlorate	0.0007	EPA IRIS (https://www.epa.gov/iris)
PCBs (Aroclor 1254)	0.00005	EPA IRIS (https://www.epa.gov/iris)
2,3,7,8-TCDD	0.0000000007	EPA IRIS (https://www.epa.gov/iris)
PFOA	0.00002	EPA (2016a)

Some efforts have been made to invoke models based on MOE (Section 6.3.8) criteria. Various interventions proposed to reduce exposures can be evaluated for their relative effectiveness in reducing risks. Decisions about tolerable residual risks are then more flexible and are not governed by a single (and quantitatively undefined) criterion for safety.

Of course, MOEs are not quantitative risk measures, and it is not until chemical risk assessments become quantitative for all toxicity endpoints that true risk-based decision making will become possible.

Food contaminants of the sort listed in Table 6.9 are unavoidable in the sense discussed in the earlier section on legal and regulatory framework.

Decisions about limits on exposures to these materials are somewhat different in character from those we have described for the various classes of intentional additives. In some instances, imposition of an ADI or negligible cancer risk standard, derived using conventional methods, would require banning of certain foods altogether or very severe restrictions on the fraction of the available supply that could be marketed. Using the carcinogenic potency derived by EPA for PCBs, for example, and a 10^{-6} upper limit on lifetime risk as the health protection standard, would require maximum fish residue levels of approximately 1–5 ppb (the limit depends heavily on data and assumptions concerning fish consumption rates). At the present time PCB residues in commercial fish in several areas of the United States contain PCBs in the range of 1–5 ppm (FDA, 1979b; Maxim, 1989). Clearly, imposition of a 5 ppb limit would mean the end of much commercial fishing until environmental levels of PCBs declined to sufficiently low levels. The regulatory approach at FDA has been to specify limits on PCBs in fish (commercial products moving in interstate commerce) at 2 ppm in edible portions. This tolerance level was chosen both to minimize health risk and to avoid an intolerable level of economic disruption in the affected industry. The risk level found tolerable by FDA exceeded by more than 100 times the 10^6 lifetime risk level used as a guide in the various "insignificant risk" decisions discussed earlier. Many state agencies have sought much stricter limits (Rodricks, 1994).

6.7 CONSTITUENTS AND CONTAMINANTS OF NATURAL ORIGIN

6.7.1 Categories of Natural Constituents and Contaminants

It is convenient to group food constituents and contaminants of natural origin into five broad categories as shown in Table 6.10 (Rodricks and Pohland, 1981). These categories include the substances that are natural components of the food itself or that have become incorporated from the plant's environment or from the animal's food supply. Other substances become a part of the food after the food is collected and stored or during preparation.

Investigations into the possible risks to health of the enormous number of chemical compounds in these groups are limited to a relatively few members of each, particularly of the first. A brief survey of some selected examples of each group reveals that the range of health risks associated with these natural substances is wide and as potentially serious as that associated with additives and industrial contaminants.

6.7.2 Intrinsic Components of Foods

6.7.2.1 Cyanogenic Glycosides Cyanogenic glycosides (CGs) are natural toxins found primarily in cassava, especially bitter cassava, and several other foodstuffs. CGs produce hydrogen cyanide when chewed or digested (Bolarinwa et al., 2016). Although the main cyanogenic plant food is cassava, CGs are also found in low levels in lima beans, almonds,

TABLE 6.10 Food Constituents and Contaminants of Natural Origin

Categories	Sources	Examples
Intrinsic food components	Natural constituents of plants and animals	Natural pesticides in plants, pufferfish (fugu) toxin
Soil and water constituents	Natural mineral sources	Nitrate; metals such as mercury and arsenic
Microbial metabolites	Toxins from bacteria and fungi	Aflatoxin B_1, botulinum toxins
Contaminants of natural origin	Toxins accumulating in marine organisms and forage plants	Ciguatera, paralytic shellfish poison
Products of storage or preparation[a]	Toxins arising in aged foods or in food preparation	Oxidized fats, polycyclic aromatic hydrocarbons

[a]Substances in this category are designated "natural" because the processes giving rise to them are generally considered "natural." This is no doubt an arguable classification.

bamboo shoot, sorghum, stone fruits, flaxseed, and elderberries (WHO/FAO, 2008). They are also found in fruit kernels including those of apples, apricots, cherries, peaches, plums, and quinces (Bolarinwa et al., 2016). Among these cyanogenic plant foods, bitter cassava has received the most international attention, as it is an important staple food for many populations worldwide and in its unprocessed form contains dangerously high levels of CGs (Bolarinwa et al., 2016). Different traditional processing methods have been identified for reducing cyanides in cassava, which include drying, soaking, boiling, fermentation, and so on (Padmaja and Steinkraus, 1995). Flaxseed has also recently gained popularity in the health food market due to their numerous nutritional benefits (Wu et al., 2012). Several researchers have been developing effective methods for reducing the amount of CGs in flaxseeds using fermentation and extrusion (Wu et al., 2012; Imran et al., 2013). In high dietary doses, CGs can cause vomiting, convulsion, stomachache, diarrhea, and even death (Bolarinwa et al., 2016). Chronic lower exposures to CGs can cause irreversible neurological conditions such as konzo (paralysis of the legs), growth impairment, and tropical ataxic neuropathy (TAN) (Bolarinwa et al., 2016). A recent study has even linked CG exposure in children to long-term cognitive impairment (Boivin et al., 2017). Some of the risk of CGs in the diet can be mitigated by inclusion of sulfur amino acid-containing foods, such as legumes and animal source protein, in the same meals, which biotransform the CGs into nontoxic compounds (Wu et al., 2014a).

6.7.2.2 Tetrodotoxin Tetrodotoxin (TTX) is a naturally occurring neurotoxin produced in the pufferfish (Japanese: fugu) by endosymbiotic bacteria. Additionally, TTX can also be found in lower levels in gastropods, crabs, starfish, newts, frogs, sea slugs, blue-ringed octopuses, ribbon worms, and bacteria (Bane et al., 2014). It causes severe human intoxication and possibly death when certain parts of the seafood are consumed; for example, in pufferfish, the liver, ovaries, and skin contain the toxin. TTX accumulates in pufferfish through a food chain that starts with marine bacteria (Noguch and Osamu, 2008). TTX causes neurotoxic effects by blocking voltage-gated sodium channels in nerve and muscle tissues in mammals, which can lead to death. Currently, there is no known antidote for TTX, which is known to be over one thousand times more toxic than cyanide (Bane et al., 2014). Until 2005, TTX was observed only in warmer waters of Southeast Asia (Japan, Taiwan, China, Korea, Thailand, and Bangladesh). However, since then, TTX has appeared in other waters worldwide. It was been reported in seafood samples harvested from European waters in the recent years (Bane et al., 2016).

6.7.2.3 Oxalate Oxalate is a compound found in many foods, including spinach, soy products, black tea, rhubarb, citrus fruits and their products, and chocolate (Holmes and Dean, 2004). Oxalate can bind with dietary calcium and make it unavailable for absorption. These foods can also cause elevated levels of urinary oxalate, which can sometimes lead to formation of kidney stones in susceptible individuals (Williams et al., 2016). The content of oxalate among these foods is known to be highly variable, depending on biological variation in cultivar, time of harvest, and growing conditions (Attalla et al., 2014). Several food processing methods can reduce oxalate component in food to prevent risks of kidney stones in susceptible individuals. Fermentation with *Saccharomyces cerevisiae* has shown some beneficial results (Huynh and Nguyen, 2017). Also, incubation with calcium chloride during juice making processes is known to form insoluble oxalate, which makes the juice safer to consume regularly.

By applying commonly used UFs, ADIs for oxalates can be calculated based on three animal studies. These studies are listed in Table 6.11. For chronic, reproductive, and developmental toxicity studies, a 100-fold UF is usually applied. This UF can be further modified to reflect the seriousness of the effect, the duration of the study (e.g., subchronic rather than chronic data), and the quality of the data (e.g., small number of animals; Kotsonis et al., 2001). Examples of possible uncertainty and modifying factors are shown in Table 6.11, along with the resulting ADIs.

The ADIs shown in Table 6.11 range from 0.2 to 3 mg/kg/day, compared with an average oxalate consumption of 3.2 mg/kg/day from oxalate-containing food. More specifically, the average U.S. intake of naturally occurring dietary oxalate is 4–17 times greater than the ADI for developmental effects and 1.6 times greater than the ADI for reproductive effects. Thus the background levels of oxalate exceed the ADI levels that are suggested from the animal toxicity data available on this substance.

It is not at all unusual for intakes of naturally occurring components of food, such as oxalate, to exceed the ADI that could be derived for them using methods applicable to food additives. Such outcomes can be interpreted in at least two ways. According to one interpretation, oxalate and other such intrinsic food components actually create a risk of chronic toxicity. Although there are no data to suggest that this is the case for oxalate, this possibility appears not to have been thoroughly examined, either clinically or epidemiologically. Some evidence suggests, however, that oxalate is only poorly bioavailable, although it is not clear that it is greatly less bioavailable in people than it is in the animal species from which the toxicity data were derived.

A second interpretation is that the methods for deriving ADIs are excessively conservative and lead to acceptable intakes that are lower than they need to be. There seems to be

TABLE 6.11 Animal Studies for Oxalate Suitable for Establishing an ADI

Study Type (Species)	NOAEL (mg/kg per day)	Uncertainty Factor	Modifying Factor[a]	ADI (mg/kg per day)	Reference
Chronic (2 years) (rat)	600	100	2	3	Fitzhugh and Nelson (1947)
Reproduction (rat)	2000	100	10	2	Goldman et al. (1977)
Developmental toxicity	175	100	2–10	0.2–0.9	Sheikh-Omar and Schiefer (1980)

[a]Introduced to compensate for data limitations and severity of toxicity.

TABLE 6.12 Some Intrinsic Components of Food of Known Toxicity

Compounds	Food Sources	Suspected or Known Toxic Endpoints[a]
Salts of oxalic acid	See text	See text
Solanine, chaconine	White potato[b]	Nervous system
HCN	Many plants, as adducts, released when plant tissue is damaged	Hemoglobin, cyanosis
Vasoactive amines	Pineapple, banana, plum	Cardiovascular system
Xanthines (caffeine, theophylline, theobromine)	Coffee[c]	CNS stimulation, other biochemical changes, cardiac effects
Myristicin	Nutmeg, mace	Nervous system
Carotatoxin	Carrots, celery	Nervous system
Lathyrus toxins	Legumes of genus *Lathyrus*	Lathyrism (neurological disease)
Tannins	Tea, coffee, cocoa	Carcinogenic in animals
Safrole and other methylenedioxy benzenes	Oil of sassafras, cinnamon, nutmeg, anise, parsley, celery, black pepper	Carcinogenic in animals (not all members of the class)
5- and 8-Methoxypsoralen (light activated)	Parsley, parsnip, celery	Carcinogenic in animals, with UV light
Ethyl acrylate	Pineapple	Carcinogenic in animals
Estragole	Basil, fennel	Carcinogenic in animals

[a]Relevance of animal carcinogenicity to humans is doubtful in some cases.
[b]Solanaceous glycoalkaloids are present in other Solanaceae, including eggplant and tomato.
[c]Coffee contains more than 600 compounds in addition to caffeine. This is typical of natural foods. Included are many different classes of organic compounds.

no way to determine definitively which of these interpretations is correct; indeed, perhaps both are to a degree. But consideration of the toxicity data on many intrinsic components of food creates dilemmas not unlike that exhibited in the case of oxalate (Committee on Food Protection, 1973; Brock et al., 2003).

Some of the more well-studied intrinsic compounds of food and their key toxicity characteristics are presented in Table 6.12.

6.7.2.4 Caffeine Caffeine (1,3,7-trimethylxanthine) is probably the most frequently ingested pharmacologically active substance in the world (Nawrot et al., 2003). It is found in common beverages (coffee, tea, soft drinks), in products containing cocoa or chocolate, and in medications (e.g., NoDoz, Excedrin). In a survey of caffeine consumption in the U.S. population, results showed that 84% of the U.S. population consumes at least one caffeinated beverage per day and the mean daily caffeine intake from all beverages was 165 ± 1 mg for all ages combined (Mitchell et al., 2014).

Following ingestion, caffeine is rapidly and essentially completely absorbed from the gastrointestinal tract into the bloodstream. The elimination half-life of caffeine ranges between 3 and 7 h in adults and can be influenced by factors such as sex, age (the half-life in newborns ranges from 50 to 100 h, but it gradually approaches that of an adult by 6 months of age), use of oral contraceptives, pregnancy (during pregnancy, the metabolic half-life increases steadily from 4 h during the first trimester to 18 h during the third trimester), and

smoking. Caffeine can cause subtle, reversible physiological effects at relatively low doses, such as increased alertness, that are clearly not adverse. Indeed, many individuals rely on this beneficial effect of caffeine, which can be demonstrated experimentally at dose levels at or below 100 mg. Of course, exaggeration of this effect due to excessive intake can interfere with normal sleep or produce other effects perceived by some individuals as undesirable. Adenosine, acting via the adenosine receptors (A1 and A2A) in the brain, plays an important role in promoting sleep. Caffeine acts as an adenosine receptor antagonist, inhibiting the sleep-promoting effects of adenosine, and appears to explain both the desirable stimulant action of caffeine and its ability, particularly when ingested at high levels close to normal sleeping time, to interfere with certain sleep parameters. Tolerance to some of the acute effect of caffeine develops with repeated and regular intake. For example, while caffeine may result in a slight increase in the blood pressure of naive individuals, regular caffeine consumers rapidly develop tolerance to such effects (Robertson et al., 1981). Partial tolerance also develops to increases in tension, anxiety, and jitteriness associated with caffeine administration (Fredholm et al., 1999; Rogers et al., 2010; Morelli and Simola, 2011).

Abrupt discontinuation of caffeine consumption results in a mild withdrawal syndrome, characterized by headache, fatigue, drowsiness, irritability, depressed mood, and anxiety, starting after 12–24 h of abstinence and peaking 20–48 h later. Symptoms of caffeine withdrawal vary considerably between different individuals. The withdrawal syndrome is usually not harmful and is self-limiting (Morelli and Simola, 2011).

6.7.2.5 Kidney Bean Toxin (Phytohemagglutinin) Phytohemagglutinin (PHA) is a lectin present abundantly in kidney beans, both red and white, and in lower concentrations in other beans. PHA is able to survive through the gastrointestinal tract in its biologically active form. In high doses, PHA stimulates contractions in the gall bladder of humans (Purhonen et al., 2008), binds to the T-cell membranes, and stimulates metabolic activity and cell division (Movafagh et al., 2011). In some animal species, PHA has been shown to cause growth impairment, weight loss, and diarrhea and reduce intestinal absorption (Rodhouse et al., 1990). One study also showed a possible contribution of PHA to causing allergic reactions (Kumar et al., 2013). In a mouse study, high concentration of PHA was found to block embryonic development (Zhang et al., 2011). PHA can be inactivated by boiling or otherwise cooking well-soaked beans thoroughly (Rodhouse et al., 1990).

6.7.2.6 Solanine and Other Glycoalkaloids Solanine is a naturally occurring glycoalkaloid that builds up in potatoes and other tubers. The main source of solanine exposure in humans is the potato—in particular, the skin. Potatoes contain two main kinds of steroidal glycoalkaloids: alpha-solanine and alpha-chaconine (more toxic). Both contribute to the flavor of potatoes, but high levels can cause a bitter taste. They play a role in protection of the potatoes when attacked by fungi, insects, and other pests (Dalvi and Bowie, 1983). In addition to potatoes, glycoalkaloids can also be found in tomatoes, eggplants, and other nightshade plants (Barceloux, 2009).

Glycoalkaloids can interfere with regulation of acetylcholine, which is responsible for conducting nerve impulses, and they can also disrupt membranes (Dalvi and Bowie, 1983). Solanine toxicity symptoms include headache, nausea, vomiting, abdominal pain, and diarrhea. In rare instances, solanine toxicity may be fatal when it causes severe gastrointestinal, nervous, and exanthematous syndromes (Cantwell, 1996). Oftentimes, storage conditions including light exposure and high temperature can increase the solanine concentration in potatoes (Dalvi and Bowie, 1983).

Glycoalkaloids are not typically destroyed during cooking processes such as boiling, baking, or frying. Transgenic potatoes have been developed to downregulate glycoalkaloid levels in potatoes (Shepherd et al., 2015). For medical purposes, however, some recent studies have also been focusing on beneficial effects of glycoalkaloids in anti-metastatic therapy and different cancers (Lu et al., 2010; Mohsenikia et al., 2013).

6.7.3 Naturally Occurring Pesticides

Substances with pesticidal properties that are found in plants are a particularly interesting subgroup of the intrinsic constituents of food. For about three decades now, several biologists, including Bruce Ames, have been acquiring, organizing, and evaluating information on food plant metabolites displaying pesticidal activity. Ames and colleagues (Ames, 1983a, 1983b; Ames and Gold, 1989) estimate daily intake of natural pesticides to be about 1.5 g, which they note is about 10,000 times the level of daily intake of man-made pesticides (Gartrell et al., 1986a, 1986b). Thousands and probably tens of thousands of compounds having insecticidal and fungicidal activity and other types of toxicity toward predators are present in plants we use as food, frequently at concentrations in the parts-per-million to parts-per-thousand range. Moreover, plant stress resulting from predator attack often induces biosynthesis of greater-than-normal concentrations. Most of these substances have not been evaluated toxicologically. Some of those that have been evaluated display the same range of toxic properties associated with synthetic chemicals. Ames and Gold (1989) list natural carcinogenic pesticides present in anise, apples, bananas, basil, broccoli, and about 40 other plant foods, herbs, and spices.

For reasons already stated, none of these findings have yet had a significant impact on regulation, either direct or indirect. Certainly there has been no attempt to regulate any of these natural carcinogens, and, until recently (see below), little effort has been devoted to evaluating the risks they may pose. Although regulation in any traditional sense would seem improbable, it may be possible to control the levels of these natural toxicants by breeding them out of plants. There seems to be little sign of interest in such an activity. Contrariwise, there are at least two instances in which plant breeders have inadvertently increased the level of natural toxicants—psoralens in celery and solanine in white potatoes—to levels sufficient to cause acute toxicity (rashes in the former case and cholinesterase inhibition in the latter). The dangerous variety of celery actually made it to market and had to be withdrawn; the problem in the new variety of white potato was discovered before marketing occurred (Ames and Gold, 1989). Surely one of the major concerns of regulators and public health officials with newer "bioengineered" foods is the potential for inadvertent introduction of dangerous levels of natural toxicants (many of which now are present at levels uncomfortably near the minimum toxic level).

Although the work of Ames and his associates and others as well has not yet had a major impact on the way health risks associated with environmental chemicals are viewed, the issue cannot forever be ignored. On the one hand, it may point to a significant role for natural food toxicants in chronic human diseases, including cancer. At the other extreme, it may suggest that our concerns over synthetic chemicals are wrongheaded, because it is clear that our risk assessment methodologies are inappropriate and greatly overstate low-dose risks (on the assumption that even the natural toxicants are not a significant health risk). It will require considerable research to untangle this issue, involving close collaboration between toxicologists and epidemiologists.

To confuse matters further, it is clear, as Ames himself has pointed out, that there are many naturally occurring dietary constituents that, probably by several mechanisms, protect against or reduce the risk of cancer. These dietary "anticarcinogens" are probably as prevalent as the carcinogens (Davis, 1989). In any event, risk evaluation requires consideration of both sets of naturally occurring food constituents.

A committee of the National Research Council (NRC) that reviewed the state of knowledge regarding dietary carcinogens and anticarcinogens (NRC, 1996) concluded that, though our understanding is relatively poor, current evidence suggested that carcinogens naturally present in food far exceed those present because of human actions. Understanding the risks associated with such agents, and the benefits associated with naturally occurring anticarcinogens, is a formidable task; the committee nevertheless stressed the need to acquire that understanding and set forth a program toward that end. Although the evidence for a significant role for natural dietary constituents in other chronic diseases was not reviewed, it seems likely that carcinogenesis is not the only disease process in which these substances play a significant role.

6.7.4 Accumulation of Chemicals from Drinking Water and Soils: Nitrate

The major members of this group are the metals and other elements that were discussed earlier in the section on industrial contaminants. A large number of metals and other elements beyond those discussed earlier are also naturally present in foods. Some are essential nutrients [copper (Cu), chromium (Cr), calcium (Ca), magnesium (Mg), iron (Fe), Zn], but many more that are present have no established nutritional value. Like Pb, Cd, As, and Hg, some of these substances can, in limited geographic areas, accumulate to excessive levels because of industrial pollution, but for most of these, natural occurrence appears to be the dominant source (Munro and Charbonneau, 1981). The health risks from nitrates pose an interesting aspect, not only because the natural occurrence of nitrate is by far the greatest source but also because nitrate itself is not the material of toxicological interest. Rather, derivatives of nitrate—nitrite and N-nitroso compounds—are formed as a result of chemical reactions and are responsible for harmful effects (NRC/NAS, 1981).

Several kinds of food plants accumulate naturally occurring nitrates from water and soil. The location of the plant and its variety, state of health, and moisture content determine how much nitrate a plant accumulates. The water and soil in the environment are the source of nitrate, and they, in turn, accumulate nitrate from nitrogen-based fertilizers as well as from nitrogenous wastes from humans and livestock. Certain food vegetables such as spinach, beets, cauliflower, lettuce, celery, radishes, kale, and mustard often contain high concentrations of nitrates (Committee on Food Protection, 1973).

Poisonings of human infants have been reported from high nitrate levels in well water used to prepare infant formulas, mostly in rural areas, and from high concentrations of nitrate in baby foods. The total daily intake of nitrate from all sources has been estimated as 75 mg/person, but in areas of high nitrate in water, this can be doubled. Although these sound like large amounts, the real concern is with the conversion products of nitrates.

Nitrates are converted into nitrites by microorganisms in the mouth and gut through reactions with ammonia and other organic nitrogen-containing compounds. Nitrites, in turn, react with hemoglobin, the Fe-containing respiratory protein in red blood cells, and convert it to methemoglobin. Methemoglobin is unable to combine with oxygen. Thus, the blood of persons with too much methemoglobin, a condition known as methemoglobinemia, has a reduced oxygen-carrying capacity as well as a decreased capacity of residual

oxyhemoglobin to dissociate and release oxygen to the tissues where it is needed. Infants are at a special risk from methemoglobinemia because their hemoglobin occurs in a form that is more easily oxidized. Furthermore, they are developmentally deficient in methemoglobin reductase, and the lower gastric acidity of infants permits nitrate-reducing microorganisms to thrive. Most episodes of methemoglobinemia in infants have been the result of high concentrations in well water (Menzer and Nelson, 1986).

Long-term carcinogenic effects of compounds formed from nitrate with other nitrogen-containing compounds are also of great concern. The reaction of nitrite with secondary amines to form nitrosamines in foods has been modeled in animal studies that show that feeding nitrate together with certain amines, for example, produces tumors in rats or mice. The nitrosamines are among the most potent animal carcinogens; single doses of certain nitrosamines are sufficient to cause cancer in experimental animals. The nitrosamines are discussed further in a later section of this chapter.

6.7.5 Mycotoxins

Mycotoxins are secondary metabolites of fungi that colonize food crops, most commonly corn, small cereal grains such as wheat, and nuts. They cause a number of adverse human and animal health effects. The most common and important mycotoxins from the perspectives of agriculture, dietary exposure, and public health are aflatoxin, fumonisin, deoxynivalenol (DON), and ochratoxin A (OTA) (Wu et al., 2014b) (Table 6.13).

Aflatoxin is the most toxic and carcinogenic of known mycotoxins. Produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, aflatoxin is classified by the IARC as a group 1 "known" human liver carcinogen (IARC, 1993). Aflatoxin is synergistic with chronic infection with hepatitis B virus (HBV) to increase liver cancer risk dramatically (Qian et al., 1994). Between 25,200 and 155,000 liver cancer cases annually worldwide may be attributable to aflatoxin exposure (Liu and Wu, 2010). In addition to liver cancer, aflatoxin has been associated with acute toxicity and liver failure, stunted growth in children, and immunomodulation (Wu et al., 2014b).

Because it is primarily produced by fungi in warm climates, aflatoxin is most commonly found in corn, peanuts, and tree nuts in tropical and subtropical areas of the world.

TABLE 6.13 Tolerable Daily Intakes (TDIs), Tolerable Weekly Intakes (TWIs), Acute Reference Doses (ARfD), or Cancer Potency Factors (CPFs) for Mycotoxins

Mycotoxin	Thresholds	Source
Aflatoxin	TDI: not established—genotoxic carcinogen CPF for individuals not infected with hepatitis B virus (HBV): 0.01 cases/100,000/yr/ng AF/kg bw/day CPF for individuals infected with HBV: 0.30 cases/100,000/yr/ng AF/kg bw/day	JECFA (1998)
Fumonisin B_1, or $FB_1 + FB_2 + FB_3$	TDI: 2 μg/kg bw/day	JECFA (2011a)
Deoxynivalenol (DON)	TDI: 1 μg/kg bw/day ARfD: 8 μg/kg bw/day	JECFA (2011b)
Ochratoxin A (OTA)	TWI: 112 ng/kg bw/week	JECFA (2008)

Multiple interventions in the field and in storage conditions—including sorting out obviously contaminated kernels, drying properly, and storing in cool and dry conditions—can help to reduce fungal growth and aflatoxin accumulation (Khlangwiset and Wu, 2010).

Fumonisin, found almost exclusively in corn, is produced by the fungi *Fusarium verticillioides, Fusarium proliferatum,* and *Aspergillus niger*. While fumonisin in animal feed has been definitively linked to adverse health effects in specific livestock species (e.g., pulmonary edema in pigs, leukoencephalomalacia in horses) (Marasas et al., 2004), the association between fumonisin exposure and adverse human health effects has been less clear. Various epidemiological studies have associated fumonisin exposure with esophageal cancer, neural tube defects in babies, and most recently growth impairment in children (Marasas et al., 2004; Shirima et al., 2015; Chen et al., 2018). While conventional breeding efforts have resulted in some success in making corn less susceptible to Fusarium head blight (and the associated fumonisin contamination), one of the most effective methods at indirectly reducing fumonisin is transgenic Bt corn, which is genetically modified to produce plant-incorporated protectants that control corn pests associated with *Fusarium* infection (Munkvold et al., 1999; Wu, 2006).

DON and other trichothecene mycotoxins such as nivalenol, fusarenon-X, and acetylated versions of DON (3-ADON, 15-ADON) are produced by *Fusarium graminearum, Fusarium culmorum,* and other *Fusarium* species in corn and small cereal grains such as wheat, barley, and oats (Pestka, 2010). When contaminated grains are processed into beer and other alcoholic beverages, DON can be present in the finished beverages (Peters et al., 2017). DON is also known as vomitoxin because of its adverse gastrointestinal impacts on humans and multiple animal species. Aside from vomiting at higher doses, DON has been associated with anorexia (feed refusal in animals), nausea, growth impairment, and immunomodulation (Bondy and Pestka, 2000).

OTA, because it is produced by both warm-weather and cool-weather fungi (*Aspergillus ochraceus* and *Aspergillus carbonarius* and *Penicillium verrucosum,* respectively), contaminates multiple diverse foodstuffs and beverages, including (but not limited to) corn, wheat, barley, oats, coffee, chocolate, wine, beer, grape juice, and dried vine fruits. In addition, because OTA bioaccumulates in blood and muscle meat at relatively higher concentrations than other mycotoxins, it can also be found in pork, pork products, and blood products such as blood sausage (Mitchell et al., 2017).

OTA exposure has been associated with renal toxicity in multiple animal species and may as well cause renal cancers (NTP, 1989). The evidence has been less well documented in humans. In the past, OTA had been associated with the disease Balkan endemic nephropathy, which is now more definitively linked to dietary exposure to aristolochic acid (Bui-Klimke and Wu, 2015).

6.7.6 Phycotoxins

Phycotoxins are toxins produced by algae and cyanobacteria that can colonize seafood, particularly during harmful algal blooms (HABs). These cause a variety of adverse health effects in the humans who consume contaminated seafood. The most important groups of phycotoxins include domoic acid, brevetoxins, azaspiracids (AZA), okadaic acid (OA), cyanotoxins, and saxitoxins (Solter and Beasley, 2013) (Table 6.14).

Domoic acid is a phycotoxin produced by the algae of the genus *Pseudo-nitzschia*, which accumulate in seafood (especially shellfish) during HABs (Ferriss et al., 2017). Domoic acid toxicity involves excitotoxic effects coupled to oxidative stress (Ramya et al.,

TABLE 6.14 Provisional Acute Reference Doses (ARfDs) for Phycotoxins (FAO/IOC/WHO 2004)

Phycotoxin (Marine Biotoxin)	Provisional ARfD (μg/kg bw/day)
AZA	0.04
Domoic acid	100
Okadaic acid	0.33
Saxitoxin	0.7
Yessotoxin	50

2017). It is known to cause amnesic shellfish poisoning (ASP), symptoms of which include confusion, nausea, and short-term memory loss. The first domoic acid poisoning event occurred in Canada in 1987, leading to multiple cases of ASP, including several deaths. Since then, due to effective seafood monitoring programs, no more such cases have been observed in humans, although marine animals have succumbed to domoic acid intoxication (Lefebvre and Robertson, 2010). Unfortunately, diatom blooms produced by *Pseudo-nitzschia* are thought to be increasing in frequency (Lefebvre and Robertson, 2010). Increase in nutrient availability in oceans from pollution and transport of iron-rich desert dust can raise the risk of HABs (Mos, 2001). Additionally, warmer ocean conditions can extend the growth period of *Pseudo-nitzschia* and contribute to increased domoic acid levels in shellfish (McKibben et al., 2017).

Brevetoxins are a group of neurotoxins produced by the marine dinoflagellate *Karenia brevis*, which cause adverse neurological and gastrointestinal effects in humans upon intoxication. Consumption of shellfish contaminated with brevetoxins cause neurotoxic shellfish poisoning (NSP). People affected by NSP experience numbness and tingling in the lips, mouth, and face and occasionally partial limb paralysis (Watkins et al., 2008). *K. brevis* is known to bloom in the Gulf of Mexico, the Caribbean Sea, and the coasts of New Zealand. Brevetoxins are known to accumulate in clams, oysters, mussels, and conch (Watkins et al., 2008). Rinsing, cleaning, cooking, or freezing the shellfish cannot remove brevetoxins. They are also odorless and tasteless. Avoidance of shellfish consumption in warmer months and when HABs are known to have occurred can reduce risk (Friedman et al., 2008).

AZA are a group of phycotoxins produced by dinoflagellates of the *Azadinium* and *Amphidoma* genera. AZA accumulates in marine organisms such as shellfish and sponges and inhibits certain potassium channels. Several hours after AZA consumption, humans can develop azaspiracid shellfish poisoning (AZP), a severe gastrointestinal illness characterized by diarrhea, vomiting, and stomach cramps (Hess et al., 2014). Several rodent studies have linked AZA with organ damage, especially to the small intestine, and long-term exposure can induce lung tumors (James et al., 2004; Furey et al., 2010). Some *in vivo* and *in vitro* studies have also associated AZA exposure with neurological effects (Chevallier et al., 2015). It is considered to be more dangerous than the other known shellfish toxins (Furey et al., 2010); therefore, detection and control is critical.

OA and its derivatives are produced by *Prorocentrum* and *Dinophysis* dinoflagellates in shellfish (Munday, 2013). OA causes epithelial damage and fluid accumulation in the gastrointestinal tract and death at elevated doses in laboratory animals. It can also promote tumor formation and neuronal damage in rodents (Munday, 2013). In a mouse study, OA caused hyperplasia of the forestomach and subacute inflammation of its submucosa (Sosa

et al., 2013). The mechanism of toxicity involves inhibition of protein phosphatases involved in cellular metabolism. The phosphatase inhibition caused by OA is known to be responsible for causing acute toxic effects, tumor-promoting activity, and neurotoxicity (Munday, 2013; Valdiglesias et al., 2013). OA can cause diarrhetic shellfish poisoning in humans; the risk is highest when individuals consume shellfish during the warm months of the year (Trainer et al., 2013).

Cyanotoxins are produced by marine cyanobacteria (blue-green algae), which can form blooms in lakes and oceans to produce high quantities of toxins. Among the toxins are microcystin (a liver cancer promoter), anatoxin-a (an acute neurotoxin), and the amino acid beta-methylamino-L-alanine (BMAA) (implicated in neurodegenerative diseases such as amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease) (Holtcamp, 2012; Solter and Beasley, 2013; Liu et al., 2017). The adverse health effects are diverse, depending on the specific cyanotoxin, as are the affected animal species (Stewart et al., 2008). In humans, cyanotoxin exposure can be cumulative through consumption of fish and shellfish. Additionally, a recent study has shown that cyanotoxins can bioaccumulate in fresh produce watered with contaminated irrigation water (Lee et al., 2017).

Saxitoxin is a neurotoxic phycotoxin that can occur in shellfish, crustaceans, molluscs, gastropods, and planktivorous fish, which accumulate the toxins by feeding on toxin-producing dinoflagellates without themselves being harmed (Wiese et al., 2010). In humans, saxitoxin intoxication causes paralytic shellfish poisoning (PSP) or saxitoxin pufferfish poisoning (SPFP) (Wiese et al., 2010), where they bind specifically and selectively with sodium channels on excitable cells (Wang, 2008). Typical symptoms of PSP include tickling sensations of the lips, mouth, and tongue, breathing difficulties, numbness of the limbs, gastrointestinal problems, and complete paralysis in severe cases (Wang, 2008). Saxitoxin is one of the most potent natural neurotoxins known: a dose of approximately 1 mg of the toxin from a single serving of contaminated shellfish is fatal to humans (Wiese et al., 2010). As of today, there is no antidote available for PSP or SPFP. The only treatments available are artificial respiration and fluid therapy. As saxitoxin is more common in shellfish during HABs, avoiding shellfish consumption when HABs are reported and during the warm months of the year can reduce risk.

6.8 COMPOUNDS PRODUCED DURING FOOD STORAGE AND PREPARATION

Substances such as oxidized fats, the polycyclic aromatic hydrocarbons, several types of heterocyclic compounds resulting from reactions taking place during cooking, the *N*-nitrosamines, and a variety of vasoactive amines are produced during food storage or preparation. The compounds that arise in processes that have been in use by man for a long time are considered here to be of natural origin, although in theory some might legally be considered food additives or contaminants of industrial origin (Roberts, 1981).

Oxidized fats, which include a variety of epoxides and peroxides, pyrolysis products of L-tryptophan and other amino acids, and polycyclic aromatic hydrocarbons resulting from heating of foods, are all well-known and well-studied examples of potential food risks created during food storage or preparation (Concon, 1988). The *N*-nitrosamines, along with the related nitrosamines and nitrosoguanidines, are of particular interest because so many members of this class are animal carcinogens, some of considerable potency, and because some can form both in foods and in the alimentary tract when the appropriate precursors

and conditions are present (Tannenbaum, 1988). The *N*-nitrosamine precursors along with nitrite are either endogenous to or formed in many foods. Included are creatine, sarcosine, proline, pyrrolidine, and piperidine (meat and meat products), methylguanidine, diethylamine, and trimethylamine (fish), diethylamine and dipropylamine (cheese), and choline and lecithin (eggs and meat). There are also reports of *N*-nitrosamine formation from certain pesticides containing secondary amine of carbamate functions (Grasso, 1984; Concon, 1988).

The nitrosamines most commonly reported in foods are dimethylnitrosamine (DMN), diethylnitrosamine (DEN), nitrosoproline (N Pro), and nitrosopyrrolidine (N Pyr). The levels of these tend to fall below 10 ppb, although levels up to several hundred micrograms per kilogram have been detected in certain smoked and nitrate or nitrite-treated foods. *In vivo* formation of *N*-nitroso compounds from precursors associated with foods apparently creates a greater exposure than that created by preformed products (Grasso, 1984).

Dietary nitrosamine formation in food can be inhibited in several ways, most particularly by limiting the acidity. But, in many situations, this is not practically achievable. Ascorbic acid is an effective inhibitor because it reacts quickly with nitrite to form nitric oxide and dehydroascorbic acid. There is some evidence that ascorbic acid also reduces *in vivo* formation of nitrosamines, although not all attempts to achieve such an effect experimentally have been successful. It is not clear whether the use of this vitamin results in a significant reduction in the human exposures to nitrosamines.

The principal reason for concern over nitrosamine formation in foods and beverages, and in other consumer and industrial products as well, is the marked carcinogenic activity exhibited by so many of these compounds in animals. Indeed, it is difficult to identify species of experimental animals that do not develop excess rates of tumors in response to exposures to nitrosamines and their chemical relatives. Typical targets following ingestion include the oral cavity, larynx, trachea, esophagus, liver, kidney, and skin. The multipotent properties of these substances are exemplified by DEN, which produces excess tumors at several sites in rats, mice, hamsters, guinea pigs, rabbits, dogs, monkeys, and several non-mammalian species. Although epidemiological evidence of nitrosamine involvement in human cancers is limited, it is difficult to imagine, given the nature of the animal evidence, that humans are somehow not among the susceptible species (Grasso, 1984; NRC, 1996). It has been suggested that red meat enhances colonic formation of *N*-nitrosamines (Lewin et al., 2006).

Acrylamide has been known for many years to be a cause of neurological disorders in excessively exposed production workers. The compound is also an animal carcinogen. In 2002 investigators in Sweden reported that the compound could be formed by reactions occurring when certain foods are heated to high temperatures. Starchy foods such as potato chips, French fries, cereals, and breads seem to be major sources; intakes from the sources seem to exceed guidelines that the EPA has established for drinking water. Levels in some heated foods have been reported to exceed 500 mg/kg. The compound seems to result from the reaction between glucose and the amino acid asparagine. Public health agencies have not published quantitative estimates of risks related to these surprising sources of acrylamide, but have made statements concerning the pressing need to understand both threats to health and control measures (www.ifst.org/acrylmd.htm).

Many other compounds are produced during food processing, particularly when it entails high temperatures. The polycyclic aromatic hydrocarbons have been most widely investigated, with many others and under increasing scrutiny.

6.9 DIETARY SUPPLEMENTS

In 1994, the U.S. Congress passed the Dietary Supplement Health and Education Act (DSHEA). DSHEA was a compromise that tried to balance FDA's need to regulate the dietary supplement industry with the public's perception that access to these products should be given without undue or excessive restraint. The definition of a dietary supplement is quite expansive and includes "a product intended to supplement the diet that bears or contains one or more dietary ingredients…a vitamin;…a mineral;…an herb or other botanical;…an amino acid;…a dietary substance for use by man to supplement the diet by increasing the total dietary intake; or…a concentrate, metabolite, constituent, extract, or combination [of any ingredient described above]" [Section 201(ff)(1) of the FDC Act, 21 U.S.C. x 321(ff)(1)]. There are additional criteria. The product must either be intended for ingestion in tablet, capsule, powder, softgel, gelcap, or liquid droplet form, or, if not intended for ingestion in such a form, not be "represented for use as a conventional food or as a sole item of a meal or the diet."

Pivotal to the regulation of dietary supplements is the provision that those containing new dietary ingredients (NDIs) are subject to premarket FDA notification. An NDI is defined as "a dietary ingredient that was not marketed in the US before October 15, 1994," presumably requiring the manufacturer or distributor to have written or other evidence that the ingredient in question is chemically identical to a dietary ingredient that was marketed in the United States before that date. A dietary supplement that contains an NDI is deemed to be adulterated unless either (1) the supplement "contains only dietary ingredients which have been present in the food supply as an article used for food in a form in which the food has not been chemically altered" or (2) there is a "history of use or other evidence of safety establishing the at the dietary ingredient when used under the conditions recommended or suggested in the labeling…will reasonably be expected to be safe."

The definition of an NDI brings up some difficult questions both for manufacturers of these products and for the FDA. The definition of "chemically altered" does not include the following physical modifications: minor loss of volatile components, dehydration, lyophilization, milling, tincture, or solution in water, slurry powder, or solid in suspension. This listing only explains what is not included in the definition and leaves much room for debate over both the definition of an NDI and the appropriate safety standard for these ingredients. As written in the regulation, safety is defined as "reasonably expected to be safe under the conditions of use defined in the labeling." The extent of scientific evidence to demonstrate safety, however, is undefined, although it is clear that FDA bears the burden of proof if it asserts that a dietary supplement is not safe under this standard. The value of understanding when an ingredient is "new," and what the appropriate standard for safety should be, is more than a semantic argument. It is operational, in that it defines when new safety information must be generated and what safety information is sufficient to establish "reasonable expectation of safety" to protect the consumer.

Interpretation of the standard of safety for NDIs was revealed in the ruling on the litigation challenging the validity of the FDA's February 2004 Final Rule that declared ephedra alkaloid dietary supplements (EDS) adulterated (unsafe) and not legally marketable in the United States. In the Final Rule, the FDA had concluded that when the minimal benefits of EDS were weighed against the substantial risks, EDS presented an unreasonable risk of illness or injury under the conditions of use recommended and were therefore adulterated under DSHEA. The issues before the court were (1) whether FDA' use of a risk–benefit analysis was appropriate under DSHEA; and (2) whether FDA had provided sufficient

evidence to support the conclusion that EDS containing 10 mg or less per day of ephedrine alkaloids posed a significant or unreasonable risk of illness or injury.

FDA had concluded that the words "significant" and "unreasonable" had two separate and independent meanings. FDA's interpretation of a significant risk involved an evaluation of risk alone, while unreasonable risk required a comparison of risks and benefits. FDA took the position that since EDS posed an unreasonable risk, it was not necessary to address the DSHEA's significant risk standard. The court found that DSHEA contains no risk–benefit provision. A dietary supplement, as with any food, is presumed to be safe and is not required to establish benefit before sale. Therefore, FDA's definition of "unreasonable" entailing a risk–benefit analysis was considered inappropriate. The court also found that although FDA could not determine a safe level, a negative inference is different from the affirmative proof of "significant or unreasonable" risk that is required to support a finding of adulteration. The court did not support FDA's position in the Final Rule, thus providing further guidance on the regulation of dietary supplements as foods.

The difficulties with the definition of an NDI may in part be responsible for the fact that a large proportion of NDI notification to FDA are rejected. For example, of 33 NDINs reviewed by FDA in 2015 for which the outcome is available, only 9 were accepted for filing by FDA. The others were rejected because FDA did not consider the data supported the safety of the substance or the substance did not qualify as an NDI (FDA, 2018b).

6.10 FOOD SAFETY INSTITUTIONS AROUND THE WORLD

Most scientific and regulatory activities in the United States that are related to food safety are used by the FDA (http://www.fda.gov), the FSIS (http://www.fsis.usda.gov), and EPA (http://www.epa.gov). FSIS is responsible for regulation of meat, poultry, and egg products; the EPA regulated pesticides used on food crops; and the FDA is responsible for all other foods. The origins of food safety regulation at the U.S. federal level in the 1906 Pure Food and Drug Act underwent several modifications since that time, and the current FDCA provides the legal foundations for most of the subjects covered in this chapter. The Food Quality Protection Act enacted in the mid-1990s brought new requirements for pesticide residues in foods, as described earlier in this chapter. The 2011 FSMA significantly increased FDA's authority to regulate food contaminants.

Similar regulatory authorities are found in the EU. The EFSA (http://www.efsa.europa.eu) provides comprehensive scientific advice to EU member states on virtually all food safe matters. A Directorate-General of the European Commission (EC) for Health and Food Safety (referred to as DG SANTE) is the primary regulatory body, and its mandate includes monitoring of natural and regional government implementation of EC directives and laws. Moreover, each EU member state has its own regulatory authority. In the United Kingdom, for example, the Food Standards Agency (FSA) (http://www.food.gov.uk) represents England, Wales, and Northern Ireland on matters of food safety regulation. The United Kingdom relied upon a number of experts and independent working groups for expert advice, including committees on toxicology, carcinogenicity, and mutagenicity. Food safety laws and regulatory practices exist in virtually every country and must be the characteristics of those developed in the Western countries.

At the international level, the Codex Alimentarius Commission (CAC) (http://codexalimentarious.org) develops and publishes food safety and quality standards that are used for all foods involved in international trade. The CAC is a joint foundation of the

WHO (http://www.who.int) and FAO (http://www.fao.org), and scientific guidance to the CAC is provided by a member of expert committees (e.g., JECFA) that are administered by FAO/WHO. The CAC develops consensus standards based in part on the scientific guidance provided by these various committees.

Much activity on food safety matters is underway in the U.S. Centers for Disease Control and Protection (CDC) (http://www.cdc.gov), which is heavily involved in the investigation of disease attributes related to foods. The International Life Sciences Institute (ILSI) (http://www.ilsi.org) brings together food safety experts from all sectors to study emerging food safety issues. The Grocery Manufacturers Association (http://www.gmaonline.org) is a source of much information and perspective on food safety, as are leading consumer advocacy groups such as the Center for Science in the Public Interest (http://www.cspinet.org) and the European Consumer Organization (http://www.beuc.eu)

The topics covered in this chapter, and many more as well, are leading areas of research and study throughout the world, and understanding and knowledge is steadily advancing, much in line with the other subjects reviewed in this book.

6.11 SUMMARY AND CONCLUSION

Because foods and beverages are so complex and variable in composition, health risks associated with them can be understood fully only through the continued pursuit of long-term epidemiological investigations. There seems little doubt that the composition of the human diet strongly influences health status, in both positive and negative ways. Current evidence suggests that the major influences on long-term health status are those associated with total caloric intake and with the natural constituents of food, both nutritive and nonnutritive. A large impact from additives and contaminants seems unlikely, though the relative importance of these constituents, especially contaminants of both industrial and natural origin, varies considerably among the geographical regions of the Earth. Recent evidence of large-scale pollution in rapidly developing countries suggests an increasing likelihood that food contamination could become a serious public health problem. As global trade in basic food commodities continues to increase, so will adverse public health impacts spread. Contamination by human pathogens—a not insignificant problem even in developed countries—is, on a global scale, almost certainly the most significant acute health problem associated with food.

A review of what is known and unknown about the risks associated with the chemical constituents and contaminants of food, as provided in this chapter, demonstrates that, on a chemical-specific basis, far more study has been devoted to substances intentionally added to food than to substances naturally present or contaminating food. This observation is confirmed by a recent NRC review of carcinogens and anticarcinogens in the diet (NRC, 1996). This state of affairs is perhaps largely explained by the fact that the laws under which foods are regulated, not only in the United States, but around the world, require much closer examination of added substances. The goal of understanding the effects on health of the diet as a whole, and of its myriad natural constituents and of its contaminants, is largely dictated by choices made in the research community and its funding agencies. If trends of the past decade are suggestive of the future, we can expect in the next decade or two a vastly increased understanding of the type of diet needed to maximize health benefits and to minimize the risks of chronic diseases. Of course, such an understanding will not, of itself, change individual behavior or that of the food production and distribution system; but without that understanding there is little hope for beneficial change.

ACRONYMS

4-MEI	4-Methylimidazole
ADI	Acceptable daily intake
ARMS	Adverse reaction monitoring system
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BMDL	Lower confidence bound on benchmark dose
CAC	Codex Alimentarius Commission
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFSAN	Center for Food Safety and Applied Nutrition
CG	Cyanogenic glycosides
CNS	Central nervous system
DEN	Diethylnitrosamine
DES	Diethylstilbestrol
DMN	Dimethylnitrosamine
DON	Deoxynivalenol
DRI	Dietary Reference Intake
DSHEA	Dietary Supplement Health and Education Act
EDI	Estimated daily intake
EDS	Ephedra alkaloid dietary supplement
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EU	European Union
FAO/WHO	Food and Agriculture Organization/World Health Organization
FCN	Food Contact Substance Notification
FDA	Food and Drug Administration
FDAC	Food, Drug, and Cosmetic Act
FEMA	Flavor and Extract Manufacturers Association
FR	Federal Register
FSIS	Food Safety and Inspection Service (USDA)
FSMA	Food Safety Modernization Act
GRAS	Generally recognized as safe
HRF	Human relevance framework
IFBC	International Food Biotechnology Council
IOM	Institute of Medicine
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOAEL	Lowest observed adverse effect level
MOA	Mode of action
NASEM	National Academy of Sciences, Engineering, and Medicine
NCI	National Cancer Institute
NDI	New dietary ingredient
NDIN	New Dietary Ingredient Notification
NOAELS	No observed adverse effect levels
N Pro	Nitrosoproline
N Pyr	Nitrosopyrrolidine
NRC	National Research Council

OTA	Ochratoxin A
PBDE	Polybrominated diphenyl ethers
PCB	Polychlorinated biphenyls
PFAS	Per- or polyfluoroalkyl substances
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PHA	Phytohemagglutinin
PST	Paralytic shellfish toxins
RfD	Reference dose
SAFEST	Safety Assessment of Food by Equivalence and Similarity Targeting
SCOGS	Select Committee on GRAS Substances
T_3	Triiodothyronine
T_4	Thyroxine
TDI	Tolerable daily intake
TSH	Thyroid-stimulating hormone
TTX	Tetrodotoxin
WHO	World Health Organization

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7

ACROLEIN AND UNSATURATED ALDEHYDES

Kifai Bein and George D. Leikauf

7.1 BACKGROUND

Human exposure to unsaturated aldehydes is extensive, resulting from endogenous formation (biogenesis through lipid peroxidation or metabolism) and exogenous sources, and can lead to several adverse health effects. The major unsaturated aldehydes of environmental concern include acrolein and crotonaldehyde and, to a lesser extent, 2-hexenal, methacrolein, and cinnamaldehyde. Other unsaturated aldehydes, also produced by lipid peroxidation, include 4-hydroxy-2-nonenal and malondialdehyde, but they have limited commercial use, and human exposure is mainly limited to endogenous production. Therefore, this chapter focuses mainly on acrolein and crotonaldehyde.

Acrolein (2-propenal, CH_2=CHCHO) and crotonaldehyde (2-butenal, CH_3CH=CHCHO) are volatile at room temperature, and both have pungent and piercing odors (Liu et al., 2012). They are bifunctional and undergo numerous chemical reactions, including as a Michael acceptor. Acrolein also is suited for use in Heck, Diels–Alder cycloaddition, aldol, and metathesis reactions. Because of its numerous industrial applications (e.g., methionine synthesis), worldwide production exceeds 500 kilotons of acrolein annually (Ghilarducci and Tjeerdema, 1995; Liu et al., 2012). Acrolein is released from a variety of sources, is encountered in the indoor and ambient atmosphere, and is listed as a hazardous air pollutant (HAP) (aka "air toxic") (Services USDOHAH, Service PH and Registry AfTSaD, 2007; Faroon et al., 2008a; Logue et al., 2010; USEPA, 2011b). Crotonaldehyde was formerly used in the manufacture of *n*-butanol and as a stabilizer for tetraethyllead, but currently, the most extensive use is in the manufacture of sorbic acid. Crotonaldehyde also is used as a warning agent in fuel gases, in the preparation of rubber accelerators, in leather tanning, and as an alcohol denaturant.

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7.1.1 Human Environmental Exposure

7.1.1.1 Acrolein Formation: Generation from Carbohydrates and Lipids Acrolein can be formed endogenously from carbohydrates, lipids, amino acids, and polyamines (Anderson et al., 1997; Uchida et al., 1998a; O'Brien et al., 2005; Stevens and Maier, 2008). Carbohydrates represent a significant source of acrolein. Although the chemical composition of plants varies greatly, typical biomass consists of ~75% carbohydrates, ~20% lignin, and ~5% other natural products, such as oils, fats, proteins, terpenes, alkaloids, and nucleic acids (Röper, 2002). Lingnert et al. (2002) hypothesized that acrolein formed through carbohydrate degradation and can be a precursor in acrylamide formation. The acrylamide level found in carbohydrate-rich foods (100–4000 μg/kg) was much higher than that detected in heated protein (5–50 μg/kg), in comparison with undetectable levels in unheated or boiled foods. Because carbohydrates can generate higher acrylamide level and acrolein is possibly an acrylamide precursor (Lingnert et al., 2002), carbohydrates are likely to represent the major source of biomass-derived environmental acrolein. Cellulose, starch, and sugars such as sucrose, lactose, and maltose can hydrolyze upon heating above 100°C at slightly acidic pH, resulting in the formation of reducing monosaccharides and levoglucosan, whose structure includes a cyclic acetal and a glycerol moiety (Antal et al., 1985; Lingnert et al., 2002). Acrolein can be formed from glucose through a series of reactions: glucose → deoxyglucose → hydroxyacetone → 2 hydroxypropanal → acrolein (Yaylayan et al., 1999; Yaylayan and Keyhani, 2000).

Stevens and Maier (2008) estimated that carbohydrates represent a significant source of acrolein in cigarette smoke. Sugars, which can be present up to 20% by weight in tobacco (Talhout et al., 2006), increase smoke-borne acrolein. For example, addition of 16% sucrose can increase acrolein in the range of 118–215 μg acrolein/cigarette. This would be in addition to 56 μg acrolein/cigarette from the addition of glycerol controls (Stevens and Maier, 2008). Estimates of acrolein in mainstream smoke vary greatly (15–468 μg/cigarette) and depend on the source and length of the cigarette, puff rate, and volume, as well as the filter breakthrough (Fujioka and Shibamoto, 2006; Al Rashidi et al., 2008; Blair et al., 2015). In addition, acrolein formation from lipid peroxidation of unsaturated fatty acids (linoleic or arachidonic acid) increases in the presence of glucose, possibly, due to free radicals generated from the glucose autoxidation process. Thus acrolein could be produced during hyperglycemic states and may lead to tissue injury (Medina-Navarro et al., 2003, 2004).

When discussing lipid peroxidation as an acrolein source, it is worth noting that the name "acrolein" refers to the pungent "acrid" smell that is released by overheating "olein," that is, oils and fats (Ho et al., 2006; Stevens and Maier, 2008; Bein and Leikauf, 2011). Although acrolein may be formed by dehydration of the glycerol backbone of triglycerides, the major source of acrolein from heated oils may be polyunsaturated fatty acids, such as linolenic acid (Endo et al., 2013). Acrolein may also be generated *in vivo* by peroxidation of polyunsaturated fatty acids by enzymatic or metal-catalyzed oxidation of low-density lipoprotein (LDL) and arachidonate, as proposed (Esterbauer et al., 1991, 1992; Uchida et al., 1998a).

Lipid peroxidation has been an area of research for over 70 years (Schneider, 2009), and yet the role of this process in endogenous acrolein generation remains controversial (Stevens and Maier, 2008). Indisputably, lipid peroxidation can produce malondialdehyde, 4-hydroxynonenal, and 4-oxo-2-nonenal, which have enjoyed wide usage as biomarkers of lipid peroxidation or "oxidative stress" (Esterbauer et al., 1991; Niki, 2009; Spickett,

2013). However, these "biomarkers of oxidative stress" should be evaluated with caution (Stevens and Maier, 2008; Zhang and Forman, 2017), because neither malondialdehyde nor 4-hydroxynonenal is solely a measure of lipid peroxidation. The classic malondialdehyde assay method is the measurement of thiobarbituric acid reactive substances (TBARS). It is often interpreted as a measure of malondialdehyde; however, this assay also measures other aldehydes in addition to malondialdehyde (Liu et al., 1997). In addition to formation by lipid peroxidation, malondialdehyde can be formed from arachidonic acid in activated platelets (McMillan et al., 1978) and by other cells that express thromboxane A synthase (Montenegro-Burke et al., 2016). Similarly, 4-hydroxynonenal can be generated enzymatically by cyclooxygenase-2 and lipoxygenase (Wang et al., 2013). In addition, inadequate sample handling can cause lipid oxidation (Dalle-Donne et al., 2006). Nonetheless, a better understanding of assay limitations and improvements in sample storage have enabled reliable measurement in most biological samples (Poli et al., 2008; Yamada et al., 2015; Tsikas, 2017; Zhang and Forman, 2017). Several methods that use antibodies [e.g., enzyme-linked immunosorbent assay (ELISA) or Western blot to measure protein adducts] or other methods (liquid chromatography and mass spectrometry) are commercially available.

Oxidation of polyunsaturated fatty acids is initiated by hydrogen abstraction from targeted atomic positions, leading to oxygenation and subsequent formation of hydroperoxides (Figure 7.1) (Fridovich and Porter, 1981; Gardner, 1989; Zhang et al., 2006; Kobeissy, 2015; Schaur et al., 2015). Hydroperoxides can propagate a reaction cascade generating reactive molecules including aldehydic products such as acrolein (Gardner, 1989; Esterbauer et al., 1991; Uchida et al., 1998a). Uchida et al. (1998a, 1998b) were among the first to investigate acrolein formation during lipid peroxidation. The investigators detected acrolein in oxidized (Cu^{2+}-modified) LDL and reported that the adduct formation was correlated with LDL peroxidation assessed by the consumption of α-tocopherol and cholesteryl ester and the concomitant formation of cholesteryl ester hydroperoxide. Free acrolein was released from the Cu^{2+}-oxidized LDL. Furthermore, acrolein was detected following Fe^{2+}/ascorbate-catalyzed oxidation of arachidonic acid, suggesting that arachidonic acid is a potential source of acrolein generated during LDL peroxidation. In addition, other polyunsaturated fatty acids, such as linoleate, *cis*-5,8,11,14,17-eicosapentaenoic acid, and *cis*-4,7,10,13,16,19-docosahexaenoic acid can generate acrolein during peroxidation. Taken together, acrolein and its conjugate with protein represent general indicators of lipid peroxidation. However, it may very well be that lipid peroxidation does not represent a major source of acrolein *in vivo* because degradation of linoleic acid-derived or arachidonic acid-derived lipid hydroperoxides yields far more 4-hydroxynonenal than acrolein (Stevens and Maier, 2008). Yet, the presence of a 100-fold greater production of acrolein metabolites compared with 4-hydroxynonenal metabolites in human urine samples indicates the presence of other processes generating acrolein (Uchida, 1999; Tomitori et al., 2005; Stevens and Maier, 2008).

7.1.1.2 Endogenous Acrolein Formation: Generation from Amino Acids The amino acid sources of acrolein include threonine and methionine. Threonine is converted to acrolein by myeloperoxidase. Myeloperoxidase is utilized by neutrophils to kill bacteria and other pathogens by forming hydrochlorous acid (HOCl) from chloride and hydrogen peroxide (H_2O_2). Activated neutrophils can also use chloride and H_2O_2 to oxidize L-threonine to 2-hydroxypropanal and its dehydration product, acrolein (Figure 7.2) (Hazen et al., 1998a, 1998b). The conversion can be inhibited by catalase (which decomposes H_2O_2)

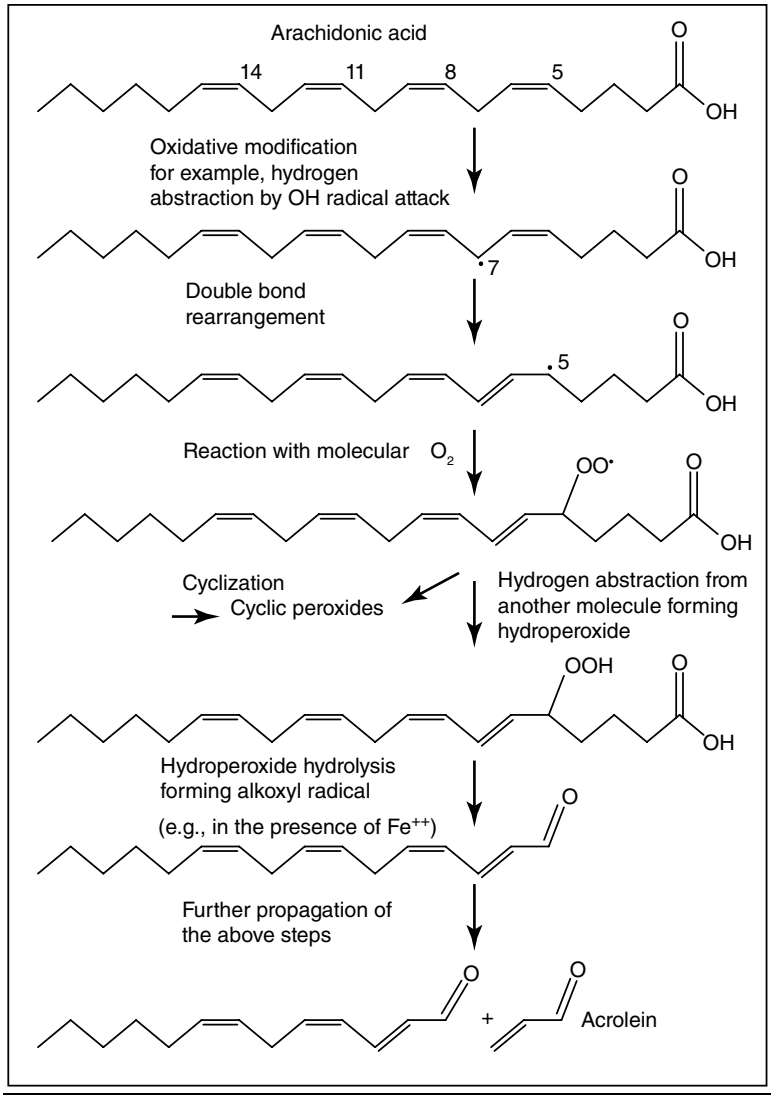

FIGURE 7.1 Acrolein formation from arachidonic acid via lipid peroxidation. *Source*: Adapted from Kobeissy (2015) and Schaur et al. (2015).

and heme poisons, providing evidence of H_2O_2 and myeloperoxidase-mediated acrolein generation (Zgliczynski et al., 1968; Anderson et al., 1997). Threonine treated with HOCl also produced 2-hydroxypropanal and acrolein.

The presence of threonine (up to 200 μM) in blood (Aoki et al., 1976) and the recruitment of phagocytic cells to sites of inflammation suggest that threonine may represent an important endogenous source of acrolein. Myeloperoxidase is prominent in regions of atherosclerotic lesions. Thus, myeloperoxidase may contribute to acrolein formation and atherogenesis by catalyzing oxidative reactions in the vascular wall (Daugherty et al., 1994). Further, in a mouse model of acute myocardial infarction, myeloperoxidase mediates acrolein production. Heart acrolein concentrations increased more in C57BL/6J mice

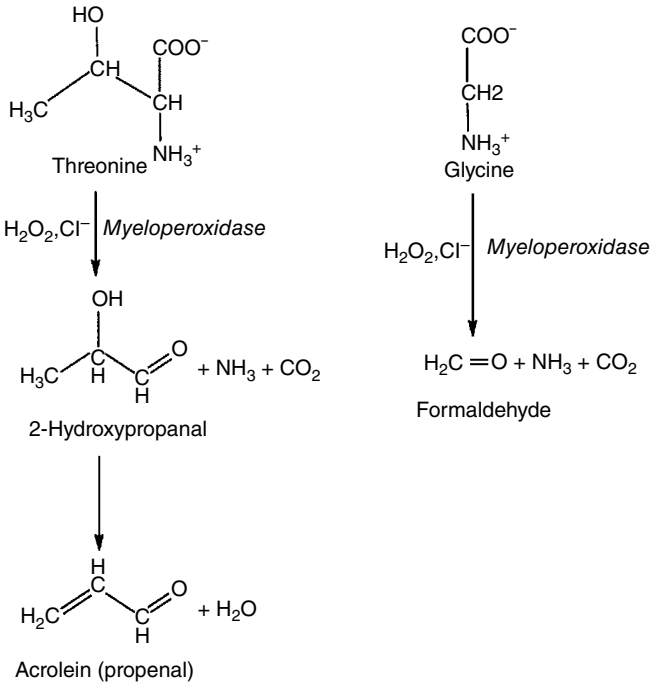

FIGURE 7.2 Activated neutrophils use myeloperoxidase, hydrogen peroxide (H_2O_2), and chloride (Cl^-) to generate acrolein from L-threonine and formaldehyde from glycine. Note the amount of acrolein produced at sites of injury is ~9–17 times more than that of formaldehyde. *Source*: Adapted from Anderson et al. (1997) and Vasilyev et al. (2005).

(298–674 nmol/mg protein) as compared with littermate myeloperoxidase null mice (15 nmol/mg protein), following induction of myocardial infarction (Vasilyev et al., 2005).

7.1.1.3 Endogenous Acrolein Formation: Generation from Polyamines Polyamines, spermine and spermidine, are present at millimolar concentrations in the cell, typically bound to RNA (Watanabe et al., 1991; Igarashi and Kashiwagi, 2000; Igarashi and Kashiwagi, 2010; Lightfoot and Hall, 2014; Wang and He, 2014). Polyamines are essential for cell growth, and their levels inside mammalian cells are regulated precisely by biosynthesis, degradation, and transport. When cells are damaged, polyamines are released from RNA and can be transported out of the cell (Kruger et al., 2013). An early discovery was that spermine, added to cell culture medium containing mature (but not fetal) bovine serum, inhibits cell proliferation (Alarcon, 1964, 1970; Dawson and Dryden, 1969; Higgins et al., 1969). Such serum contains serum amine oxidase. Serum amine oxidase and spermine oxidase catalyze the oxidative deamination of spermine to produce an aminoaldehyde [*N′*-(4-aminobutyl)-aminopropionaldehyde] or an aminodialdehyde [*N,N′*-bis (3-propionaldehyde)-1,4-butanediamine] plus H_2O_2 and ammonia and spermidine plus 3-aminopropanal and H_2O_2, respectively. Acrolein is then spontaneously formed from the aldehydes (Figure 7.3) (Tabor et al., 1964; Sakata et al., 2003a).

The physiological role of polyamines in cellular functions is significant (Igarashi and Kashiwagi, 2000, 2010; Lightfoot and Hall, 2014). A number of studies, in particular those conducted studying plant physiology, have found that polyamine level correlated with

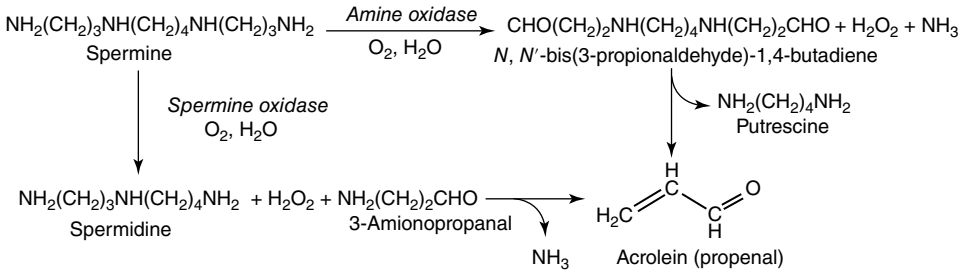

FIGURE 7.3 Amine oxidase and spermine oxidase use the polyamine spermine to generate acrolein, hydrogen peroxide (H_2O_2), and NH_3. *Source*: Adapted from Sakata et al. (2003a) and Tabor et al. (1964).

stress resistance. Cellular polyamines in plants increase under both short- and long-term abiotic stress conditions and are implicated in cell protection from, but also enhancement of, stress damage. Elevated polyamine content by exogenous treatment with polyamines, or through genetic engineering of genes encoding polyamine biosynthetic enzymes, enhanced plant tolerance to abiotic stress. However, polyamine increase can also potentially cause cellular harm because catabolic metabolism of polyamines generates H_2O_2 and acrolein (Hussain et al., 2011).

Although spermine degradation forms H_2O_2 and acrolein, addition of aldehyde dehydrogenase, but not catalase, reversed inhibition of mouse mammary carcinoma FM3A cell growth by spermine (Sakata et al., 2003a). Further, increased polyamines are associated with inflammation, and an increase in the catabolic enzyme polyamine oxidase was observed in aged rats (Ferioli et al., 1998; Puntambekar et al., 2011). In addition, although polyamines, which are derived from arginine (ornithine) and decarboxylated *S*-adenosylmethionine (Stevens and Maier, 2008), occur in all cells (Hoet and Nemery, 2000), high micromolar concentrations can be measured in brain tissue (Shaw and Pateman, 1973). In addition, lung alveolar epithelial cells appear endowed with a much higher polyamine uptake system (Hoet and Nemery, 2000).

Polyamines, which produce acrolein in the presence of amine oxidase, can react with acrolein to produce 1,5-diazacyclooctane produced from acrolein and 2 spermine molecules (Figure 7.4) (Tsutsui et al., 2014). The proposed reaction mechanism by Tanaka et al. (2001) involves OH^- and amine-mediated "head-to-tail" imino [4+4] reaction of unsaturated imines. This dimerization occurs in the presence of hydroxylated alkyl groups on the imino nitrogen. Although diazacyclooctane formation effectively neutralized acrolein toxicity, further polymerization by excessive acrolein initiates sequential diazacyclooctane polymerization reactions that produce a cationic hydrogel. The hydrogel was cytolytic and proposed to accelerate oxidative stress, and thus, is highly cytotoxic (Figure 7.4). The resulting hydrogel could also be formed from acrolein-spermidine conjugates as well. In HeLa cells, the spermine (IC_{50} = 0.14 μM) and spermidine conjugates (IC_{50} = 4.6 μM) are more cytotoxic than acrolein (IC_{50} = 17.2 μM) and could induce heme oxygenase 1, an enzyme associated with cell stress response. This study suggested that diazacyclooctane formation is one possible mechanism underlying acrolein-mediated oxidative stress. Thus, enhanced polyamine catabolism and myeloperoxidase action on threonine represent key endogenous sources of focal acrolein generation in areas of cell damage in inflammation.

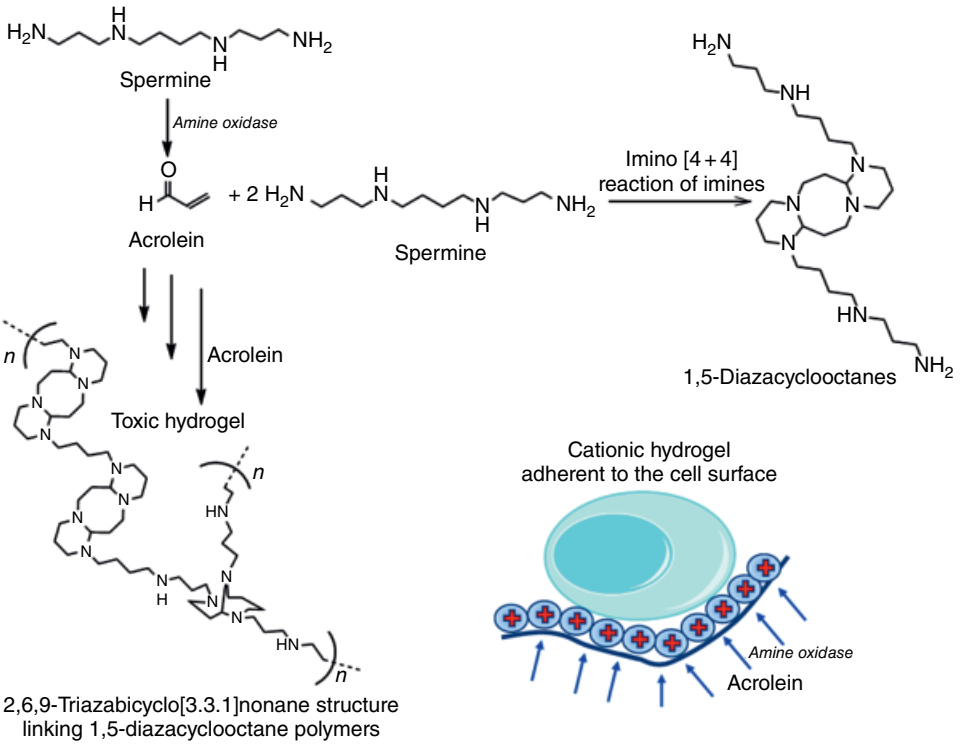

FIGURE 7.4 Acrolein generation from spermine mediated by amine oxidases. Spermine can be a source of acrolein, but it also can act as a sink by forming 1,5-diazacyclooctanes. However, whenever acrolein is in excess, it initiates a further polymerization of the 1,5-diazacyclooctanes, which forms a positively charged hydrogel that accelerates oxidative stress and is cytotoxic. *Source*: Adapted from Tsutsui et al. (2014).

7.1.1.4 Endogenous Acrolein Formation: Generation by the Intestinal Microbiome *In vivo* acrolein increase, in part, may result from formation in the gut or exogenous source, for example, ingestion of oxidized polyunsaturated fatty acids in food (Beretta et al., 2008; Gorelik et al., 2008; Guillen and Goicoechea, 2008; Sirota et al., 2013). Glycerol is an abundant carbohydrate source in the gut, and members of the bacterial genera *Klebsiella*, *Enterobacter*, *Citrobacter*, *Clostridium*, and *Lactobacillus* expressing glycerol dehydratase are capable of reductive glycerol metabolism to produce 3-hydroxypropionaldehyde (3-HPA). This is a member of the reuterin system, which includes 3-HPA, hydrate 1,3-propanetriol, and dimeric 2-(2-hydroxyethyl)-4-hydroxy-1,3-dioxane. Most bacteria convert 3-HPA to 1,3-propanediol by 1,3-propranediol dehydrogenase (1,3-PD) during growth. However, some bacteria lack adequate 1,3-PD and secrete 3-HPA, which is subsequently converted to acrolein. Acrolein formation provides the antimicrobial and heterocyclic amine transforming capability of reuterin (Engels et al., 2016). Heterocyclic amines are formed in food preparation (e.g., grilled meat or bacon) and have been associated with colon cancer. By the conjugation of heterocyclic amine, bacterially formed acrolein can thus be viewed as protective, since these metabolites have been proposed to have limited absorption (Vanhaecke et al., 2008; Nicken et al., 2015). In addition, acrolein improves the competitiveness of reuterin producing strains while serving to limit reuterin

sensitive bacteria by inducing an imbalance in intracellular redox status. Thus, acrolein has a dual function in metabolism of the gut microbiome.

7.1.1.5 Ambient Acrolein Exposure Acrolein is highly reactive and remains a public health hazard because exposure is unavoidable as a result of various human activities. The underlying reason for the human activities associated with acrolein exposure can be basic necessity (e.g., cooking and food preparation, emissions from vehicles for transportation and from fire places for heating), personal choice/addiction (e.g., smoking), occupational exposures, or occasional incidents (e.g., forest and house fires). A portion of ambient acrolein exposure originates from mobile sources (USEPA, 2011a). The EPA reported mean ambient acrolein concentrations of 14 μg/m^3 (6.1 ppb), ranging from 8 to 24 μg/m^3 (3.5–10.4 ppb), for two urban locations based upon data from 1961 to 1980 (USEPA, 2003). In contrast, the average ambient acrolein concentration across the United States for the year 1999 ranged from 0.008 to 0.4 μg/m^3 (<1 ppb), and the concentration was projected to increase absent any regulatory measures (Woodruff et al., 2007). However, more recent studies, using an analytical method designed to detect low levels, noted ambient air acrolein concentration of 0.26 μg/m^3 (0.11 ppb) near the Peace Bridge Plaza in Buffalo, NY. Sites in Northern California chosen to reflect the hemispheric background, a region dominated by biogenic resources, and an urban development, had acrolein concentration of 0.05 (0.02 ppb), 0.09 (0.04 ppb), and 0.29 μg/m^3 (0.13 ppb), respectively (Seaman et al., 2006). In another study, the median natural summertime background of acrolein in coastal and remote inland areas was near 0.04 μg/m^3 (0.017 ppb), while in urban areas, the concentration was approximately three- to eightfold higher (Cahill, 2014).

The introduction of reformulated gasoline, together with the advances in new motor vehicle exhaust catalysts and fleet turnovers, may counter the projected increases (Destaillats et al., 2002). Although different fuels produced modest differences in emission rates, engine technology may be more important in controlling emissions than the different fuels (Cahill and Okamoto, 2012). Interestingly, other studies focused on diurnal and seasonal analyses of acrolein concentrations that range from none to 0.625 μg/m^3 (0.27 ppb) in several busy roadways suggested that vehicles are not the major source of ambient acrolein through primary emissions or secondary oxidation products. Rather, wintertime acrolein concentrations correlated well with 2-furaldehyde, a tracer of biomass burning (Spada et al., 2008). Thus, acrolein released from smoking, traditional cooking, house fires, grass and forest fires, or chemical storage tanks could pose greater sources of exposure.

7.2 CELLULAR EXPOSURE AND METABOLISM

7.2.1 Acrolein's Mode of Action

α-β-Unsaturated electrophilic aldehydes react with a variety of biological nucleophilic targets (Table 7.1) and may induce a variety of responses including: cell signaling, oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum stress (Mohammad et al., 2012). Although acrolein and crotonaldehyde react preferentially with the sulfhydryl group of cysteine (Seiner et al., 2007; Martyniuk et al., 2011; LoPachin and Gavin, 2014), the imidazole moiety of histidine, the ε-amino group of lysine, the guanidine group of arginine, and the deoxyguanosine moiety of DNA also are targeted (Lambert et al., 2007; Stevens and Maier, 2008). It has been suggested that despite the widespread occurrence of cysteine

TABLE 7.1 Acrolein Reactions with Biomolecular Targets

Target Protein/Cell/Organelle	Acrolein Concentration (μM)	Incubation Condition Medium/Solution	Endpoint Determination	Reference
Studies employing cell-free systems				
hMMP9, MMP14	0.1–1	4 h, 37°C, tris-HCl containing reaction buffer	MMP cleavage and activation: acrolein (>300 nM)	Deshmukh et al. (2008, 2009)
Aldehyde dehydrogenase I	0.5–60	5 min, 37°C, potassium phosphate buffer, pH 7.4	Aldehyde dehydrogenase 1 enzyme activity: 35.4 + 13.8% and 85.3 + 4.1% inhibition by 10 or 50 μM acrolein, respectively	Ren et al. (1999)
Human erythrocyte GAPDH	10–100	0–30 min, 30°C, tris-acetate, sodium arsenate, pH 7.4 or 8.5	GAPDH enzyme activity: inhibition correlated with adduction at Cys152 and electrophilicity Ki = 38 μM	Martyniuk et al. (2011)
Bovine protein disulfide isomerase	10–200	16 h, 37°C, sodium phosphate buffer, pH 7.4	Enzyme activity: 200 μM acrolein inhibited protein disulfide isomerase by 60%	Carbone et al. (2005)
Preproenkephalin fragment, BSA	20–1000	0–3 h, 37°C, water, phosphate buffer, pH 7.0	Adduct formation: SDS PAGE of 2,4-dinitrophenylhydrazine-derivatized molecules, mono- and bis-adducted within 30 min; >20 μM acrolein increased carbonylation	Kaminskas et al. (2005)
DNA polymerase alpha-primase	100–1000	0–30 min, potassium phosphate buffer, pH 7.5	Enzyme activity: >40% inhibition of synthase activity within 5 min; IC_{50} = 100 μM	Catalano and Kuchta (1995)
Antithrombin	100–2000	0–4 h, 37°C, sodium phosphate buffer, pH 7.4	Enzyme activity: IC_{50} = 250 μM acrolein; >100 μM acrolein-enhanced slower migration	Gugliucci (2008)
Neurofilament-L	100–2000	24 h, 37°C, potassium phosphate buffer, pH 7.4	Protein carbonylation, aggregation: >100 μM increased aggregation	Jeong and Kang (2008)

(continued)

TABLE 7.1 *(Continued)*

Target Protein/Cell/Organelle	Acrolein Concentration (μM)	Incubation Condition Medium/Solution	Endpoint Determination	Reference
Paraoxonase 1	120–10,000	2 h, 37°C, sodium phosphate buffer, pH 7.4	Enzyme activity: IC_{50} = 1 mM at 2 h; protein modification: high mol. wt. bands observed at >0.12 mM	Gugliucci et al. (2007)
BSA and microsome	200–2000	1–3 h, potassium/ sodium phosphate buffer, pH 7.4	Carbonylation: >50 μM acrolein increased carbonylation	MacAllister et al. (2013)
Lipoproteins	200–10,000	24 h, 37°C, tris-EDTA, pH 7.4	Lipoprotein modification: >200 μM acrolein increased gel migration of acrolein modified apolipoprotein B	Watanabe et al. (2013)
Insulin	300	0–4 h, 37°C, tris base, pH 7.4, 8.0	Carbonylation higher at pH 8.0 than pH 7.4	Medina-Navarro et al. (2007)
Rnase A	750–12,000	0–3.5 h, 37°C, sodium phosphate buffer, pH 7.0	Protein cross-linking: observed as early as 30 min; starting at 750 μM	Burcham and Pyke (2006)
Human hemoglobin	5000	20 h, 37°C, bis-tris buffer, pH 7.3	Protein conjugation: heterogeneous mixture of derivatives	Hoberman and San George (1988)
Fibrinogen	9000	0–8 h, 37°C, PBS, pH 7.4	Protein aggregation: began within 30 min, a-fibrinogen appearing the first target	Xu et al. (2012)
9-Ethyladenine, 2-deoxyadenosine	0.1×10^6	2 days, 37°C, sodium phosphate buffer, pH 4.6	Acrolein reacted with 9-ethyladenine forming two compounds	Pawlowicz et al. (2006)
Studies employing tissue culture cells				
Rat cortical neurons, astrocytes	0.001–25	4 h, 37°C, Locke's solution	Within 6 h of treatment, 10 μM acrolein decreased survival to 20%; 1 nM to 1 μM decreased 2-[^{3}H] deoxyglucose transport; 0.75–10 μM decreased ^{3}H-labeled glutamate (3,4-[^{3}H]glutamic acid) uptake	Lovell et al. (2000)
EA.hy926 cells, human lung microvascular endothelial cells	0.001–0.3	4 h, 37°C, DPBS	Claudin 5 transcript: acrolein (up to 30 nM) increased, whereas 100 and 300 nM decreased claudin 5 transcript	Jang et al. (2011)

NCI-H292 cells	0.01–0.1	4 h, 37°C, PBS, pH 7.4	MUC5AC transcript: acrolein (0.01–30 nM) increased MUC5AC transcript	Deshmukh et al. (2005) and Borchers et al. (1999a)
NCI-H292, NHBE cells	0.01–0.3	4 h, 37°C, PBS	MMP-9 transcript: acrolein (>10 nM) increased hMMP9 transcripts; matrix metalloproteinase-14 transcript: acrolein (>30 nM) increased hMMP14 transcripts	Deshmukh et al. (2008, 2009)
Human bronchial epithelial cells	0.1–10	24 h, 37°C, LHC-D/RPMI	Acrolein >0.1 μM induced IL-8 release	Mio et al. (1997)
Rat brain microglia, astrocytes	0.1–10	24 h, 37°C, DMEM + 10% FBS	Cytotoxicity: EC_{50} = 0.3 μM in microglia, but not astrocytes	Takano et al. (2005)
RAW 264.7 macrophages	0.1–100	8, 24 h, 37°C, DMEM + 10% FBS	Cytotoxicity: in LPS-treated cells >1 μM acrolein decreased viability; ROS and NO production: negatively regulated in LPS-treated cells	Ambrozova et al. (2011)
Retinal pigment epithelial ARPE19 and primary human fetal cells	0.1–100	DMEM-F12 + 10% FBS	Cytotoxicity: >50 μM acrolein (24 h) or 0.1–5 μM (8 or 32 days) decreased cell viability, mitochondrial potential, GSH, antioxidant capacity, Nrf2 expression, and enzyme activity while it increased oxidant, protein carbonyls, and calcium	Jia et al. (2007) and Li et al. (2013)
Rat primary hippocampal neurons	0.5–10	3–24 h, 37°C, Locke's solution	Cytotoxicity: within 6 h of treatment, 0.5 μM acrolein led to ~25% decrease in neuron survival, and all cells were dead at a concentration of 10 μM; treatment of hippocampal cultures with as little as 2.5 μM acrolein led to nearly complete neuron death within 24 h; >0.75 μM increased intracellular Ca^{2+}	Lovell et al. (2001)
Male germ cell-derived GC-1 cells	0.5–100	4–48 h, DMEM + 10% FBS?	Cytotoxicity: >2 μM decreased viability; >10 μM decreased GSH	He et al. (2014)
Human neutrophils	0.5–500	3–13 min, 37°C, HBSS	Eicosanoid synthesis: >0.5 μM acrolein decreased formation of 5-lipoxygenase by GM-CSF/fMLP-stimulated neutrophils	Berry et al. (2008)

(continued)

TABLE 7.1 *(Continued)*

Target Protein/Cell/Organelle	Acrolein Concentration (μM)	Incubation Condition Medium/Solution	Endpoint Determination	Reference
Rat lung type II alveolar cells, clone 14	1–5	20 min, 37°C, HBSS	GSH depletion: EC_{50} = 2 μM	Meacher and Menzel (1999)
Toad urothelial TBM-54 cells	1, 10	16, 64 h, 37°C, Coon's F-12-Leibovitz's L-15 medium + 10% BSA	Muscarinic receptor function: 10 μM acrolein reduced change in extracellular acidification rate in response to carbachol	Giglio et al. (2008)
Retinal pigment epithelial R-50 cells	1–25	20 min to 7 days, Ham's F-12 medium + 10% FBS	Acrolein >1 μM inhibited phagocytic function as early as 20 min after exposure	Sheu et al. (2010)
Human neuroblastoma SH-SY5Y cells	1–25	24 h, 37°C, DMEM	Acrolein (>1 μM) decreased cell viability and >5 μM increased oxidative stress	Ansari et al. (2008)
Human gingival fibroblasts	1–100	3 h, 5 days, 37°C, DMEM + 10% FBS	Cell attachment: inhibition at >30 μM acrolein; proliferation: inhibition at >10 μM—removal of treatment medium after 3-day reversed proliferation inhibition	Cattaneo et al. (2000) and Anand et al. (2011)
Mouse cardiomyocytes	1–100	1 h, 37°C, MEM, Ca^{2+} Tyrode's solution	Cytotoxicity: >25 μM acrolein decreased viability; intracellular Ca^{2+} and oxygen free radicals: increased within 1 min after 1 μM acrolein addition	Wang et al. (2011)
PC12 cells	1–100	1–12 h, 37°C, PBS	Cytotoxicity: threshold of 10 μM acrolein; 100 μM induced cytoskeletal changes in 4 h	Liu-Snyder et al. (2006)
HT22 murine hippocampal neuronal cells; Sprague–Dawley rats	1–100, 2.5 mg/kg/day	3–48 h, 37°C, DMEM + 0.5% FBS, 8 days gavage-fed in tap water	Cytotoxicity: 25 μM acrolein for 12–24 h reduced viability by 40%, dysregulated amyloid-B protein metabolism; Alzheimer's disease-like pathology in brain	Huang et al. (2013, 2014)
hTRPA1-expressing HEK293 cells, trigeminal neurons, frog oocytes	1–100		Ca^{2+} influx: increase in TRPA1 transfected cells by 20 μM acrolein; EC_{50} = 5 μM in oocytes	Bautista et al. (2006)

Human peripheral blood lymphocytes	1–200	3-h treatment, 24-h incubation, RPMI-1640 + 10% FBS	Inhibition of cytokine production: IC_{50} = 3 μM acrolein; 10 μM decreased viability	Lambert et al. (2005)
Mammary carcinoma FM3A and neuroblastoma Neuro2a cells	1–200	72 h, 37°C, DMEM + 2 or 10% FBS	Cytotoxicity: IC_{50} = 2.6 μM, 4.2 μM	Tomitori et al. (2012)
Bovine aortic endothelial cells	1–300	30 min to 24 h, DMEM	Acrolein induced heme oxygenase-1 expression at 1–50 μM and cytotoxicity at 100 μM and above	Wu et al. (2006)
Murine nasal septal epithelia	1–500	0–30 min, 37°C, PBS, pH 7.3	Transepithelial chloride transport: altered transport 100–500 μM	Alexander et al. (2012)
Murine FL5.12 proB lymphocytes	2.5–40	30 min, EBSS	Cytotoxicity: >10 μM acrolein increased oncosis/necrosis; >20 μM circumvented etoposide or interleukin-3 withdrawal induced apoptosis	Kern and Kehrer (2002)
Rat aortic smooth muscle A10 cells	2.5–60	24 h, 37°C, DMEM + 0.5% FBS	Cytotoxicity: >20 μM (LC_{50} = 17 μM) acrolein decreased viability	Cao et al. (2003)
Human bronchial epithelial HBE1 cells	3–30	30 min, HBSS	>10 μM acrolein reduced thioredoxin reductase 1 enzyme activity	Randall et al. (2013b)
Human bronchial smooth muscle cells, small airway epithelial cells, normal human bronchial epithelial cells and alveolar macrophages	3–60	2–24 h, 37°C, DMEM	IL-8 protein secretion and mRNA: although not toxic, 30 and 60 μM acrolein increased IL8 mRNA expression and stability and protein secretion	Moretto et al. (2012)
Bovine artery pulmonary endothelial cells	3–300	0–18 h, RPMI 1640 + 10% FBS	^{51}Cr release (cytotoxicity): 30 and 300 μM acrolein induced cytotoxicity	Kachel and Martin (1994)
Human small airway epithelial cells	5–20	2–72 h, 37°C, small airway basal medium	Cytotoxicity: >5 μM acrolein decreased viability	Yadav et al. (2013)
Rat aortic smooth muscle A10 cells	5–25	24 h, 37°C, DMEM + 0.5% FBS	Acrolein (>5 μM) decreased cell viability	Zhu et al. (2008)

(continued)

TABLE 7.1 *(Continued)*

Target Protein/Cell/Organelle	Acrolein Concentration (μM)	Incubation Condition Medium/Solution	Endpoint Determination	Reference
A549 lung cells	5–200	3.5–6 h, 37°C, DPBS, F12K/0.5%FBS	Cytotoxicity: decreased cell viability following exposure to >100 μM acrolein dependent on medium composition; 5–50 μM acrolein increased cytosolic cytochrome C protein	Burcham et al. (2010a, 2010b) and Thompson and Burcham (2008)
Rat pneumocyte II L2 cells, lung slices	5–200	24 h, 37°C, DMEM/ Ham F12 + 10% FBS or HITES	Cytotoxicity: ATP decrease By >10 μM acrolein in L2 cells and >50 μM in slices; phospholipid synthesis: decreased by 30 μM in L2 cells and 200 μM in slices; GSH level: bell-shaped response	Monteil et al. (1999)
Chinese hamster cell line	8–25	Modified Eagle's MEM culture medium	Radioactive precursor incorporation	Alarcon (1972)
Human sinonasal epithelial cells	10–50	30 min	10–50 μM acrolein decreased IL-8 and human beta-defensin 2 expression in primary but not differentiated cells	Lee et al. (2007)
Human neuroblastoma SH-SY5Y cells	10–50	24 h, 37°C, DMEM + 0.5% FBS	Cytotoxicity: >10 μM acrolein increased toxicity and decreased GSH content	Jia et al. (2009b)
Bovine trachial epithelial cells	10–100	20 min, 37°C, Krebs–Henseleit buffer	Eicosanoid release: 30 μM was the threshold for acrolein induced release	Doupnik and Leikauf (1990)
Human bone marrow-derived osteoblastic cells	10–120	7, 14, 21, 28 days, a-MEM + 10% FBS	Cytotoxicity: reduced viability and consumption of ionized calcium, that is, matrix mineralization, IC_{50} = 60 μM	Pereira et al. (2010)
Rat primary astroglial cells	15–20		Acrolein (>15 μM) is cytotoxic; oxidative stress	Dang et al. (2011)

Human proximal tubule IHKE cells	15–300	24–72 h, DMEM/ Ham's F12 medium, pH 7.2	Cytotoxicity, protein content, cytoplasmic Ca^{2+}: 150 and 300 μM acrolein decreased protein content and induced mild apoptosis, 15–300 μM increased LDH release	Schwerdt et al. (2006)
Renal carcinoma Caki cells	20–60	24 h, 37°C, DMEM + 10% FBS	Cell apoptosis: 20 μM acrolein promoted TRAIL-induced apoptosis, DNA fragmentation, caspase-3 activation, and PARP cleavage	Yang et al. (2011)
Dorsal root ganglion neurons	25	Neurobasal medium + 2% B27	Acrolein (25 μM)-induced intracellular Ca^{2+} in TRPA1 wild type but not in knockout cells	Shang et al. (2016)
Rat brain C6 glioma cells	25–150	4, 24 h, 37°C, DMEM + 10% FBS, Krebs-HEPES solution	Cytotoxicity: membrane damage and reduced viability, EC_{50} = 76 μM	Noya et al. (2013)
A549 lung cells, Hsp90 in cells; RNase in cell-free system	25–200	0–4 h, DPBS, phosphate buffer, pH 7.0	Cytotoxicity: acrolein conc higher than 100 μM; protein cross-linking: high mass Hsp90 detected at 50 μM acrolein exposure	Burcham et al. (2007)
Rat vascular smooth muscle cells	40–180	2–24 h, 37°C, DMEM + 10% FBS	Cytotoxicity: >89 μM acrolein; acrolein induced MAPK signaling independent of cytotoxicity	Ranganna et al. (2002)
Renal epithelial cell line LLC-PK1	50–100	1 h, DMEM	Protein content, DNA and RNA synthesis reduced by 50–75 μM after 1 h; 100 μM effect after 1 min	Mohrmann et al. (1994)
Human colon cancer HT-29 cells	50–200	24 h, DMEM + 10% FBS	Acrolein-derived 1,*N*(2)-propanodeoxy guanosine formation correlated with apoptosis measured by Annexin V staining at 100 μM acrolein and higher conc	Pan et al. (2009)
Human keratinocytes	50–500	5 min to 2 h, 37°C, Humedia-KG2, supplement-free	50 μM acrolein-induced EGFR phosphorylation and activity, within 1 min of treatment, led to phosphorylation of MAPK signaling proteins	Takeuchi et al. (2001)
Human lymphoid Raji cells	50–500	10 min, RT or 37°C, PBS-glucose followed by 1–3 days in medium	Bromodeoxyuridine incorporation: 50 μM acrolein inhibited BrdU incorporation while 100 and 500 μM acrolein induced low-level DNA damage	Yang et al. (1999)

(continued)

TABLE 7.1 *(Continued)*

Target Protein/Cell/Organelle	Acrolein Concentration (μM)	Incubation Condition Medium/Solution	Endpoint Determination	Reference
Human spermatozoa	50–800	2–24 h, 37°C Biggers, Whitten, and Whittingham (BWW) medium	Mitochondrial ROS: 50–200 μM acrolein-induced production; lipid peroxidation: 200 μM acrolein-induced lipid peroxidation and DNA fragmentation	Aitken et al. (2012)
Rat hepatocytes	75, 150	Krebs–Henseleit buffer	Cytotoxicity: toxic at 75 and 150 μM acrolein, augmented by oxidative stress	MacAllister et al. (2013)
HaCaT-keratinocytes	100–1000; cigarette smoke puff	10 min to 1 h following puff exposure, DMEM + 10% FBS	Intracellular protein carbonylation: observed within 10 min of exposure	Avezov et al. (2015)
Mouse fibroblast sarcoma S180 cells		Medium + 10% horse serum	Cell inhibitory activity: ID_{50} = 26–35 μM	Alarcon (1964)
A549 lung cells			8-Oxoguanine in total RNA	Baldridge et al. (2015)
Studies employing tissues, organelles, organs, and organism				
Rat spinal cords and brain mitochondria	0.01–10	5 min, 37°C, respiration buffer	Acrolein decreased mitochondrial respiration in spinal cord (at >0.01 μM) and brain (at >0.1 μM)	Vaishnav et al. (2010)
Human bronchus, rat tracheal rings	0.01–10	5 min to 1 h, 37°C, Krebs–Henseleit solution, pH 7.4	Acrolein (0.1–1 μM) increased tracheal ring smooth muscle reactivity to carbachol, histamine, and neurokinin A-induced contractility	Ben-Jebria et al. (1993, 1994)
Pig spinal cord strips, synaptosomes	1–200	15 min to 4 h, Krebs' solution, Locke's buffer	Cytotoxicity: ethidium bromide uptake increased 15 min after exposure to 200 μM and 2 h after 10 μM acrolein exposure; LDH increased following 200 μM exposure for 4 h; lipid peroxidation increased after 4 h exposure to 25 μM or higher; 10 μM exposure for 1 h increased protein carbonylation and decreased mitochondrial electron transport	Luo and Shi (2004)
Guinea pig spinal cord segments	100–500	6–12 h, Krebs solution	Myelin damage: acrolein conc greater than 200 μM	Shi et al. (2011)

Striatal synaptosomes and vesicles	1–10,000	15 min, 30°C, Krebs-HEPES buffer, pH 7.4	The calculated IC_{50}'s for nerve terminal functional parameters included: dopamine release from synaptosomes 811 µM (579 µM–1.2 mM), uptake by synaptosomes 53 µM (16–81 µM), and vesicular transport into vesicles 213 µM (86–488 µM)	LoPachin et al. (2007)
Fetal mouse fore- or hindlimb	179–893	20 h	Limb growth parameters: malformed appearance	Hales (1989)
Brain			Acrolein in Alzeimer's disease amygdala (2.5 6+ 0.9 nmol/mg protein) compared with controls (0.3 + 0.05 nmol/mg protein) and in hippocampus and parahippocampal gyrus (5.0 + 1.6 nmol/mg protein) compared with controls (0.7 + 0.1 nmol/mg protein)	Lovell et al. (2001)
Rat embryos	0.5–160	42 h, 37°C, 90% rat serum; 10% Tyrode's solution, Waymouth's medium	Embryolethality: EC_{50} = 8.3 µM in serum-free and 115 µM in the presence of serum; teratogenicity: EC_{50} = 2.8 µM in serum-free and 137 µM in the presence of serum	Slott and Hales (1986, 1987a, 1987b)

residues in biomolecules, electrophile–nucleophile reactions exhibit a significant degree of selectivity, as defined by the hard and soft acids and bases (HSAB) theory (Schultz et al., 2006; Seiner et al., 2007; LoPachin et al., 2012). In addition to other parameters (e.g., steric features, solubility), the molecular state of the targeted cysteine sulfhydryl group is a critical determinant of reactivity. At physiological pH (7.0–7.4) the sulfhydryl groups are in the non-nucleophilic state due to the pK_a (8.4) of the cysteine sulfhydryl side chain. Yet, anionic thiolate groups do exist in pK_a-lowering microenvironments, as in cysteine-centered catalytic triads (LoPachin and Decaprio, 2005; LoPachin and Barber, 2006; LoPachin and Gavin, 2014) that are located within the critical sites of regulatory, transport, structural, enzymatic, and signaling proteins. Indeed, α-β-unsaturated aldehyde reactivity is extensive, and a variety of cell plasma membrane, cytoskeletal, cytosolic, and nuclear proteins are targeted by these compounds and the resulting adducted proteins (Shao, 2012; Zarkovic et al., 2013) (Table 7.2). For example, protein-acrolein adduct formation (Burcham et al., 2010a) can alter protein function and as a consequence affect cell growth, membrane integrity, differentiation, and thiol status. The extent of protein modification depends on acrolein concentration. While a lower subacute concentration modifies cytosolic proteins, a higher concentration also damages nuclear, membrane, and cytoskeletal proteins.

An important protective mechanism against damage by electrophiles is direct or glutathione-*S*-transferase (GST)-mediated conjugation with the antioxidant glutathione (GSH) (Grafstrom et al., 1988; Pal et al., 2000; Kazi and Ellis, 2002; Reddy et al., 2002). Acrolein exposure of two lung carcinoma cell lines (A-427 and SK-LU-1) with differing GSH content resulted in A-427, the cell line with lower GSH content, being more sensitive to growth inhibition compared with SK-LU-1 cells (Rudra and Krokan, 1999). GSH's protective activity is further complemented by acrolein reactivity with GST and acrolein-elicited responses. Although the effect of acrolein binding to GST on enzyme activity is not clear because both inhibitory and stimulatory effects have been reported (Ansari et al., 1987; Haenen et al., 1988), GST possibly serves as a molecular scavenger (Berhane and Mannervik, 1990; Mano et al., 2017). Further, acrolein induces transcription of phase II genes including the GSH synthesis rate-limiting enzyme glutamate–cysteine ligase, catalytic subunit (GCLC), and other genes, such as NAD(P)H:quinone oxidoreductase-1 and heme oxygenase-1 (HMOX1) (Tjalkens et al., 1998, 1999; Tirumalai et al., 2002; MacLeod et al., 2009; Sthijns et al., 2014; Que et al., 2016). Acrolein adducts kelch-like ECH-associated protein 1 (KEAP1), resulting in activation of nuclear factor erythroid 2-related factor 2 (NFE2L2 aka NRF2), and therefore enhanced transcriptional activity of gene promoters with antioxidant response element (ARE) (Tirumalai et al., 2002; Spiess et al., 2013). Thus, the initial acrolein-induced GSH depletion can be followed by a recovery phase with GSH returning to control levels or above by 12-h posttreatment, likely due to a feedback *de novo* synthesis (Horton et al., 1997).

Acrolein (3–30 μM) can deplete reduced glutathione, protein thiols, and antioxidants (Grafstrom et al., 1988; Rudra and Krokan, 1999; Nardini et al., 2002; Reddy et al., 2002; Deshmukh et al., 2005; Jia et al., 2009a). In addition, acrolein inactivates glutathione reductase, an enzyme involved in recycling oxidized GSH (Iuchi et al., 2004). GSH depletion has been implicated in acrolein-induced cellular toxicity (Horton et al., 1997). However, although GSH depletion may render cells more sensitive to toxicity (Horton et al., 1997), GSH depletion, in itself, may not cause cytotoxicity (apoptosis and/or necrosis). Supplementation of acrolein-treated cells, with either alpha-tocopherol or ascorbic acid, inhibited apoptosis and the increase of intracellular oxidant generation, although GSH depletion was unaffected (Nardini et al., 2002). Also, treatment of

TABLE 7.2 Acrolein Modifications of Biomolecular Targets

Target Biomolecule	Species/Sample	Modified Site	Impacted Region	Reference
Salivary amylase	Human saliva from healthy male and female nonsmokers	–SH groups implicated		Weiner et al. (2008)
Albumin	Human plasma from patients with silent brain infarction and normal subjects	Lys-557, Lys-560	Domain III with lys residues on the surface of HSA	Yoshida et al. (2010a)
Low-density lipoprotein	Human plasma of a healthy donor	Lys residues		Uchida et al. (1998a)
Apolipoprotein E	Rat, recombinant	Aldimine adduct at Lys149 and Lys155, a propanal adduct at Lys135 and Lys138, an *N*(ε)-(3-methylpyridinium)lysine (MP-lysine) at Lys64, Lys67, and Lys254, and an *N*(ε)-(3-formyl-3,4-dehydropiperidino)lysine (FDP-lysine) derivative at position Lys68		Tran et al. (2014)
Apolipoprotein A-I	Human blood collected from healthy subjects	Lys 226	Center of helix 10 of apoA-I, a region with a critical role in cellular interactions and ability of apoA-I to transport lipid	Shao et al. (2005a, 2005b)
Aldose reductase	Human, recombinant	Cys 298	Catalytic active site	Srivastava et al. (1999)
Thioredoxin-1	Human	Cys-73	Nonactive site thiol	Go et al. (2007)
Carnosine	Human	Amino group		Bispo et al. (2016)
Glutathione		Thiol group		van der Toorn et al. (2007)
Synaptosomal proteins: tropomyosin-3-gamma isoform 2, tropomyosin-5, beta-actin, mitochondrial Tu translation elongation factor (EF-Tu(mt)) and voltage-dependent anion channel	Gerbil	ND		Mello et al. (2007)

(continued)

TABLE 7.2 *(Continued)*

Target Biomolecule	Species/Sample	Modified Site	Impacted Region	Reference
Alpha-synuclein	Human samples prepared from the substantia nigra of control and Parkinsonian patients and recombinant aSYN	ND		Shamoto-Nagai et al. (2007)
Paired helical filament—tau protein	Human neuropathologically diagnosed Alzheimer's disease and control cases	ND		Calingasan et al. (1999)
Skeletal muscle G-actin	Rabbit	Cys374 the primary target, His87, His173, and, minimally, His40	ATP-binding site	Dalle-Donne et al. (2007)
Beta-actin and protein disulfide isomerase	Human			Chung et al. (2009)
Vimentin, keratin-18, keratin-7, and keratin-8	Human	ND		Burcham et al. (2010a)
Alpha-actin, desmin, myosin light polypeptide 3, creatine kinase-2, ATP synthase	Mouse	ND		Luo et al. (2007)
Malate dehydrogenase, NADH dehydrogenase (ubiquinone) flavoprotein 1, cytochrome c oxidase subunit VIb isoform 1, ATP synthase d chain, ADP/ATP translocase 1	Rat	Cys residues		Wu et al. (2011)
NF-κB1 (p50)	Human, recombinant	Cys-61 mainly, but also Cys-87, Cys-118, Cys-123, Cys-261, Cys 272, Arg-230, His-306, and Arg-307	DNA binding domain; interaction with p65	Lambert et al. (2007)

PKCepsilon	Mouse	ND		Wang et al. (2008)
NADPH oxidase	Human	ND	p47phox subunit of NADPH oxidase	Nguyen et al. (2001)
Cytochrome c oxidase subunit VIa isoform 2	Rat	His		Han et al. (2012)
NADH dehydrogenase 1 alpha subcomplex subunit 5, succinate dehydrogenase (ubiquinone) flavoprotein subunit, ubiquinol–cytochrome c reductase complex core protein I cysteine, ubiquinol–cytochrome-c reductase complex core protein II cysteine, Long-chain specific acyl-CoA dehydrogenase, Acetyl-CoA acetyltransferase, ADP/ATP translocase 1	Rat	Cys		Han et al. (2012)
DNA polymerase alpha-primase complex	Calf	Thiols	Polymerase a synthase and primase	Catalano and Kuchta (1995)
ADP/ATP translocase 1	Rat	Cys-256	Loop containing the ADP binding site	Han et al. (2007)
Arylamine N-acetyltransferases	Human	Cys	Active site	Bui et al. (2013)

ND, not determined.

hepatocytes with a ferric iron chelator, deferoxamine, or an antioxidant, *N,N′*-diphenyl-*p*-phenylenediamine (DPPD), prevented cell killing by allyl alcohol, which is metabolized to acrolein. The protection was not due to altered allyl alcohol metabolism, nor due to GSH depletion (Miccadei et al., 1988). The cell killing with allyl alcohol was preceded by the peroxidation of cellular lipids, as evidenced by malondialdehyde accumulation in the culture. Besides, despite buthionine sulfoximine's (BSO) effect on GSH depletion, BSO-treated macrophages maintain normal viability (Rouzer et al., 1981; Buchmuller-Rouiller et al., 1995). It is also not clear how acrolein treatment in a micromolar concentration can deplete intracellular GSH concentrations, which range from 0.5 to 10 mM (Kehrer and Biswal, 2000). In human JURKAT T lymphocytes treated with anti-Fas/APO-1 antibody to induce apoptosis, GSH depletion was attributed to a specific export mechanism (van den Dobbelsteen et al., 1996).

The impact of acrolein reactivity is far more extensive than GSH level decrease-based redox imbalance. The maintenance of cellular thiol redox balance also is controlled by the thioredoxin (TXN) system (Myers and Myers, 2009). In bronchial epithelial cells, acrolein irreversibly inhibited cytosolic TXN reductase 1 (TXNRD1) and mitochondrial TXNRD2 enzyme activities, leading to oxidation of the cytosolic peroxiredoxin 1 and mitochondrial peroxiredoxin 3, which was reversible. Thus, TXN inhibition was consequent to acrolein binding, whereas peroxiredoxin oxidation resulted from lack of reducing equivalents (Myers and Myers, 2009). However, the initial inactivation of TXNRD1 is followed by induction of *TXNRD1* gene expression (Park et al., 2005), and other studies suggested that TXNRD1 and TXN alkylation are reversible (Randall et al., 2013a).

Interestingly, in *Escherichia coli*, YqhD (aldehyde reductase, NADPH-dependent) expression was proposed as a GSH-independent, NADPH-dependent protective response to lipid peroxidation (Perez et al., 2008). *In vitro*, purified YqhD catalyzed the reduction of a number of aldehydes, including acrolein. In primates and higher plants, reductases could catalyze the reductive detoxification of aldehydes, notably acrolein, and thereby prevent its detrimental effects (Kurahashi et al., 2014; Shimakawa et al., 2014). Reduction in endoplasmic reticulum stress and protein carbonylation induced by acrolein treatment was demonstrated in mouse embryonic fibroblasts expressing human AKR1A. It is possible that redox imbalance due to lipid peroxidation and other oxidative injury following GSH depletion and general thiol conjugation may contribute to acrolein toxicity (Miccadei et al., 1988; Kehrer and Biswal, 2000; Furuhata et al., 2002). Thiol depletion in human fetal lung fibroblasts, by culturing in cysteine-free medium, or in the presence of thiol-depleting agents, induced oxidant accumulation, leukotriene production, and MAPK14 phosphorylation and its nuclear substrate activating transcription factor 2 (ATF2), leading to apoptotic cell death (Aoshiba et al., 1999).

Acrolein and its protein adducts and the depletion of GSH further potentiate cellular oxidative stress (Furuhata et al., 2002). In the yeast *Saccharomyces cerevisiae*, allyl alcohol exposure increased the level of thiobarbituric-acid-reactive substances, but not reactive oxygen species (ROS) (Kwolek-Mirek et al., 2009). An increase in protein carbonyl and TBARS has also been reported in acrolein-treated hepatocytes (Arumugam et al., 1997; Truong et al., 2009; Shah et al., 2015) and thymocytes (Tokarchuk and Zaitseva, 2014). Despite its lack of specificity, the increase of TBARS is suggestive of lipid peroxidation. *In vivo*, acrolein increased the generation of lipid hydroperoxide in plasma and aortic tissue (Yousefipour et al., 2005). Further, acrolein-induced lipid peroxidation, especially in mitochondria, has been suggested as a likely basis of allylamine's cardiovascular toxicity via acrolein formation (Awasthi and Boor, 1994). Thus, while lipid peroxidation may not fully

account for acrolein's toxicity, a better understanding is needed of the toxic lipid peroxidation products that may contribute to acrolein toxicity.

Oxidative stress due to acrolein exposure is also associated with altered mitochondrial respiration (Picklo and Montine, 2001; Vaishnav et al., 2010; Fabisiak et al., 2011; Agarwal et al., 2013). Acrolein can inhibit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity, leading to cellular pyruvate decrease. As a result, the cell (e.g., alveolar type II cells) may respond by activation of glucose-6-phosphate dehydrogenase, the regulatory control of the pentose phosphate pathway, and utilization of palmitate as an alternative fuel source for mitochondrial respiration (Agarwal et al., 2013). In isolated mitochondria, acrolein inhibited mitochondrial complex I, II, pyruvate dehydrogenase, and alpha-ketoglutarate dehydrogenase suggesting that acrolein may act as a mitochondrial toxicant (Sun et al., 2006). However, the exact contribution of oxidative stress and lipid peroxidation to acrolein toxicity is still not clear. Treatment of A549 cells with ascorbate and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid exhibited antioxidant actions but failed to counter cell ATP depletion by acrolein (Burcham et al., 2012).

Acrolein interacts with numerous cellular targets, and the cell responds via complex molecular and enzymatic pathways to counter acrolein's toxicity. Exposure to a low dose of acrolein protected cells against the toxic effect of a second higher dose of acrolein via NFE2L2-mediated gene expression of γ-glutamylcysteine synthetase, leading to elevated GSH levels (Sthijns et al., 2014). However, due to the presence of a large number of potential cellular molecular targets, the damaging impact of acrolein exposure is not limited to the redox system. Low acrolein doses can inhibit cell proliferation and colony forming potential (Grafstrom et al., 1988; Horton et al., 1997). *In vitro* studies suggested that acrolein irreversibly inactivated the DNA synthetic capacity of DNA polymerase alpha through modification of sulfhydryl groups (Catalano and Kuchta, 1995). Acrolein can also interact with DNA forming two major adduct isomers of 1,N^2-propano-deoxyguanoside: α-hydroxy-acrolein-deoxyguanosine and γ-hydroxy-acrolein-deoxyguanosine (Chung et al., 1984; Feng et al., 2006; Wang et al., 2009). Acrolein-derived 1,*N*(2)-γ-hydroxypropano deoxyguanosine (γ-HOPdG) can inhibit mammalian pol δ and pol ε DNA polymerases (Kanuri et al., 2002). Further, acrolein can interact with histones and compromise acetylation and chromatin assembly (Fang et al., 2016). It is possible that polymerase and DNA substrate modifications underlie the antiproliferative effect of acrolein. Further, O^6-alkylguanine-DNA alkyltransferase (AGT) may play a protective function by removal of the acrolein adduct from DNA (Friedman et al., 1999).

As a result of acrolein's reactivity, and of the structural, enzymatic, and metabolic changes that ensue, the expression pattern of a large number of gene families is altered (Kehrer and Biswal, 2000; Bein and Leikauf, 2011; Leikauf et al., 2011). The alterations include NFE2L2-mediated oxidative stress response, cell death, transforming growth factor beta, glucocorticoid receptor, lipid metabolism, and retinoic acid receptor signaling pathways (Bein and Leikauf, 2011). Thus, cellular toxicity occurs consequent to direct adduct formation by acrolein, which directly and indirectly alters subcellular structural and functional systems.

7.2.2 Acrolein Use *In Vitro*

Acrolein, diluted in aqueous solutions and media, has been extensively used to treat a variety of cell types and proteins (Table 7.1). In most studies, acrolein has been found to be more toxic to cells than ROS such as hydroxyl radical or H_2O_2 (Yoshida et al., 2009a). The

areas investigated include protein modification, enzyme activity, cell signaling, gene expression, metabolic state, and cytotoxicity. The amount of acrolein applied ranges from nanomolar to millimolar concentrations. Although many of the studies that used high acrolein concentrations (up to 1–100 mM) focused on cell-free systems, other studies used low acrolein concentrations (less than 3 μM, down to nM range) and demonstrated the effect of acrolein on protein structure and function (Table 7.1). The targeted nucleophilic residues of biomolecules would be broader with increased acrolein concentration and thus less selective than with lower concentrations being used (Cai et al., 2009). The divergence in acrolein concentrations used in *in vitro* studies may be due to differences in the experimental approaches, including the endpoint determined, the period of incubation, and the treatment/reaction culture system used. For example, to treat cells with acrolein, some studies replaced tissue culture medium with defined physiological solutions in order to preclude side reactions with medium components (e.g., cysteine containing thiols), whereas others used tissue culture medium without or with varying fetal calf serum and other supplements (Table 7.1). Extracellular biomolecules in the culture medium may react with the administered acrolein and neutralize its cellular impact. Yet, a number of studies have determined that acrolein imparts a wide range of cellular responses, even when acrolein is added to supplemented tissue culture medium. These findings may indicate that (1) the cells used are sensitive to low acrolein concentration, (2) extracellular biomolecular adducts contribute to direct action of acrolein on cells, or (3) the acrolein reaction with medium components modulates cell physiology. Interestingly, a close examination of the listing of the cell types and acrolein concentrations (Table 7.1) suggests that neural cells, in general, appear more sensitive than other cell types. If these *in vitro* observations reflect similar differential cell sensitivity in *in vivo* settings, neural cell sensitivity to acrolein may suggest a link to the implicated role of acrolein in neurodegenerative diseases.

7.2.3 Acrolein Concentration *In Vivo* and Metabolism

The steady state of acrolein concentration in healthy human exhaled breath condensate and plasma/serum ranges from 1 nM to 2.8 μM (Andreoli et al., 2003; Sakata et al., 2003b; Corradi et al., 2004; Deshmukh et al., 2008; El-Maghrabey et al., 2014), while increased concentrations can be measured in the samples of patients with COPD and renal failure. Determination of free acrolein is difficult because of acrolein's high reactivity and volatility and the presence of various endogenous detoxifying mechanisms. One alternative method used to assess plasma acrolein has been application of FDP-lysine (N^{α}-acetyl-N^{ε}-(3-formyl-3,4-dehydropiperidino)lysine) detection (Uchida et al., 1998a) to measure protein-conjugated acrolein (Sakata et al., 2003b; Yoshida et al., 2009b, 2010b; Igarashi et al., 2015). Acrolein measured as FDP-lysine conjugates in normal plasma ranged from 1 to greater than 100 μM. It is not clear whether the wide range of protein-conjugated acrolein concentration is indicative of individual subject variability or is due to experimental variables. FDP-lysine is formed upon acrolein reaction with the ε-amino group of lysine. However, although cysteine is the most acrolein-reactive residue, the adduct formed is not stable (Cai et al., 2009). Thus, while FDP-lysine detection has been useful as a biomarker tool, its use to determine acrolein level may underestimate acrolein concentration.

Acrolein reacts with the thiol group of the principal peptidyl intracellular thiol GSH (Stevens and Maier, 2008) (Figure 7.5). GSH is a tripeptide consisting of a glutamic acid, cysteine, and glycine. Once acrolein binds to the thiol moiety in the cysteine, the resulting *S*-(2-formylethyl)-glutathione is enzymatically cleaved. The γ-glutamic acid is removed by

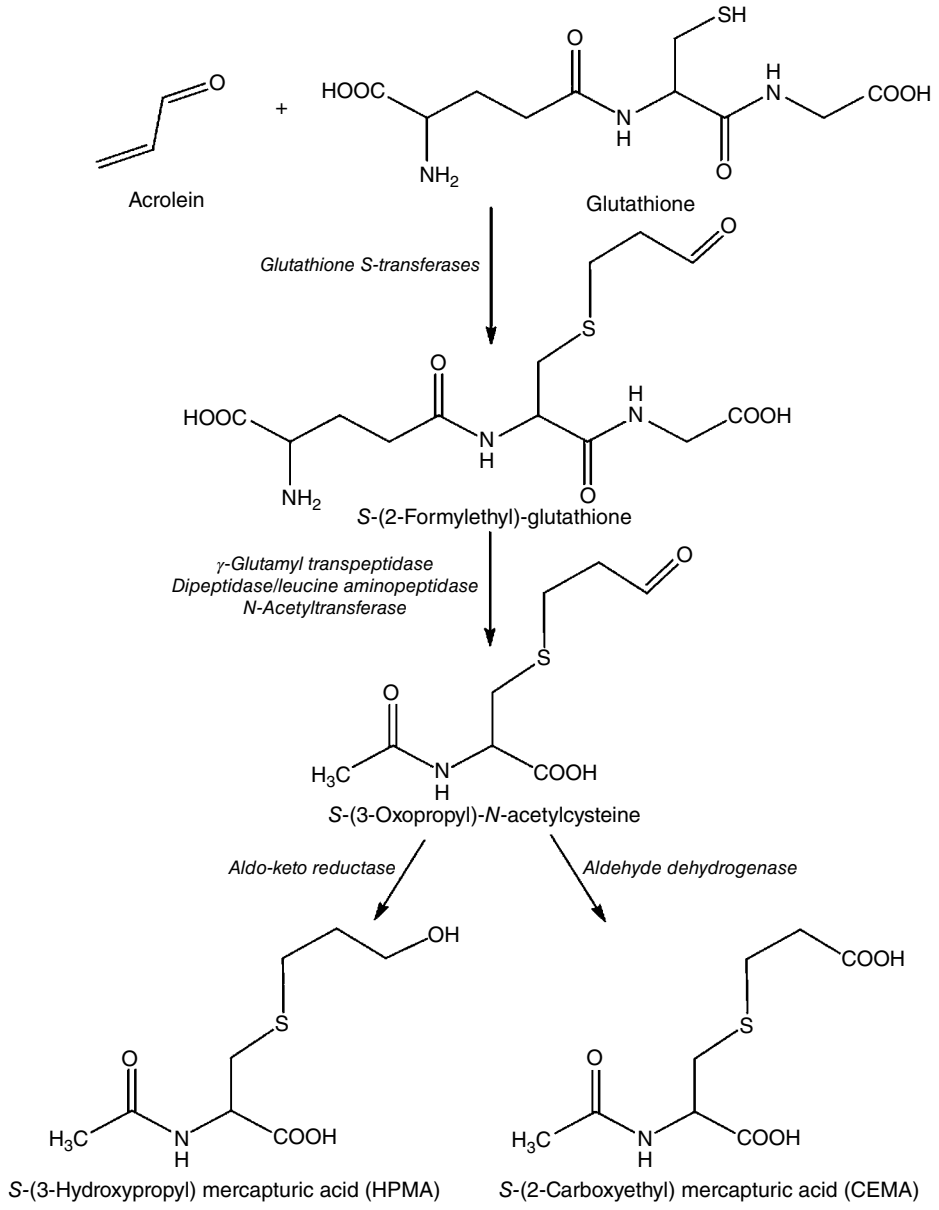

FIGURE 7.5 Metabolism of acrolein. The main pathway for elimination of acrolein is conjugation with glutathione (GSH) in the liver, followed by enzymatic cleavage of the γ-glutamic acid and glycine residues, respectively, in the liver and in the kidney (Lieberman et al., 1995; Josch et al., 2003) and N-acetylation of the resultant cysteine conjugate to form *S*-(3-oxopropyl)-*N*-acetylcysteine in the kidney. Reduction of this aldehyde yields *S*-(3-hydroxypropyl)-*N*-acetylcysteine (HPMA), the main metabolite of acrolein found in urine, while oxidation of the aldehyde group produces *S*-carboxyethyl-*N*-acetylcysteine [*S*-(2-carboxyethyl) mercapturic acid, CEMA].

γ-glutamyltranspeptidase in the liver. The glycine residue is removed by leucine aminopeptidase in the liver or by dipeptidase in the kidney (Lieberman et al., 1995; Josch et al., 2003). N-acetylation of the resultant cysteine is conjugated by N-acetyl transferase to form *S*-(3-oxopropyl)-*N*-acetylcysteine in the kidney. Reduction of this aldehyde yields *S*-(3-hydroxypropyl)-*N*-acetylcysteine (3-HPMA), the main metabolite of acrolein found in urine (Kaye, 1973; Carmella et al., 2007; Schettgen et al., 2008; Carmella et al., 2013). In addition, oxidation of the *S*-(3-oxopropyl)-*N*-acetylcysteine aldehyde group produces *S*-carboxyethyl-*N*-acetylcysteine [carboxyethyl mercapturic acid (CEMA)]. In addition, acrolein can be epoxidated to glycidaldehyde. Glycidaldehyde reacts with water to yield glyceraldehyde (Patel et al., 1980), or it can form a conjugate with glutathione (Parent et al., 1996, 1998). Additional metabolites including 3-hydroxypropionic acid and *N*-acetyl-*S*-(2-carboxy-2-hydroxyethyl)-cysteine (the metabolite of glycidaldehyde-glutathione addition) were found in urine of rats following oral administration of 2.5 mg/kg [2,3-^{14}C] acrolein (Parent et al., 1996, 1998). The radioactivity was also found in oxalic acid, but no radioactive oxalic acid could be detected after intravenous injection, suggesting that it is formed in the gastrointestinal tract by the gut microflora (Parent et al., 1998).

Levels of urine 3-HPMA and CEMA are higher among tobacco smokers (cigarettes, cigars, and pipe users) than among nontobacco users (Alwis et al., 2015). The median 3-HPMA levels for tobacco smokers and nontobacco users were 1089 and 219 μg/g creatinine, respectively. Similarly, median CEMA levels were 203 μg/g creatinine for tobacco smokers and 78.8 μg/g creatinine for non-tobacco users. Regression analysis indicated that serum cotinine was a significant positive predictor ($p < 0.0001$) of both 3-HPMA and CEMA among tobacco smokers. Crotonaldehyde is also a major constituent of tobacco smoke, and its exposure can be quantified using its urinary metabolite *N*-acetyl-*S*-(3-hydroxypropyl-1-methyl)-L-cysteine (HPMM). Sample-weighted, median urinary HPMM levels for smokers and nonusers were 1.61 and 0.31 mg/g creatinine, respectively (Bagchi et al., 2018). Multivariable regression analysis among smokers showed that HPMM was positively associated with serum cotinine, after controlling for survey year, urinary creatinine, age, sex, race, poverty level, body mass index, pre-exam fasting time, and food intake. Other significant predictors of urinary HPMM include sex (female > male), age (children > nonuser adults), and race (non-Hispanic blacks < non-Hispanic whites).

7.3 SINGLE EXPOSURE HEALTH EFFECTS

7.3.1 Accidental Acrolein Exposure

While acrolein production in industrial settings is a potential health hazard to workers and the general public, the relative health hazards attributable to such sources are not known. Engineering controls in industrial settings that ensure a closed system, and adherence to stringent requirements for personal protective equipment, may limit or minimize possible health hazards due to direct acrolein exposure. Yet, illness resulting from occupational exposure to acrolein used as an aquatic herbicide in Washington and California has been reported (Centers for Disease Control and Prevention, 2013). In Washington State, a licensed pesticide applicator was acutely exposed while investigating a leak in the connection between the acrolein tank and the metal assembly through which acrolein flowed. Following the exposure, the worker had, at different times, burning and watery eyes, throat tightness, difficulty breathing, inability to swallow, moderate phlegm production, vomiting,

inability to talk because of dyspnea, facial droop, ventricular fibrillation, and a grand mal seizure. Seven cases of acute acrolein-related illness occurred in California during 1993–2009. Symptoms ranged from low to moderate severity and included eye irritation (5 workers), headache (3), dyspnea (2), and skin irritation or burns (2). Acrolein exposure also represents a significant occupational inhalation hazard to firefighters, workers in bars and restaurants, and people cooking with biomass fuels (Siegel, 1993; Spada et al., 2008; Reijula and Reijula, 2010; Wilson et al., 2010; Gleich et al., 2011).

7.3.2 Skin Exposure

The major factors influencing the dermal impact of acrolein exposure are concentration, frequency, duration of exposure, and location with respect to the source of acrolein production (urban vs. rural, indoor vs. outdoor, smoking vs. nonsmoking, dense traffic vs. light traffic, etc.). Acrolein reacts at the point of contact. Thus, it can be toxic to exposed body surfaces including the skin, eyes, and mucous membranes (ATSDR, 2014). The skin is exposed to a variety of environmental insults, including UV radiation and cigarette smoke. Cigarette smoke and the oxidative compounds that derive from the combustion of cigarettes can affect the skin (Egawa et al., 1999; Fortino et al., 2007). The outer epidermal layer of the skin contains mainly differentiated keratinocytes (corneocytes) embedded in a lipid matrix rich in ceramides, cholesterol and free fatty acids forming the stratum corneum (SC) (Sticozzi et al., 2012). Carbonylation of cornified envelopes (Hirao and Takahashi, 2005), photodamaged skin of aged individuals (Tanaka et al., 2001), and skin of children with atopic dermatitis (Tsukahara et al., 2003) have been detected using antibodies against aldehyde-bound proteins, including anti-acrolein antibodies. Protein carbonylation due to acrolein exposure decreased the water-holding capacity of stratum corneum *in vitro* (Iwai and Hirao, 2008). Damage to the skin is likely localized and limited to the exposed region of the body because estimated dermal uptake of acrolein is small compared with inhalation (Weschler and Nazaroff, 2014).

7.3.3 Eye Exposure

Eye irritation, perceived as rapid-onset mild to moderate stinging accompanied by increased blinking, is one of the most sensitive responses to low-level acrolein exposure (≤0.25 ppm), and the degree of irritation becomes more pronounced in a concentration-dependent manner (Grigsby et al., 2012). Lacrimation occurred within 20 s in individuals exposed to 0.81 ppm and within 5 s at 1.22 ppm (Sim and Pattle, 1957; Faroon et al., 2008b). In rabbit eyes, acrolein vapor exposure caused immediate eye closure with excess tearing, corneal erosions, and severe inflammation of the anterior chamber, followed by corneal neovascularization starting as early as 1 week post-exposure (Dachir et al., 2015).

7.3.4 Oral Exposure

The acrolein content in many food categories varies greatly. For example, the acrolein content can be <10 μg/kg in fruits, whereas in wine the level can be up to 3800 μg/L. Further, the acrolein content in food can vary depending on the food-processing recipe and the quantity of food intake (Feron et al., 1991; Sauvageot et al., 2000; Abraham et al., 2011). The acrolein content varied from 1.44 ng/g (range from 0.68 to 2.99 ng/g) in french fries fried in soybean, canola, sunflower, corn, and palm oil to 4.08 ng/g (range from 2.25

to 4.85 ng/g) in potato fried in soybean oil. The acrolein content also changed depending on the number of times that the frying oil was recycled (Osorio and de Lourdes Cardeal, 2011). Despite such variations, and the limited available data, the acrolein concentration in food is considered within the range of the provisional tolerable concentration for ingestion, that is, 1.5 µg/L, corresponding to 7.5 µg/kg body weight per day (Gomes et al., 2002). However, acrolein-derived 1,*N*(2)-propanodeoxyguanosine (AdG) was detected by immunohistochemical techniques using a monoclonal antibody in human oral cells collected from the cheeks and tongue by the cytobrush technique (Greenspan et al., 2012). In addition, higher AdG levels were detected in gingival tissue of smokers compared with nonsmokers (1.36 ± 0.90 µmol/mol guanine in smokers versus 0.46 ± 0.26 µmol/mol guanine in nonsmokers) indicating that the gastrointestinal tract may be impacted by acrolein exposure.

In animal studies employing oral administration of acrolein, although there was no clear dose-related effect upon mortality or weight gain in female mice, increased mortality was reported in male mice dosed via oral intubation at 4.5, but not 0.5 and 2, mg/kg/day (Parent et al., 1991). Based on the metabolism and distribution of [2,3-^{14}C]acrolein in Sprague–Dawley rats orally gavaged with 2.5 mg/kg, radioactivity analysis of urine, feces, expired air, organic volatiles, and tissues revealed that 75–86% of the orally administered acrolein was absorbed (Parent et al., 1996).

7.3.5 Acute Inhalation Exposure and Immune Responses

Acrolein inhalation is likely the most significant route of human exposure (Linhart et al., 1996). Acrolein is a common ambient air pollutant because it is continuously introduced into the environment from multiple sources (Bein and Leikauf, 2011; Burcham, 2017). Inhalation of acrolein, as the name indicates (pungent "acrid" smell from "oleum"—Latin for "oil"), elicits odor perception and can cause nose irritation accompanied by eye irritation and tearing. Because of acrolein's volatility, the respiratory system is the most common target. Acrolein's odor threshold is 0.16 ppm (0.37 mg/m^3) (Amoore and Hautala, 1983), although this can vary between individuals. Rapid binding of acrolein to respiratory sensory nerve endings in the nasal mucosa, including Ca^{2+}-permeable transient receptor potential cation channel, subfamily A, member 1 (TRPA1) may result in rapid depolarization of the associated neurons to produce nasal irritation (Alarie, 1973; Grace and Belvisi, 2011). In addition to nasal irritation, acrolein-activated TRPA1 signaling can lead to cough and inflammation (Andre et al., 2008; Birrell et al., 2009). In animal studies, short-term exposure of rats to >0.6 ppm (>1.5 mg/m^3) acrolein injured respiratory and olfactory epithelium (Feron et al., 1978; Leach et al., 1987; Cassee et al., 1996). Further, the impact of inhaled acrolein is not limited to respiratory surfaces because it can accumulate systemically (Sithu et al., 2010; Tully et al., 2014a). Acrolein exposure may cause or contribute to a number of respiratory complications including acute lung injury, bronchial hyperreactivity and asthma, and chronic obstructive pulmonary diseases (Bein and Leikauf, 2011).

Exposure may occur due to acrolein in the ambient air as well as that arising from endogenously produced acrolein. Higher acrolein concentrations were measured in the expired breath condensate and induced sputum (exceeding 100–150 nM) in ex-smoking subjects with COPD or subjects with asthma, when compared with those of healthy nonsmoking control subjects (~1 nM) (Corradi et al., 2004; Deshmukh et al., 2008). In addition to exogenous sources, because acrolein may be generated endogenously in various body tissues under oxidative stress, it has been implicated in numerous pathologies including

Alzheimer's disease, Parkinson's disease, multiple sclerosis, and spinal cord injury (Shamoto-Nagai et al., 2007; LoPachin et al., 2008; Singh et al., 2010; Leung et al., 2011; Tully and Shi, 2013; Tully et al., 2014b), atherosclerosis (Uchida et al., 1998b), renal failure (Sakata et al., 2003b), and stroke (Tomitori et al., 2005).

Decreased respiratory rate has been studied in mice (Steinhagen and Barrow, 1984). In this experimental system, α,β-unsaturated aliphatic aldehydes, acrolein and crotonaldehyde, decreased respiratory rate at half-maximal concentration (ED_{50}) of 1000 and 3500 ppb as compared with 3100 ppb for formaldehyde. In contrast, the half-maximal dose for acetaldehyde was much greater (>2.85 ppm). Acrolein is consistently more potent than formaldehyde in multiple assays. Saturated aliphatic aldehydes with two or more carbons (e.g., butyraldehyde or propionaldehyde) had half-maximal concentrations of 0.75–4.2 ppm, whereas cyclic aldehydes (e.g., 3-cylcohexane-1-carboxyldehyde or benzaldehyde) exerted their effects in intermediate doses ranging from 60,000 to 400,000 ppb. Thus, the relative potency was acrolein > crotonaldehyde ≥ formaldehyde > benzaldehyde >> acetaldehyde. This apparent relationship holds for most other toxic endpoints including measures of pulmonary function in humans (Cullumbine and Pattle, 1956); half-maximal lethal dose in mice, guinea pigs, and rabbits (Salem and Cullumbine, 1960); and nasal pathology in rats (Lam et al., 1985; Roemer et al., 1993; Cassee et al., 1996).

Acrolein, at doses less than 1.0 ppm, can produce a number of pulmonary effects. Murphy et al. (1963) reported increases in pulmonary resistance and decreases in respiratory rate in guinea pigs exposed to 400–1000 ppb acrolein. As with exposure to formaldehyde, these changes were rapid in onset, remained somewhat constant ("response plateau") during exposure, and reversed within 60 min after exposure. Atropine inhibited the acrolein-induced change in resistance, suggesting involvement of a vagal cholinergic pathway. That this response is so readily reversible with cessation of exposure also suggests that continuous occupancy of an irritant receptor by acrolein during inhalation is necessary for the initiation of this reflex. Microelectrode recordings of trigeminal nerve fibers during inhalation of aldehydes (Kulle and Cooper, 1975) are consistent with involvement of this neural pathway in the decrease in respiratory rate (Kane and Alarie, 1977). The role of specific sensory afferent pathways was further examined by Lee et al. (1987), who reported that acrolein inhalation evoked an inhibitory effect on breathing with a prolongation of expiration and bradycardia. As determined by a number of interventions, acrolein activated both vagal C-fiber active afferents and rapidly adapting irritant receptors and suggested that the elongation of expiration was due to stimulation of the former afferent pathway. Similar to acrolein, formaldehyde stimulates C-fiber nerves and can stimulate the release of substance P, which may induce a neurogenic inflammatory response. In rat airways, this is marked by an increase in vascular permeability that is mediated predominantly by stimulation of the tachykinin NK1 receptor (Ito et al., 1996).

High acrolein concentrations (>20 ppm) are an irritant component of smoke and are thought to be a causative agent in pulmonary edema, pulmonary hypertension, and acute lung injury that result from such exposures (Hales et al., 1988; Barrow et al., 1992; Hales et al., 1992). This process is dependent, in part, on eicosanoid formation, and inhibitors of specific pathways may be beneficial therapeutic approaches (Janssens et al., 1994; Hales et al., 1995).

Because acrolein has greater penetration of the upper respiratory tract than formaldehyde, effects of acrolein on lung defense mechanisms have been examined. Exposure to 1.0–2.0 ppm acrolein significantly suppressed intrapulmonary bacterial killing in mice challenged with bacteria (Jakab, 1977), suggesting that host defense mechanisms of distal

airway and alveolus were impaired. Macrophage activation and dysfunction induced by acrolein have also been observed *in vitro* (Leffingwell and Low, 1979; Grundfest et al., 1982; Sherwood et al., 1986; Jakab, 1993; Li et al., 1997). Thus, damage to the lower respiratory tract is more likely after exposures to unsaturated aldehydes than to formaldehyde, and these compounds have equal to or greater toxicity.

Even though acrolein can penetrate to the distal lung more than formaldehyde, the effects of acrolein (2.5 ppm) can be enhanced by co-exposure with carbon black particulate matter (PM) at 10 mg/m^3. Following exposure to each agent or co-exposures of 4 h/day for 4 days, Jakab (1993) challenged Swiss mice with different infectious agents: *Staphylococcus aureus* to evaluate alveolar macrophage (AM) surveillance, *Proteus mirabilis* to evaluate AMs and polymorphonuclear leukocytes (PMNs) surveillance, *Listeria monocytogenes* to evaluate lymphokine-mediated cellular immunity, and *influenza A* virus to evaluate the cytotoxic T-cell-mediated cellular immunity. Only co-exposures suppressed the intrapulmonary killing of *S. aureus* a day after exposure with a return to control levels by day 7. In contrast, the co-exposure enhanced the intrapulmonary killing of *P. mirabilis* possibly resulting from a significant increase in PMNs recovered in lung lavage fluid (also only noted following co-exposure with infection). Combined exposure to carbon black and acrolein also impaired elimination of *L. monocytogenes* and *influenza A* virus from the lungs. Exposure of alveolar macrophages to these concentrations directly suppressed Fc-receptor-mediated phagocytosis for up to 11 days (Jakab and Hemenway, 1993), which agrees with diminished *S. aureu*s surveillance. However, the effect was short-lived *in vivo*. These studies suggested that the carbon black particle acted as a carrier for acrolein to enhance penetration to the distal airway and alveolar regions of the lung.

The mechanisms of diminished innate immunity following acrolein exposure are not fully understood. Witz et al. (1987) noted the reactive aldehydes diminish superoxide anion production by neutrophils. In addition, α,β-unsaturated aldehydes were found to alter NADH activity and macrophage and neutrophil membrane function, fluidity, and sulfhydryl status. In an extension of these studies, Li et al. (1997) demonstrated that acrolein increased cell death (necrosis) and programmed cell death (apoptosis) of macrophages. These effects were thought to be mediated by secondary intracellular oxidant formation (Nardini et al., 2002; Yousefipour et al., 2005; Misonou et al., 2006) and activation of nuclear factor kappa B subunit 1 (NFκB). Acrolein also inhibited endotoxin-induced NFκB activation and decreases the basal level NFκB activity, which may be responsible for the inhibition of cytokine release and the induction of apoptosis in human alveolar macrophages (Li et al., 1999). The induction of apoptosis may be mediated by caspase-7 and -9 activation that induces cytochrome c release from the mitochondria (Luo et al., 2005; Tanel and Averill-Bates, 2005). In addition, acrolein and crotonaldehyde may diminish innate immunity by diminishing T-cell cytokine production, which can be reversed by the thiol compound, *N*-acetylcysteine (Lambert et al., 2005). Lastly, *in vivo* acrolein exposure of mice suppressed LPS-induced Th1 cytokine responses without affecting acute neutrophilia (Kasahara et al., 2008). This finding suggested that cytokine signaling can be disrupted by acrolein and may represent a mechanism by which smoking contributes to chronic disease in chronic obstructive pulmonary disease and asthma.

In contrast, acrolein diminishes apoptosis of neutrophils (Finkelstein et al., 2001). The inhibition of constitutive neutrophil apoptosis is mediated by common mechanisms, involving changes in cellular-reduced GSH status, resulting in reduced activation of initiator caspases as well as inactivation of caspase-3 by modification of its critical cysteine residue (Finkelstein et al., 2005). Diminished apoptosis in leukocytes at site of injury could lead to persistent inflammation.

7.4 REPEATED EXPOSURE HEALTH EFFECTS

7.4.1 Acrolein's Role in Chronic Obstructive Pulmonary Disease (COPD)

Acrolein represents a significant inhalation hazard and has been implicated in various diseases. Acrolein reactivity with target biomolecules is the initiator of and underlies its toxicity. As evidenced by protein carbonylation determination, the reactivity is rapid and extensive (Burcham and Fontaine, 2001; Burcham et al., 2007). As a result of acrolein's reactivity, loss of metabolic, enzymatic, transport, or structural function may ensue (Levine, 2002), and the net impact of such loss depends on the severity of the exposure. At high exposure, acrolein's action on biomolecules can be the effector of cell death. However, at moderate and low levels of exposure, the timing of the onset of cell death varies indicating that steps occurring subsequent to the initial interaction contribute to and dictate the outcome.

Persistent decrements in pulmonary function, consistent with those observed in patients with COPD, were noted by Costa et al. (1986) who exposed rats to 4.0 ppm acrolein (62 days × 6 h/day × 5 days/week). Airflow dysfunction was accompanied by focal peribronchial lesions and alterations of structural proteins (elastin). In tests by Lyon et al. (1970) with rats, guinea pigs, dogs, and monkeys, 0.7 ppm acrolein produced squamous metaplasia of the lungs in monkey. This study suggested that obligatory nasal breathers (mice, rats, and guinea pigs) are somewhat less responsive to chronic lower respiratory effects than oronasal breathers (dogs and monkeys). For example, Lyon et al. reported histopathological effects of 0.22 ppm acrolein (24 h × 90 days) in the lungs of dogs and monkey that were only apparent after 1.0–1.8 ppm exposure in rats and guinea pigs (Lyon et al., 1970). However, others have reported that rats do respond to lower acrolein exposure. Rats, hamsters, and rabbits were compared after exposures to 0.4, 1.4, and 4.9 ppm acrolein (6 h/day × 5 days/week × 13 weeks) by Feron et al. (1978) and found rats to be more susceptible, responding at 0.4 ppm, whereas hamsters and rabbits were nonresponsive at this level (Feron et al., 1978).

The USEPA recommended chronic inhalation reference concentration (RfC) for acrolein is 0.02 μg/m^3 (0.008 ppb) based on nasal lesions in rats. The RfC is an estimate concentration of lifetime inhalation exposure likely to be without an appreciable risk of deleterious effects in humans (including susceptible subpopulations). Even the lowest ambient acrolein concentration in the United States, 0.05 μg/m^3 (0.02 ppb) (Woodruff et al., 2007), exceeded the RfC. Indoor acrolein concentrations in homes, commercial establishments, and taverns permitting smoking have often been higher than outdoor levels due to food cooking, wood burning, and smoking and can be 150–1200 times the ambient RfC (Bein and Leikauf, 2011).

Cigarette smoking and several nonsmoking-related exposures including inhalation of secondhand smoke, biomass fuel smoke, and polluted air are among the causes of COPD (Bein and Leikauf, 2011). The impact of acrolein released from waterpipe smoking (Daher et al., 2010) on COPD remains to be determined. The COPD attributable to smoking ranges from 9.7 to 97.9% (Eisner et al., 2010). Of the various toxic smoke constituents, acrolein could account for approximately 90% of the noncancer hazard index associated with cigarette mainstream smoke (Haussmann, 2012). Acrolein interaction with cell-associated and cell-free molecules elicits a spectrum of responses that can contribute to COPD development and progression. Mucus hypersecretion from surface epithelial (goblet) cell and submucosal glands is one of the clinical features of COPD (Jeffery, 2000; Rogers, 2007). Acrolein

can induce excessive mucus production either directly by acting on lung epithelial cells or indirectly via inflammation.

Acrolein increases airway mucin 5, subtypes A and C (MUC5AC), and MUC5B, the predominant mucin proteins expressed in the lung (Hovenberg et al., 1996a, 1996b; Thornton et al., 1997; Sheehan et al., 1999; Voynow, 2002). MUC5AC mRNA and protein increased in the trachea and lungs of rats exposed to acrolein (3.0 ppm, 6 h/day, 5 days/week, 2 weeks) (Borchers et al., 1998). Increased MUC5AC was associated with macrophage/monocyte accumulation indicating that acrolein-induced monocytic inflammation contributes to mucus hypersecretion (Borchers et al., 1999b). Although the impact of acrolein exposure is multifaceted, due to acrolein's reactivity, interestingly, acrolein induced MUC5AC mRNA in NCI-H292 cells through epidermal growth factor receptor (EGFR) activation and downstream signaling mediators, which was linked to increased metalloproteinase (MMP) 9 and MMP14 expression and activity (Deshmukh et al., 2005, 2008, 2009).

Increased MMP activity not only promotes mucus production, but it also, in conjunction with prolyl endopeptidase, MMP activation, causes collagen degradation generating proline–glycine–proline (PGP) fragments (Noerager et al., 2015). The tripeptide PGP, which is structurally homologous to the SGP region of interleukin-8 (CXCL8), serves as a chemoattractant for neutrophils through CXC receptors (Weathington et al., 2006; Jackson et al., 2011). The enzyme leukotriene A4 hydrolase (LTA4H) can cleave the PGP peptide, negating its chemoattractant function. However, when present, acrolein inhibits the LTA4H peptidase activity (Snelgrove et al., 2010), in addition to acetylating the PGP to form N-acetylated PGP, which is insensitive to degradative action of LTA4H (Hardison et al., 2012). Thus, accumulation of acrolein and N-acetylated PGP may attract more inflammatory cells, establishing a positive feedback loop that can exacerbate the COPD state.

7.4.2 Acrolein's Role in Asthma

A hallmark of asthma is airway hyperreactivity, in which bronchoconstriction occurs at lower doses of inhaled methacholine than that observed in non-asthma control subjects. In addition to the transient increase in baseline pulmonary resistance, acrolein exposure of ≥800 ppb (2 h) can produce bronchial hyperreactivity in guinea pigs (Leikauf et al., 1989). Hyperreactivity occurred as early as 1 h after exposure to 1300 ppb became maximal at 2–6 h and lasted for longer than 24 h. This response was accompanied by an increase in three bronchoactive eicosanoids (prostaglandin F2α, thromboxane B2, and leukotriene C4) in bronchoalveolar lavage (BAL) fluid. Inhibition of 5-lipoxygenase diminished the response (Leikauf et al., 1989), indicating lower respiratory tract epithelial injury. An increase in leukocyte infiltration was also noted, occurring 6–24 h after exposure. These findings suggested that acrolein-induced hyperreactivity occurred by a pathway dependent on acute injury to the airway epithelium and mediator release. In addition, migration of leukocytes into the airway did not precede hyperreactivity, suggesting that injury to cells normally present in the lung during exposure produced the mediators responsible for hyperreactivity. Ben-Jebria et al. (1994, 1995) reported that lumenal exposure of isolated ferret trachea to 300 ppb acrolein for 1 h decreased the contractile dose of cholinergeric agonists (carbachol or acetylcholine), and increased the maximal contraction indicative of increased smooth muscle reactivity. Subsequent studies with human and rat tracheal smooth muscle exposed to acrolein (effective dose typically 0.1–0.2 mM) demonstrated that cholinergic enhancement of contraction was accompanied by increased membrane current (Hyvelin et al., 2001) and oscillations in intracellular calcium (Roux et al., 1998; Hyvelin et al., 2000).

Turner et al. (1993) examined airway responses to intravenous substance P following acrolein exposure. Guinea pigs were exposed twice to 1600 ppb acrolein (7.5 h/day on 2 consecutive days) and followed for up to 28 days. Pulmonary inflammation and epithelial damage were prominent 1 day after exposure. Neutral endopeptidase (NEP) activity was decreased in the lungs, trachea, and liver 1 and 7 days after exposure. At 28 days after exposure, NEP activity in the lungs and liver was not significantly different in vehicle- and acrolein-exposed guinea pigs, but was still reduced in tracheal tissue. Acrolein increased airway reactivity to substance P and lasted for up to 28 days following exposure. Thiorphan, a NEP inhibitor, potentiated this response. To further investigate the role of neuropeptides in acrolein-induced airway responses, a subsequent study with capsaicin-treated guinea pigs exposed to acrolein was performed (Turner et al., 1993). Capsaicin depletes neuropeptides from C-sensory fibers and resulted in 100% mortality (12 of 12 guinea pigs) within 24 h of two 7.5 h × 1600 ppb exposures. This compared with only 14% mortality in guinea pigs exposed to acrolein alone. Pretreatment with capsaicin also exacerbated pulmonary inflammation and epithelial necrosis and denudation. Thus, acrolein activates airway C-fibers, which release neuropeptides, and alters breathing. The resulting shallow breathing patterns may be protective by reducing deposition in the distal airways.

O'Brien et al. (2016) examined the effect of acrolein inhalation on allergic sensitization to the inhaled antigen ovalbumin (OVA), as well as pulmonary inflammation during subsequent OVA challenge. Adult male C57BL/6NCrl mice were exposed to OVA aerosol (1%, 30 min/day, 4 days/week) with or without acrolein (5 ppm, 4 h/day, 4 days/week) over 2 weeks. This was followed by challenging with OVA aerosol (1%, 30 min/day) over 3 consecutive days. Serum anti-OVA immunoglobulin G1 levels and BAL neutrophils increased in mice exposed to both OVA and acrolein, compared with animals exposed to either OVA or acrolein alone.

In the National-Scale Air Toxics Assessment 2005, chronic outdoor acrolein exposure estimates at the census tract were linked with residences of adults (≥18 years old) in the National Health Interview Survey (NHIS) 2000–2009 (n =271,348 subjects) (USEPA, 2011a; deCastro, 2014). A sample-weighted logistic regression model characterized the association between the prevalence of reporting at least one asthma attack in the 12 months prior to survey interview and quintiles of exposure to outdoor acrolein, controlling for potential confounders. In the highest quintile of outdoor acrolein exposure (0.05–0.46 μg/m^3), asthma attack risk was marginally increased [prevalence odds ratio (95% CI) = 1.08 (0.98:1.19)] relative to the lowest quintile. The highest quintile was also associated with a marginally significant increase in prevalence odds [1.13 (0.98:1.29)] in a model limited to never smokers (n = 153,820). If confirmed by additional studies, these findings suggest that chronic exposure to outdoor acrolein of 0.05–0.46 μg/m^3 may increase the prevalence odds of having at least one asthma attack in the previous year by 8% in a representative cross-sectional sample of the adult U.S. population.

7.4.3 Acrolein's Role in Neurologic Disorders

Acrolein has been implicated in the development/progression of age-related neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, and acute trauma, such as spinal cord injury (Shi et al., 2002; Reed, 2011). This proposition is supported by acrolein elevation in the hippocampus and temporal cortex of the brain of patients with neurodegenerative disorders (Lovell et al., 2001; Shamoto-Nagai et al., 2007; Dang et al., 2010) that

may be consequent to polyamine metabolism (Wood et al., 2007). The brain is also rich in polyunsaturated fatty acids (Shaw and Pateman, 1973) that may serve as acrolein precursors, although the extent of the contribution of unsaturated fatty acids to acrolein formation has been questioned (Stevens and Maier, 2008). Increased acrolein is not limited to end-stage Alzheimer's or Parkinson's disease because acrolein reactivity may represent a critical determinant of early events in the neuropathogenic progression (LoPachin et al., 2008).

Alzheimer's disease and Parkinson's disease are characterized by the deposition of extracellular amyloid plaques whose main constituent is aggregated amyloid beta protein and intraneuronal neurofibrillary tangles and intracellular inclusions called Lewy bodies that consist of synuclein alpha (SCNA) aggregates in the brain (Galloway et al., 1992; Calingasan et al., 1999; Shepherd et al., 2002; Jomova et al., 2010). Acrolein can react with SCNA (Shamoto-Nagai et al., 2007), synaptosomal proteins (Mello et al., 2007; Shamoto-Nagai et al., 2007), actin, intermediate filament proteins, neurofilament light (Dalle-Donne et al., 2007; Jeong and Kang, 2008; Burcham et al., 2010a), and DNA (Dang et al., 2010). In turn, these reactions can alter the structure and function of the modified molecules. Further, acrolein can alter protein state, exemplified by hyperphosphorylated tau protein, a component of neurofilament tangles (Gomez-Ramos et al., 2003; Liu et al., 2005; Kuhla et al., 2007). The extensive reactions of acrolein with neuronal target molecules could thus possibly result in altered energy production, mitochondrial dysfunction, metabolism, neurotransmission, and cytoskeletal integrity, leading to decline in neurological functions.

The brain is thought susceptible to oxidative stress as the brain represents for only ~2% of total body weight but consumes 20% of the body's oxygen (Smith et al., 2007). The high concentration of PUFA that is susceptible to lipid peroxidation and relatively high redox transition metal ions but relatively low antioxidant capacity compared with other organs may further contribute to acrolein generation and its consequent neurodegenerative impact (Shaw and Pateman, 1973; Markesbery and Lovell, 2007).

7.4.4 Acrolein's Role in Renal Failure

The polyamine putrescine is a uremic solute listed by the European Work Group on Uremic toxins (toxins EWGoU, 2017, accessed March 2019). Because polyamine oxidase action on the polyamines spermine and spermidine may produce acrolein in diabetic nephropathy, chronic glomerulonephritis, and nephrosclerosis, acrolein has been proposed as a uremic toxin (Sakata et al., 2003a; Igarashi et al., 2006). Both free acrolein and protein-conjugated acrolein increase in the plasma of renal patients, the conjugated acrolein being about six-fold higher in the plasma of renal failure patients compared with normal subjects. Further, the level of protein-conjugated acrolein correlated with the severity of renal failure (Sakata et al., 2003b). However, it should be noted that the steady state of acrolein concentration in control plasma/serum varies (Andreoli et al., 2003; Sakata et al., 2003b; Corradi et al., 2004; Deshmukh et al., 2008; El-Maghrabey et al., 2014). The use of the antineoplastic agents cyclophosphamide and ifosfamide has been associated with hemorrhagic cystitis attributed to drug metabolism-induced acrolein generation (Brock et al., 1981). Acrolein also inhibits organic cation transporter activity in human proximal tubule epithelial cells, suggesting that acrolein reaction with tubular molecules may hamper xenobiotic clearance (Schophuizen et al., 2013). One of the health risks associated with renal dysfunction is cardiovascular complications (Sindhu, 2016).

7.4.5 Acrolein's Role in Cardiovascular Dysfunction

Inhaled and endogenously produced acrolein has been linked to adverse cardiovascular responses (Conklin, 2016; Kurhanewicz et al., 2017; Thompson et al., 2017). One of the immediate responses to acrolein inhalation is activation of the respiratory sensory nerve endings, including Ca^{2+}-permeable transient receptor potential cation channel, subfamily A, member 1 (TRPA1) (Grace and Belvisi, 2011). In addition to nasal irritation, which can serve as a warning signal, acrolein-activated TRPA1 induces cough reflex, neurogenic inflammation, and downstream autonomic dysfunction (Andre et al., 2008; Birrell et al., 2009; Kurhanewicz et al., 2017). Aside from the TRPA1-induced chemoreflexes, the blood pressure-sensing baroreceptors, located in the carotid sinuses and aortic arch, may also be altered. The autonomic dysfunction and baroreceptor dysregulation can cause heart rate variability, arrhythmia, and mean arterial blood pressure changes (Hazari et al., 2014; Moghe et al., 2015; Kurhanewicz et al., 2017). Besides inhalation, high therapeutic doses of cyclophosphamide that generate acrolein have been implicated in cardiotoxicity (Dorr and Lagel, 1994; de Jonge et al., 2005; Nakamura et al., 2010; Nishikawa et al., 2015; Reis-Mendes et al., 2015). Cardiac ventricular fibrillation was determined as the cause of death of cyclophosphamide-administered Fischer rats (Friedman et al., 1990). Myofilament impairment due to acrolein reactivity may be related to the modification of proteins involved in myocardial contraction and energy metabolism (Luo et al., 2007). Also, in acrolein-fed C57BL/6 mice, left ventricular dilatation, contractile dysfunction, and impaired relaxation were reported (Ismahil et al., 2011).

Acrolein may also contribute to atherosclerosis. Indeed the pioneering work of Uchida et al. (1998a, 1998b) on acrolein as a lipid peroxidation product was conducted in connection with atherosclerosis. Acrolein may interfere with HDL cholesterol transport by modifying apolipoprotein A1 (apoA-I). Acrolein-conjugated apoA1 was associated with decreased cholesterol efflux from cells via the ATP-binding cassette transporter A1 pathway, a modification that might impair cholesterol removal from artery wall cells (Shao et al., 2005a, 2005b; Shao, 2012). Further, acrolein increases metalloproteinase activity and expression (Deshmukh et al., 2008; O'Toole et al., 2009; Lemaitre et al., 2011). In advanced atherosclerotic lesions, increased metalloproteinase activity may destabilize plaques and lead to infarction. Acrolein level determination, along with acetylpolyamine oxidase plus spermine oxidase or interleukin-6 plus C-reactive protein determination, has been investigated as possible markers of stroke or silent brain infarction (Tomitori et al., 2005; Yoshida et al., 2009b). The possible utility of 3-HPMA as one of the biomarkers for the diagnosis of brain infarction has also been investigated (Yoshida et al., 2012; Higashi et al., 2016).

7.5 CONCLUSION

Human exposure to unsaturated aldehydes remains extensive and results from ambient, indoor, personal, and occupational sources. Unsaturated aldehydes are contained in environmental tobacco smoke and cooking fumes and produced in substantial amounts during grassland fires and biomass burning. Acrolein is the unsaturated aldehyde of greatest concern. Currently, less is known about other unsaturated aldehydes, although processes that lead to acrolein exposure often can lead to crotonaldehyde exposure and crotonaldehyde

has similar toxic mechanisms to those of acrolein. Of the numerous HAPs, acrolein is one of the most often found in ambient concentrations exceeding RfCs. Furthermore, indoor exposure levels typically exceed ambient levels. In addition to environmental exposure, acrolein exposure can result from endogenous formation during lipid peroxidation. Because endogenous acrolein also can be generated by additional mechanisms including amino acid degradation by neutrophil myeloperoxidase and oxidative polyamine degradation during inflammation, chronic acrolein formation often exceeds that of 4-hydroxynonenal and malondialdehyde, which are generated mainly from lipid peroxidation alone. Acrolein is a key component of carbonyl stress and avidly binds to cellular macromolecules, leading to acute and chronic toxicity. For instance, although controversial, acrolein may be carcinogenic inasmuch as *in vitro* studies suggest that acrolein can disrupt DNA repair mechanisms. Acrolein exposures have been associated with a wide range of diseases including chronic obstructive pulmonary disease, asthma exacerbations, and Alzheimer's, renal, and cardiovascular diseases.

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8

CHEMICAL WEAPONS

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8.1 OVERVIEW

We are living in a world of chemical threats. Indeed, over the centuries, many toxic chemicals were turned into chemical weapons (Szinicz, 2005; Johnson et al., 2009). In more recent times, these include not only industrial and agricultural chemicals but also toxicants specifically generated for chemical warfare (Bajgar et al., 2009). Although attempts have been made to limit their production and use, many are low cost and easy to synthesize in bulk, making them attractive for use as weapons of war and terrorism. Complicating the problem is the fact that stockpiles of these chemicals remain in many countries around the world.

Chemical weapons fall into distinct categories and include respiratory toxicants (e.g., phosgene and halogenated chemicals, such as chlorine and bromine), vesicants (e.g., sulfur mustard and lewisite), nerve agents (e.g., sarin, VX, and many pesticides), metabolic poisons (e.g., cyanide and hydrogen sulfide), rodenticides (phosphine and warfarin), and both inorganic and organic arsenicals. Common sites of injury following exposure to these agents are the skin, lung, and eyes; significant systemic toxicity has also been described. However, the specific toxic manifestations depend on the nature of the chemical, the conditions during exposure (e.g., temperature and relative humidity), the dose, and the duration of exposure and route of entry into the body. Some agents, like sarin, VX, and various derivatives, rapidly induce toxicity, while others, such as sulfur mustard, exhibit toxicity of delayed onset. The health effects resulting from exposures to chemical warfare agents are also multifactorial and are influenced by age, sex, nutritional status, and overall health of exposed victims. Vulnerable populations, including the very young, the elderly, and individuals with preexisting diseases, are particularly susceptible to chemical exposures.

Biochemical aspects of the chemical, including metabolism and tissue and cellular repair processes, may also contribute to toxicity. Genetic susceptibility is also an important factor

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TABLE 8.1 Examples of Chemical Threat Agents

Vesicants	**Metabolic poisons**
Dichloroformoxime (phosgene oxime)	Cyanogen chloride (CK)
Bis-2-chloroethyl sulfide (sulfur mustard)	Hydrocyanic acid (AC)
Lewisite (J-chlorovinyldichloroarsine)	Hydrogen sulfide
Arsenicals (inorganic and organic)	**Nerve agents**
Arsenic trioxide	*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonofluoridate (VX)
Arsine	Isopropyl methylphosphonofluoridate
Lewisite	Trimethylpropyl methylphosphonofluoridate
	Cyclohexyl methylphosphonofluoridate
	Ethyl *N*,*N*-dimethyl phosphoramidocyanidate (Tabun)
Rodenticides	**Pesticides**
Warfarin	Parathion
Superwarfarins	Disulfoton
Tetramethylenedisulfotetramine	Malathion
Phosphine–metal phosphides	Azinphos-methyl
	Dichlorvos
	Diazinon
Pulmonary agents	
Carbonyl chloride (CG, phosgene)	
Trichloromethyl chloroformate (DP, diphosgene)	
Hydrogen sulfide	
Halogenated toxicants (chlorine, bromine)	
Aldehydes and related irritants	

influencing toxic responses to chemical threat agents. For example, chemicals requiring metabolic activation, including organophosphorus nerve agents such as parathion, diazinon, and chlorpyrifos, are dependent on expression of specific cytochrome P450 genes, while active metabolites (oxons) of these compounds are hydrolyzed by paraoxonases (Povey, 2010; Mackness and Mackness, 2015). Genetic polymorphisms in these enzymes control turnover of the active metabolites and, consequently, susceptibility to these nerve agents (Costa et al., 2013; Tawfik Khattab et al., 2016; Dardiotis et al., 2019). Similarly, in humans, genetic variants of acetylcholinesterase (AChE) and butylcholinesterases, targets of nerve agents and organophosphorus pesticides, may limit susceptibility to these agents (Lockridge et al., 2016).

This chapter provides an overview of chemical threat agents, with a focus on vesicants and nerve agents that have recently been used (Gulland, 2017; John et al., 2018, Kaadan and Cranmer, 2018) and of other chemical threats (e.g., metabolic poisons, arsenicals, rodenticides, pulmonary agents, and halogenated gases) (Table 8.1). Several classes of these agents (e.g., arsenicals, aldehydes, and pesticides) are discussed in more detail in other chapters in this volume.

8.2 NERVE AGENTS

The development of nerve agents arose first through the efforts of Gerhard Schrader to develop organophosphorus compounds into insecticides at IG Farben in Germany during the 1930s (Lopez-Munoz et al., 2009). In 1936, Schrader synthesized Tabun (ethyl

dimethylphosphoramidocyanidate), also known as GA. Upon synthesis, Schrader started to exhibit signs of what now is known as cholinergic crisis, which presented as headache, difficulty breathing, and pinpoint pupils. This discovery was reported to the German military by IG Farben, where it was renamed Tabun. Schrader and colleagues subsequently developed an even more toxic compound that was named sarin (isopropyl methylphosphonofluoridate; GB). Along with soman (trimethyl propoxyfluoromethyl phosphine oxide; GD) and cyclosarin (cyclohexyl methylphosphonofluoridate; GF), these compounds comprised the G-series of nerve agents. Although the German military worked to weaponize these compounds and were prepared to use them during World War II (WWII), Hitler declined to use them in the war effort for reasons that are still not clear.

Soon after the discovery of sarin and soman, many countries, including the United States, England, and Russia, as the former Soviet Union, began synthesizing these nerve agents and experimenting with the development of new agents (Wiener and Hoffman, 2004). Similar to the development of the G-series agents in Germany, the V-series was discovered by chemists at ICI in the early 1950s that were researching insecticides. The first compound discovered was VG (diethyl diethylaminoethyl phosphorothioate), which was approximately as toxic as sarin, and VX, which is the best-known V-series nerve agent. During the 1970s through the early 1990s, the Soviet Union and Russia developed the Novichok series of agents. Novichok translated as "newcomer" and the series was designed to be undetectable using chemical detection equipment, defeat protective gear, be safer to handle, and circumvent the Chemical Weapons Convention list of banned chemicals (Nepovimova and Kuca, 2018; Kloske and Witkiewicz, 2019). Novichok agents were a totally new way of designing nerve agents, as many were developed as binary agents that, once combined, would form an extremely toxic nerve agent. The first binary formulation and "Novichok" developed was VR, which was structurally similar to VX and differed only in the alkyl substituents on oxygen and nitrogen atoms.

The various classes of organophosphate nerve agents share a similar mechanism of toxicity but differ significantly in their physiochemical properties. The G-series agents are liquids in their pure state and are characterized by high boiling points and low melting points. Once vaporized, they give off colorless vapors that are heavier than air and can persist up to several hours depending on conditions (Abou-Donia et al., 2016). The V-series of compounds are oily liquids with low volatility and typically a higher boiling point than the G-series, leading to them being used primarily in aerosol form. Unlike the G-series, the V-series compounds are much more stable, leading to persistence in the environment for weeks to months, depending on the weather conditions (Wiener and Hoffman, 2004). Very little is currently known about the physiochemical properties of Novichok agents, as most of their structures are only suspected and not confirmed (Nepovimova and Kuca, 2018).

The toxicity of all nerve agents is primarily related to the inhibition of AChE. Organophosphate compounds such as nerve agents have a high affinity for the serine hydroxyl residue in the esteratic or active site of AChE, leading to inhibition of activity and buildup of the neurotransmitter acetylcholine. G-series and V-series compounds are highly potent at inhibition of AChE, with IC_{50} values in the low nanomolar to high picomolar range in guinea pig brain extracts, plasma, red blood cells, and whole blood (Fawcett et al., 2009). Importantly, the IC_{50} values for AChE inhibition in the brain were demonstrated to be good predictors of lethality of G- and V-series agents. Once inhibited by an organophosphate compound, AChE activity can be regenerated through spontaneous hydrolysis in a relatively slow (hours) reaction whose efficacy depends on the nature of the leaving group, with less alkylated compounds being hydrolyzed more rapidly than more alkylated (Casida and Quistad, 2004). The phosphorylated AChE can also undergo a related nonenzymatic

time-dependent process termed "aging," a dealkylation of the phosphorylating compound (Millard et al., 1999). In the case of nerve gases, the half-time of aging can vary from a few minutes for soman to a few hours for sarin (Milatovic et al., 2009). Aging results in permanent inactivation of AChE, with *de novo* synthesis being the only way to recover enzyme action.

AChE is present throughout the central and peripheral nervous system, and its inhibition leads to an accumulation of Ach in the synaptic cleft and overstimulation of cholinergic receptors, both muscarinic and nicotinic. Signs and symptoms of toxicity depend on severity of and route of exposure. As muscarinic receptors are found on smooth muscle and glands, inhibition of AChE and stimulation of these receptors typically result in smooth muscle contraction and excessive secretion, particularly in the lung, gastrointestinal system, sweat and salivary glands, cardiovascular system, pupils, and bladder. These classic effects of massive AChE inhibition are clinically known through the acronym DUMBELS, which stands for increased diarrhea, urination, miosis, bronchospasm, emesis, lacrimation, and salivation. Hyperstimulation of nicotinic ACh receptors in skeletal muscle and the neuromuscular junction is associated with muscle fasciculations, twitching, weakness, and cramps (Jett and Richardson, 2009).

Standard-of-care pharmacological treatment for nerve agent exposure consists of three primary approaches. The first is administration of atropine to block the effects of overstimulation of muscarinic receptors (Leifkin, 2002). This is accompanied by administration of an oxime, typically pralidoxime (2-PAM), that regenerates AChE that is not aged through breaking the phosphorylation of AChE by the nerve agent (Kassa, 2002). Finally, anticonvulsant agents are administered to stop seizure activity, with diazepam previously being the drug of choice that has recently been replaced by diazepam (Reddy and Reddy, 2015). Although necessary as antidotes, there is evidence of toxicity related to these therapies, include anticholinergic toxicity as the result of aggressive atropinization.

Although Germany did not use nerve agents during WWII, there are several incidents of their use in wartime and terrorist incidents. During the Iran–Iraq War in the 1980s, Iraq is believed to have used sarin several times against Iranian soldiers and ethnic Kurds in the north of Iraq. Based on translation of writings from the Iranian physician Dr. Syed Abbas Foroutan (Newmark, 2004), individuals exposed first developed miosis, sweating, and increased respiratory secretions, indicative of effects on muscarinic receptors. Nicotinic receptor hyperstimulation was observed, with "muscle debility and flabbiness." The primary means of treatment was atropine injections, as oximes were in short supply in Iran, and diazepam. Due to lack of follow-up, the long-term effects of these exposures are not clear.

More recently, the Japanese religious sect Aum Shinrikyo released a form of sarin in a Tokyo Metro station in 1995, leading to more than 10 deaths and thousands of injuries. Although the injured recovered initially, there is evidence of long-term effects in some patients (Nishiwaki et al., 2001; Miyaki et al., 2005). In addition to carrying diagnosis of posttraumatic stress disorder (PTSD), significant declines in psychomotor and memory function were reported, and reductions in brain volume by MRI were observed in several regions, including the cortex, hippocampus, and amygdala (Yamasue et al., 2003; Abe et al., 2006). Although it is difficult to disentangle the effects of PTSD and chemical exposure, the MRI findings did correlate with serum ChE levels (Abe et al., 2006). Similar structural brain alterations were observed in veterans thought to be exposed to low levels of sarin and cyclosarin during the 1991 Gulf War (Chao et al., 2010, 2011). In a recent report that followed children exposed during the Tokyo Metro attack, there were several

neuropsychiatric symptoms reported (Talabani et al., 2018) suggesting long-term effects from the incident. There have also been several instances of suspected and confirmed uses of sarin during the Syrian Civil War over recent years. Most recently, a Novichok compound was used to murder Sergei Skripal in England. The long-term effects of these most recent exposures remain to be determined, although Skripal eventually succumbed to the exposure.

8.3 RESPIRATORY TOXICANTS

Chlorine, bromine, and phosgene gases are among the most toxic of chemical agents that affect the respiratory tract. The short-term effects of exposure to these agents are concentration dependent, with the greatest number of casualties occurring in the immediate vicinity of their release. They all act rapidly, causing airway injury after inhalation (Woolf and Shannon, 1999; Carlisle et al., 2016). The first report of pulmonary toxicant use was on April 22, 1915, during World War I (WWI), when thousands of pressurized canisters were opened on the battlefront at Ypres, releasing several hundred tons of chlorine. This discharge killed an estimated 800–1400 soldiers and left an additional 2000–3000 wounded (Greenhalgh, 2014).

Since then, there have been multiple reports of accidental and intentional exposures to pulmonary agents (Mackie et al., 2014; Sydnes, 2018). Chlorine has been the most commonly used respiratory toxicant. It reacts rapidly with water in airway and ocular surface liquids, forming hydrogen chloride and hypochlorous acid. Clinical symptoms after mild inhalation include eye, nose, and throat irritation (Schäfer, 1915). This progresses to bronchoconstriction, cough, wheezing, and dyspnea after moderate exposure (Carlisle et al., 2016). Inhalation of massive doses of chlorine causes immediate asphyxiation as a consequence of destruction and sloughing of the epithelial lining of the airways. The resulting sloughed cellular debris and concomitant edema interfere with the oxygen diffusing capacity of the alveoli. Due to pulmonary edema, blood concentrates and CO_2 accumulates. Chlorine has also been reported to diffuse from the lung into the bloodstream, affecting the heart and reducing cardiac output (Samal et al., 2010). The presence of chlorine or its reactive products may also interfere with blood chemistry, leading to acidosis and impaired cardiac function (Szerlip and Singer, 1984; Zaky et al., 2015). Acute pulmonary injury after chlorine inhalation is also associated with bronchoconstriction, hypoxemia, and acute lung injury (ALI). Experimental studies have suggested that a single exposure to chlorine may lead to chronic complications, including reactive airway disease and possibly fibrosis (Hoyle and Svendsen, 2016; Govier and Coulson, 2018).

Bromine is another weaponized halogen that was deployed during WWI; more recent industrial accidents have been reported (Woolf and Shannon, 1999). The symptoms observed in exposed victims suggest a pathophysiologic mechanism similar to that of inhaled chlorine, including airway hyperreactivity, lung edema, and death from respiratory failure (Woolf and Shannon, 1999). Derivatives of bromine including bromoacetone, benzyl bromide, ethyl bromoacetate, xylyl bromide, and α-bromobenzyl cyanide have been used as tear gas agents (Schwenk, 2018).

Another halogenated agent used during WWI was phosgene ($COCl_2$). Unlike the visible green haze characteristic of chlorine gas, phosgene is colorless. As WWI progressed, phosgene was commonly discharged as a mixture with other chemicals including chlorine to reduce its density and improve its battlefield distribution. It is estimated that as many as 91,000 deaths attributed to gas in WWI were a result of phosgene or a similar

agent, diphosgene. Industrial accidents releasing phosgene have also occurred. In 1924, 11 tons of phosgene gas escaped from a war surplus store in central Hamburg, poisoning 300 people, 10 of whom died (Ryan et al., 1996).

In contrast to chlorine and bromine, phosgene has low solubility in aqueous solutions, enabling it to penetrate the alveolar spaces, without any appreciable losses in the proximal airways (Li and Pauluhn, 2017). Inhalation of phosgene results in distal lung damage and life-threatening pulmonary edema. The reaction of phosgene with water is much slower than the reaction with more nucleophilic chemical moieties, including alveolar surfactants and cell membranes. Its slower reactivity in the airway relative to chlorine is evident from the delayed onset of edema in exposed victims (Li and Pauluhn, 2017). Consistent with these mechanistic differences, symptoms of battlefield exposure often require 24 h or longer to manifest. Damage to cells and critical airway proteins following phosgene exposure is due to acylation reactions with primary and secondary amines, as well as hydroxyl and sulfhydryl groups, resulting in inflammation and tissue destruction. Phosgene can also enter the capillary circulation following inhalation, enabling it to exert toxic effects on peripheral tissues and blood constituents (Holmes et al., 2016). Investigational studies in animals indicated that therapies that improve vascular tone may limit phosgene mortality if administered early following exposure (Holmes et al., 2016). Survival following phosgene inhalation is also improved by using ventilatory strategies. A recent study, performed in pigs, demonstrated improved survival by using an over-the-counter continuous positive airway pressure (CPAP) device (Graham et al., 2018). Beneficial effects of CPAP are likely due to improved tissue oxygenation and redistribution of lung fluids from the alveoli to the perivascular space as a result of increased mean airway pressure. Use of CPAP could aid in the short-term treatment of mass casualties when critical care facilities are overburdened.

Halogenated oximes were first synthesized during the late 1920s. Related to phosgene, dichloroformoxime, commonly known as phosgene oxime, is the most irritating of these chemicals. It is not a true gas, but rather a colorless crystalline solid that becomes a liquid at 40°C. It remains in liquid form when returned to room air temperature while also releasing a vapor that is extremely irritating to skin and sinus cavities. While commonly listed alongside chemical vesicants, phosgene oxime and other halogenated oximes are considered "nettle" gases rather than actual blistering agents. Military interest in phosgene oxime stemmed from its rapid irritating properties, which was thought to thwart protective gear, by forcing soldiers to remove or adjust protective masks in response to exposure (Augerson, 2000; Patocka et al., 2018). Despite its production and stockpiling during the 1930s, there are no reports of phosgene oxime deployment. Therefore, little clinical data is available to investigate its pathophysiology or mechanism of action.

8.4 VESICANTS

Vesicants including sulfur mustard (bis-2-chloroethyl sulfide), nitrogen mustard, and lewisite are cytotoxic blistering agents (Graef et al., 1948; Weinberger et al., 2016). They are known to cause incapacitating injuries to the skin, lung, and eyes (Smith et al., 1995; Weinberger et al., 2016). As observed with other chemical warfare agents, the severity of injury due to these agents often depends on routes of exposure, concentrations of the blistering agent, time following exposures, and environmental conditions (Graham and Schoneboom, 2013). The principal pathophysiological feature of vesicant toxicity is

blistering, which involves extravasation of the agent and leakage of plasma constituents into interstitial regions of affected target organs.

Despite its name, sulfur mustard or mustard gas is not a true gas, but rather a pale-yellow liquid. Its effectiveness as a weapon stems from its unique chemical properties. As depicted in Fig. 8.1, the initial step in sulfur mustard reactivity is the formation of a heterocyclic sulfonium ion. This intramolecular reaction occurs when unpaired electrons from the central sulfur atom initiate a nucleophilic attack on either one of the terminal chlorine molecules. After an exchange of electrons, a single chlorine atom leaves the molecule, resulting in the formation of an unstable cyclic immonium ion intermediate that is capable of alkylating biomolecules (Fig. 8.2). Formation of this intermediate is a nonenzymatic process that occurs rapidly in aqueous solution (half-life in water ~5 min at 37°C), contributing to the sensitivity of moist skin regions, the eyes, and the upper bronchi of the airways (Shakarjian et al., 2010).

Nitrogen mustard undergoes a similar reaction scheme. Despite its reactivity in water, sulfur mustard retains potency for days or even years in an environmental setting. For example, there have been several incidents where improper disposal, including burning,

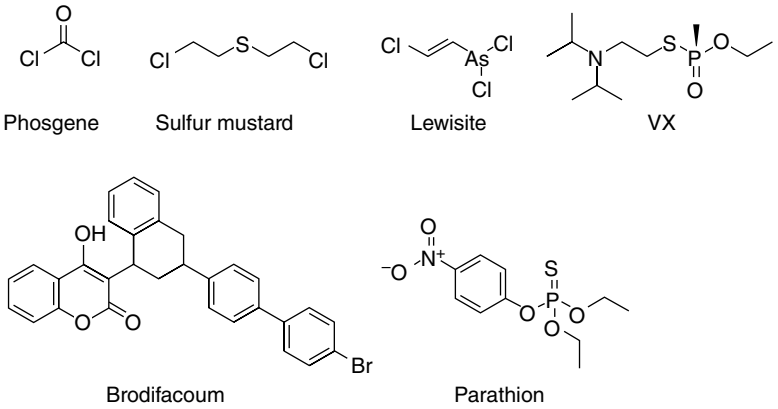

FIGURE 8.1 Structures of several chemical threat agents.

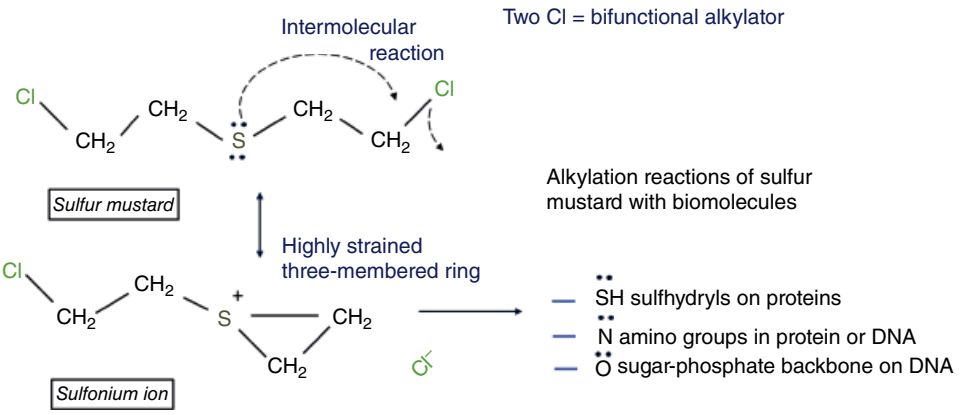

FIGURE 8.2 Sulfonium ion formation from sulfur mustard and its interaction with biomolecules.

burying, placement in "mustard pits" (sulfur mustard is deposited and reacted with bleach), or dumping at sea, has led to accidental human exposures (ATSDR, 2003; Ashmore and Nathanail, 2008; Greenberg et al., 2016). This incongruity in sulfur mustard half-life relates to its poor solubility in water, permitting hydrophobic droplets to coalesce, rather than disperse into an aqueous solution. Low solubility also results in the formation of a sulfonium salt interface between a sulfur mustard droplet and the surrounding water phase (Yang et al., 1992). Accumulation of this product at the sulfur mustard/water boundary slows further sulfur mustard dissolution, enabling it to retain potency below ground or in water for decades (Ashmore and Nathanail, 2008). Stability, long shelf life, and persistence after deployment are the principal reasons why sulfur mustard became a preeminent battlefield vesicant, rather than alkylating agents such as nitrogen mustard (Monteiro-Riviere and Inman, 1997).

Structurally, mustards can be divided into two groups: monofunctional and bifunctional. Monofunctional mustards, including 2-chloroethyl ethyl sulfide (CEES), possess only one chlorine atom and, therefore, can only cause a single alkylation reaction. Bifunctional mustards, including authentic sulfur mustard and nitrogen mustard, have two chlorine atoms, one at either terminus. Mustards can react with a variety of biomolecules, the most reactive being nucleophilic centers on thiol groups present in glutathione and proteins, as well as amines present in peptides, free amino acids, and DNA (thiol > amine > phosphate > carboxyl). The main reaction between DNA and sulfur mustard involves alkylation of the 7-nitrogen of guanine (Warwick, 1963). Because sulfur mustard and nitrogen mustard are bifunctional, they can participate in two alkylation reactions leading to cross-linking of cellular components such as DNA with proteins, DNA with small thiols, or two bases within the same DNA strand or on opposite DNA strands (Batal et al., 2015). DNA cross-links are particularly cytotoxic due to their ability to block replication, transcription, and repair pathways.

Another important property of sulfur mustard, as it relates to toxicity, is its lipophilicity. Sulfur mustard has low water solubility and high solubility in oils, fats, or organic solvents. High lipid solubility enables sulfur mustard to penetrate clothing and fabric and rapidly move across cell membranes. Due to these chemical properties, sulfur mustard can overwhelm the innate xenobiotic barriers of the skin, lung, or eye.

Current understanding of sulfur mustard toxicity comes from clinical evaluation of two cohorts of victims—those exposed during WWI (1917–1918) and those exposed during the Iran–Iraq War (1980–1988) (Balali-Mood and Hefazi, 2006). Most of these individuals received a single exposure. Physicians examining victims during WWI considered sulfur mustard skin lesions to be a new type of chemical burn, distinct from burns caused by heat or corrosives (Warthier and Weller, 1918). Others viewed sulfur mustard skin injury as simply the response of skin to a slow-acting toxicant (Sinclair, 1949). As the initial dermal contact with sulfur mustard liquid or vapor does not cause immediate overt responses, exposed individuals were less likely to avoid or decontaminate affected skin. As a result, exposures were potentially increased, leading to exacerbation of the toxic response.

Latency following sulfur mustard exposure distinguishes it from other chemical weapons, such as chlorine and phosgene oxime, which cause rapid irritation and pain in the skin and respiratory tract (Goswami et al., 2016; Goswami et al., 2018). The onset of symptoms from sulfur mustard exposure ranges from 1 to 24 h, depending on its concentration. Following sulfur mustard exposure, itchy sensations develop about 2 h after the latency period ends; these sensations are followed almost immediately by signs of erythema. With moderate to high exposure, small vesicles filled with a pale-yellow fluid appear at the margins of the

erythematous area and, with time, coalesce to form sack-like blisters. These vesicating properties are progressive, with the pinnacle of skin necrosis occurring after 5–10 days (Warthier and Weller, 1918). Blisters subsequently burst, resulting in a necrotic skin layer. Typically, mustard wounds resolve over the course of 2 months, leaving pigmentation changes that may persist for months or years. Additional subacute effects of mustards have been reported to include the presence of cherry angiomas, eczema, hypertrophic scarring, and dry and sensitive skin (Dacre and Goldman, 1996; Balali-Mood and Hefazi, 2006; Ghabili et al., 2010). Topical sulfur mustard can also penetrate the skin, leading to systemic toxicity, including bone marrow depression, and effects on other tissues.

Skin blistering in response to sulfur mustard and other vesicants distinguishes this class of chemicals from most other chemical threat agents. The stratum corneum, the outermost layer of skin, consists of cornified layers of dead cells and acts as the first line of defense against environmental chemicals (Brisson, 1974). Due to its lipid solubility however, sulfur mustard readily diffuses through the superficial epidermal layers and enters into the dermis and subcutaneous regions, causing injury and vascular toxicity (Riviere et al., 1995). The destruction of inner skin anatomy by sulfur mustard can have acute and chronic consequences. Damage to the extracellular matrix and hemidesmosomes at the dermal-epidermal junction allows separation of the keratinocyte layer from its basolateral attachment point on the basement membrane. Additional damage to skin vasculature permits leakage of plasma exudate into injured regions, further distending the epithelium away from basement membrane. Destruction of basal cells, a major stem cell of the skin, coupled with basement membrane erosion, limits effective epidermal renewal. Consequently, healing is delayed, making the skin prone to infection and the formation of fibrotic scars (Pechura and Rall, 1993). The clinical management of sulfur mustard dermal injury is essentially symptomatic and supportive. Systemic anti-inflammatories and local emollients can be administered to reduce itching and dryness of skin during convalescence. Recent advances designed to improve the rate of wound healing including dermabrasion and stem cell therapy are presently being investigated for clinical application.

Injury to the lung is the principal cause of acute mortality and morbidity following sulfur mustard poisoning. Whereas moderate inhalation exposure causes acute edema and destruction of the airway epithelial lining, higher exposure levels result in injury to the sinuses, larynx, trachea, bronchi, and alveolar regions of the respiratory tract. Sulfur mustard has the capacity to injure vasculature in the interstitial areas beneath the airway epithelial lining. Vessel "leakiness" leads to an exudative process, culminating in the formation of obstructive airway casts. Failure to remove the obstructing matter from large airways can cause death by asphyxiation or secondary pneumonia. Additional airway complications, including chronic bronchitis, recurrent pneumonia, bronchiectasis, and tracheobronchomalacia, also have been reported (Ghanei et al., 2008).

Among gassed individuals from the Iran–Iraq conflict, 75% of the survivors developed some type of long-term respiratory complication. Structurally, these included tracheal and/or bronchial deformities or strictures, bronchiolitis obliterans, and/or pulmonary fibrosis. Additional airway complications, including chronic bronchitis, recurrent pneumonia, bronchiectasis, and tracheobronchomalacia, also have been reported (Ghanei et al., 2008). A number of patients from the Iran–Iraq cohort also presented with constrictive bronchiolitis on lung biopsy, even with normal pulmonary function tests and chest high-resolution computed tomography (HRCT) scans.

Epidemiological studies indicate a link, albeit weak, between cancer and sulfur mustard exposure among exposed victims. A greater incidence of malignancies, including

leukemia, Bowen's disease, and laryngeal and bronchial cancer, was observed in German, Japanese, and British factory workers involved with the production of sulfur mustard (Easton et al., 1988). Potential treatments being investigated to mitigate acute sulfur mustard lung injury include anticoagulants or "clot-busting" drugs to restore airway patency, along with anti-inflammatory agents including steroids, cytokine antagonists (e.g., anti-TNFα antibody), antioxidants, and protease inhibitors (Rancourt et al., 2014; Weinberger et al., 2016; Malaviya et al., 2019). A number of these drugs are also being investigated for use in the treatment of chronic pathologies associated with mustard poisoning.

Another important target of mustard is the eye. After an initial latency period, ocular "burning," tearing, and photophobia were reported (Shakarjian et al., 2010). As the injury progresses, there is severe ocular pain, dry eyes, and impaired vision. Although large blisters are not formed in the cornea, microbullae develop, and once enough have accumulated, the epithelium detaches from the basement membrane, causing epithelial sloughing (DeSantis-Rodrigues et al., 2016). Histological and clinical evaluations of victims of mustard poisoning also showed corneal erosions, vascular injury, and edematous plasma influx (Panahi et al., 2017). These effects dissipate following low sulfur mustard exposure, resulting in complete healing after several weeks. In contrast, following high-level exposure or direct ocular contact, more profound injury is observed. As a consequence, during subsequent attempts at tissue repair, corneal opacifications and collagen deposition develop, with neovascularization emanating from the limbal area migrating toward the center of the cornea (Vidan et al., 2002). Vision loss can occur in response to these structural deformities. Latent onset of ulcerative keratitis a decade after a long asymptomatic period has also been described in WWI veterans and in Iranian veterans exposed during the 1980s (Etezad-Razavi et al., 2006). These lesions recurred even after corneal transplants and led to late-onset blindness. Currently, there are no effective treatments for ocular injury due to mustard exposure, although research aimed at modifying protease activity appears promising.

Chemical arsines possessing an $–ASCl_2$ group are also potent vesicants. These organic arsenicals were produced as weapons between WWI and WWII and are largely variants of arsenic trichloride or trichloroarsine (see further below) (Marrs et al., 2007). Lewisite [dichloro(2-chlorovinyl)arsine] is the best-known and most widely produced agent in this class of chemical threats. It was first synthesized in 1918 by Dr. Wilford Lee Lewis; it has not been used in warfare, although it may remain stockpiled in some nations. However, it is uncertain whether it retains long-term activity. The arsenic in lewisite is trivalent and can react with thiol groups in many enzymes. Because pathophysiological data on human exposure are lacking, the exact mechanism of lewisite toxicity is uncertain. Horse, swine, hairless guinea pig, and mice have been used to investigate the pathogenesis of skin lesions in response to dermal exposure to lewisite (Li et al., 2016a). The pattern of injury in these models is similar and is characterized by the early onset of erythema and edema. In contrast to mustards, symptoms of lewisite exposure occur rapidly, with almost no latency period. Exposed skin exhibits a gray discoloration, which becomes necrotic by 4 days' post-exposure, a response that persists until day 7. Subsequently, there is gradual recovery and complete healing after 4 weeks.

Histological analysis has shown that inflammatory cell infiltration, microvesication, and vascular thrombosis are early features of lewisite-induced injury (Li et al., 2016b). Molecular changes include elevated expression of cytokines and matrix metalloproteinases, which are generally consistent with other types of chemically induced cutaneous injury (Li et al., 2016a). During the acute phase of injury, discolored skin lesions show

rapid progression to necrosis and extensive edema, inflammation, and vascular thrombosis (Rice and Brown, 1999). During this phase of injury, lewisite also penetrates the skin and can elicit systemic toxicity, which is typical of arsenic poisoning. Lewisite also has the capacity to perturb enzymatic pathways, interfering with cellular metabolism and energy production. Like arsenic, lewisite can also cause vascular endothelial dysfunction, resulting in increased permeability of systemic capillaries, organ congestion, and hypovolemic shock. Hepatic or renal failure can occur, with prominent gastrointestinal effects (vomiting and diarrhea) thought to be of greater severity than the response to sulfur mustard poisoning.

The eye is extremely vulnerable to lewisite, and ocular exposure can result in permanent blindness if decontamination is not accomplished within 1 min (Li et al., 2016a). Studies in rabbits exposed to lewisite vapor exhibited edema of the eyelids, inflammation, corneal necrosis, and blindness (Tewari-Singh et al., 2016). A dose-dependent increase in corneal opacity is evident as early as 6-h post-exposure and gradually increases up to day 3. These alterations were associated with prominent neovascularization during the subacute period, which remained persistent over the course of the 28-day experiments.

The sequelae of airway injury in response to lewisite are similar to mustard inhalation in experimental settings, except that its vapor is extremely irritating to mucous membranes in the sinus (McManus and Huebner, 2005). Unlike mustard exposures, lewisite-exposed individuals seek immediate evacuation and decontamination, which helps to mitigate injury. Inhalation exposure in canines elicits massive nasal secretions, retching, and lacrimation, which continues until death (Harrison and Ordway, 1946). Histological evaluation revealed airway edema, destruction of the epithelium, and pseudomembrane formation extending from the nostrils to the bronchi. Such an exudative process is reminiscent of obstructive airway cast production observed in response to mustard inhalation and may be the cause of acute mortality.

There are no presently FDA-approved antidotes for lewisite poisoning. Early decontamination is the only way of preventing or lessening lewisite damage. Chelation through use of dimercaprol can mitigate injury when applied to the eye or skin within minutes of an exposure. Dimercaprol has also been shown to reduce mortality when administered 100 min after a lethal inhalation exposure in canines (Peters et al., 1945).

8.5 RODENTICIDES

A large number of rodenticides were developed to control rodent populations. Because of their ease of manufacture, low cost, and ready availability, many of these chemicals are of concern as potential weapons. Examples include phosphine and the related metal phosphides including aluminum, magnesium (Mg_3P_2) and zinc phosphide (Zn_3P_2), various anticoagulants, and the neurotoxin tetramethylenedisulfotetramine (TMDT) (Proudfoot, 2009; Bumbrah et al., 2012). Metal phosphides are reactive with moisture, particularly under acidic conditions, wherein they liberate phosphine, and this is likely their major mechanism of toxic action (Bogle et al., 2006). Phosphine, a colorless and odorless gas, is used as a grain fumigant for insect and rodent control. It is readily absorbed through the skin, eyes, lung, and gastrointestinal tract where it can induce systemic toxicity (Nath et al., 2011). Phosphine and various metal phosphides are known to disrupt the sympathetic nervous system. In nerve cells and many other cell types, they suppress energy metabolism, which can alter the redox balance of cells and induce oxidative stress. This occurs via

suppression of mitochondrial function, which is due to inhibition of cytochrome oxidase activity, cytochrome c, and mitochondrial respiration (Kashi and Chefurka, 1976; Singh et al., 2006; Sciuto et al., 2016). This in turn can cause injury in many tissues including the lung, liver, gastrointestinal tract, and cardiovascular system (Bogle et al., 2006; Gurjar et al., 2011; Mehrpour et al., 2012). Cardiac tissue is a major target for phosphine and phosphides; injury to the heart can result in circulatory failure and shock (Wander et al., 1990; Siwach et al., 1998; Akkaoui et al., 2007; Karimani et al., 2018). In rodent models, metal phosphides cause pericarditis and myocardial necrosis, dysrhythmic abnormalities, and cardiac conduction anomalies, resulting in abnormal impulse propagation; conduction blockage and ectopic beats have also been observed (Lall et al., 1997).

Anticoagulants, particularly the superwarfarins, represent an important class of chemical threats. Early studies demonstrated that dicoumarol, a plant product derived from coumarin, was the agent active in clover that induces hemorrhage in cattle [for reviews see Mueller and Scheidt (1994) and Wardrop and Keeling (2008)]. Warfarin, a derivative of dicoumarol, was first developed in the late 1940s as a rodenticide and later used clinically as an anticoagulant. As an inhibitor of vitamin K epoxide reductase, warfarin suppresses the synthesis of vitamin K, an essential cofactor in the blood-clotting cascade (Feinstein et al., 2016). Resistance to warfarin due to mutations in vitamin K epoxide reductase led to the development of more potent "superwarfarins" that have profound anticoagulation actions (Feinstein et al., 2016). These chemically modified forms of warfarin, including difenacoum and brodifacoum, are highly lipophilic derivatives active against warfarin-resistant rodents (Hadler and Shadbolt, 1975; Rennison and Hadler, 1975). This is presumably due to the fact that the hydrophobic nature of superwarfarins leads to retention in fatty tissues, a factor that markedly increases their half-life (O'Reilly et al., 1963; Gebauer, 2007). Moreover, these rodenticides have a much greater affinity for vitamin K epoxide reductase when compared with warfarin (Gebauer, 2007). As large quantities of superwarfarins are available for use as rodenticides, this raises the possibility of their intentional use on the battlefield, as well as in acts of terrorism. Although vitamin K can be an effective countermeasure, it is costly and requires prolonged treatment (King and Tran, 2015). Superwarfarins also possess anticoagulant-independent effects including regulation of energy metabolism, inflammation, bone remodeling, and fertility (Ma et al., 2015; Li et al., 2016a).

Another high-priority chemical threat is TMDT, a polyhedral organic compound related to the antiviral agent adamantine (Ticku and Olsen, 1979; Banks et al., 2014). Originally synthesized as a rodenticide (Haskell and Voss, 1957; Casida et al., 1976) and later used as a pesticide, it is currently banned due to its extreme toxicity. As an odorless and colorless neurotoxin, TMDT acts in the central nervous system by binding to $GABA_A$ chloride ionophore complexes (Whitlow et al., 2005; Zhao et al., 2014). By impeding chloride entry into neurons, TMDT interferes with the inhibitory actions of GABAergic receptors in the brain (Zhao et al., 2014; Patocka et al., 2018). This disrupts neuronal depolarization and allows unregulated calcium entry into neurons (Cao et al., 2012; Banks et al., 2014). $GABA_A$ receptors are structurally heterogeneous, and selectivity among $GABA_A$ receptor subtypes for TMTD has been reported. Thus, using whole cell patch clamping, Pressly et al. (2018) reported that the $\alpha2\beta3\gamma2$ and $\alpha6\beta3\gamma2$ $GABA_A$ receptor subtypes were most sensitive to TMTD and most likely to be important in mediating the seizure-inducing activity of TMTD.

TMDT exposure most often results in seizures and cardiotoxicity (Sobotka and Safanda, 1973). Electrocortical changes, particularly those associated with a seizure phenotype, have been observed in rodent models (Sobotka and Safanda, 1973; Dray, 1975; Shakarjian et al., 2012); electrocortical changes have also been observed in cases of

poisoning in humans (Chau et al., 2005; Lu et al., 2008; Li et al., 2012). High doses can result in death due to multi-organ failure (Chau et al., 2005; Patocka et al., 2018). Although there is no known antidote for TMDT poisoning in humans, signs of toxicity may be controlled by anticonvulsants and appropriate supportive care (Chau et al., 2005; Whitlow et al., 2005; Patocka et al., 2018). Of interest are studies in rodents showing that combined treatment with diazepam, a $GABA_A$ receptor agonist, and an *N*-methyl-D-aspartate receptor antagonist such as dizocilpine maleate or allopregnanolone effectively inhibits or reverses TMDT-induced seizures (Bruun et al., 2015). In rats, TMDT-induced seizures are also generally more severe in females than males (Shakarjian et al., 2012; Lauková et al., 2018). Moreover, younger animals are more vulnerable to TMDT. Thus, specific sex- and age-specific treatment strategies may be needed to mitigate TMDT-induced toxicity.

8.6 ARSENICALS

As highly reactive organic and inorganic arsenic derivatives, arsenicals are known to cause significant tissue injury (Swaran et al., 2009; Flora, 2015). The most notable organic derivative is lewisite, and its actions are described above. Of the many inorganic derivatives, arsenic trioxide and arsine are of major concern, as they are readily available as industrial chemicals and/or by-products of industrial processes. Arsenic trioxide is absorbed upon skin contact, after inhalation, and via the digestive system (Flora et al., 2009; Li et al., 2016). Signs of arsenic trioxide poisoning in humans have been reported largely as a result of accidental, suicidal, or homicidal use and include gastrointestinal, cardiovascular, and nervous system toxicity; systemic toxicity can result in death (Alamolhodaei et al., 2015; Vineetha and Raghu, 2019). Because of its antiproliferative actions, lower doses of arsenic trioxide are also used for the treatment of certain forms of cancers including hematologic malignancies and solid tumors (Hoonjan et al., 2018).

Mechanisms underlying arsenic trioxide toxicity are multifactorial. Arsenic trioxide and related arsenicals are known to disrupt cellular metabolism, in part by binding with sulfhydryl residues in cells including proteins and intracellular sulfhydryls such as glutathione (Li et al., 2016a). Protein modifications can disrupt their functional activity and compromise metabolism, while glutathione depletion can cause oxidative and nitrosative stress (Flora, 2015; Madhyastha et al., 2018). Oxidative stress as a result of disruption of mitochondrial membrane proteins can lead to alterations in transmembrane potential and energy metabolism (Pace et al., 2017; Madhyastha et al., 2018). It has also been shown that arsenicals can induce endoplasmic reticulum (ER) stress, a process by which unfolded proteins accumulate in the ER (Li et al., 2016a). This can generate an unfolded protein response (UPR) signaling cascade, which can lead inflammation and apoptosis (Zhang and Kaufman, 2008; Li et al., 2011; Srivastava et al., 2013). Toxicity may also be due to the ability of arsenicals to modulate the immune system, a process that may also be mediated the by ER stress response (Li et al., 2016a; Haque et al., 2017).

Arsine is a colorless and flammable gas with strong reducing activity; in the presence of water and light, it readily transforms into a variety of oxidized forms of arsenic that contribute to its toxicity (Flora et al., 2009). Since it is often found in occupational settings, accidental exposures are a significant health concern (Landrigan et al., 1982). Because of its physiochemical properties, including the fact that it is more than twice as dense as air, it is considered a chemical threat agent (Henriksson et al., 1996; Thomas and Young, 2001; Flora et al., 2009). At high doses, inhalation of arsine is lethal (Hocken and Bradshaw, 1970; Klimecki and Carter, 1995; Pullen-James and Woods, 2006; Flora, 2015), while at

lower dosages, it is hemolytic (Hocken and Bradshaw, 1970; Risk and Fuortes, 1991). It can cause injury to the kidneys, nervous system, liver, heart, and lung (Pullen-James and Woods, 2006). Metabolism is thought to play a key role in arsine toxicity; in many tissues, injury is caused by arsenic dihydride intermediates and elemental arsenic (Carter et al., 2003). The precise mechanism of cytotoxic action of the inorganic arsenicals is not known. They readily suppress mitochondrial oxidative metabolism (Pace et al., 2017). Their ability to generate reactive oxygen species is likely to cause DNA damage and result in the initiation of the carcinogenic process (Huang et al., 2004; Shi et al., 2004). Oxidative stress may be responsible for the hemolytic activity of arsine as hemoglobin can be degraded by arsenic as well as adducts derived from reactive oxygen species (Hatlelid et al., 1996).

8.7 METABOLIC POISONS

Metabolic poisons are noted for their ability to disrupt cellular respiration and include agents such as hydrocyanic acid, cyanogen chloride, and hydrogen sulfide (Table 8.1). Hydrogen cyanide (cyanide anion) and cyanogen chloride (cyanic chloride) are often found in industrial synthetic processes; cyanide is a combustion by-product of many synthetic products containing cyanogens (Bhattacharya and Flora, 2009). In the blood, cyanogen chloride is rapidly converted to hydrogen cyanide (Schwenk, 2018). It also decomposes in water to form hypochlorous acid, hydrochloric acid, and ammonia, which are irritating to the eyes and upper airways (Munro et al., 1999). Most often, unintentional cyanide poisoning results from inhalational exposures or following contamination of the skin (Seidl et al., 2003).

Cyanide is rapidly taken up into tissues; detoxification takes place through a variety of mechanisms including enzymatic transsulfuration reactions (Bhattacharya and Flora, 2009). Cyanide toxicity, which can result in respiratory depression, convulsions, and coma, is largely attributed to anoxia; binding of cyanide to ferric iron in iron-containing enzymes disrupts their functional activity. A key target for cyanide is the iron-containing enzyme cytochrome oxidase in the terminal mitochondrial respiratory chain (Solomonson, 1981). This impairs oxidative metabolism and suppresses mitochondrial respiration resulting in hypoxia. Under hypoxic conditions, pyruvate generated in cells that is normally used for oxidative metabolism for the production of adenosine triphosphate (ATP) is instead reduced to lactate. This causes lactic acidosis, a condition associated with many of the signs and symptoms to cyanide poisoning (Solomonson, 1981). It should be noted that in addition to cytochrome oxidase, cyanide also reacts with many metalloenzymes that disrupt metabolic and physiologic activity. Cyanide also targets ferric iron in hemoglobin, a process that reduces its ability to carry oxygen in red blood cells (Way, 1984). Cyanide can also react with sulfhydryl and carbonyl groups in proteins; additional sulfhydryl-containing cellular metabolites such as mercaptopyruvate, glutathione, and cysteine are also reactive with cyanide (Way, 1984; Ballantyne, 1987; Baskin and Brewer, 1997; Baud, 2007). Inhibition of cellular antioxidants by cyanide also results in oxidative stress. Reactive oxygen species are cytotoxic and can initiate lipid peroxidation, a contributing factor in cyanide-induced neurotoxicity (Bhattacharya and Flora, 2009).

Hydrogen sulfide (H_2S) is an industrial/agricultural chemical that also binds metalloproteins (Guidotti, 2010). Like cyanide, H_2S binds cysteine residues in proteins including cytochrome c (Williams et al., 2015). As a result, mitochondrial energy production is reduced (Guidotti, 2010). Thus, life-threatening doses of H_2S can suppress breathing and induce shock and coma (Almeida and Guidotti, 1999; Woolf and Shannon, 1999; Struve

et al., 2001; Almeida et al., 2008; Guidotti, 2010). Acute cardiac depression significantly contributes to long-term neurologic deficits following exposure to H_2S (Haouzi et al., 2019). Prolonged exposure to H_2S can cause pulmonary edema, likely due to alveolar injury (Guidotti, 2010). Of interest is the fact that methylene blue has been shown to counteract many of the signs of toxicity in animal models including H_2S-induced alterations in cardiac and metabolic functions (Sonobe and Haouzi, 2015; Sonobe et al., 2015; Judenherc-Haouzi et al., 2016; Cheung et al., 2018). The actions of methylene blue may be due to its redox active properties, suggesting that H_2S perturbs cellular redox balance (Hancock and Whiteman, 2016). In this regard, Haouzi et al. (2019) have recently shown that methylene blue can restore mitochondrial dysfunction in mouse cardiac myocytes treated with water-soluble sodium hydrosulfide hydrate.

8.8 SUMMARY

Chemical weapons represent a diverse group of threat agents, some of which were synthesized for use in warfare, while others are industrial or agricultural products. As weapons of war and terrorism, these agents can be stockpiled or produced on demand. Toxic industrial or agricultural chemicals are also readily available, for example, as industrial intermediates or for use as rodenticides or pesticides, or they are relatively easy to manufacture in bulk and at low cost. A major effort is underway to define the mechanism of action of many of these chemical threats with the aim of developing countermeasures for use either on the battlefield or in an emergency situation for civilian populations (Jett, 2016; Jett and Spriggs, 2018). The U.S. government has published information in case of exposures to communities and/or individuals for many chemical threats. Many of these documents are available from the Agency for Toxic Substances and Disease Registry (ATSDR) at the Centers for Disease Control and Prevention (CDC), a federal agency under the Department of Health and Human Services. The list below gives representative examples and is not comprehensive. This includes profiles for blister agents/vesicants (https://emergency.cdc.gov/agent/vesicants/index.asp), nerve agents (https://emergency.cdc.gov/agent/nerve/index.asp), superwarfarins (https://emergency.cdc.gov/agent/superwarfarin/index.asp), respiratory agents (https://emergency.cdc.gov/agent/pulmonary/index.asp), and metabolic poisons (https://emergency.cdc.gov/agent/cyanide/index.asp).

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9

AMBIENT AIR PARTICULATE MATTER

Morton Lippmann

9.1 INTRODUCTION

Particulate matter (PM) suspended in ambient air contains a broad range of chemical components, which vary both temporally and spatially. The mixture is also highly variable in terms of the concentrations of both inorganic and organic gas-phase air pollutants originating from both natural and anthropogenic sources. It includes fine PM, that is, PM in particles <2.5 μm in aerodynamic diameter ($PM_{2.5}$) emitted into the ambient air by a broad variety of sources, such as carbonaceous particles in wood smoke, soot, and Diesel engine exhaust PM (DEP), containing elemental carbon (EC), aka black carbon (BC). Such source-category-related PM also includes (1) organic carbonaceous (OC) PM formed within the atmosphere during the photochemical reaction sequence that also leads to ozone (O_3) formation (see Chapter 21), (2) acidic sulfur (S) and nitrogen (N) oxides resulting from the oxidation of sulfur dioxide (SO_2) and nitrogen oxide (NO_x) vapors released during fuel combustion (see Chapters 20 and 25), and (3) compounds resulting from the neutralization of acidic compounds by ammonia (NH_3) emitted into the atmosphere during biological decay of plant and animal wastes. The mixture also includes coarse-mode PM with aerodynamic diameters >2.5 μm, which are mostly created by communition, that is, mechanical processes that break up larger solid masses and liquids into smaller particles and droplets. The fine and coarse PM differ not only in particle size but also in chemical composition and their ability to elicit functional and/or adverse biological responses and diseases. It has long been known that inhalation of ambient concentrations of PM attributable to fossil fuel combustion in power plants and motor vehicles is associated with concentration-related adverse health effects. We also know that population impacts vary with both particle size and chemical composition. However, we still do not know the full extent that physicochemical factors govern the health-related responses, and therefore we have had to rely, for public health protection, on reducing PM exposures on the basis of mass-based concentration limits of PM in specific particle size ranges.

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Since reliance on the concentration of major mass fraction limits the utility of current routine ambient air monitoring programs for efficient public health protection, this chapter is, of necessity, still incomplete. There is a rapidly growing peer-reviewed literature that addresses the knowledge gaps with respect to the roles of particle size and chemical components as causal factors for the PM-associated human health effects.

This chapter focuses on (1) the quite extensive body of epidemiological literature that has demonstrated statistically significant associations between the mass concentrations of airborne PM particle size fractions and the rates of mortality, morbidity, and functional decrements in human populations, (2) the emerging literature that relates to the still limited networks in the United States and Europe that have provided a means of determining associations of specific chemical components in ambient air with adverse health effects, and (3) important supplemental information obtained in controlled inhalation exposures of human volunteers and laboratory animals to PM in specific size ranges and compositions that has proven useful in the interpretation of the epidemiological literature. Due to space limitations, (1) it omits many publications that compare responsiveness to PM mass indices with those of other criteria pollutants (O_3, NO_2, and SO_2); (2) it omits observational studies whose attribution of responsibility for causation among PM and other criteria pollutants within the mixture is speculative, leaving such discussion to the chapters on O_3, NO_x, and SO_x; and (3) it introduces but does not thoroughly review the extensive and rapidly growing literature on the *in vitro* toxicology of PM that has focused on the biological mechanisms underlying responses to PM inhalation.

9.2 BACKGROUND

Comprehensive documentation of knowledge concerning ambient air PM in the United States, and its known and likely health effects, has been provided periodically, initially in EPA criteria documents (CDs) and in their successor documents, that is, Integrated Science Assessments (ISAs). In some studies of the associations between health effects and PM, the strength of the association has increased as one passes from PM mass concentrations expressed in terms of (1) total suspended particulate matter (TSP) to (2) PM mass in particles less than 10 μm in aerodynamic diameter (PM_{10}) to (3) fine PM, that is, PM <2.5 μm ($PM_{2.5}$). Furthermore, recent research has demonstrated that health-related responses to PM exposures have often correlated better with the concentrations of some specific PM chemical components than with others. Thus, the choice of the most suitable index of PM concentration for use in future studies of the toxic effects of inhaled PM, and/or most appropriate exposure limits, needs to consider the acquisition of data on the concentrations of chemical components. $PM_{2.5}$ and $PM_{10–2.5}$ are the major mass fractions of PM_{10}. However, particles with diameters below ~0.1 μm, which usually constitute only a minor mass fraction of PM_{10}, often account for most of the PM number concentration and are known as ultrafine particles (UFP). The influence of a sampling system inlet on the sample mass collected is illustrated in Fig. 9.1.

Most of the coarse-mode particles, less than 10 μm but greater than 2.5 μm in aerodynamic diameter, can penetrate beyond the larynx into the thorax during inhalation and cause health effects after deposition within lung airways. These $PM_{10–2.5}$ are largely composed of mechanically dispersed soil and mineral ash. Both of the thoracic particle size fractions, $PM_{2.5}$ and $PM_{10–2.5}$, are complex chemical mixtures in dynamic equilibrium within the ambient air, that is, they enter the air at about the same rate as they are removed.

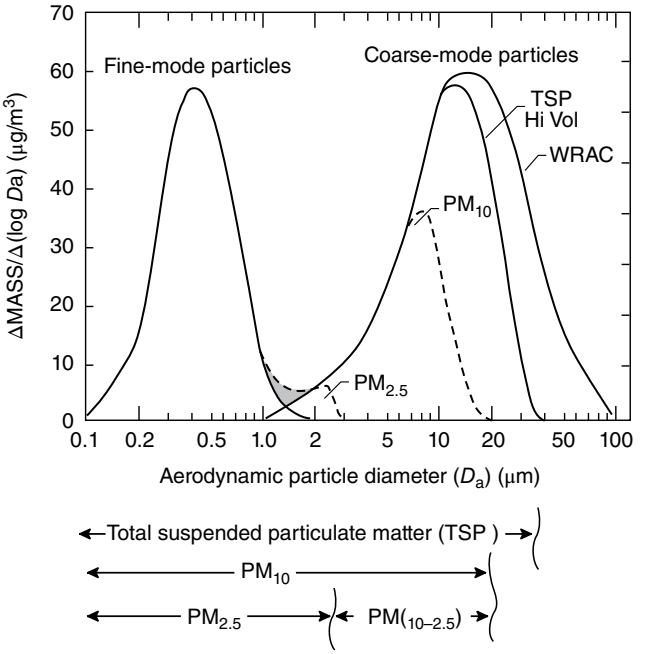

FIGURE 9.1 Representative bimodal mass distribution as a function of aerodynamic particle diameter for Phoenix, AZ, showing effect of size-selective sampling inlet on mass collected for (a) wide-ranging aerosol classifier (WRAC), (b) standard total suspended particulate (TSP) high-volume sampler, (c) sampler following EPA's (PM_{10}) criteria for thoracic dust, and (d) sampler following EPA's criteria for fine *particulate* matter ($PM_{2.5}$). *Source*: U.S. EPA (1996a, 1996b).

In dry weather, the concentrations of $PM_{10–2.5}$ are balanced between dispersion into the air, mixing with air masses, and gravitational fallout, while the concentrations of $PM_{2.5}$ are determined by rates of formation and transformation and by meteorological factors. Ambient air PM concentrations of both fractions are periodically and effectively depleted by rainout and washout. Further elaboration of these distinctions is provided in Table 9.1.

The knowledge of health-related responses to inhaled PM summarized in this chapter comes from research reported in the peer-reviewed literature describing studies of exposure–response relationships in people who have been exposed (1) in natural environments, (2) as human volunteers inhaling defined pollutants or mixtures for short periods, and (3) from laboratory animals exposed to PM and/or its components in either short- or long-term exposure studies. Unfortunately, the literature has been difficult to fully interpret because it involves so many variables in terms of temporal variations in exposures, the composition of the materials to which the subjects have been exposed, and the multiplicity of biological responses. Human responses to natural exposures depend on (1) temporal and spatial variations in pollutant concentrations, (2) the correspondence of the concentrations at a monitoring site to those that people actually inhale, (3) inhaled volumes, and (4) age, genetic, and constitutional factors affecting responsiveness. For animal responses to controlled laboratory exposures, interpretation is complicated by species and strain differences from humans with respect to (1) body sizes, (2) lifespans, (3) ventilation rates, (4) metabolic pathways and rates, and (5) pollutant dosimetries, including variations in deposition within the airways, clearance from the airways, translocation to other organs, and toxicant residence times at critical sites.

TABLE 9.1 Comparison of Ambient Particles, Fine Particles (Ultrafine Plus Accumulation Mode), and Coarse Particles

	Fine		
	Ultrafine	Accumulation	Coarse
Formation processes	Combustion, high-temperature processes, and atmospheric reactions	Breakup of large solids/droplets	
Formed by	Nucleation	Condensation	Mechanical disruption (crushing, grinding, abrasion of surfaces)
	Condensation	Coagulation	Evaporation of sprays
	Coagulation	Reactions of gases in or on particles	Suspension of dusts
		Evaporation of fog and cloud droplets in which gases have dissolved and reacted	Reactions of gases in or on particles
Composed of	Sulfate	Sulfate, nitrate, ammonium, and hydrogen ions	Suspended soil or street dust
	Elemental carbon	Elemental carbon	Fly ash from uncontrolled combustion of coal, oil, and wood
	Metal compounds	Large variety of organic compounds	Nitrates/chlorides/sulfates from HNO_3/HCl/SO_2 reactions with coarse particles
	Organic compounds with very low saturation vapor pressure at ambient temperature	Metals: compounds of Pb, Cd, V, Ni, Cu, Zn, Mn, Fe, etc.	Oxides of crustal elements (Si, Al, Ti, Fe)
		Particle-bound water	$CaCO_3$, $CaSO_4$, NaCl, sea salt
			Pollen, mold, fungal spores
			Plant and animal fragments
			Tire, brake pad, and road wear debris
Solubility	Probably less soluble than accumulation mode	Largely soluble, hygroscopic, and deliquescent	Largely insoluble and nonhygroscopic
Sources	Combustion	Combustion of coal, oil, gasoline, diesel fuel, wood	Resuspension of industrial dust and soil tracked onto roads and streets

TABLE 9.1 *(Continued)*

	Fine		
	Ultrafine	Accumulation	Coarse
	Atmospheric transformation of SO_2 and some organic compounds	Atmospheric transformation products of NO_x, SO_2, and organic compounds, including biogenic organic species (e.g., terpenes)	Suspension from disturbed soil (e.g., farming, mining, unpaved roads)
	High-temperature processes	High-temperature processes, smelters, steel mills, etc.	Construction and demolition
			Uncontrolled coal and oil combustion
			Ocean spray
			Biological sources
Atmospheric half-life	Minutes–hours	Days–weeks	Minutes–hours
Removal Processes	Grows into accumulation mode	Forms cloud droplets and rains out	Dry deposition by fallout
	Diffuses to rain drops		Scavenging by falling rain drops
Travel distance	<1–10s of km	100–1000s of km	<1–10s of km (small size tail, 100–1000s in dust storms)

Source: Adapted from Wilson and Suh (1997).

In consideration of the remaining interpretive challenges, and the great volume of the relevant literature, this chapter is focused on mortality, morbidity, and functional responses in humans and laboratory animals exposed to measured concentrations of ambient air pollutants and their components. Unfortunately, most of the epidemiological literature that is reviewed herein lacks detailed information on the specific chemical components that have been associated with the health effects of exposures to ambient air PM. In many cases, the exact composition of the material to which people have been exposed has simply not been determined.

The U.S. Clean Air Act (CAA) requires that EPA set, and periodically revise, primary (health-related) National Ambient Air Quality Standards (NAAQS) for criteria pollutants, including PM. In doing so for PM, EPA has relied on a large, and generally consistent, body of epidemiological evidence associating mass concentrations of ambient air PM size fractions with mortality and morbidity that cannot be explained by potential confounders and in unlikely to be explained by chance. To date, EPA has established PM NAAQS based only on mass concentrations expressed as PM_{10} and $PM_{2.5}$. In the future, consideration may be given to establishing separate PM NAAQS for the acute and chronic effects of coarse thoracic size fraction, that is, the $PM_{10–2.5}$.

The first part of the discussion that follows summarizes the nature and extent of the health effects that have recently been associated with gravimetric ambient air PM

concentrations and with a limited number of specific chemical components, that is, those making up relatively large mass fractions of $PM_{2.5}$, that is, OC, EC, sulfate ion ($SO_4^=$), and nitrate ion (NO_3^-), as well as those of minor mass fractions that have been considered to be important with respect to causing health effects, such as nickel (Ni), copper (Cu), arsenic (As), and selenium (Se). Further discussions of the health effects of some other specific components of the ambient air PM are provided in Chapter 11 on asbestos and other mineral fibers, Chapter 14 on diesel engine exhaust particles (DEP), Chapter 17 on lead (Pb), Chapter 20 on nitrogen oxides (NO_x), Chapter 25 on sulfur oxides (SO_x), and Chapter 19 on nanoparticles.

9.3 SOURCES AND PATHWAYS FOR HUMAN EXPOSURE

As indicated in Table 9.1, $PM_{2.5}$ and $PM_{10–2.5}$ generally have distinct sources and formation mechanisms, although there is usually some overlap. Primary $PM_{2.5}$ forms from condensation of high temperature vapors formed during fuel combustion. Secondary $PM_{2.5}$ forms from gaseous precursors.

By contrast, most coarse-mode particles are emitted directly as PM and result from mechanical disruption such as crushing, grinding, evaporation of spray droplets, or suspensions of dust from construction and agricultural operations. Most airborne coarse-mode particles have been recently formed by breaking up bigger masses into smaller ones, and the energy needed to reduce coarse particles limits the individual particles to sizes >1.0 μm in diameter.

PM_{10} and $PM_{10–2.5}$ have distinctly different sources and chemical compositions and differ in terms of solubility, acidity, and biological effects. $PM_{2.5}$ is mainly composed of varying proportions of inorganic ions (H^+, NH_4^+, NO_3^-, and $SO_4^=$), EC and OC, trace elements, and condensed water vapor. By contrast, $PM_{10–2.5}$ is primarily crustal in origin and includes compounds of silicon (Si), aluminum (Al), iron (Fe), and potassium (K), although some of Fe and K in $PM_{2.5\ is}$ from non-crustal sources, including biological material, such as bacteria, pollen, and spores. As a result of the fundamentally different chemical compositions and sources of $PM_{2.5}$ and $PM_{10–2.5}$, the chemical composition of the sum of these two fractions, that is, PM_{10}, is more heterogeneous, in both particle size and chemical composition, than either size mode alone.

Concentrations of wind-borne $PM_{10–2.5}$ are generally higher than those of $PM_{2.5}$ in arid areas. With the exception of EC and some of the OC compounds that account for most of the soiling properties, $PM_{2.5}$ components are largely soluble in water and hygroscopic, and, except under fog conditions, the $PM_{2.5}$ is acidic. By contrast, $PM_{10–2.5}$ is mostly insoluble, nonhygroscopic, and basic. These differences affect (1) exposures, (2) the representativeness of central-site concentration values, and (3) the behavior of PM formed outdoors that penetrates into homes and buildings where people spend most of their time.

$PM_{2.5}$ typically has a longer atmospheric lifetime (i.e., days to weeks) than $PM_{10–2.5}$ and tends to be more uniformly dispersed across an urban area or large geographic region, such as the Eastern United States. Atmospheric chemical transformations of $PM_{2.5}$ can take place locally, during atmospheric stagnation, or during transport over long distances. For example, the formation of $SO_4^=$ from SO_2 emitted by power plants with tall stacks can occur over distances exceeding 500 km and 12 h of transport time; therefore, the resulting $PM_{2.5}$ is well mixed within an airshed. Once formed, the very low dry deposition velocities of $PM_{2.5}$ contribute to its persistence and uniformity throughout a large air mass.

$PM_{10–2.5}$ generally deposits more rapidly than $PM_{2.5}$; as a result, $PM_{10–2.5}$ is less uniform in concentration across a region than $PM_{2.5}$. Coarse-mode particles >10 μm in aerodynamic diameter tend to fall out of the air most rapidly within the proximate area downwind of their emissions and have atmospheric lifetimes of only minutes to hours. The characteristics of $PM_{10–2.5}$ as regards atmospheric transport and deposition are intermediate between those of PM_{10} and $PM_{2.5}$.

9.4 AMBIENT AIR PM CONCENTRATIONS

9.4.1 Gravimetric PM Mass Concentration Monitoring

This chapter is focused primarily on $PM_{10–2.5}$ and $PM_{2.5}$, since human health considerations are of primary concern. Up until the mid-1980s, ambient air PM in the United States was generally measured as TSP, which included PM up to ~50 μm. An approximately fivefold reduction in TSP occurred during the decades preceding 1970, with further reduction in the late 1970s. Further reductions in both PM_{10} and $PM_{2.5}$ have occurred since then. Since TSP mass concentration can be dominated by particles too large to penetrate into the human thorax during inhalation, it provided a poor index of inhalation hazard. The mass concentration varies with season and climate, being especially severe in the arid portions of the Western United States. The major downward historical trend in TSP in the Eastern United States was due to the transition from burning coal for heating domestic and commercial buildings and its replacement by fuels with much lower ash contents, that is, light oil and natural gas.

9.4.2 Non-gravimetric PM Mass Concentration Estimation

Some PM monitoring systems determined PM concentrations by measuring the optical properties of the particles collected on a filter disk in terms of light reflectance (as for black smoke, aka "British Smoke" [BS], or for a similar index of blackness, known as KM) or in terms of light transmission through the filter [coefficient of haze (CoH)]. These metrics underestimate the mass concentration of light-colored ash particles and/or overestimate the particle mass of sample containing DEP. Such errors have complicated the interpretation of historic epidemiological data.

Major changes in pollution sources are generally accompanied by somewhat parallel reductions in all emitted pollutants insofar as new sources tend to be less polluting than their predecessors. Parallel reductions in ambient $PM_{2.5}$ concentrations have helped us to gain both a historic perspective on the effects of local fossil fuel combustion pollution impacts and, in recent years, an appreciation of PM impacts on regional scales.

In terms of historic perspective, Fig. 9.2 shows that, as coal smoke came under control in London, England, between 1960 and 1980, there were essentially proportionate reductions in BS, SO_2, and H_2SO_4 in ambient air PM.

However, even with relatively precise gravimetric measurements of sampled PM, there can be significant measurement artifacts. Positive artifacts can occur when the PM sampling filters, or the PM collected on them, are hygroscopic and extract water vapor- and gas-phase pollutants from the sampling stream by chemical reaction or sorption. Negative artifacts occur when sampled components of PM volatilize from the sampling substrate during or after sample collection. Semi-volatile constituents of the ambient air PM include

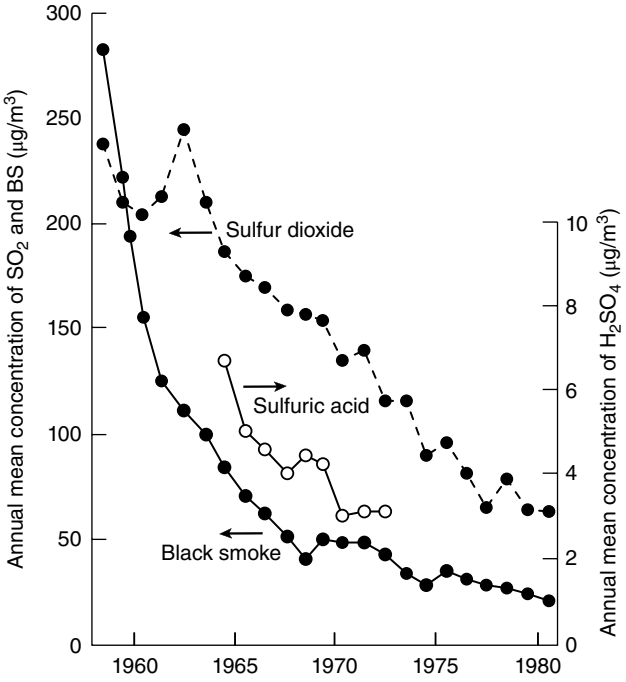

FIGURE 9.2 Long-term trends in annual mean atmospheric conditions of BS and SO_2 at seven stations in Greater London, and annual mean concentration of H_2SO_4 at St. Bartholomew's Hospital in Central London.

ammonium nitrate (NH_4NO_3) and volatile OC components that are formed by photochemical reactions. In Southern California, especially on hot summer days, NH_4NO_3 and OC volatilization from the sampling filter have accounted for substantial underestimations of the true ambient air PM_{10} concentrations and even greater underestimations of $PM_{2.5}$, since the more volatile components are largely within the $PM_{2.5}$ fraction.

Despite the inherent limitations of ambient air PM mass concentration limits, including (1) the assumption of equivalent toxicity of all sampled PM and (2) the sampling and analytical artifacts that limit the accuracy and precision of measured mass concentrations, there is a substantial body of epidemiological evidence that shows statistically significant associations between airborne PM mass concentrations and excess mortality, morbidity, and functional decrements. Furthermore, these effects appear to be coherent and not explicable on the basis of known potential confounding factors or coexisting gas-phase pollutants (Bates, 1992; Pope et al., 1995a; U.S. EPA, 2009). A discussion of this evidence follows a discussion of ambient air quality data and the relationships between ambient air PM concentrations and actual human exposures.

9.5 POPULATION EXPOSURES TO AMBIENT AIR PM

The concentrations of PM components in ambient air are important determinants of health-related responses to the inhalation of PM of outdoor origin, even when exposure, and thus the PM inhalation, occurs indoors. Factors influencing exposure include (1)

limited penetrability of PM of outdoor origin into indoor spaces, which varies with particle size and composition, building size, type of construction, heating and cooling systems, and wind velocity, (2) PM deposition on indoor surfaces, and (3) chemical transformations occurring while the PM is airborne. Each of these factors tends to reduce indoor exposures to some PM of outdoor origin. There will also be variations by season, based on minimal convective air exchange in midwinter and, for air-conditioned homes, in midsummer as well. Particle size affects penetrability, especially when infiltration pathways are reduced in order to save thermal energy. Under such conditions $PM_{10-2.5}$ penetration can be greatly reduced.

Once PM penetrates indoors, particle size and chemical composition become major determinants of its fate. Acidic particles will be more rapidly neutralized by NH_3 released into the indoor air by people, pets, tobacco smoking, and household product usage. Thus, the indoor/outdoor concentration ratio can be close to unity for a component such as $SO_4^=$, which is (1) almost entirely in the accumulation mode (0.1–2.5 μm), (2) chemically nonreactive, and (3) without indoor sources. EPA's Particle Total Exposure Assessment Methodology (PTEAM) study in Riverside, CA, reported a ratio of personal to outdoor $SO_4^=$ of $0.78 + 0.02$. For a chemically reactive PM component, such as hydrogen ion (H^+), the ratio is much lower.

When components of outdoor PM have significant indoor sources, the ratio of inhaled air to outdoor concentration can be much greater than unity. Major indoor PM sources include smoking, cooking, and cleaning activities that create a personal cloud based upon the release or resuspension of PM as we engage in dusting, sweeping, and conventional vacuum cleaning or when we generate personal clouds as we engage in routine activities whereby our motions resuspend settled dust from floors, furniture, and other surfaces. The PTEAM Study showed that elements in settled dust can be enriched in the personal cloud by a factor of about 2. On average, personal activities were associated with 37% of overall personal PM exposure, as compared with 21% for identifiable indoor sources (cooking, environmental tobacco smoke, and other) and 42% for PM of outdoor origin.

There are some extensive data sets for locations other than Riverside, CA, that indicate that the PTEAM results were not atypical (U.S. EPA, 1996a). Gravimetric PM concentrations measured indoors may bear little relation to ambient mass PM, and the compositions of indoor and outdoor PM can be very different. If the objective is to determine total exposure to $PM_{2.5}$ of outdoor origin, then one can use a conservative tracer of PM from outdoor sources, such as $SO_4^=$ (by ion chromatography) or S [by X-ray fluorescence (XRF) analysis], along with data on the ratio of $SO_4^=$ or S to $PM_{2.5}$.

9.6 EVIDENCE FOR ADVERSE HUMAN HEALTH EFFECTS DUE TO THE INHALATION OF AMBIENT AIR PM

9.6.1 Mortality Associated with Coal Smoke (When Measured as BS)

Quantitative information on adverse health effects associated with ambient air PM dates back to the 1873 London fog episode. A summation of bronchitis mortality during and following the December 1873 fog episode was tabulated in the U.K. Ministry of Health (1954) report of the subsequent December 5–9, 1952 episode. As shown in Table 9.2, various nineteenth-century fog episodes produced excesses in bronchitis deaths that were comparable with that reported for the more famous 1952 episode. There was a high baseline

TABLE 9.2 Excess Bronchitis Deaths Associated with Historic London Fogs

Dates of Fog	Av. Weekly Bronchitis Mortality	Excess Bronchitis Deaths in Week of Fog and During Succeeding 3 Weeks				Total 4 Week Excess in Bronchitis Deaths
December 9–11, 1873	in Previous 10 Years	Dec. 7–13	Dec. 14–20	Dec. 21–27	Dec. 28–Jan. 3	
	228	133	424	129	102	788
Jan. 26–29, 1880		Jan. 25–31	Feb. 1–7	Feb. 8–14	Feb. 15–21	
	294	258	939	453	167	1817
Feb. 2–7, 1882		Jan. 29–Feb. 4	Feb. 5–11	Feb. 12–18	Feb. 19–25	
	357	14	324	186	31	555
Dec. 21–24, 1891		Dec. 20–26	Dec. 27–Jan. 2	Jan. 3–9	Jan. 10–16	
	375	35	583	333	437	1388
Dec. 28–30, 1892		Dec. 25–31	Jan. 1–7	Jan. 8–14	Jan. 15–21	
	451	−55	208	154	2	309
Nov. 26–Dec. 1, 1948		Nov. 21–27	Nov. 28–Dec. 4	Dec. 5–11	Dec. 12–18	
	65	14	84	33	20	151
Dec. 5–9, 1952		Dec. 1–6	Dec. 7–13	Dec. 14–20	Dec. 21–27	
	86	−3	621	308	92	1018

Source: Adapted from Ministry of Health (1954).

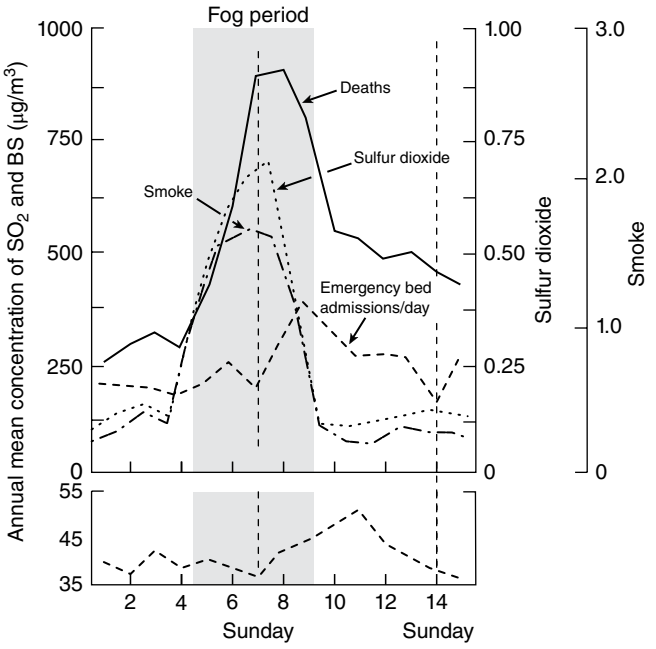

FIGURE 9.3 Metropolitan London total mortality and emergency bed admissions during the 1952 pollution episode in relation to black smoke, expressed as mg/m^3, and sulfur dioxide, expressed as parts per million by volume.

bronchitis mortality rate for London in the late nineteenth century, when the population was below 3 million (compared with about 8 million in 1952) and cigarette smoking was not yet common.

Two other notable early pollution episodes produced excess mortality before December 1952. Firket (1936) reported that the December 1930 fog episode in the Meuse Valley in Belgium was associated with 60 deaths from a population of ~6000. Schrenk et al. (1949) described a fog episode in Donora, PA, a valley town of about 10,000 people at a bend in the Monongahela River south of Pittsburgh within a valley containing steel mills, wire mills, zinc works, and a sulfuric acid plant along the riverbank. A persistent valley fog was associated with 20 excess deaths as well as acute morbidity among 43% of the population. About 10% were reported to have severe effects requiring medical attention. In a 10-year follow-up of the affected population, Ciocco and Thompson (1961) reported greater mortality rates and incidences of heart disease and chronic bronchitis among those residents who had reported acute illness in December 1948 than among residents who did not report such illness.

As shown for London in 1952 (Fig. 9.3), the daily death rate rose rapidly with the onset of the fog on December 5 and peaked one day later as indexed by the available daily pollutant measurements of BS and SO_2. The rise in hospital emergency admissions peaked 2 days after the pollutant peaks. Both the deaths and hospital admissions remained elevated for several weeks after the fog lifted (see Table 9.2). There were declines in hospital admissions on Sundays, a finding consistent with the known practices for hospital admissions.

The U.K. Ministry of Health (1954) report attributed an excess of ~4000 deaths from all causes to the fog exposures during the 1952 episode. The daily deaths peaked in the first full

week but were still above baseline levels 2 weeks later. The specific causes of excesses over 4 weeks were bronchitis (1156), with a relative risk (RR) = 6.7, and heart disease with 737 excess deaths (RR = 1.8). Most excess deaths were among those over 55 years of age (2616), but there were also excesses for all age groups beyond 4 weeks of age and were concentrated among the elderly with preexisting disease. The Bell et al. (2004) London mortality reanalysis for 1952 daily mortality extended the time interval of excess death counts out to 3 months and suggested that the overall impact of this episode included 12,000 excess deaths.

While more recent daily mortality studies show much lower absolute and RRs from contemporary PM pollution, elevated RRs are most marked among the very young and eldest, and the risk rankings among causes of death are quite similar today to those of December 1952. The 1954 report also noted clear associations between chronic air pollution and the annual incidence of bronchitis and other respiratory diseases. The bronchitis death rate from in England and Wales (with high coal smoke pollution) was much higher than in other northern European countries with much lower levels of coal smoke pollution. Also, it is likely that the high chronic exposure and the high prevalence of chronic bronchitis had created a large pool of individuals susceptible to "harvesting" by an acute pollution episode. The U.K. Clean Air Act of 1956 led to the mandated use of smokeless fuels in towns and cities, and annual mean BS levels declined, by 1962, to about half of the 1958 level. While the annual average SO_2 concentration had not declined by 1962, it dropped off markedly thereafter, along with a further marked decline in BS levels. For the period between 1964 and 1972, the measured levels of H_2SO_4 followed a similar decline. The December 1962 London fog episode was the last to produce a clearly evident acute short-term harvest of excess deaths, albeit a much smaller one than that of December 1952.

Commins and Waller (1963) developed a technique to measure H_2SO_4 in urban air and made daily measurements of H_2SO_4 at St. Bartholomew's Hospital in Central London during the 1962 episode. As shown in Fig. 9.4, the airborne H_2SO_4 rose rapidly during the 1962 episode, with a greater relative increase than that for BS.

In the first major time-series analysis of daily London mortality for the winter of 1958–1959, Martin and Bradley (1960) and Lawther (1963) reported that daily BS and SO_2 data were both associated with excess daily mortality for concentrations exceeding 750 µg/m^3. However, later analyses led to quite different conclusions. Ware et al. (1981) concluded that there was no demonstrable lower threshold for excess mortality down to the lowest

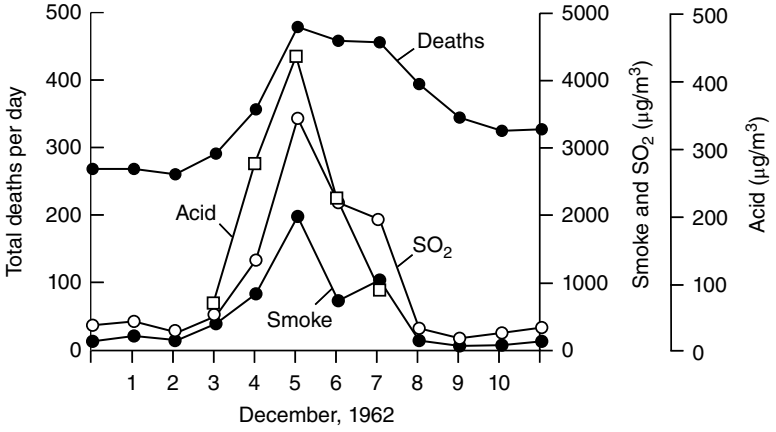

FIGURE 9.4 London pollution episode, December 1962.

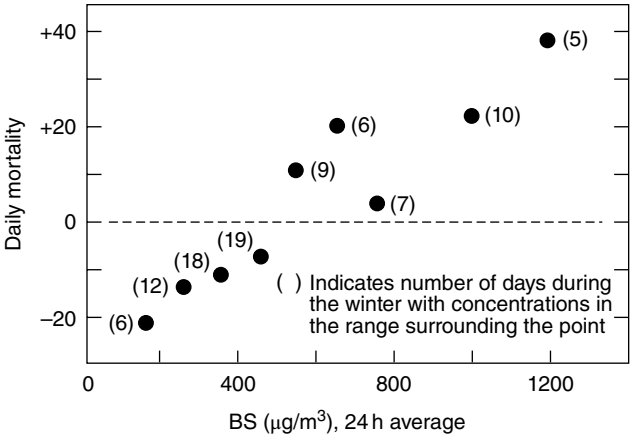

FIGURE 9.5 Martin and Bradley (1960) data for winter of 1958–1959 in London as summarized by Ware et al. (1981), showing average deviations of daily mortality from 15-day moving average by concentration of black smoke (BS).

TABLE 9.3 Standardized Annual Mortality Rate Regression Coefficients on Smoke[a] for 64 U.K. County Boroughs

Sex	Ages	Mortality in Year	Cancer of Trachea, Bronchus, and Lung	Chronic Bronchitis
Males	45–64	1969–1973	0.07	0.02
		1958–1964	0.53**	0.32*
		1948–1954	0.71***	0.48***
	65–74	1969–1973	0.15	−0.06
		1958–1964	0.68***	0.31
		1948–1954	0.87***	0.37*
Females	45–64	1969–1973	−0.02	−0.02
		1958–1964	−0.64**	0.33*
		1948–1954	0.49*	0.49**
	65–74	1969–1973	0.07	0.03
		1958–1964	0.25	0.40*
		1948–1954	0.61**	0.31

Source: Adapted from Chinn et al. (1981).
[a]Based on index of black smoke pollution 20 years before death of Daly.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

range of observation (BS ≈ 150 μg/m^3), as illustrated in Fig. 9.5. Although 150 μg/m^3 is now near the upper end of observed concentrations rather than at the lower end, time-series analyses still indicate effects with an increasing concentration–effect slope as concentrations decrease.

The marked reduction in U.K. BS pollution levels during the 1960s was shown to be associated with a marked reduction in annual mortality in County Boroughs by Chinn et al. (1981). As shown in Table 9.3, mortality rates in middle-aged and elderly men and women

for the 1969–1973 period were no longer associated with BS pollution. By contrast, for both the 1948–1954 and 1958–1964 periods, the index of BS exposure correlated strongly with annual mortality rates for both chronic bronchitis and respiratory tract cancers.

9.6.2 Morbidity Associated with Coal Smoke Pollution (as BS)

Lawther et al. (1970) reported the daily symptom scores of a panel of patients with chronic bronchitis in relation to the daily concentrations of BS and SO_2. There was a close correspondence between symptom scores and both pollutant indices. Chronic exposure also affected baseline lung function. Holland and Reid (1965) analyzed spirometric data collected on British postal workers in 1965 when pollution levels were well below their earlier peaks, but most postal workers had been chronically exposed out of doors for many years when pollution levels were higher. The London postal workers had lower forced expiratory volumes in one second (FEV_1) and peak expiratory flow rates (PEFR) than their country town counterparts.

9.6.3 Mortality and Morbidity Associated with Other Historic PM Indices

9.6.3.1 TSP TSP levels in the United States declined markedly between the 1950s and 1970s. The first U.S. National Health and Nutrition Examination Survey (NHANES I) was conducted between 1971 and 1975. Chestnut et al. (1991) described the relationship between pulmonary function and quarterly average levels of TSP for adults. Statistically significant relationships were observed between TSP levels and forced vital capacity (FVC) and $FEV_{1.0}$ for "never" smokers. A one standard deviation increase (about 34 $\mu g/m^3$) in TSP from the sample mean of 87 $\mu g/m^3$ was associated with an average FVC decrease in of 2.25%.

Other results from NHANES I included diagnoses of respiratory illness by examining physicians. After controlling for age, race, sex, and cigarette smoking, a 10 $\mu g/m^3$ increase in annual average TSP was associated with increased chronic bronchitis odds ratio (OR) = 1.07, with a 95% confidence interval (CI) = 1.02–11.2, while for a respiratory diagnosis, there was an OR = 1.06 and CI = 1.02–1.11. When the analyses were restricted to never smokers, the associations remained, with slight increases in the ORs.

In a study of the influence of chronic PM exposure on lung function of children and young adults (ages 6–24), Schwartz (1989) used TSP and NHANES II data 1976–1980. TSP, NO_2, and O_3 were all significantly associated with reduced FVC, FEV_1, and PEFR, but SO_2 was not. For TSP and O_3, there appeared to be thresholds for response to O_3 at ~40 ppb (daily average) and 90 $\mu g/m^3$ TSP. The relationships held whether or not children with respiratory conditions or smokers were included. Demographic and geographic variables had little or no impacts on the pollution relationships, which also held when only persons still residing in their state of birth were considered.

9.6.3.2 PM_{10}, $PM_{2.5}$, and $SO_4^=$ As shown in Table 9.4, beginning in the 1990s, there were many peer-reviewed papers describing time-series studies of the associations between daily mass concentrations of measured and/or estimated ambient air concentrations of major mass fractions of PM and daily rates of mortality and hospital admissions for respiratory diseases were published. For morbidity, there was also a rapid growth of the literature showing associations between airborne PM and exacerbation of asthma, increased symptom rates, decreased respiratory function, and restricted activities.

TABLE 9.4 Human Health Effects Associated with Human Exposures to $PM_{2.5}$, PM_{10}, and/or SO_4 or NO_2

$PM_{2.5}$- and PM_{10}-Associated Increased Effects	Population	Reference
Long-term exposures		
Mortality increase		
Annual mortality	U.S. cities	Lave and Seskin (1970)
Annual mortality	Six cities cohort	Dockery et al. (1993)
Annual CVD mortality	Six cities cohort	Lepeule et al. (2012)
Annual mortality	ACS cohort	Pope et al. (1995b, 2002)
Annual IHD mortality	ACS cohort	Pope et al. (2004b)
Annual CVD, COPD, and LC mortality	Beijing, PRC	Yin et al. (2017)
Annual mortality	U.S. cities	Fann et al. (2017)
Fatal and nonfatal CVD events	WHI cohort	Miller et al. (2007)
Annual CVD, resp., and lung cancer mortality	Dutch cohort	Fischer et al. (2015)
Annual mortality	New England	Shi et al. (2016)
Morbidity increase		
Incident kidney disease	U.S. VA cohort	Bowe et al. (2018)
Amyotrophic lateral sclerosis	The Netherlands	Seelen et al. (2017)
Low birth rate	Beijing, PRC	Xu et al. (1995)
Low birth rate	Beijing, PRC	Wang et al. (1997)
Low birth rate	Southern California	Ritz et al. (2000)
Asthma incidence	GA birth cohort	Pennington et al. (2018)
Neurological hospital admissions	Northeastern United States	Kioumourtzoglou et al. (2016)
Chronic bronchitis	U.S. women's cohort	Hooper et al. (2018)
Blood pressure	Taiwanese adults	Zhang et al. (2018)
Short-term exposures		
Short-term mortality increase		
Daily CVD mortality	100 NMMAPS counties	Dominici et al. (2007a)
Daily CVD mortality	Nine California counties	Ostro et al. (2007)
Daily CVD mortality	Barcelona, Spain	Ostro et al. (2011)
Daily IHD mortality	Canadian cohort	Crouse et al. (2012)
Daily CVD mortality	Barcelona residents	Perez et al. (2009)
Daily cerebrovascular mortality	Barcelona residents	Perez et al. (2009)
Daily mortality	New England	Shi et al. (2016)
Hospital admission increase		
CVD admissions	Southern Europe	Stafoggia et al. (2013)
CVD admissions	U.S. Medicare	Powell et al. (2015)
CVD, MI, and CHF admissions	26 U.S. communities	Zanobetti et al. (2009)
CVD and all-cause admissions	U.S. Medicare	Makar et al. (2017)
Daily COPD admissions	Beijing, PRC	Tian et al. (2018)
Pediatric ED admissions	Georgia (USA)	Strickland et al. (2016)
Dementia admissions	Northeastern United States	Kioumourtzoglou et al. (2016)
Morbidity and functional responses		
Cardiorespiratory morbidity	City of Atlanta	Sarnat et al. (2008)
Cardioverter defibrillator discharges	Eastern Massachusetts	Peters et al. (2000)

(continued)

TABLE 9.4 *(Continued)*

$PM_{2.5}$- and PM_{10}-Associated Increased Effects	Population	Reference
Ischemia, S-T, and T-segment depressions	WHI cohort	Zhang et al. (2009)
S-T segment depression Helsinki	Cardiac patients	Pekkanen et al. (2002)
S-T segment depression	Boston residents	Gold et al. (2005)
QT prolongation	MESA cohort	Van Hee et al. (2011)
Intraventricular conduction delay	MESA cohort	Van Hee et al. (2011)
Adverse ventricular repolarization	APACR study	Liao et al. (2010)
Exercise-induced ischemia	Amsterdam, Erfurt, and Helsinki	Lanki et al. (2006)
Pulmonary embolism	Nurses cohort	Pun et al. (2015)
HRV	Utah residents	Pope et al. (1999)
HRV	Boston residents	Gold et al. (2000)
HRV	Chapel Hill elderly	Devlin et al. (2003)
Pulse rate, HRV, peripheral basophils	Los Angeles elderly	Gong et al. (2004a)
HR, HRV	Los Angeles asthmatics	Gong et al. (2004b)
HRV	Taipei, Taiwan, CHD patients	Chuang et al. (2007)
HRV (patients with metabolic syndrome)	MESA cohort	Park et al. (2010)
HRV	Beijing taxi drivers	Wu et al. (2011)
CIMT	MESA cohort	Diez-Roux et al. (2008)
CAC	MESA cohort	Kaufman et al. (2016)
FMD	Ottawa, Canada	Dales et al. (2007)
Flow-mediated dilatation	Type 2 diabetics	Schneider et al. (2008)
Small artery elasticity	Type 2 diabetics	Schneider et al. (2008)
Vascular reactivity	270 Boston residents	O'Neill et al. (2005)
Fibrinogen, platelet, and WBC counts	NHANES III cohort	Schwartz (2001)
Plasma fibrinogen	London office workers	Pekkanen et al. (2000)
Platelets, thrombin, fibrinogen, and CRP	Rotterdam	Rudez et al. (2009)
Plasma tissue plasminogen activator	Edinburgh cardiac patients	Mills et al. (2008)
Blood flow	Edinburgh cardiac patients	Mills et al. (2008)
WBC	NHANES III cohort	Chen and Schwartz (2008)
SBP and PP	MESA cohort	Auchincloss et al. (2008)
SBP and DBP	Heinz Nixdorf cohort	Fuks et al. (2011)
Exhaled breath 8-isoprostane	Edinburgh cardiac patients	Mills et al. (2008)
hs-CRP	German cohort	Hoffmann et al. (2009)
Prothrombin time	Lombardy, Italy	Baccarelli et al. (2006)
Wheeze and shortness of breath	United Kingdom and the Netherlands	Doiron et al. (2017)
Wheeze exacerbation and asthma	LBW children in Massachusetts	Khalili et al. (2018)

CAC, coronary artery calcification; CIMT, carotid intimal-medial thickness; WBC, white blood cell count; SPB, systolic blood pressure; PP, pulse pressure; HR, heart rate; HRV, heart rate variability; hs-CRP, high sensitivity C-reactive protein; FMD, flow-mediated brachial artery dilatation.

Pope et al. (1995a) summarized literature from the early 1990s. They converted historically measured values for CoH and TSP to PM_{10} estimates and reported very similar coefficients of response for the PM_{10}-daily mortality associations for all locations. In Thurston's (1995) analysis of acute mortality studies in nine communities with measured PM_{10} concentrations, including 4 of the 10 studies cited by Pope et al. (1995a), the coefficients of response tended to be higher when the PM_{10} was expressed as a multiple-day average rather than a daily peak concentration and lower when other air pollutants were included in multiple regression analyses.

Zanobetti and Schwartz (2009) regressed daily mortality for 112 U.S. cities against both $PM_{2.5}$ and PM_{10} and reported statistically significant associations for total, CVD, MI, stroke, and respiratory mortality for both $PM_{2.5}$ and PM_{10}, but with smaller effects for $PM_{10-2.5}$.

Crooks et al. (2016) focused on daily mortality on dust storm days in the United States as a whole, using PM_{10} concentrations as an index of exposure. They reported that dust storms are associated with lagged non-accidental and CVD mortality.

As discussed in the SO_x chapter, $SO_4^=$ was often a relatively large fraction of $PM_{2.5}$ during the coal smoke era. It is non-volatile component, stable on filters used for air sampling, and easily extracted from filters and can be accurately analyzed with relatively simple and inexpensive procedures. Furthermore, it generally correlated with mortality and indices of morbidity as well as, or better than, other historic PM indices, such as TSP, BS, CoH, and PM_{10} studies.

9.6.3.3 TSP, BS, CoH, and PM_{10} Fischer et al. (2015) evaluated the associations between long-term exposures to PM_{10} with annual mortality in the Dutch Environmental Longitudinal Study (DUELS). The annual average ambient air concentrations of PM_{10} and NO_2 as modeled in high-resolution maps (100 × 100 m grids) in 2001 was correlated with mortality rates by disease cause (circulatory, respiratory, and lung cancer) for a cohort of 7.1 million Dutch residents greater than 30 years of age over 7 years (2004–2011). For each 10 μg/m^3 of PM_{10}, the respiratory mortality HR was 1.8 (1.07–1.09), while for NO_2 it was 1.03 (1.02–1.03). For lung cancer the PM_{10} HR was 1.26 (1.21–1.30), and for NO_2 was 1.10 (1.09–1.11). For circulatory, it was 1.06 (1.04–1.08) for PM_{10}, but 1.0 for NO_2. PM_{10} associations were robust to adjustment for NO_2.

The 1996 and 2004 PM CDs and the 2009 PM ISA concluded that the associations between PM_{10} and daily mortality were not seriously confounded by weather variables or the presence of other criteria pollutants. Figure 9.6 from the 2004 PM CD shows that the calculated relative acute mortality risks for PM_{10} are relatively insensitive to the concentrations of SO_2, NO_2, CO, and O_3.

9.7 HEALTH EFFECTS OF SPECIFIC PM COMPONENTS

The studies summarized in Tables 9.4–9.6, and discussed in the text that follows, are focused on PM components that are currently used or being considered to be possible future candidates for being index pollutants for ambient air sampling networks and/or ambient air standards and guidelines.

The findings summarized in Table 9.4 demonstrate that a broad range of studies of human health effects, ranging from excess mortality to admissions to critical care facilities to functional impairments. These responses have been significantly associated with

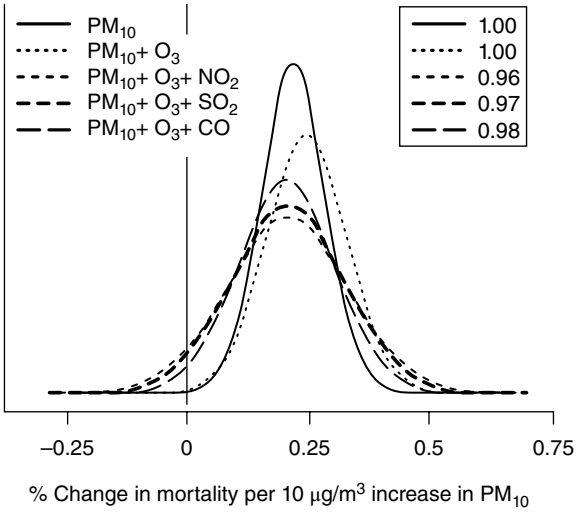

FIGURE 9.6 Marginal posterior distributions for effect of PM_{10} on total mortality at lag 1, with and without control for other pollutants, for the NMMAPS 90 cities. The numbers in the upper right legend are the posterior probabilities that the overall effects are greater than 0. *Source*: Dominici et al. (2007a).

exposure to $PM_{2.5}$ of outdoor origin, including both short-term exposures with acute effects and long-term exposures with chronic effects. The effects have not been limited to the respiratory tract, where the inhaled PM is deposited, and have often been greater in the cardiovascular system. Furthermore, studies exploring effects in other organs and systems have recently extended our concern to the renal, and neurological, and pediatric responses, indicating that PM constituents are dissolving in bodily fluids, reach other organs via the circulating bloodstream, and have other adverse effects.

Since the exposure data available for the studies summarized in Table 9.4 have been limited to on PM mass concentrations, these studies cannot inform us on the nature and extent of the concentrations of the components contributing to the effects. It is highly unlikely that all of the PM components were equally soluble in bodily fluids or had similar toxicities at sites of biological actions. Thus, it is important to identify the components that are most toxic and their dose–response relationships.

As shown in Table 9.5, there have been many recent studies in human populations that have used data on PM components to tease out evidence that specific PM components have had significant associations with health-related responses. It is notable that some of these studies focused on source categories and/or major mass fractions of the PM (in $\mu g/m^3$), while others focused on the mass concentrations of some of the trace fractions (in ng/m^3 or particle number concentrations). The high frequency of statistically significant associations of responses with extremely low concentrations of metals, primarily transition metals, is remarkable, since the measurements in almost all of the studies were made by X-ray diffraction. Such measurements indicate total rather than the soluble fraction, which can affect non-respiratory tract responses. Furthermore, for those trace metals that were measured, their concentrations were often below the detection limits on many of the days, limiting the ability of the investigators to define exposure–response relationships from their regressions with responses. Some responses to ambient air PM may also be influenced by exposures to freshly generated reactive organic PM components, but these may be

TABLE 9.5 Associations of $PM_{2.5}$ Components (Including Trace Elements) and Health Indices in Human Populations, Panels, and CAPs Exposure Studies

Effect	Components or Sources Associated	Reference
Long-term exposures		
Annual mortality	Coal comb., traffic, metals industry	Ozkaynak and Thurston (1987
Annual mortality	Coal combustion, soil factors	Laden et al. (2000)
Annual mortality	Traffic factor, Ni, V	Lipfert et al. (2006)
Annual mortality	Traffic factor, SO_4, Mn, Cl, As, EC, Ni	Lipfert et al. (2009)
Annual mortality	S, Ni, K	Beelen et al. (2015)
Annual CVD mortality	Ni, V	Hedley et al. (2002, 2004)
Annual CVD mortality	EC, OC, SO_4, Cu, Fe, Mn, V, Zn	Ostro et al. (2007)
Annual IHD mortality	K, OC, SO_4, NO_3, Fe, Si, Zn, EC	Ostro et al. (2010)
Annual IHD mortality	S, Ni, V	Cahill et al. (2011)
Annual IHD and lung cancer mortality	Coal comb., Se, As	Thurston et al. (2016b)
Annual lung cancer incidence	Cu, Zn, S, Ni, K	Raaschou-Nielsen et al. (2016)
Annual chronic bronchitis and emphysema	House dust endotoxin	Mendy et al. (2018)
Low birth weight	Ni, Al, and EC	Ebisu and Bell (2012)
Annual coronary events	K, Ni, Si, S, K	Wolf et al. (2015)
Short-term exposures		
Mortality increase		
Daily mortality	SO_4, Fe, Ni, Zn	Burnett et al. (2000)
Daily mortality	Ni, V	Lippmann et al. (2006)
Daily mortality	Al, Ni, SO_4, As	Franklin et al. (2008)
Daily mortality	Traffic, Fe, Ni, Zn, V, Cu, Cr	Ito et al. (2013)
CVD mortality	Se, SO_4, EC, OC, Si	Ito et al. (2011)
CVD mortality	SO_4, NO_3, NH_3	Son et al. (2012)
CVD mortality	Traffic, oil comb., SO_4, NO_3, soil, road dust	Ostro et al. (2011)
Hospital admission increase		
Daily admissions	Traffic, Fe, Ni, Zn, V, Cu, Cr	Ito et al. (2013)
CVD admissions	Traffic, oil and coal comb., metals	Janssen et al. (2002)
CVD admissions	Ni, V, EC	Bell et al. (2009)
CVD and resp. admissions	Ni, V, EC	Bell (2012)
CVD and resp. admissions in elderly people	Ni, V, EC	Bell et al. (2014)
CVD and resp. admissions	Mn, Zn, Ni	Basagana et al. (2015)
CVD hosp. admissions	Se, Zn, OC, EC, SO_4, Ni, V	Ito et al. (2011)
CVD hosp. admissions	Transition metals	Suh et al. (2011)
CVD and resp. admissions	EC, OC, $SO_4^=$, NO_3^-	Kim et al. (2012)
CVD admissions	Fe, V, $SO_4^=$, EC, OC	Ye et al. (2018)
Stroke admissions	BC, Ni, S	Mostofsky et al. (2012)
Emergency department visits		
CVD ED visits	Traffic, biomass burning	Sarnat et al. (2008)

(continued)

TABLE 9.5 *(Continued)*

Effect	Components or Sources Associated	Reference
CVD and resp. visits	EC, OC, and Ni for CVD: EC and OC for resp.	Ostro (2016)
Other short-term responses		
Pediatric otitis media	EC, NO_2	Brauer et al. (2006)
Pediatric resp. symptoms	Ni, V, and Zn	Patel et al. (2009)
Asthma symp—adolescents	BC, NO_2	Patel et al. (2010)
HR	Ni	Hsu et al. (2011)
HR	As, Ca, Ce, Fe, Mg, Mn, P, Rb, S, Se, Ti	Morishita et al. (2015)
HRV	Oil combustion, long-range transport	de Hartag et al. (2009)
HRV	Ca, Ni, Fe	Wu et al. (2011)
HRV	Ni, Zn, Cr	Wu et al. (2012)
Plasma fibrinogen	Cu/Zn/V factor	Ghio et al. (2000)
PMNs and platelets	Fe, Se, SO_4 factor	Ghio et al. (2000)
Syst. blood pressure	Ni	Dai et al. (2016)
WBCs and IL-6	Cr	Riediker et al. (2004) and Riediker (2007)
BUN, R-R interval	Cu	Riediker et al. (2004) and Riediker (2007)
Uric acid and vWF	Ca	Riediker et al. (2004) and Riediker (2007)
Oxidative stress	V, Cr	Sorensen et al. (2005)
Reactive hyperemia	Cr, Mn, Ni, Cu, Fe, V, BC	Zhang et al. (2016)
FEV_1 and CVD function	$PM_{2.5}$ and UFP	Sinharay et al. (2018)

BUN, blood urea nitrogen; CVD, cardiovascular disease; ED, emergency department; HR, heart rate; HRV, heart rate variability; PMN, polymorphonuclear neutrophils; vWF, von Willebrand factor; WBC, white blood cells; UFP, ultrafine PM.

short-lived in the atmosphere, and few studies have had data on the airborne concentrations of reactive as well as aged organic species.

In Table 9.6, associations between trace metals in ambient air and responses in animals exposed under controlled conditions in laboratory settings at different locations are too few to draw firm general conclusions, but do show at least some consistency with those summarized in Table 9.5.

9.7.1 Studies Based on $PM_{2.5}$ and/or $PM_{10–2.5}$

Stafoggia et al. (2013) found significant associations between both $PM_{2.5}$ and $PM_{10–2.5}$ and cardiovascular and respiratory hospital admissions in Southern Europe. For cardiovascular, a 10 μg/m^3 increase in $PM_{2.5}$ and a 6.3 μg/m^3 increase in $PM_{10–2.5}$ were associated with associated with increases in 0.51% (0.12–0.90), and 0.46% (0.1–0.82), respectively. For respiratory, the increases were 1.4% for $PM_{2.5}$ and 1.2% for $PM_{10–2.5}$.

TABLE 9.6 Associations of $PM_{2.5}$ Components and CVD Effects in CAPs Exposure Studies in Laboratory Animals

	Species	Location	Components	Reference
Short-term effects				
CVD effects of short-term inhalation exposures to $PM_{2.5}$ and $PM_{2.5}$ components				
HR	Rats	Boston		Wellenius et al. (2004)
HR, cardiac oxidative stress	Rats	Boston		Ghelfi et al. (2008)
Lipid peroxidation, edema	Rats	Boston		Ghelfi et al. (2008)
QT, RT, Pdur, Tpe intervals	Rats	Boston		Ghelfi et al. (2008)
Blood flow	Dogs	Boston		Bartoli et al. (2009)
Cardiac vascular resistance	Dogs	Boston		Bartoli et al. (2009)
WBC and lymphocyte counts	Dogs	Boston	Al/Si factor	Clarke et al. (2000)
Small pulmonary arteries	Rats	Boston	Si, Pb, SO_4, EC	Batalha et al. (2002)
Oxidative stress	Rats	Boston	Fe, Al, Si, Ti	Gurgueira et al. (2002)
S-T segment elevation	Rats	Boston	Si, Al, Fe, Ti	Wellenius et al. (2003)
HR and HRV	Rats	Boston	Al, Si, Fe	Rhoden et al. (2005)
HR and HRV	Mice	Tuxedo, NY	Ni	Lippmann et al. (2006)
Long-term effects				
CVD effects of long-term inhalation exposures to $PM_{2.5}$				
Vasomotor tone	Mice	Tuxedo, NY		Sun et al. (2005)
Vascular inflammation	Mice	Tuxedo, NY		Sun et al. (2005)
Potentiated atherosclerosis	Mice	Tuxedo, NY		Sun et al. (2005)
Macrophage infiltration	Mice	Tuxedo, NY		Sun et al. (2008a)
Tissue factor expression	Mice	Tuxedo, NY		Sun et al. (2008a)
Aortic vasoconstriction	Rats	Tuxedo, NY		Sun et al. (2008b)
Nonalcoholic fatty liver	Mice	Tuxedo, NY		Tan et al. (2009)
Glucose metabolism	Mice	Columbus, OH		Zheng et al. (2013)
Insulin resistance	Obese mice	Tuxedo, NY		Sun et al. (2009)
Insulin resistance	Mice	Tuxedo, NY		Xu et al. (2011b)
Insulin resistance	Mice	Columbus, OH		Xu et al. (2012)
Altered vasomotor tone	Mice	Tuxedo, NY		Ying et al. (2009a)
Enhanced atherosclerosis	Mice	Tuxedo, NY		Ying et al. (2009a)
Potentiated hypertension	Mice	Columbus, OH		Ying et al. (2009b)
Allergic airway disease	Mice	Los Angeles, CA		Kleinman et al. (2007)

Powell et al. (2015) studied Medicare hospital admissions associated with ambient air $PM_{10–2.5}$ concentrations in 110 U.S. communities. There was a statistically significant association of $PM_{10–2.5}$ with for CVD admissions, but not for respiratory admissions.

Fann et al. (2017) estimated historical $PM_{2.5}$ concentrations, and the effects of changes in exposures on mortality in adults (age ≥30 years), and on life expectancy at birth, in the contiguous United States during 1980–2010. Between 1980 and 2010, population-weighted

exposures fell by about half, with the estimated excess deaths declining by about a third, with CA, VA, NJ, and GA having some of the largest estimated reductions in $PM_{2.5}$-attributable deaths. Relative to a counterfactual population with exposures held constant at 1980 levels, people born in 2050 would experience an ~1-year increase in life expectancy at birth, with a cumulative gain of 4.4 million life years among adults ≥30 years of age.

Hooper et al. (2018) regressed 5–7 years of the ambient air concentrations of PM_{10}, $PM_{2.5}$, and NO_2, as estimated from long-term mean concentrations, determined using a national land use regression model, for the residence locations of individual women participating in a study of sisters of women with breast cancer for their incidence and prevalence of chronic bronchitis and cough and phlegm by questionnaire. Prevalent chronic bronchitis was associated overall with PM_{10} (IQR = 5.8 μg/m^3, RR = 1.07; CI: 1.01–1.13) and with $PM_{2.5}$ in never smokers, $PM_{2.5}$ (IQR = 4.4 μg/m^3; RR = 1.18; CI: 1.04–1.34).

Tian (2018) examined 2 years of associations of short-term outpatient and inpatient visits for COPD in Beijing, PRC, and $PM_{2.5}$ (IQR = 90.8 μg/m^3). There were increases in both outpatient visits per IQR (2.4%, CI: 2.2–2.5) and inpatient (6.0%, CI: 5.2–6.9).

9.7.2 Aerosol Optical Density (AOD) as an Index of $PM_{2.5}$ over Large Spatial Regions

Kloog et al. (2014) developed a hybrid method for assessing temporally and spatially resolved $PM_{2.5}$ concentrations for epidemiological studies by combining 1 km × 1 km resolution satellite-retrieved AOD measurements with land use terms, meteorological variables, and their interactions. Shi et al. (2016) used this technology for the New England states to estimate both daily and annual mortality attributable to $PM_{2.5}$ during the years 2003–2008, as shown in Fig. 9.7. For each 10 μg/m^3, daily mortality increased by 2.1% (CI: 1.4–2.9), and annual mortality increased by 7.5% (2.0–13.4), with the rate of increase increasing at concentrations >6 μg/m^3.

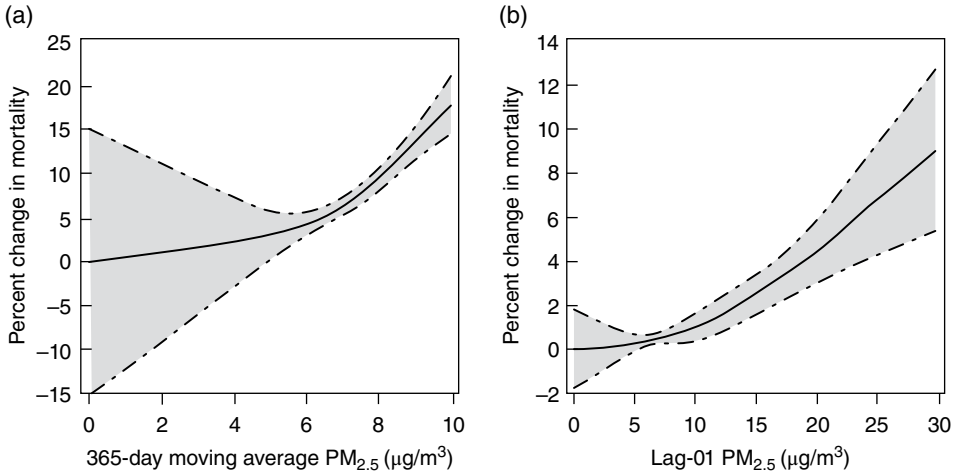

FIGURE 9.7 Exposure–response relationship between annual mortality and mean annual Lag-01 $PM_{2.5}$ ambient air concentration with: (a) mutual adjustment and (b) without mutual adjustment shaded areas show 95% CI. *Source*: Adapted from Shi et al. (2016).

Yin et al. (2017) studied ~190,000 men from 45 Chinese areas in relation to $PM_{2.5}$ concentration estimates based on models developed by Global Burden of Disease. The mean $PM_{2.5}$ for 2000–2005 was 44 μg/m^3 (ranging from 4.2 to 84 μg/m^3). Mortality hazard ratios (HRs) per 10 μg/m^3 increase for overall, CVD, COPD, and lung cancer were 1.09 (CI: 1.08–1.09), 1.12 (1.10–1.13), 1.12 (1.07–1.13), and 1.12 (1.07–1.14).

Zhang et al. (2018) regressed the mean ambient air concentrations of $PM_{2.5}$, as estimated from long-term mean concentrations and determined using a national land use regression model, for the residence locations of ~360,000 adults in Taiwan against systolic blood pressure (SBP) and diastolic blood pressure (DBP) and pulse pressure (PP). Each 10 μg/m^3 of $PM_{2.5}$ was associated with increases of SBP, DBP, and PP of 0.45, 0.07, and 0.38 mmHg, respectively (CIs: 0.40–0.50, 0.04–0.11, and 0.33–0.42). Also, there was an increase of 3% in the risk of developing hypertension.

9.7.3 Daily Mortality Effects in Adults

Table 9.5 lists studies of significant associations of $PM_{2.5}$ components on short- and long-term exposures on short- and long-term health effects. The first study to examine the use of chemical components to identify sources of PM and their associations with daily mortality was the Harvard Six Cities Study, with Laden et al. (2000) reporting that the mobile-source component accounting for a 3.4% (CI: 1.7–5.2%) increase, while the coal combustion source accounted for a 1.1% (CI: 0.3–2.0%) increase. The crustal components were not associated with excess daily mortality.

Chemical speciation network (CSN) data were also used in the studies relating PM components to available daily mortality data from the NMMAPS study (Dominici et al., 2007a). Other studies also supported $PM_{2.5}$ as a useful index of excess mortality risk. Those having access to CSN data implicated transition metals (Burnett et al., 2000; Hedley et al., 2002, 2004; Lipfert et al., 2006; Lippmann et al., 2006; Franklin et al., 2008; Bell et al., 2009) and traffic markers (Pekkanen et al., 2000; Ebelt et al., 2005; Gold et al., 2005; Schwartz et al., 2005; Ostro et al., 2007, 2008) or both metals and traffic markers (Janssen et al., 2002) within the $PM_{2.5}$ as being especially likely to be causal factors.

Ito et al. (2011) studied the associations of $PM_{2.5}$ and its constituents in NYC for those over 40 years of age for 2000–2006. Daily excess cardiovascular mortality was associated with EC, OC, Ni, V, Zn, Se, Br, $SO_4^=$, NO_3^-, Na^+, NO_2, SO_2, and CO.

Ostro et al. (2011) used speciation data collected every sixth day in factor analyses to determine the contributions of source categories to $PM_{2.5}$ and daily total and CVD mortality risks in a case-crossover study of Barcelona, Spain, for 2003–2007. The statistically significant increases for cardiovascular mortality for 2-day lags were traffic (10.3%), $SO_4^=$ (7.2%), road dust (6.7%), minerals (6.6%), NO_3^- (5.5%), and fuel oil combustion (4.6%).

Son et al. (2012) studied the associations of 14 months of 1-h average concentrations of $PM_{2.5}$, EC, OC, Mg, Na, K, Ca, Cl^-, NH_4^+, $SO_4^=$, and NO_3^- with total, cardiovascular, and respiratory mortality in Seoul, South Korea. Of 92 daily deaths, 22.4 were classified as cardiovascular and 5.4 as respiratory. For cardiovascular mortality, there were associations ($p = 0.1$) for an IQR in NH_4^+, $SO_4^=$, and NO_3^-, while for respiratory mortality, the only components with $p < 0.1$ were Cl^- and Mg.

In NYU's National Particle Component Toxicity (NPACT) study, Ito et al. (2013) used factor analysis of the 2000–2005 nationwide EPA CSN data on the composition of $PM_{2.5}$ at 167 sites in 102 metropolitan statistical areas (MSAs) throughout the United States to identify major elemental groupings, interpretable as being associated with specific $PM_{2.5}$ source

categories, at many U.S. locations. The major source categories identified, and their key elements, were metals industry (Pb, Zn), soil (Ca, Si), motor vehicles (OC, EC, NO_2), steel industry (Fe, Mn), coal combustion (As, Se), oil combustion (V, Ni), and salt (Na, Cl). There were close associations of daily mortality with exposure to traffic-related effluents (EC, OC, and road dust) and with one or more transition elements (Fe, Ni, Zn, V, Cu, Cr). The only significant mortality associations were (1) with the traffic source factor and with constituents associated with traffic, such as EC, OC, Cu, and Pb, and with those associated with resuspended road dust (Si and Fe). In the second-stage analyses, which identified the $PM_{2.5}$ constituents that have the greatest influence on the associations of $PM_{2.5}$ mass concentrations for the second-stage analysis for daily mortality, $SO_4^=$ was the most influential constituent, and Se and As showed positive, albeit nonsignificant associations.

Ostro et al. (2016) performed case-crossover regressions of source-specific 2005–2009 $PM_{2.5}$ estimates (vehicular, NO_3^-, $SO_4^=$, biomass burning, soil, and trace metals) for eight major CA metro areas against ED visits for CVD, asthma, and all respiratory visits. For CVD, there were significant associations for EC, K, and Zn, while for respiratory ED visits, there were significant associations for EC, OC, NO_3^-, Zn, V, Cu, Fe, K, Si, and Mn. In terms of sources, the only significant associations were for vehicular for all CVD, acute myocardial infarction, and dysrhythmia.

9.7.4 Effects on Infant Mortality

Another aspect of the influence of PM on short-term mortality is its role in sudden infant death syndrome (SIDS). Woodruff et al. (1997) examined the relationship between post-neonatal infant mortality (28 days to 1 year) and PM_{10} in the United States for approximately 4 million infants born between 1989 and 1991 in 86 MSAs in the United States. They combined data from the National Center for Health Statistics (NCHS) birth/infant death records with measurements of PM_{10}. Infants were categorized as having high, medium, or low exposure. After adjustment for other covariates, the odds ratio (OR) and 95% confidence intervals (CI) for total post-neonatal mortality for the high exposure versus the low exposure group was 1.10 (1.04, 1.16). In normal birth weight infants, high PM_{10} exposure was associated with respiratory causes (OR = 1.40 [1.05, 1.85]) and SIDS (OR = 1.26 [1.14, 1.99]). In a follow-up study of post-neonatal deaths in relation to $PM_{2.5}$ in California, Woodruff et al. (2006) reported that the OR for a 10 μg/m^3 increase in $PM_{2.5}$ was 1.07 (0.93–1.24) overall, 2.13 (1.12–4.05) for respiratory causes, and 0.82 (0.55–1.23) for SIDS.

Studies in Mexico City and in the Czech Republic have also reported associations between PM and infant mortality. For Mexico City (average $PM_{2.5}$ of 27 μg/m^3), Loomis et al. (1999) reported an 18.2% excess in infant mortality per 25 μg/m^3 (CI: 6.4–30.7). Bobak and Leon (1999) reported that long-term exposure to PM for the whole Czech Republic was associated with excess neonatal and post-neonatal deaths.

9.7.5 Effects on Daily Hospital Admissions

9.7.5.1 Cardiovascular and Respiratory Hospital Admissions Suh et al. (2011) examined average daily hospital admissions for Medicare patients in Atlanta, GA, in relation to the concentrations of groups of $PM_{2.5}$ components. Significant increases in CVD admissions were associated with (1) the transition metal oxides group (for those with >90% of the concentrations above the lower limit of detection, i.e., Cu, Mn, Zn, Ti, and Fe) and

(2) the alkane group. In terms of specific disease categories, the transition metals were significantly associated with admissions for IHD, congestive heart failure (CHF), and atrial fibrillation (AFib). The alkane group was associated with respiratory-related admissions. In contrast, the aromatic group, and As, Br, Se, Pb, and Si, were associated with decreased CVD- and respiratory-related hospital admissions.

Ito et al. (2011) studied the associations of $PM_{2.5}$ and its components (EC, OC, Ni, V, Zn, Se, Br, $SO_4^=$, NO_3^-, and Na^+) and also with NO_2, SO_2, and CO with daily excess cardiovascular hospitalizations in NYC for those over 40 years of age for 2000–2006. There were excess risks for CVD hospitalizations with 0-day lag in the colder 6 months for $PM_{2.5}$, EC, OC, Ni, Zn, Si, Se, Br, NO_3^-, NO_2, SO_2, and CO, but none for longer lags. In the warmer 6 months, the only components associated with statistically significant risks were EC, OC, NO_2, and CO.

Mostofsky et al. (2012) used data from 1060 patients admitted to a Boston medical center with ischemic stroke in 2003–2008. The relative rankings of the associations between constituents and ischemic stroke were fairly consistent across models. The strongest associations were for EC, with Ni being second strongest with an average concentration of only 2.3 ng/m^3 (0.02% of $PM_{2.5}$). The association with Ni at such a low concentration may seem unlikely from a toxicological perspective, but the fact that it also has been associated with CVD effects in other cited studies suggests that it, or the other pollutants emitted, with it from fossil fuel combustion sources, could be causal factors.

Kim et al. (2012) examined the temporal lag structure of the associations of CVD and respiratory hospitalizations in Denver, CO, for 2003–2007 for $PM_{2.5}$, but only for its major mass components, that is, EC, OC, $SO_4^=$, and NO_3^-. For IHD, the only significant associations were for EC and OC concentrations at 0-day lag, and for EC at both 0- and 1-day lags. For CHF, only OC had significant associations (at lag days 2 and 3). For cerebrovascular disease, there were significant negative associations with $PM_{2.5}$ for lag days 1–4. For asthma, there were significant associations with $PM_{2.5}$ for lag days 4–12, with EC for lag days 2–11, with OC and $SO_4^=$ for lag days 4–11, and with NO_3^- for lag days 5–12. For pneumonia, there were significant negative associations with OC for lag days 5–11.

Bell (2012) found that $PM_{2.5}$ component concentrations and hospitalizations for cardiovascular and respiratory diseases varied across the United States as well as over seasons. There were (1) statistically significant increases in CVD admissions associated with same-day $PM_{2.5}$ in spring and fall, which were largest in winter; (2) regional differences that were greatest across 108 Northeastern U.S. counties; and (3) increases in respiratory hospitalizations that were most pronounced on the second day. EC, the largest fraction of $PM_{2.5}$ mass, explained the variation. Among minor mass components, Ni and V were associated with largest effect estimates for both cardiovascular and respiratory hospitalizations. PM_{10} associations with mortality were larger in regions and seasons with higher fractions of V, and particularly of Ni. EC, a mass component with concentrations near or above 1 μg/m^3, was implicated as a cause of respiratory hospitalizations, while Ni and V, which were typically below 10 ng/m^3, had the largest effect estimates for both respiratory and cardiovascular hospital admissions.

In the Ito et al. (2013) component of the NYU NPACT study, there were close associations of daily hospital admission rates with exposure to traffic-related effluents (EC, OC, and road dust) and with one or more transition elements (Fe, Ni, Zn, V, Cu, Cr) in most of the prior short-term exposure studies. The short-term hospital admissions findings indicated that the only significant associations were (1) with the traffic source factor, and with components associated with traffic, such as EC, OC, Cu, and Pb, and with those associated

with resuspended road dust (Si and Fe). In the second-stage analyses, which identified the $PM_{2.5}$ components that have the greatest influence on the associations of $PM_{2.5}$ mass concentrations with short-term health effects and accounted for pollutant concentration correlations, the pollutants and other variables most closely associated with cardiovascular hospital admissions included residual oil combustion components (Ni, V, S, and seaport berth volume), as well as traffic-related factors (Cu and the lengths of major roads), with $SO_4^=$ showing a nonsignificant negative association. There was little evidence for an association of hospital admissions with markers for coal combustion effluents ($SO_4^=$, As, and Se). On the other hand, for the second-stage analysis for daily mortality, $SO_4^=$ was the most influential component, and Se and As showed positive, albeit nonsignificant associations. These findings suggested that, even for short-term peak exposures, the components causing hospital admissions (OC and transition metals) differed from those associated with daily mortality (coal combustion effluents).

Bell et al. (2014) examined associations of $PM_{2.5}$ components (Ca, BC, V, and Zn) and sources (traffic, road dust, oil combustion, sea salt, and coal combustion) with hospital admissions for four counties in Connecticut and Massachusetts for persons >65 years of age. As shown in Fig. 9.8, for cardiovascular admissions, there were significant associations with road dust, BC, V, and Zn. For respiratory, there were significant associations with road dust, BC, Al, Ca, Si, Ti, Ni, and V, and the effect estimates were generally robust to adjustments for other components.

Basagana et al. (2015) studied daily hospital admissions in five cities in Southern Europe (Barcelona, Madrid, and Huelva in Spain and Rome and Bologna in Italy). There were increases in both CVD and respiratory admissions associated with EC, $SO_4^=$, SiO_2, Ca, Fe, Zn, Cu, Ti, Mn, V, and Ni, but only Mn, Zn, and Ni remained significant after adjustment for PM mass.

Zhang et al. (2016) studied a panel of 93 elderly nonsmoking adults in Los Angeles in terms of microvascular function, as represented by reactive hyperemia index (RHI), in relation to daily mean concentrations of $PM_{2.5}$, $PM_{0.18}$, $PM_{0.18–0.25}$, $PM_{0.25–10}$, BC, OC, ROS, CO, NO_x, and O_3. There were significant reductions in RHI associated with daily exposure to obese subjects (BMI > 30) for BC, EC, OC, NO_x, CO, and ROS; for former smokers with fine EC, OC, and ROS; and for transition metals (Cr, Mn, Ni, Cu, and Fe), but an increase in RHI for V.

Ye et al. (2018) regressed the measured concentrations of O_3, NO_2, SO_2, CO, $PM_{2.5}$, a variety of $PM_{2.5}$ major components (OC, EC, $SO_4^=$, NO_3^-), and minor components (water-soluble metals), against cardiovascular hospital ED admissions for Atlanta, GA, for 2008–2013. Among the metals, the strongest association was for water-soluble Fe (IQR = 20.5 ng/m^3, RR = 1.012; CI: 1.005–1.019). When controlling for soluble Fe, soluble V had an IQR = 0.19 ng/m^3 (RR = 1.102, CI: 1.00–1.025).

Respiratory Emergency Responses Sarnat et al. (2008) studied the influence of $PM_{2.5}$ source factors (gasoline-powered engines, diesel powered engines, wood smoke, resuspended soil (RS), cement kiln, railroad, and metal processing, as well as $SO_4^=$, NO_3^-, on cardiorespiratory morbidity in Atlanta, GA, for 1999–2002). There were positive associations between $PM_{2.5}$ attributed to mobile sources and biomass burning for emergency department (ED) visits for CVD. For the summer months, $SO_4^=$ was significantly associated with respiratory ED visits.

Strickland et al. (2016) studied associations in Georgia of short-term $PM_{2.5}$ concentrations at zip codes with pediatric ED admissions for asthma or wheeze (2–18 years of age),

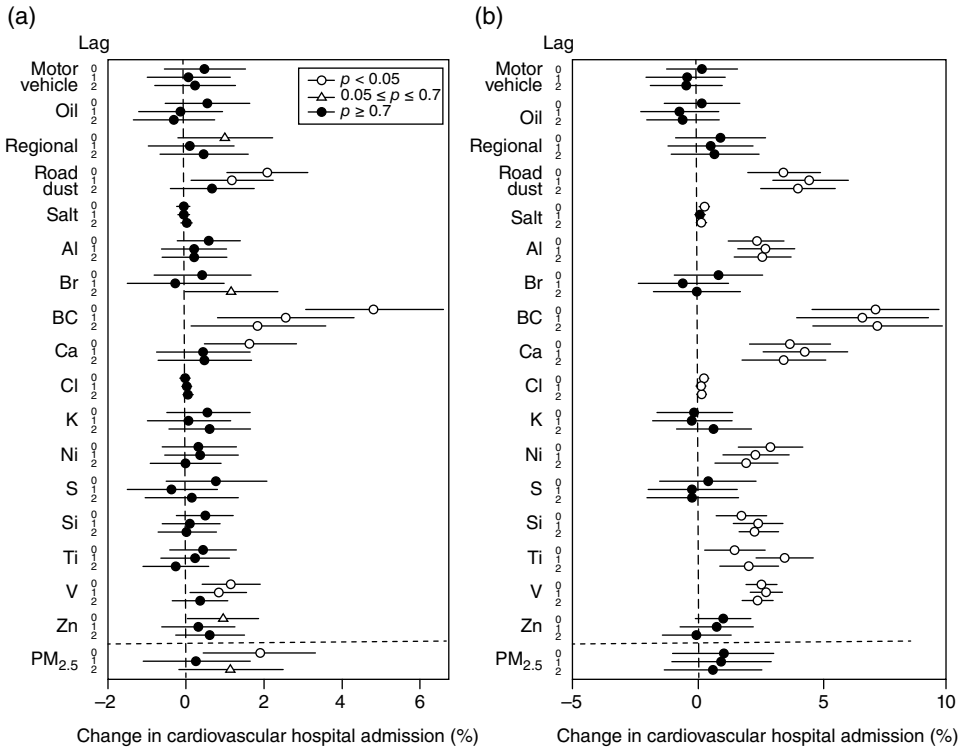

FIGURE 9.8 Change (%) in risk of hospital admissions for (a) cardiovascular or (b) respiratory causes associated with $PM_{2.5}$ and its components. The horizontal lines represent 95% CIs. *Source*: Adapted from Bell et al. (2014).

bronchitis (0–18 years), chronic sinusitis, otitis media, pneumonia, and upper respiratory infections. A 10 μg/m^3 increase in $PM_{2.5}$ was associated with ED visits for asthma or wheeze with an OR = 1.103 (1.003–1.023), and an OR = 1.015 (1.008–1.022) for upper respiratory infections.

Ostro et al. (2016) studied associations of source-specific $PM_{2.5}$ with ED visits in eight CA metropolitan areas for 2005–2009. The soil source (IQR = 0.9 μg/m^3), which includes road dust, had the highest excess risk (for asthma with a 2-day lag) at 4.5% (1.1–8.0). The next highest risk, for asthma, was vehicular emissions (IQR = 2.8 μg/m^3) was 1.9% at 1-day lag (0.5–3.5). Among cardiovascular risks, the highest were for IHD with vehicular emissions (IQR = 2.8 μg/m^3) for a 0-day lag at 1.7% (−0.1–2.3) and dysrhythmia for a 2-day lag at 2.0% (0.0–4.0). For individual $PM_{2.5}$ components, the highest excess risks for cardiovascular were associated with EC and OC, while for respiratory, they were EC, OC, and Cu.

In the Ito et al. (2013) component of the NYU NPACT study, there were close associations of daily hospital admission rates with exposure to traffic-related effluents (EC, OC, and road dust) and with one or more transition elements (Fe, Ni, Zn, V, Cu, Cr) in most of the prior short-term exposure studies. For short-term hospital admissions, the only significant associations were (1) with the traffic source factor, and with components associated with traffic (EC, OC, Cu, and Pb), and those associated with resuspended road dust (Si and Fe). The second-stage analyses identified the $PM_{2.5}$ components having the greatest influence on the associations of $PM_{2.5}$ mass with short-term health effects and accounted for pollutant

concentration most closely influenced the cardiovascular hospital admissions including residual oil combustion components (Ni, V, S, and seaport berth volume), as well as traffic-related factors (Cu and road lengths), with $SO_4^=$ showing a nonsignificant negative association. There was little evidence for an association of hospital admissions with markers for coal combustion effluents ($SO_4^=$, As, and Se). On the other hand, for the second-stage analysis for daily mortality, $SO_4^=$ was the most influential component, and Se and As showed positive, albeit nonsignificant associations. These findings suggested that, even for short-term peak exposures, the components causing hospital admissions (OC and transition metals) differed from those associated with daily mortality (coal combustion effluents).

Khalili et al. (2018) examined the associations between short-term $PM_{2.5}$ estimates (IQR = 5.9 μg/m^3 from remote sensing) and asthma or wheeze exacerbations among 33,000 children with asthma in Massachusetts. The subset with low birth weight (LBW) (<2500 g) had clinical encounters associated with $PM_{2.5}$.

In summary, the $PM_{2.5}$ components most closely associated with adverse health effects varied considerably from study to study, and significant associations were also seen in those studies that included gaseous criteria pollutants as well as $PM_{2.5}$ components. However, before concluding that causal $PM_{2.5}$ components cannot be identified, one must consider the limitations of most of cited the studies in terms of their variations of (1) study designs and statistical powers; (2) variable atmospheric compositions from city to city, within each city, and over time; (3) the detection limits for many of the trace components that were measured; and (4) the choices of components included in the analyses. It does seem clear that some major mass $PM_{2.5}$ components ($SO_4^=$, EC, and OC), as well as some trace components (Ni, V, Cu, Zn), need to be considered as likely causal factors or at least as markers for influential source categories.

9.8 CHRONIC EXPOSURES TO $PM_{2.5}$ AND COMPONENTS ON ANNUAL MORTALITY

9.8.1 Studies Relying on $SO_4^=$ and $PM_{2.5}$

There were statistically significant associations between annual average $PM_{2.5}$ and $SO_4^=$ concentrations and annual mortality in early prospective cohort studies of annual mortality rates in relation to long-term $PM_{2.5}$ concentrations in the Harvard Six Cities Study (Dockery et al., 1993; Laden et al., 2006), the American Cancer Society (ACS) cohort (Pope et al., 1995b, 2002, 2004a, 2004b; Krewski et al., 2000), the Adventist Health Study on Smog (AHSMOG) (Beeson et al., 1998; Abbey et al., 1999; McDonnell et al., 2000), and the Veterans study (Lipfert et al., 2000).

If the bulk of the excess daily mortality associated with $PM_{2.5}$ mass concentration had been due to "harvesting" of terminally ill people who would have died within a few days, then the public health impact would be much less than if it led to prompt mortality among acutely ill persons who, if they had not died then, would have recovered and lived productive lives for years or decades longer. Summaries of these key studies and their findings follow, along with the findings of thorough reanalyses performed by Krewski et al. (2000) for the Health Effects Institute (HEI). Dockery et al. (1993) described a 14- to 16-year annual mortality follow-up of 8111 adults in the Harvard Six Cities Study in relation to average annual ambient air concentrations of TSP, $PM_{2.5}$, $SO_4^=$, O_3, SO_2, and NO_2, which were available for 14–16 years. The mortality rates were adjusted for cigarette smoking, education, body mass index (BMI), and other influential factors not associated with

pollution. The pollutants best correlated with annual (mostly cardiopulmonary) mortality were $PM_{2.5}$ and $SO_4^=$. The RRs and 95% CIs for both $PM_{2.5}$ and $SO_4^=$ were 1.26 (1.08–1.47) overall and 1.37 (1.11–1.68) for cardiopulmonary, expressed in terms of the concentration range found across the cities. The mean lifespan shortening was in the range of 2–3 years.

Pope et al. (1995b) linked 1980 $SO_4^=$ data from 151 U.S. metropolitan areas with individual risk factor on 552,138 adults in the ACS cohort residing in these areas in 1982, as well as $PM_{2.5}$ data for 295,223 adults in 50 of those communities. Deaths were ascertained through December 1989. The all-cause, lung cancer, and cardiopulmonary mortalities were controlled for smoking, education, and other risk factors. $PM_{2.5}$ was associated with cardiopulmonary and lung cancer mortality, but to other causes. Adjusted RRs of all-cause mortality (ACM) for the most polluted areas compared with the least polluted equaled 1.15 (1.09–1.22) and 1.17 (1.09–1.26) for $SO_4^=$ and $PM_{2.5}$, respectively. The mean lifespan shortening was between 1.5 and 2 years. The results for $SO_4^=$ were similar to those for previous cross-sectional studies of Ozkaynak and Thurston (1987) and Lave and Seskin (1970). Thus, the results of these earlier cross-sectional studies provided some confirmatory support for the findings of Pope et al. (1995b, 2002) and indicated that the concerns about the credibility of the earlier results, due to their inability to control for potentially confounding personal factors such as smoking and socioeconomic variables, could be eased. The Dockery et al. (1993) study had multiple PM metrics. As shown in Fig. 9.9, the association became stronger as the PM metric shifted from TSP to PM_{10}. Within the PM_{10},

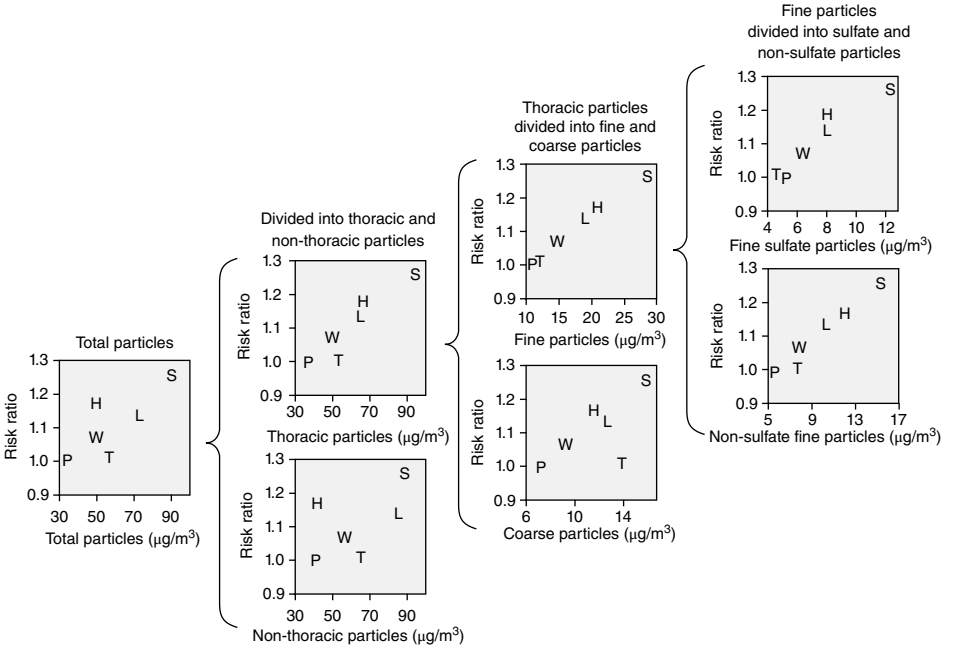

FIGURE 9.9 Adjusted relative risks for annual mortality are plotted against each of seven long-term average particle indexes in the Six-City study, from largest size range—total suspended particulate matter (*lower left*)—through sulfate and nonsulfate fine particle concentrations (*upper right*). Note that a relatively strong linear relationship is seen for fine particles, and for its sulfate and nonsulfate components. Topeka, which has a substantial coarse particle component of thoracic particle mass, stands apart from the linear relationship between relative risk and thoracic particle concentration. *Source*: Adapted from fig. V-5 of PM Staff Paper; U.S. EPA (1996b).

the association was much stronger for $PM_{2.5}$ than for $PM_{15-2.5}$. Within the $PM_{2.5}$ fraction, both the $SO_4^=$ and non-$SO_4^=$ fractions correlated strongly with annual mortality.

The findings of these prospective cohort studies, indicating that mean lifespan shortening was of the order of 2 years, implied that many individuals in the population had lives shortened by many years and that there is excess premature mortality associated with $PM_{2.5}$ exposure greater than that implied by the cumulative results of the time-series studies of daily mortality.

An extensive reanalysis (Krewski et al., 2000) of the Six Cities and ACS cohort studies indicated that the findings of Dockery et al. (1993) and Pope et al. (1995b) were substantially valid even when considering a much wider range of confounding variables. The Pope et al. (2002) analyses of ACS data for more extended time periods not only substantiated the original findings but also provided strong evidence for an ambient PM exposure relationships for increased lung cancer risk, which was suggested by the AHSMOG study (Beeson et al., 1998).

For the Six Cities Study, Krewski et al. (2000) reported the excess mortality RR from all causes (ACM) associated with an increase in $PM_{2.5}$ of $10\,\mu g/m^3$ to be 14% versus 13% for the original investigation. For the ACS Study, the RR for ACM for a $10\,\mu g/m^3$ increase in $PM_{2.5}$ was 7.0% in the reanalysis versus the original 6.6% value.

For both the Six Cities and ACS studies, Krewski et al. (2000) investigated the associations of $PM_{2.5}$ and $SO_4^=$ for potentially susceptible population subgroups. They did not find differences in $PM_{2.5}$-mortality associations among subgroups based on gender, marital status, smoking status, or exposure to occupational dusts and fumes. However, estimated effects of $PM_{2.5}$ did vary with educational level: the association between an increase in $PM_{2.5}$ and mortality was highest for individuals without a high school education. Krewski et al. (2000) also tested for linearity between ambient concentrations and mortality and found some indications of both linear and nonlinear relationships, depending upon the analytic technique used. When the decline in $PM_{2.5}$ was included as a time-dependent variable, the association between $PM_{2.5}$ and ACM was reduced.

Both the original ACS and the newly constructed data sets contained $SO_4^=$ levels inflated by ~50% due to SO_2 vapor collection by the sampling filter. For the Six Cities Study, the mortality RR was essentially the same for adjusted or unadjusted $SO_4^=$. For the ACS Study, adjusting for $SO_4^=$ artifact resulted in slightly higher RRs for ACM and cardiopulmonary mortalities, while the lung cancer mortality RR was lower after data adjustment. Thus, they found essentially the same results as the original Harvard Six Cities and ACS studies, even after using independently developed pollution data sets and adjustment for $SO_4^=$ sampling artifact.

The Pope et al. (2002) reanalyses of Pope et al. (1995a, 1995b) and that of Krewski et al. (2000) involved (1) doubling of the follow-up time from 7 to 16 years and tripling of deaths; (2) including 2 additional years of $PM_{2.5}$ data; (3) having data on gaseous co-pollutants; (4) additional statistical adjustments for occupational exposure; (5) incorporating data on dietary covariates (consumption of total fat, vegetables, citrus fruit, and high-fiber grains); (6) using nonparametric spatial smoothing for random effects; and (7) using the $PM_{2.5}$ means for both 1999–2000 and 1979–1983. For 51 cities, the concentrations of $PM_{2.5}$ were lower in 1999–2000 than 1979–1983 and were highly correlated ($r = 0.78$), with the rank order of the cities nearly the same.

Based on these results, the authors concluded the following: (1) The association of $PM_{2.5}$ and mortality persisted as the participants in the cohort grow older and more of them had died. (2) The $PM_{2.5}$ associations with cardiopulmonary and cancer mortality were

stable to adjustment for smoking status and were robust after inclusion of many additional covariates: education, marital status, BMI, alcohol consumption, occupational exposure, and dietary factors. (3) Education was an effect modifier, with larger and more statistically significant $PM_{2.5}$ effect estimates for persons with less education. Because the ACS cohort had a much higher percentage of well-educated persons than the general public, the education effect modification seen suggests that the overall $PM_{2.5}$ effect estimates underestimated the effects for the general public. (4) While $PM_{2.5}$ was associated with elevated total, cardiopulmonary, and lung cancer mortality risks, it was not for other mortality causes. (5) Neither $PM_{10-2.5}$ nor TSP were associated with total or with any cause-specific mortality. (6) The excess risk for $PM_{2.5}$ was much smaller than that for cigarette smoking for current smokers in the same cohort (Pope et al., 1995b): RR = 2.07 for total mortality, RR = 2.28 for cardiopulmonary mortality, and RR = 9.73 for lung cancer mortality. (7) In the more polluted areas of the United States, the RR for substantial obesity was larger than that for $PM_{2.5}$, but the RR from moderately overweight was somewhat smaller.

Jerrett et al. (2005) restricted the analysis of annual mortality rates to the ACS cohort members living in Los Angeles through 2000. They interpolated $PM_{2.5}$ concentration data from multiple monitors to get better exposure estimates for individual members. The RR was 1.17 for an increase of 10 $\mu g/m^3$ in $PM_{2.5}$, which was much larger than that for the whole ACS cohort.

Laden et al. (2006) extended the annual mortality analysis of the Six Cities cohort by 8 years (to 1998) and reported that total, cardiovascular, and lung cancer mortality rates were each positively associated with ambient $PM_{2.5}$ concentrations. Furthermore, there was a substantial reduction in $PM_{2.5}$ concentrations in the later years, and, very importantly, the reductions in concentrations were associated with reduced mortality risks.

The results of these pioneering studies provided substantial evidence for positive associations between long-term ambient $PM_{2.5}$ exposure and mortality, and a peer-reviewed EPA-sponsored expert elicitation study (http://www.epa.gov//ttn/ecas/ria.html), focused on the size of the coefficient for excess annual mortality, reported a consensus judgment that the best estimate for the coefficient was closer to those of the Six Cities Study of Laden et al. (2006) and Jerrett et al. (2005) than that of Pope et al. (2002), perhaps because both of reliance on better exposure assessments and of the Six Cities Study using a more representative population.

Thurston et al. (2016a) examined the associations of ambient air $PM_{2.5}$ with annual mortality in the NIH-AARP Diet and Health Cohort, a U.S. cohort with detailed personal-level risk factor information and post-2000 (lower) $PM_{2.5}$ exposures. The cohort, assembled by the National Institutes of Health (NIH), consisted of members of the American Association of Retired Persons (AARP). It included residents over 50 years of age residing in CA, FL, LA, NJ, NC, and PA, plus the metropolitan areas of Atlanta, GA, and Detroit, MI. The air quality data came from EPA's Air Quality System (AQS) and relied on census tract centroid estimates of monthly average concentrations. $PM_{2.5}$ was significantly associated with total HR = 1.03 (1.00–1.05) and CVD mortality HR = 1.10 (1.05–1.15). For never smokers, there was a significant for respiratory mortality HR of 1.27 (1.03–1.56). The associations were similar when adjusted for ambient O_3 concentrations.

Baccarelli et al. (2016) extended the age range for studies of the association of ambient air $PM_{2.5}$ and longevity in the continental United States. On average, for each 10,000 in the 55–64 age range in 1980, 2295 were in the 85–94 age range in 2010, and 71.4 were in the 100–104 age range in 2010. For an increase in the $PM_{2.5}$ IQR of 4.2 $\mu g/m^3$, there 182 fewer in the 85–94 age range ($p < 0.001$), and 6.4 fewer in the 100–104 age range ($p < 0.001$).

Penn et al. (2017) estimated state-specific contributions to $PM_{2.5}$- and O_3-related health burden from residential combustion (RC) and electricity generating unit emissions (EGUs) in the US. They attributed 21,000 premature mortalities per year to EGUs, of which half were traceable to SO_2 emissions and 10,000 to RC, with much of it related to wood combustion.

9.8.2 Ambient Air $PM_{2.5}$, Its Major Components, and Pulmonary Function

Doiron et al. (2017) described the association of NO_2, $PM_{2.5}$, PM_{10}, and $PM_{10–2.5}$ with the prevalence of wheeze and shortness of breath in two large European cohorts, that is, Lifelines, with 152,180 adults, 18–93 years of age (2006–2013), and 502,655 adults in UK Biobank, 40–69 years of age (2006–2010). All exposure variables were associated with the symptoms, with the strongest association being for $PM_{2.5}$.

9.8.3 Ambient Air $PM_{2.5}$ and Disease Incidences

Yuan et al. (2017) investigated the associations the concentrations of a variety of metals in blood plasma on the incidence of coronary heart disease (CHD) in an ongoing prospective cohort study of 27,000 retired employees of the Dongfeng Motor Corp. in Shiyan, PRC. There were high correlations of the concentrations in plasma, whole blood, and urine and a lack of interaction with other metals or dietary risk factors for only of the metals (Ti, As, and Se). Of these, the only one whose OR rose steeply with plasma concentration was As, a marker for coal combustion. Se, another marker for the coal combustion source, had a modest decrease in OR with plasma concentration, but that could have been due to its antioxidant effect.

Bowe et al. (2018) described the association of $PM_{2.5}$ with the risk of incident chronic kidney disease (CKD) and of its progression to end-stage renal disease (ESRD) in a cohort of 2.5×10^6 U.S. veterans followed for a median of 8.5 years in exposure quartiles $PM_{2.5}$ exposure at 5–9, 9–11, 11–13, and 13–22 $\mu g/m^3$. They reported linear relationships between $PM_{2.5}$ and incident CKD and progression to ESRD. The results were consistent with time-variation reflecting movement of cohort participants and changes in $PM_{2.5}$ over the years.

9.8.4 Dementia, Alzheimer's Disease (AD), and Parkinson's Disease

Kioumourtzoglou et al. (2016) investigated the impact of long-term $PM_{2.5}$ exposure on time to first hospital admission for dementia, Alzheimer's disease (AD), or Parkinson's disease in an Medicare population in 9.8 million elderly residents 50 cities across Northeastern United States. For a $1\,\mu g/m^3$ increment in annual average $PM_{2.5}$, there was a HR = 1.08 (1.05–1.11) for dementia, 1.15 (1.11–1.19) for AD, and 1.08 (1.04–1.12) for PD.

9.8.5 Amyotrophic Lateral Sclerosis

Seelen et al. (2017) conducted a population-based case–control study in the Netherlands (Jan 2006–Jan 2013) with 917 ALS patients and 2662 controls. Mean air concentrations (NO_2, NO_x, $PM_{2.5}$, $PM_{10–2.5}$, and PM_{abs}) were assessed by land use regression (LUR). Conditional logistic regression analysis models were adjusted for age, gender, education, smoking status, alcohol use, BMI, socioeconomic status, and urbanization. Risk of ALS

was significantly increased for the upper exposure quartile of PM_{abs} (OR = 1.67; CI: 1.27, 2.18), NO_2 (OR = 1.74; CI: 1.32, 2.30), and NO_x (OR = 1.38; CI: 1.07, 1.77).

9.8.6 Annual Human Mortality

Hoek et al. (2002) assessed the effect of traffic pollutants on mortality in a Dutch cohort study on diet and cancer in 5000 randomly selected adults (aged 55–69) and found that traffic markers and BS were significantly associated with annual mortality.

Gehring et al. (2006) studied long-term exposure in Germany among 4800 women participating in a series of cross-sectional studies. They reported that cardiopulmonary mortality was significantly associated with living within 50 m of a major road and with the concentrations of PM_{10} and NO_2.

Lipfert et al. (2006) examined the influence of $PM_{2.5}$ components on annual mortality in the Veterans cohort, finding that traffic density was the strongest predictor of survival, with significant contributions from some specific $PM_{2.5}$ components, that is, NO_3^-, EC, Ni, and V. Lipfert et al. (2009), using two-pollutant models found that traffic density remained significant, but the reduction in survival effect was notably greater when both traffic and other pollutants were included (NO_x, EC, and Ni).

Ostro et al. (2010) studied the associations between annual mortality and long-term exposure to $PM_{2.5}$ (IQR = 6.1 $\mu g/m^3$) and some of its components EC (0.16 $\mu g/m^3$), OC (1.0 $\mu g/m^3$), $SO_4^=$ (1.3 $\mu g/m^3$), NO_3^- (3.6 $\mu g/m^3$), Fe (0.06 $\mu g/m^3$), K (0.05 $\mu g/m^3$), Si (0.05 $\mu g/m^3$), and Zn (0.01 $\mu g/m^3$) among a prospective cohort of 7888 active and retired female teachers in California. There were significant IHD mortality risks in single pollutant models for all the measured pollutants, with the highest risks being for K, OC, NO_3^-, and $SO_4^=$. For pulmonary mortality, there were significant risks only for OC, $SO_4^=$, and Si. For all but EC, the risks were higher for average exposure over the 3 years preceding death than those for the last year only.

Cahill et al. (2011) described a substantial drop in annual IHD mortality between 1989 and 1991 in Bakersfield, CA, located at the southern end of the 400 mile-long Central Valley attributable to the replacement, by natural gas, of crude oil combustion for generating steam to enhance heavy petroleum recovery at the local oil refinery in 1990. Measured concentrations of S, Ni, V, Zn, and Pb at Bakersfield in 1974–1976, in ng/m^3, averaged 1685 for S, 38 for Ni, 19 for V, 61 for Zn, and 1714 for Pb and were much lower in the more northern parts of the Valley. By 2009, the corresponding concentrations in 2009 in Bakersfield were 505, 2.3, 0.2, 32, and 9.4 ng/m^3. Although there was relatively little variation, within the Central Valley, of climate, elevation, or population demography, the IHD mortality in 1989–1991 was 60% higher in the area around Bakersfield than in areas further north within the Valley. By 2003–2007, the IHD mortality around Bakersfield had dropped by 30%. The mortality reductions, associated with the substantial reductions in the concentrations of one or more of the trace metal components, suggest causality, given the lack of any other known exposures that varied so greatly over that time span. This association is consistent with the other studies that found excess mortality and morbidity being associated with trace concentrations of the transition metals despite their extremely low concentrations.

In NYU's NPACT Study of the ACS Cohort, Thurston et al. (2013, 2016b) used factor analysis of annual average $PM_{2.5}$ based on the 2000–2005 nationwide EPA CSN data to identify $PM_{2.5}$ major elemental groupings, interpretable as being associated with specific $PM_{2.5}$ source categories, at many locations around the United States, including 167 sites in

102 MSAs. The major source categories identified and their key elements were metals industry (Pb, Zn), soil (Ca, Si), motor vehicles (OC, EC, NO_2), steel industry (Fe, Mn), coal combustion (As, Se), oil combustion (V, Ni), and salt (Na, Cl). In terms of $PM_{2.5}$ concentration, Fig. 9.10, adapted from Baccarelli et al. (2016), shows that chronic exposure to $PM_{2.5}$ reduced longevity in the elderly. Figure 9.11 shows that most of the excess annual mortality was attributable to cardiovascular causes.

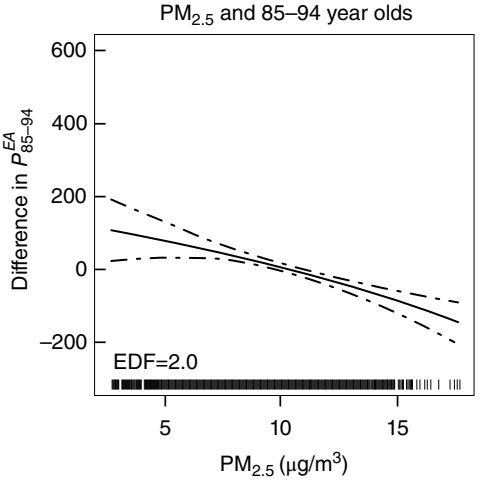

FIGURE 9.10 Association between annual mean $PM_{2.5}$ and difference in mortality between those aged 70–74 in 1980 and those aged 85–94 in 2010. *Source*: From Baccarelli et al. (2016).

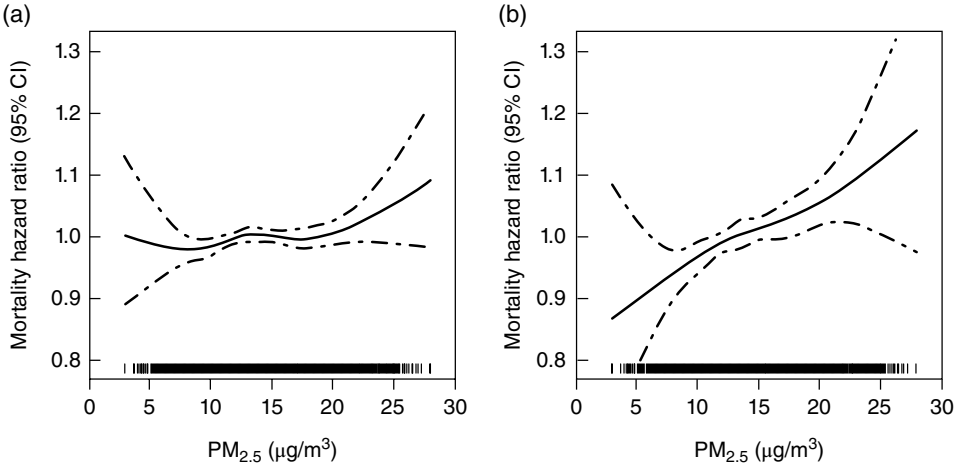

FIGURE 9.11 Association between annual mean $PM_{2.5}$ and mortality in the ACS cohort for (a) all causes and (b) cardiovascular causes. For 4 degrees of freedom, as adjusted for all individual-level covariates (race, education, marital status, BMI, alcohol consumption, and smoking). *Source*: Adapted from Thurston et al. (2016b).

- Source-related exposures to motor vehicle-related emissions were highest in Southern California; soil exposures were highest in the Southwest; steel related exposures were highest in cities with steelworks (e.g., Detroit, MI, and Birmingham, AL); coal combustion exposures were highest in the Ohio River Valley region (e.g., Pittsburgh, PA); and residual oil burning exposures were highest in cities with wintertime residual fuel oil burning (e.g., NYC) or having deepwater ports (e.g., Los Angeles and Long Beach, CA; Savannah, GA; and Newark, NJ-NYC, NY), consistent with exposures by emissions from oceangoing ships burning highly polluting "bunker fuel." The analysis reveals the same major U.S. source factors and spatial distribution as those previously reported for the 1979–1983 IP network (Ozkaynak and Thurston, 1987), albeit at lower levels than in the earlier period, indicating a qualitative consistency in the spatial representativeness of these results over the past 30 years.
- Individual risk factor data for approximately 446,000 adults in the ACS cohort from 100 MSAs across the United States were linked with $PM_{2.5}$, trace element, and source factor exposure data throughout the United States. As shown in Fig. 9.12, the strongest $PM_{2.5}$, trace element, and source factor associations were found for IHD and lung cancer deaths (LCD), consistent with past ACS analyses (Pope et al., 1995b, 2002).

  $PM_{2.5}$ and the trace elements associated with coal combustion (i.e., Se, As, and S) were most significantly associated with ACM, consistent with results derived when considering individual trace components. The inclusion of contextual variables did not change the rankings or statistical significance of the various source-related contributions to annual IHD mortality, nor confound the associations. IQRs were used to normalize the exposure variable the various RRs to make them more comparable (so the effect size is not dependent on the relative concentrations of the source impacts). The IQR for each is provided on the figure, so the reader can convert (for example) to a per mg/m^3 effect size.
- While $PM_{2.5}$ pollution from most industrial and fossil fuel combustion categories had RR estimates above 1.0 for annual IHD deaths, coal combustion $PM_{2.5}$ and its correlated trace elements (e.g., As, Se, S) were most strongly and consistently associated with annual IHD mortality across all the various model specifications considered.
- $PM_{2.5}$ and EC attributable to traffic were associated with annual IHD in some models. $PM_{2.5}$ originating from windblown soil or from biomass burning (e.g., wood combustion) was generally not associated with increased risk of annual IHD mortality in this cohort, while associations with other sources were more equivocal across models.
- Respiratory mortality was most significantly associated with long-term exposure to secondary OC, but not with any specific source.
- The coal-burning source factor and its associated tracer (Se, S) were significantly associated with increased risk of death from lung cancer.
- Source-specific $PM_{2.5}$ associations with health impacts were consistent with and more easily interpreted than those of individual trace constituents from a variety of sources.

Overall, modeling results from NYU's main analyses indicated that long-term exposures to $PM_{2.5}$ from one key source, and its elemental tracers, were most explanatory of the $PM_{2.5}$–mortality associations found in past ACS cohort studies that were limited to PM mass and $SO_4^=$ concentrations. In particular, the coal combustion source factor was most consistently associated with increased risk of IHD mortality across

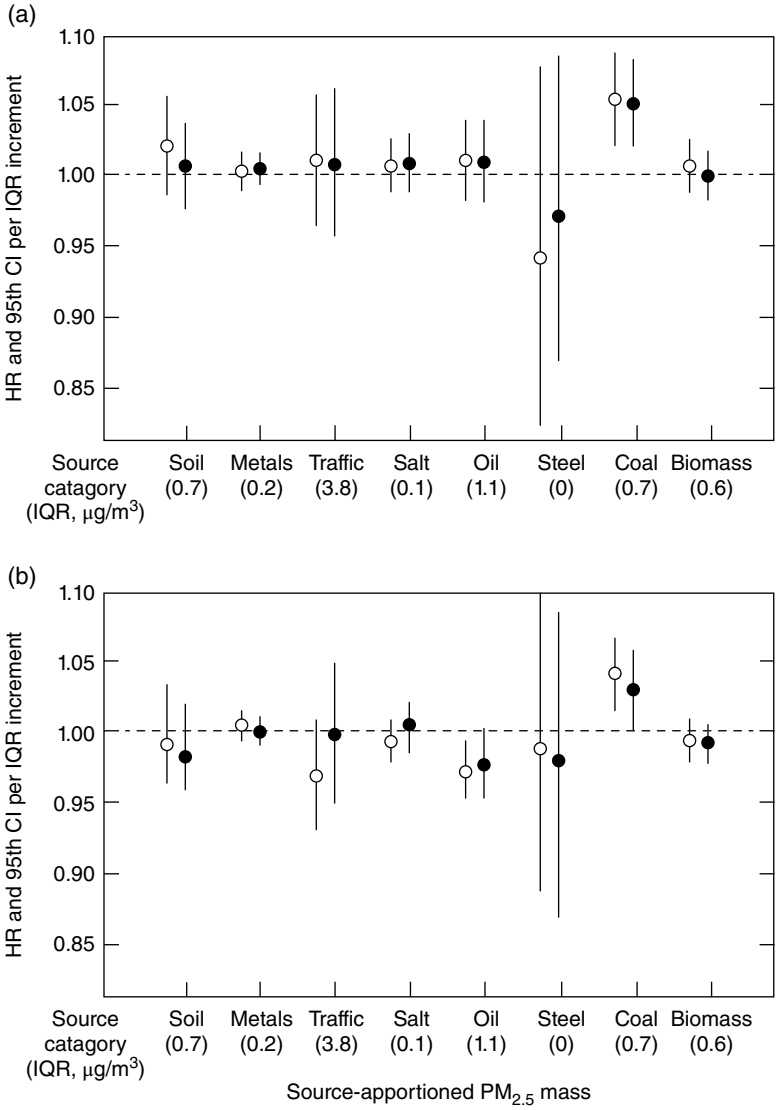

FIGURE 9.12 Association between annual mean $PM_{2.5}$ and its source apportioned mass concentrations with mortality using random effect model in the ACS cohort for (a) IHD and (b) lung cancer. The vertical lines represent 95% CIs. *Source*: Adapted from Thurston et al. (2016b).

models considered. The soil or wood combustion source factors were not associated with any causes of mortality across models considered. The traffic and salt source factors, and especially their respective tracers, EC and Cl, showed significant associations with IHD mortality in some models. There were associations between $PM_{2.5}$ and lung cancer mortality for the coal combustion source factor and its tracers, Se and S, but not by other components of $PM_{2.5}$.

Beelen et al. (2015) conducted an integrated collaborative study of associations of long-term exposure to $PM_{2.5}$ and its components with annual mortality in 19 European

cohorts in England, Denmark, Norway, Sweden, Finland, the Netherlands, Germany, France, Switzerland, Italy, and Greece. There were 291,816 participants, of whom 25,466 died during the follow-up period of 14.3 years. HRs were positive for almost all elements and were significant for $PM_{2.5}$ S, at 1.14 (1.06–1.23). The lowest *p*-values were for S (0.003), Ni (0.08), and K (0.08).

Ostro et al. (2015) observed statistically significant associations of annual IHD mortality in California with $PM_{2.5}$, NO_3^-, EC, Cu, S, meat cooking, and diesel fuel combustion.

Raaschou-Nielsen et al. (2016) reported a study of PM air pollution components and risk for lung cancer. They assessed the associations between the concentrations of PM_{10} and $PM_{2.5}$ components (Cu, Fe, K, Ni, S, Si, V, and Zn) and lung cancer in 245,782 members of 14 cohorts from 8 European countries over 13 years. For members who did not move, there were statistically significant associations with lung cancer for $PM_{2.5}$ Cu, PM_{10} Zn, PM_{10} S, PM_{10} Ni, and PM_{10} K, and two-pollutant model analyses indicated the strongest associations for $PM_{2.5}$ S.

Mendy et al. (2018) studied the association between house dust endotoxin and chronic bronchitis and emphysema (CBE) in 3393 participants ≥20 years old from the National Health and Nutrition Examination Survey (NHANES) 2005–2006, a sample representative of the U.S. population. The median endotoxin concentration in house dust was 14.61 EU/mg dust, and 8.2% of participants had CBE. In the adjusted analysis, one EU/mg increase in $\log_{10}$-transformed endotoxin concentrations was associated with a 27% increase in the odds of CBE diagnosis [OR = 1.27 (CI: 1.00, 1.61)] and a 78% increase in chronic bronchitis symptoms (defined as cough and phlegm for ≥3 months in a year for ≥2 years) [OR = 1.78 (CI: 1.01, 3.12)]. Sensitization to inhalant allergens ($p = 0.001$) modified the relationship between endotoxin and CBE diagnosis, with stronger associations observed in sensitized participants [OR = 2.46 (95% CI: 1.72, 3.50)].

Pennington (2018) studied the "association between exposure to $PM_{2.5}$, NO_x, and CO during pregnancy and infancy and asthma incidence by ages 2–6 in the Kaiser Air Pollution and Pediatric Asthma Study, a racially diverse birth cohort of 24,608 children born between 2000 and 2010. They estimated concentrations at the maternal and child residence. Asthma was defined using diagnoses and medication dispensings from medical records. They used binomial generalized linear regression to model the impact of exposure continuously, and by quintiles on asthma risk. Controlling for covariates and modeling log-transformed exposure, a 2.7-fold increase in first year of life $PM_{2.5}$ was associated with an absolute 4.1% (CI: 1.6–6.6%) increase in risk of asthma by age 5. Quintile analysis showed an increase in risk from the first to second quintile, but similar risk across quintiles 2–5. Risk differences increased with follow-up age. Results were similar for NO_x and CO and for exposure during pregnancy and the first year of life owing to high correlation."

9.9 PEDIATRIC RESPONSES TO LONG-TERM PM EXPOSURES

9.9.1 Preterm Birth and Low Birth Weight

PM exposure, albeit at high ambient levels, has been associated with preterm delivery and LBW. Xu et al. (1995) followed all registered pregnant women who lived in four residential areas of Beijing, PRC. The analysis included 25,370 first live births in 1988 and very high SO_2 concentrations (mean = 102, maximum = 630 μg/m^3) and TSP

(mean = 375, maximum = 1003 μg/m^3), and there was a significant dose-dependent association between gestational age at birth and TSP. The estimated reduced duration of gestation was 7.1 h for each 100-μg/m^3 increase in TSP, and the OR for preterm delivery was 1.10 (1.01–1.20) for each 100-μg/m^3 increase in TSP. In addition, the gestational age to distribution of high-pollution days was more skewed toward the left tail compared with low-pollution days.

In a follow-on study, Wang et al. (1997) examined the relationship between maternal air pollution exposure during periods of pregnancy (entire and specific periods) and birth weight in a well-defined cohort between 1988 and 1991 of all pregnant women living in four residential areas of Beijing. The sample for analysis included 74,671 first-parity live births with gestational age 37–44 weeks. Multiple linear regression and logistic regression were used to estimate the association of TSP with birth weight and LBW (<2500 g), adjusting for gestational age, residence, birth year, maternal age, and infant gender. There was a significant exposure–response relationship between maternal exposures during the third trimester and birth weight. The adjusted odds ratio for LBW was 1.10 (1.05–1.14) per 100 μg/m^3 increase in TSP. The corresponding estimated reduction in birth weight was 6.9 g. The birth-weight distribution of the high-exposure group was more skewed toward the left tail (i.e., with higher proportion of births <2500 g) than that of the low-exposure group.

Ritz et al. (2000) studied the association between pollutant exposures and LBW in 97,518 neonates in Southern California, after adjustment for maternal age, race, smoking during pregnancy, and so on. A 50 μg/m^3 increase in PM_{10} exposure averaged over the first month of pregnancy was associated with a 16% increase in preterm delivery, while a corresponding increase averaged over 6 weeks prior to birth was associated with a 20% increase. However, a study by Maisonet et al. (2001) of the association between LBW and PM_{10} in Eastern U.S. cities (Boston and Springfield, MA; Hartford, CT; Philadelphia and Pittsburgh, PA; and Washington, DC) was negative.

Ebisu and Bell (2012) studied the associations of $PM_{2.5}$ components with LBW in the Northeastern and mid-Atlantic U.S. regions for 2000–2007. There were statistically significant excess risks for IQR concentrations ranges of Ni (5.7%), Ti (5.0%), aluminum (Al) (4.9%), and EC (4.7%).

Hao et al. (2016) examined the associations of preterm births in Georgia (USA) with ambient air PM_{10}, $PM_{2.5}$, $SO_4^=$, NO_3^-, NH_4^+, EC, OC, CO, NO_2, O_3, and SO_2 in 2002–2006. The ORs per IQR for preterm birth were greatest for OC, PM_{10}, $PM_{2.5}$, $SO_4^=$, NH_3^+, and EC, but not significant for NO_3^- and O_3.

Pedersen et al. (2016) examined LBW and head circumference in relation to elemental components of $PM_{2.5}$ and PM_{10} (Cu, Fe, K, Ni, S, Si, V, and Zn) in Sweden, Denmark, Lithuania, the Netherlands, Germany, Italy, and Spain for 34,923 singleton births between 994 and 2008. A 200 ng/m^3 increase in S, adjusted for $PM_{2.5}$, was associated with an increased OR for LBW of 1.36 (1.17–1.58). There were also increased ORs for LBW for Ni and Zn. All components other than K were associated with reduced head circumference.

Schembari et al. (2015) reported that a 5 μg/m^3 increase in third trimester $PM_{2.5}$ was significantly associated with LBW and smaller head circumference in white British mothers, but not for British mothers of Pakistani origin.

Nachman et al. (2016) measured the association of ambient air $PM_{2.5}$ with intrauterine inflammation (IUI), a risk factor for preterm birth and neurodevelopment outcomes, in a Boston birth cohort. For the whole pregnancy, the OR for IUI was 1.92 (1.55–2.37).

9.9.2 Other Pediatric Effects

Brauer et al. (2006) studied the association of average concentrations of air pollutants in the first 2 years of life in the Netherlands and Germany with otitis media, a common childhood infection. Increases of $3\,\mu g/m^3$ $PM_{2.5}$, $0.5\,\mu g/m^3$ EC, and $10\,\mu g/m^3$ NO_2 were associated with ORs of 1.13 (CI: 1.00–1.27), 1.10 (1.00–1.22), and 1.14 (1.03–1.27) in the Netherlands, and 1.24 (0.84–1.83), 1.10 (0.86–1.41), and 1.14 (0.87–1.49) in Germany.

Patel et al. (2009) studied the associations of 3-month averages of $PM_{2.5}$ and the Ni, V, Zn, and EC within the $PM_{2.5}$ on longitudinal reports of symptoms in a birth cohort in Manhattan and Bronx (in NYC) who lived near EPA speciation sites. Symptom reports for the prior 3 months were collected every 3 months from 3 to 24 months of age. About 90% of the children were on Medicaid, and 30% were reported to have or might have asthma based on a doctor's questionnaire entry at 24 months. Symptoms of wheeze and cough were significantly associated with Ni, V, and Zn, whereas traffic-related EC was associated with only cough, and $PM_{2.5}$ was not associated with either symptom. In contrast, when Patel et al. (2010) studied symptoms among 249 adolescent students in a panel study in NYC and its suburbs in relation to peaks in conventional traffic markers ($PM_{2.5}$, NO_2, and BC), there were significant associations with BC and NO_2, but not with $PM_{2.5}$, and they were largest among urban dwellers and asthmatic subjects.

$PM_{2.5}$ is a risk factor for subnormal respiratory function in children, as illustrated in the Children's Health Study (CHS) in 12 Southern California communities. There were significant associations between the percentage of children with $FEV_1 < 80\%$ of predicted and the mass concentrations of $PM_{2.5}$, PM_{10}, and EC, but not for O_3 (Gauderman et al. 2004).

9.10 OTHER MORBIDITY RESPONSES AFFECTED BY $PM_{2.5}$ COMPONENTS

9.10.1 Cardiovascular Responses

Most recent morbidity studies, described below, have focused on cardiovascular responses and have often gone well beyond the earlier studies in terms of the more complex aspects of the relationships between PM exposures and responses, with greater attention being given to (1) chemical composition within particle size ranges and the compositional variations associated with different source strengths; (2) response characterization in terms of organ system affected, disease categories, acute and chronic responses, time lags between exposures and responses, and host factors affecting responsiveness; and (3) the possible roles of gaseous co-pollutants on responses to PM exposures. The section below that summarizes those studies reports both cardiovascular and pulmonary effects. It is followed by a brief section that summarized studies showing associations of ambient air components on pulmonary and other non-cardiovascular effects.

Lanki et al. (2006) found an influence of $PM_{2.5}$ component exposures in on exercise-induced ischemia in 45 elderly nonsmokers with stable CHD in Amsterdam, Erfurt, and Helsinki. The traffic and long-range transport sources were associated with ST-segment depression during submaximal exercise testing. In a multi-pollutant model, only the traffic source was significantly associated with the effect.

Auchincloss et al. (2008) studied associations among ambient air concentrations of 24-h average $PM_{2.5}$, SO_2, NO_2, and CO, averaged over the previous 1, 2, 7, 30, and 60 days, and blood pressure (BP) in six U.S. cities as part of the Multi-Ethnic Study of

Atherosclerosis (MESA). They found (1) no evidence of clear threshold/nonlinear effects for $PM_{2.5}$, (2) significant associations of $PM_{2.5}$ with SBP and PP, (3) that associations of $PM_{2.5}$ with SBP and PP became stronger with increasing averaging time up to 30 days, (4) that associations of $PM_{2.5}$ with SBP and PP became stronger after adjustment for gaseous air pollutants; (5) that associations of $PM_{2.5}$ with traffic exposure indicators were significantly negative; (6) that $PM_{2.5}$ associations with BP were not modified by age, sex, diabetes, smoking, study site, SO_2, CO, season, or distance from a roadway; and (7) that associations of $PM_{2.5}$ with BP were stronger for persons on BP medications or having hypertension, during warmer weather, with higher NO_2, living 5300 m from a major road, or surrounded by a high road density.

de Hartag et al. (2009) measured SDNN and HF power of HRV in female subjects with CHD biweekly in Amsterdam (11 subjects), Erfurt (4), and Helsinki (21) over 6 months in relation to the concentrations of $PM_{2.5}$, S, V, Zn, Ca, Cl, Fe, Cu, BC, and their source categories (local traffic, long-range transport, oil combustion, industry, and salt) and their medication usage. Beta blockers were used by 13 of the 39 subjects. There were no significant associations of SDNN or HF for those subjects using β blockers. For those not using them, there were significant depressions of SDNN with BC and V (lag day 1), S (lag days 2 and 3), local traffic and long-range transport sources (lag day 3), and the oil combustion source (lag day 1). For HF, there were with 2- and 3-day lags for S, and a 3-day lag for the oil combustion source.

Aaron et al. (2016) reported that long-term $PM_{2.5}$ exposures in the MESA in six U.S. communities were associated with greater right ventricle (RV) mass and RV mass/end-diastolic volume ratio. In this MESA study, Kaufman et al. (2016) showed a significant $PM_{2.5}$-associated increase in coronary artery calcium (CAC), but did not find a pollutant associated change in common carotid intima–media thickness (CIMT). In a cross-sectional analysis of 4 European cohorts in the ESCAPE study, Perez et al. (2015) did report CIMT increases, but they did not reach statistical significance.

Dai et al. (2016) identified $PM_{2.5}$ components associated with BP in elderly men in the Veterans Affairs Normative Aging Study (NAS) in the Boston, MA, area. For SBP, an IQR increase of 2 ng/m^3 of Ni was associated with a 2.5 (1.5–3.5) mmHg increase in SBP and a 2.2 (1.7–2.8) mmHg increase in DBP.

Sinharay et al. (2018) recruited 40 healthy men and women >60 years old, 39 with angiographically proven stable IHD and 40 with stage 2 COPD who were clinically stable for 6 months. All had abstained from smoking for at least 12 months and continued their doctor-recommended medications. They were randomly assigned to a 2-h walk, either along a heavily traveled commercial street in London (Oxford Street) or in an urban park (Hyde Park). Baseline measurements were taken before the walk in the hospital laboratory. During each walk session, BC, $PM_{2.5}$, UFP, and NO_2 concentrations were higher on Oxford Street than in Hyde Park. Participants with COPD reported more cough [odds ratio (OR) = 1.95, CI: 0.96–3.95; $p < 0.1$], sputum (3.15, 1.39–7.13; $p < 0.05$), shortness of breath (1.86, 0.97–3.57; $p < 0.1$), and wheeze (4.00, 1.52–10.50; $p < 0.05$) after walking on Oxford Street compared with Hyde Park. In all participants, walking in Hyde Park led to an increase in FEV_1 and FVC and a decrease in pulse wave velocity (PWV) and augmentation index up to 26 h after the walk. By contrast, such beneficial responses were attenuated after walking on Oxford Street. In participants with COPD, a reduction in FEV_1 and FVC was associated with an increase in during-walk exposure to NO_2, UFP, and $PM_{2.5}$.

9.11 CONTROLLED SHORT-TERM HUMAN INHALATION EXPOSURE STUDIES

Ghio et al. (2000) exposed 38 healthy volunteers exercising intermittently at moderate levels of exertion for 2 h to either filtered air (FA) or concentrated ambient PM (CAPs) (23–311 $\mu g/m^3$) in Chapel Hill, NC. Cells and fluids obtained 18 h after exposure showed a mild increase in polymorphonuclear neutrophils (PMNs) in the bronchial and alveolar fractions of bronchoalveolar lavage (BAL) in subjects exposed to the highest quartile concentration (mean of 206.7 $\mu g/m^3$). Lavage protein did not increase, and there were no other indicators of pulmonary injury. No respiratory symptoms or decrements in pulmonary function were found after exposure to CAPs. The 38 human volunteers were also examined for changes in host defense and immune parameters in BAL and blood (Harder et al., 2001). There were no changes in the number of lymphocytes or macrophages, subcategories of lymphocytes, cytokines IL-6 and IL-8, or macrophage phagocytosis in BAL or lymphocyte subsets in blood. Thus, a mild inflammatory response to CAPs was not accompanied by an effect on immune defenses. The increase in PMNs may have represented an adaptive response of the lung to $PM_{2.5}$, although the presence of activated PMNs may release biochemical mediators that produce lung injury.

Other human CAPs inhalation studies have had limited power because of the small numbers of subjects studied. Petrovic et al. (1999) exposed four healthy volunteers (aged 18–40) via facemask under resting conditions to FA and three CAPs concentrations (23–124 $\mu g/m^3$) for 2 h. The exposure was followed by 30 min of exercise. No cellular signs of inflammation were observed in induced sputum samples 2 or 24 h after exposure. There was a trend toward an increase in nasal lavage PMNs, but not statistical significance. The only change in pulmonary function was a 6.4% decrease in thoracic gas volume after exposure to $PM_{2.5}$ at 124 $\mu g/m^3$ versus a 5.6% increase after FA. A similar small pilot study (Gong et al., 2000) found no changes in pulmonary function or symptoms in four subjects aged 19–41 after a 2-h exposure to FA or CAPs at 148–246 $\mu g/m^3$ in Los Angeles, CA. In a follow-up study, Gong et al. (2004b) exposed 12 mildly asthmatic and 4 healthy adults to FA and $PM_{10-2.5}$ supplied via a coarse particle concentrator in a Los Angeles suburb with high levels of motor vehicle pollution for 2 h with intermittent exercise. Mean $PM_{10-2.5}$ was 157 $\mu g/m^3$ (range: 56–218 $\mu g/m^3$). Relative to FA, $PM_{10-2.5}$ exposure did not significantly alter respiratory symptoms, spirometry, arterial O_2 saturation, or airway inflammation. After $PM_{10-2.5}$ exposure, Holter electrocardiograms (ECG) showed small ($p < 0.05$) increases in heart rate (HR) and decreases in HRV, which were larger in healthy than in asthmatic subjects.

A review of human CAPs inhalation studies by Ghio and Huang (2004) summarized some other recent studies. Huang et al. (2003) applied principal component analysis of the CAPs aerosol. They linked specific water-soluble PM components to both the PMN influx and elevation in blood fibrinogen (Huang et al., 2003). A $SO_4^=$/Fe/Se factor, attributed to photochemical air pollution, was associated with the PMN increase in the lavage, while a Cu/Zn/V factor, related to various combustion processes, was linked to increases in blood fibrinogen. In another study, healthy and asthmatic individuals (18–45 years of age) were exposed (with 2 h of exercise) to both CAPs (mean concentration = 174 $\mu g/m^3$) and FA (Gong et al., 2003). There were no changes in symptomatology, pulmonary function, and hematologic measurements attributable to CAPs. CAPs decreased columnar epithelial cells in induced sputum in both healthy and asthmatic subjects. There were also small changes in mediators of blood coagulability, inflammation, and HRV.

CAPs have also been used to compare the vascular responses between exposures to CAPs/O_3 versus FA in Toronto (Brook et al., 2002). Nonsmoking adults were exposed to CAPs and FA separated by 2 days. CAPs exposure was 150 μg/m^3 while O_3 exposure was 120 ppb. High-resolution vascular ultrasonography was used to measure alterations in brachial artery diameter (BAD), endothelial-dependent flow-mediated dilatation, and endothelial-independent nitroglycerine-mediated dilatation. Exposure to CAPs and O_3 was associated with small statistically significant BAD constriction compared with FA. There were no differences in flow-mediated dilatation or BP responses between exposures. In a follow-up paper, Urch et al. (2004) examined the relationship between total and constituent $PM_{2.5}$ mass concentrations and the acute vascular response, finding a significant negative association between both the OC and EC concentrations and the difference in the post-exposure change in the BAD (ΔBAD) between and CAPs + O_3 and FA exposure days.

Devlin et al. (2003) studied individuals between 60 and 80 years of age exposed to both CAPs and FA for 2 h without exercise. There were significant decrements in HRV in both time and frequency domains immediately following exposure to CAPs, and some changes persisted for at least 24 h. These results contrasted with those of Ghio et al. (2000) in which young, healthy subjects exposed to CAPs with exercise had no changes in HRV relative to subjects inhaling FA.

9.12 ANIMAL INHALATION STUDIES WITH CONCENTRATED PM (CAPS)

These studies are grouped by study location on the basis that the CAPs chemical compositions differ by location. Some of the findings are summarized in Table 9.6.

9.12.1 Boston

Studies in normal dogs exposed to Boston CAPs by inhalation (Clarke et al., 2000) showed increases in pulmonary inflammation and circulating blood PMNs associated with specific ambient PM components. Mean concentrations were 203 and 361 μg/m^3. Saldiva et al. (2002) studied rats with chronic bronchitis and normal rats exposed by inhalation either to FA or CAPs and induced significant increases in BAL PMNs in normal and bronchitic animals exposed to CAPs. They concluded that (a) short-term exposures to CAPs from Boston induce a significant inflammatory reaction in rat lungs and (b) the reaction is influenced by PM composition.

Gurgueira et al. (2002) exposed adult Sprague–Dawley rats to either CAPs (300 ± 60 μg/m^3) or FA for periods of 1–5 h. CAPs exposure for 5 h induced significant oxidative stress in the lung and heart, but not in the liver. Increases in chemiluminescence showed strong associations with the CAPs content of Fe, Mn, Cu, and Zn in the lung and with Fe, Al, Si, and Ti in the heart. CAPs inhalation also led to tissue-specific increases in the activities of the antioxidant enzyme superoxide dismutase and catalase, suggesting that PM air pollution not only has a potential for oxidant injurious effects but many also trigger adaptive responses.

Cheng et al. (2003) exposed male Sprague–Dawley rats with implanted radiotelemetry devices to CAPs for 6 h/day for 3 days, with rest for 4 days in each week during the experimental period of 5 weeks (CAPs during weeks 2, 3, and 4 and FA during weeks 1 and 5). PM concentrations (range 108–338 μg/m^3) were associated with changes in HR and mean BP immediately after exposure. HR decreased, reaching its low during the first and second

hours, with decreases of 14.9 ($p < 0.01$) and 11.7 ($p = 0.01$) beats per minute, respectively. The hourly mean BP also decreased after the CAPs exposure, with a maximal decrease of 3.3 ($p < 0.01$) and 4.1 ($p < 0.01$) mmHg.

The effects of PM on myocardial ischemia have also been studied. Inhaled PM exacerbated ischemia in a model of coronary arterial occlusion in conscious dogs. Exposures to Boston CAPs significantly increased peak ST-segment elevation during a 5-min coronary artery occlusion compared with FA exposures in two different protocols (Godleski et al., 2000; Wellenius et al., 2003).

9.12.2 New York City

Nadziejko et al. (2003) exposed old Fischer 344 rats with implanted EKG transmitters at 18 months of age for 4 h to FA or NYC CAPs at 160 and 200 μg/m^3 57 to determine the effects of PM on the frequency of spontaneous arrhythmias. There was a significant increase in the frequency of supraventricular arrhythmias after exposure to CAPS compared with FA.

Zelikoff et al. (2003) reported effects on pulmonary or systemic immune defense mechanisms in Fischer 344 rats exposed to NYC CAPs at 0 or 90–600 μg/m^3 for 3 h prior to intratracheal (IT) instillation of *Streptococcus pneumoniae*. The number of lavageable macrophages and PMNs increased in both FA and CAPs groups but was elevated faster and reached twice the concentrations, in addition to staying elevated longer in the CAPs-exposed group. Lymphocytes and white blood cells were significantly increased 24- and 72-h post-infection in both groups. CAPs exposure significantly increased bacterial burdens at 24-h post-infection. Thereafter, CAPs-exposed animals exhibited significantly lower bacterial burdens.

Zelikoff et al. (2003) also evaluated the effects of a single 5-h exposure to CAPs in rats following an IT instillation of *S. pneumoniae*. CAPs exposure significantly reduced percentages of lavageable neutrophils 24 h following CAPs exposure. Lavageable macrophages were significantly increased in the CAPs-exposed animals. CAPs exposure reduced the levels of TNF, IL-1, and IL-6. The bacterial burden decreased in both exposed groups over time; however, CAPs-exposed animals had a significantly greater burden after 24 h than did FA rats. Lymphocyte and monocyte levels were unaffected by CAPs exposure.

In contrast to the nonsignificant decrease in HR observed in dogs exposed to Boston CAPs (Godleski et al., 2000), statistically significant HR increases (~5%) were observed by Gordon et al. (1998) in both normal and MCT rats exposed to NYC CAPs, but not on all exposure days. Thus, extrapolation of the HR changes in these animal studies to human health effects is difficult, although the increase in HR in rats is similar to that observed in some human population studies.

Gordon et al. (1998) reported other cardiovascular effects in animals exposed to NYC CAPs. Increases in peripheral blood platelets and PMNs were observed in control and MCT rats at 3 h, but not 24 h, after exposure to 150–400 μg/m^3 of CAPs. This PMN effect did not appear to be dose-related and did not occur on all exposure days, suggesting that day-to-day changes in PM composition may have played an important role in the systemic effects of CAPs. The number of studies reported was small; and it is therefore not possible to statistically determine if the day-to-day variability was truly due to differences in PM composition or even to determine the size of this effect.

Nadziejko et al. (2003) exposed healthy rats to NYC CAPs at a range of 95–341 μg/m^3 for 6 h and sampled blood at 0-, 12-, and 24-h post-exposure. They found no consistent

differences in counts of platelets and blood cells or in levels of proteins in the blood coagulation system that included fibrinogen, thrombin–antithrombin complex, tissue plasminogen activator, plasminogen activator inhibitor, and factor VII.

Ying et al. (2009a) exposed ApoE$^{-/-}$ mice on an HF diet to FA or $PM_{2.5}$ CAPs for 6 h/day, 5 days/week, for 4 months in northern Manhattan, at a mean concentration of 173 μg/m^3, to test the hypothesis that exposure to CAPs enhances atherosclerosis through induction of vascular reactive oxygen and nitrogen species. Manhattan CAPs were characterized by higher concentrations of OC and EC than Tuxedo, NY, CAPs. Analysis of vascular responses revealed significantly decreased phenylephrine-induced vasoconstriction in CAPs-exposed mice, which was restored by a soluble guanine cyclase inhibitor ODQ (1H-[1,2,4]oxadiazole[4,3-a]quinoxalin-1-one). Vascular relaxation to A23187, but not acetylcholine, was attenuated in CAPs-exposed mice. Aortic expression of NADPH oxidase subunits (p47phox and rac1) and inducible nitric oxide synthase (iNOS) were markedly increased, paralleled by increases in superoxide generation and extensive protein nitration in the aorta. The composite plaque area of the thoracic aorta was significantly increased, with pronounced macrophage infiltration and lipid deposition in the CAPs-exposed mice. Thus, CAPs exposure in Manhattan altered vasomotor tone and enhances atherosclerosis through NADPH oxidase-dependent pathways.

9.12.3 Tuxedo, NY

Lippmann et al. (2005a) conducted a 6-month CAPs inhalation study in normal mice and a mouse model of atherosclerotic disease. The primary objective was to determine whether cumulative daily exposures would cause progressive changes in cardiac function in an animal model for a susceptible human population. In both groups of mice, there were FA exposures following the same protocols used to expose animals to CAPs.

Other coordinate objectives were to look for other $PM_{2.5}$-related responses including:

(1) Short-term changes in cardiac function associated with daily peak $PM_{2.5}$ concentrations and/or specific air trajectories.
(2) Aortic plaque formation and/or plaque size at the end of exposures.
(3) Gene activation at the end of the exposures.
(4) Morphologic changes in the heart, lungs, and brains at the end of the exposures.

Protocols for the analyses of the huge volume of cardiac function data that were developed specifically for this study were described by Chen and Hwang (2005) and Hwang et al. (2005). Groups of normal mice (C57) and knockout mice that develop artherosclerotic plaques (ApoE$^{-/-}$ and ApoE$^{-/-}$ LDLr$^{-/-}$) were exposed for 6 h/day, 5 days/week for 5 or 6 months during the spring/summer of 2003 to either FA or 10-fold CAPs in Tuxedo, NY (mean $PM_{2.5}$ = 110 μg/m^3). Some mice had implanted electocardiographic monitors. As described by Lippmann et al. (2005b): (1) This complex interdisciplinary study was technically feasible in terms of daily exposures, collection of air quality monitoring data; the collection, analysis, and interpretation of continuous data on cardiac function; and the collection and analyses of tissues of the animals sacrificed at the end of the study. (2) The daily variations in CAPs were significantly associated, in ApoE$^{-/-}$ mice, with daily variations in cardiac function. (3) There were significant differences between CAPs- and FA-exposed ApoE$^{-/-}$ mice in terms of cardiac function after the end of the exposure period, as well as

small differences in atherosclerotic plaque density, coronary artery disease, and cell density in the substantia nigra in the brain in the ApoE$^{-/-}$ mice. (4) Suggestive indications of gene expression changes for genes associated with the control of circadian rhythm in the ApoE$^{-/-}$ LDLr$^{-/-}$ double knockout (DK) mice.

Lippmann et al. (2005c) also examined temporal variations in HR and HRV responses during the 24 h beginning with the start of the 6-h CAPs exposure in relation to the components of the CAPs as determined by source apportionment. Daily 6-h $PM_{2.5}$ air samples were also collected and analyzed by XRF, permitting attribution to major $PM_{2.5}$ source categories. For HR, there were significant transient associations with RS during the CAPs exposures and for $SO_4^=$ in the afternoon after exposure. For HRV, there were significant transient associations with residual oil combustion effluents in the afternoon and for $SO_4^=$ late at night.

In a follow-up subchronic $PM_{2.5}$ CAPs inhalation study of ApoE$^{-/-}$ mice at 85 μg/m^3 (Lippmann et al., 2006), there was a dramatic change in cardiac function in the fall months in the ApoE$^{-/-}$ mice. The 14 days with northwest winds carried more Ni, Cr, and Fe, but less of the other elemental tracers than the 89 days with winds from all other directions, and were associated with significant increases in HR and significant decreases in HRV (Lippmann et al., 2006). V was lower than normal on the 14 days with unusually high levels of Ni, Cr, and Fe. Back trajectory analyses from Sterling Forest for the 14 days with northwest winds led through lightly populated areas to Sudbury, Ontario, the location of the largest Ni smelter in North America. Daily 6-h $PM_{2.5}$ air samples permitted attribution to major $PM_{2.5}$ source categories, that is, $SO_4^=$, suspended soil, residual oil combustion, and a remainder category, which was largely due to long-range transported motor vehicle traffic. Examination of associations between components and both HR and HRV for three different daily time periods indicated that for HR, there were significant associations for $SO_4^=$, during exposure and for residual oil combustion in the afternoon. For HRV, there were comparable associations with suspended soil in the afternoon and for both residual oil combustion and traffic late at night. One important parameter that was not addressed in the above study, but that could influence metals' ability in mediating biological response, is the extent of soluble metal components present in the $PM_{2.5}$ mass. In a follow-up subchronic $PM_{2.5}$ CAPs inhalation study of ApoE$^{-/-}$ mice at 85 mg/m^3 (Lippmann et al., 2006), there was a dramatic change in cardiac function in the fall months in the ApoE$^{-/-}$ mice.

At the end of the 6 months of exposure in this study, Sun et al. (2005) compared mice in the CAPs-exposed subgroup on an HF diet with those exposed to FA. For the CAPs-exposed mice, the plaque area in the aorta was 41.5 versus 26.2% in the FA group ($p < 0.001$), whereas for the subgroup on a normal diet, CAPs exposed versus FA exposed was 19.2 versus 13.2% ($p = 0.15$). Lipid content in the aortic arch in the HF group versus normal chow (NC) group exposed to CAPs was 30 versus 20% ($p = 0.02$). Responses to vasoconstrictor challenges in the thoracic aorta were increased in the CAPs-exposed HF mice versus the FA mice ($p = 0.03$), and relaxation in response to acetylcholine was greater ($p = 0.04$). In addition, HF mice exposed to CAPs had marked increases in macrophage infiltration, expression of inducible NO synthase, ROS generation, and immunostaining for 3-nitrotyrosine (all with $p < 0.001$). Thus, the 30-h/week subchronic CAPs exposure of ApoE$^{-/-}$ mice at 85 μg/m^3 altered vasomotor tone, induced vascular inflammation, and potentiated atherosclerosis.

In a third 6-month CAPs inhalation exposure study in Tuxedo, NY, Chen and Lippmann (2013) examined associations between these $PM_{2.5}$ components and both HR and HRV during three different daily time periods: (1) during exposure, (2) the afternoon following

exposure, and (3) late at night. For HR, there were significant transient associations ($p < 0.01$) for $SO_4^=$ during exposure and for residual oil combustion in the afternoon. For HRV, there were comparable associations with suspended soil in the afternoon and for both residual oil combustion and traffic late at night. The biological bases for these associations and lags may relate to the differential solubility of the $PM_{2.5}$ components at the respiratory epithelia and their access to cells that release mediators that reach the cardiovascular system. One important parameter not addressed was the extent of soluble metal components present in the $PM_{2.5}$ mass.

Results of additional assays on $ApoE^{-/-}$ mice in a 6-month CAPs exposure study were reported by Sun et al. (2008a). They described the results of *in vivo* measurements of plaque in the aorta by ultrasound biomicroscopy (UBM) prior to sacrifice, as well as macrophage infiltration (CD68) and tissue factor (TF) expression in the sections of the aorta. UBM-derived plaque areas were 7% larger in the CAPs-exposed HF mice than in the FA-exposed controls ($p = 0.04$), whereas the comparison among NC mice was not statistically significant ($p = 0.07$). Based on immunochemistry, TF expression was increased in the HF mice by CAPs exposure (15 versus 8%, $p < 0.01$), as was macrophage infiltration (19 versus 14%, $p < 0.01$).

In a 10-week, 30-h/week CAPs inhalation exposure study in SD rats conducted in Tuxedo, NY, at an average concentration of 79 μg/m^3, Sun et al. (2008b) implanted minipumps for angiotensin II (A-II) after 9 weeks of exposure. Following the infusion, the mean arterial pressure was elevated in the CAPs-exposed rats compared with the FA-exposed rats ($p < 0.001$). Aortic vasoconstriction to phenylephrine was potentiated, with exaggerated relaxation to the Rho-kinase (ROCK) inhibitor Y-27632 and increase in ROCK-1 mRNA levels in the CAPs-exposed A-II rats. In addition, superoxide levels in the aorta were increased in the CAPs-exposed A-II rats. Based on these findings and some coordinate *in vitro* PM exposures, they concluded that the CAPs exposure exaggerates hypertension through superoxide-mediated upregulation of the Rho/ROCK pathway.

In a CAPs inhalation study, Tan et al. (2009) exposed 4 groups of C57BL/6 male mice fed NC or HF chow (HFC) to either FA or CAPs at 85 μg/m^3 for 6 h/day, 5 days/week, for 6 weeks at Tuxedo. After sacrifice, nonalcoholic fatty liver disease (NAFLD) was evaluated. Stellate cell activation was detected, and collagen I staining was quantified by morphometric analysis. The *in vitro* exposures used a reference PM sample (NIST SRM1649a). Wild-type (wt) and Toll-like receptor 4 (TLR4) knockout ($TLR4^{-/-}$) C57BL/6 mice were sacrificed 24 h after a 500-mg IV injection. For the mice exposed to CAPs by inhalation, there was no significant steatosis in mice on an NC diet. Activated stellate cells were detected in the HFC groups, with mean steatohepatitis grade and stage being significantly higher in the CAPs-exposed group. The mean collagen I staining was significantly greater in the CAPs-exposed HFC group. For the mice exposed by IV injection, SRM particles were detected only in Kupffer cells from livers of SRM-injected mice. In cell culture studies, incubation with 0–200 mg/mL SRM for 24 h induced a dose-dependent increase in pro-inflammatory cytokine mRNA levels, particularly IL-6 ($p < 0.01$). Supernatant analysis confirmed increased IL-6 protein secretion ($p = 0.03$). Similarly, $PM_{2.5}$ exposure (0–100 mg/mL) increased IL-6 secretion in a dose-dependent manner in wild-type Kupffer cells ($p = 0.08$), but not for $TLR4^{-/-}$ Kupffer cells ($p = 0.29$). $PM_{2.5}$ exposure (0–400 mg/mL, 24 h) did not affect collagen 1A1 mRNA or protein levels in the LX2 stellate cell line. $PM_{2.5}$ up to 400 mg/mL did not enhance collagen 1A1 levels in wild-type or $TLR4^{-/-}$ mouse stellate cells. However, collagen 1A1 mRNA levels increased significantly in wild-type and $TLR4^{-/-}$ stellate cells when incubated with conditioned medium from SRM exposed RAW

cells ($p < 0.01$). Thus, direct exposure to $PM_{2.5}$ activates IL-6 production by Kupffer cells in a TLR4-dependent manner. Therefore, enhanced inflammation and fibrosis in NAFLD via direct activation of Kupffer cells may be caused by inhaled PM that enters the circulation, and exposure to ambient air PM may be a significant risk factor for NAFLD progression.

In a study of the effects of CAPs exposure on an obese mouse model, Sun et al. (2009) used male C57BL/6 mice fed HFC for 10 weeks before being exposed to CAPs at 73 μg/m^3 at Tuxedo, NY, for 6 h/day, 5 days/week, for 24 weeks. Compared with the FA-exposed controls, the CAPs-exposed mice had insulin signaling abnormalities that were associated with abnormalities in vascular relaxation to insulin and acetylcholine and increased adipose tissue macrophages (F4/80 cells) in visceral fat expressing higher levels of TNFα/IL-6 and lower IL-10/Mgl1 (macrophage activation marker galactose-*N*-acetylgalactosamine specific lectin). In coordinate *in vitro* tests, PM induced cell accumulation in visceral fat and potentiated cell adhesion in the microcirculation. They concluded that CAPs exposure exaggerated insulin resistance and visceral inflammation/adiposity, providing a link between CAPs exposure and type 2 diabetes mellitus and metabolic syndrome (ms). The biological plausibility of ambient air PM contributing to ms in the CAPs-exposed obese mice was enhanced by a report by Chen and Schwartz (2008) on NHANES III data showing an association of PM_{10} with WBC count.

Blum et al. (2017) exposed timed-pregnant B6C3F1 mice to $PM_{2.5}$ CAPs at 160 μg/m^3 or FA throughout pregnancy for 6 h/day from gestational day (GD) 0.5–GD16.5. A follow-up study examined the effects of exposure during discrete gestational periods aligning to milestones during human development. Exposure throughout pregnancy decreased gestational term by 0.5 day (~1.1 week decrease for humans) and birth weight by 11.4% compared with FA. The follow-up experiment investigated timing of mean concentrations at 178, 193, 171, and 173 μg/m^3 for gestational periods 1–4, respectively. Pregnancy was significantly shortened (vs. FA) by ~0.4 day when exposure occurred during gestational periods 2 and 4 and by ~0.5 day when exposure occurred during period 3. Exposure during periods 1, 2, and 4 reduced birth weight by ~10% vs. FA, and placental weight was reduced (~8%) on GD17.5 when exposure occurred only during period 3.

9.12.4 Columbus, OH

In a follow-up study of the effects of CAPs exposure in Tuxedo, NY, on the Rho/ROCK pathway, Ying et al. (2009b) exposed C57BL/6 male mice that had been fed NC to either FA or CAPs at 74 μg/m^3 for 6 h/day, 5 days/week, for 12 weeks at Columbus, OH. Following the inhalation exposures, the mice were implanted with minipumps for A-II or vehicle for 14 days. One day after that, they were treated with fasudil, a Rho-kinase inhibitor or vehicle. The CAPs exposure potentiated A-II-induced hypertension, and this effect was abolished by fasudil treatment. Cardiac and vascular RhoA activation was enhanced by CAPs exposure, along with increased expression of the guanine exchange factors PDZ, RhoGEF, and p115Rho- GEF, increased A-II-induced cardiac hypertrophy, and collagen deposition, which were all normalized by fasudil treatment. These findings help to explain the chronic cardiovascular effects of $PM_{2.5}$ exposures.

In a further CAPs inhalation study focused on effects on the liver, Laing et al. (2010) exposed C57BL/6 male mice that had been fed NC to either FA or CAPs for 6 h/day, 5 days/week, for 10 weeks at a mean concentration of 76 μg/m^3. They also exposed a murine monocytic/macrophage cell line (Sigma-Aldrich RAW264.7) to Columbus PM *in vitro* at 300 mg/mL. The CAPs inhalation induced endoplasmic reticulum (ER) stress and activation

of a unique unfolded protein response (UPR) in the liver. The *in vitro* exposures demonstrated that macrophage ingestion of PM and the selective activation of the UPR components rely on ROS and Ca signals. In the liver tissue of the CAPs-exposed mice, the selective activation of the UPR components is coordinated with the activation of NF-κB and c-Jun amino-terminal kinase (JNK) and reduced expression of paraoxonase 1 (PON-1) and peroxisome proliferator-activated receptor gamma (PPAR-γ), which favors the development of cardiovascular diseases.

Zheng et al. (2013) exposed C57BL/6 mice to Columbus, OH, CAPs for 6 h/day, 5 days/week at a mean concentration of 75 μg/m^3 for 3 or 10 weeks. Significant effects were seen in mice exposed for 10 weeks, in terms of a nonalcoholic steatohepatitis (NASH-like phenotype, characterized by hepatic steatosis), inflammation, and fibrosis. They displayed impaired hepatic glycogen storage, glucose intolerance, and insulin resistance.

9.12.5 Los Angeles, CA

Kleinman, as described by Lippmann et al. (2003), exposed ovalbumin (OVA)-sensitized mice in a specially equipped van that was located 50 m downwind of a Los Angeles freeway. Groups of mice were exposed to CAPs at 400 and 800 μg/m^3 for 5 or 10 days. Control mice were FA exposed. All mice received an inhalation challenge of OVA 2 weeks after their last CAPs or FA exposure. Eosinophils and OVA-IgE were increased, relative to FA exposed, after the 5- and 10-day CAPs exposures at 400 μg/m^3.

Kleinman et al. (2007) exposed OVA pretreated BALB/c mice to $PM_{2.5}$ CAPs at 300–400 μg/m^3, $PM_{<0.18}$ CAPs at 200–300 μg/m^3, or FA for 5 day/week, for 2 weeks, at 50 and 150 m (meters) downwind from a heavily traveled freeway in Los Angeles. Measurements were made of IL-5, IL-13, immunoglobulin E (IgE), IgG1, and pulmonary infiltration of PMNs and eosinophils. Mice exposed at a distance of 50 m, compared with FA mice, had significant increases in IL-5 and IgG1, whereas those exposed at 150 m did not. The changes at 50 m were significantly associated with the EC and OC in the $PM_{2.5}$ and $PM_{<0.18}$ CAPs, suggesting that freshly formed OC could exert adjuvant affects and promote the development of allergic airway disease.

Araujo et al. (2008) exposed $ApoE^{-/-}$ mice to CAPs for 5 h/day, 3 days/week, for 5 weeks in two size ranges (<0.18 and <2.5 mm) at a site in Los Angeles that was 300 m from the 110 Freeway. The UFP-exposed mice had greater early atherosclerotic lesions than the mice exposed to $PM_{2.5}$ CAPs or FA, and UFP exposure also inhibited the anti-inflammatory capacity of plasma high-density lipoprotein and produced greater oxidative stress as evidenced by a significant increase in hepatic malonaldehyde levels and upregulation of Nrf2-regulated antioxidant genes.

9.13 EFFECTS OF PM SOURCE MIXTURE INHALATION EXPOSURES IN LABORATORY ANIMALS

This section summarizes some studies that used source-related PM components found in ambient air that are relevant to current or recent human exposures. Each subsection begins with studies conducted at PM concentrations occurring in occupational environments and then describes some studies conducted using complex mixtures somewhat higher PM concentrations (up to about 1 mg/m^3), on the basis that they may be relevant to subsequent comparisons of results with those of the CAPs studies.

9.13.1 Diesel Engine Exhaust

In a study that compared the effects of diluted WDE with $PM_{2.5}$ CAPs, Quan et al. (2010) exposed male $ApoE^{-/-}$ mice 5.2 h/day, 5 day/week, for 5 months to five different exposure atmospheres: (1) FA; (2) Tuxedo, NY, $PM_{2.5}$ CAPs (mean = 110 μg/m^3); (3) WDE, containing DEP at 436 μg/m^3; (4) diesel exhaust gases (DEG) equivalent to gases in WDE; and (5) CAPs + DEG. Atherosclerotic plaques were quantified for brachiocephalic artery cross sections (BACs), after 3 and 5 months of exposure using (1) serial ultrasound imaging, (2) hematoxylin and eosin (H&E) histology, and (3) en face Sudan IV stain. All three methods indicated that (1) DEG did not exacerbate progression, (2) there were no interactive effects between DEG and CAPs, and (3) CAPs, despite having a much lower PM concentration, caused more plaque progression than WDE, indicating that some components in ambient PM, not present in WDE, are most responsible for the exacerbation of plaque progression by CAPs.

Campen et al. (2003) examined the cardiac effects of diesel exhaust exposure in SH rats. They were exposed to 0, 30, 100, 300, and 1000 μg DEP/m^3 for 6 h/day for 7 days. FA-exposed rats displayed a reduced daytime HR from the beginning of the protocol, whereas DEP-exposed rats maintained a significantly elevated HRs. This difference persisted during the evenings of the exposure period but was not observed at any time during the pre- or post-exposure periods. The PQ interval, an index of atrioventricular node conduction velocity, was significantly prolonged among DEP-exposed rats in a concentration-dependent manner. Increased HR with prolongation of the PQ interval may represent a substrate for ventricular arrhythmias.

Anselme et al. (2007) exposed adult male WKY rats with and without IHD to 500 μg/m^3 of DEP for 3 h. They were evaluated for ventricular arrhythmia during and after the exposure by ECG telemetry. There was an immediate decrease in root mean square of the standard deviation of normal-to-normal beat (RMSSD) in both groups of rats and an immediate increase in premature ventricular beats in the IHD rats only. The changes in HRV were over after 2.5 h, whereas the pro-arrythmic effects in the IHD rats persisted for 5 h after the end of DEP exposure.

Saxena et al. (2009) exposed WKY and SH rats to 0, 500, or 2000 μg/m^3 of DEP for 4 h/day, 5 day/week, for 4 weeks to determine pulmonary retention of EC. While SH rats had 50% higher minute volumes, they had 16% less EC at the end of the exposures at 500 μg/m^3 and 32% less at 2000 μg/m^3 than the WKY rats. For the same exposures, Gottipolu et al. (2009) noted PMN influx in the lung lavage in both strains, with only minimal changes in injury markers. This study provided evidence that WDE produced a hypertensive-like cardiac gene expression pattern associated with mitochondrial oxidative stress in the WKY rats, but not in the SH rats.

Hazari et al. (2011) exposed SH rats to either 150 or 500 μg/m^3 of DEP for 4 h to WDE or the same concentration of DEG and assessed arrhythmogenesis 24 h later by continuous intravenous infusion of aconitine, an arrhythmogenic drug, while HR and ECG were monitored. Rats exposed to WDE or DEG had slightly higher HRs and increased LF/HF ratios (sympathetic modulation) than did controls; ECG showed prolonged ventricular depolarization and shortened repolarization periods. Rats exposed to WDE developed arrhythmia at lower doses of aconitine than did controls; the required dose was even lower in rats exposed to DEG. Pretreatment of low WDE-exposed rats with a TRPA1 antagonist or sympathetic blockade prevented the heightened sensitivity to arrhythmia. Lamb et al. (2012) extended this line of research and exposed both SH and WKY rats to WDE

containing either 150 or 500 μg/m^3 of DEP for 4 h or to DEG (the filtered stream having the same gas concentrations). Exposure to WDE, but not DEG, caused post-exposure ST depression and increased sensitivity to the pulmonary C fiber agonist capsaicin in SH rats, while exposure to DEG caused immediate ECG alteration in cardiac repolarization (ST depression) and atrioventricular conduction block (PR prolongation) as well as bradycardia in SH rats. For WKY rats, the only notable effect of WDE exposure was a decrease in HR.

Hazari et al. (2012) continued this line of research and exposed both SH and WKY rats to WDE to 150 μg/m^3 of DEP for 4 h. Increasing doses of dobutamine, a β_1-adrenergic agonist, were administered to conscious unrestrained rats 24 h later to elicit the cardiac response observed during exercise while HR and ECG were monitored. The WDE exposure potentiated the HR response of WKY and SH rats during dobutamine challenge and prevented HR recovery at rest. During peak challenge, WDE-exposed SH rats had lower overall HR variability when compared with controls, in addition to transient ST depression. All WDE-exposed animals also had increased arrhythmias.

Carll et al. (2012) exposed SH rats to WDE containing 150 μg/m^3 of DEP for 4 h or to DEG having the same gas concentrations and measured HR and HRV. WDE increased BP and decreased HRV. DEG and WDE decreased HR in the 4 h after exposure. Thus, WDE and DEG differentially alter CV and autonomic physiology and may increase risk through divergent pathways. Carll et al. (2013) extended their research to a 4-h exposure to 500 μg/m^3 of DEP in WDE and the associated DEG. The WDE increased HRV during exposure. In the 4-h post-exposure, WDE increased cardiac output, left ventricular volume, stoke volume, HRV, and atrioventricular block arrhythmias, ST and T amplitudes, ST area, T-peak to T-end duration, but not HR. Changes in HRV were correlated with bradyarrhythmia frequency, repolarization, and echocardiographic parameters. At 24-h post-exposure, WDE-exposed rats had increased serum CRP and pulmonary eosinophils.

9.13.2 Motor Vehicle Engine Exhaust (MVE) and Other Ambient Air Components

As reported by Campen et al. (2013), the Lovelace Respiratory Research Institute (LRRI) part of the HEI-sponsored NPACT study was focused on the cardiovascular responses in ApoE$^{-/-}$ mice that were fed HFC. The mice were exposed by inhalation for 6 h/day for 50 day to 100 and 300 μg/m^3 of $PM_{2.5}$ components found in ambient air. The eight different exposure atmospheres consisted of (1) diluted MVE (50 μg/m^3 of $PM_{2.5}$ from a spark ignition engine and 250 μg/m^3 from a diesel engine) and pollutant vapors in the exhaust stream, (2) diluted emission gases (MVEG), (3) 300 μg/m^3 of resuspended road dust, (4) 300 μg/m^3 of $SO_4^=$, (5) 300 μg/m^3 of NO_3^-, (6) MVEG + $SO_4^=$, (7) MVEG + NO_3^-, and (8) MVEG + road dust. Measured responses were lipid peroxidation (TBARS), plaque growth, plaque inflammation, vascular gelatinase activity, NO pathway components, MMP expression, vasoconstriction, and oxidized LDL. The selection of the eight pollutant mixtures provided a basis for determining the effects of $PM_{2.5}$ components accounting for most of the $PM_{2.5}$ mass (traffic and stationary fossil fuel combustion) and the extent to which fossil fuel combustion-related gases and vapors may enhance the toxicity of the airborne PM.

For vascular (aortic) lipid peroxidation, there were significant effects (in decreasing order of potency) for whole MVE, $SO_4^=$ + MVEG, NO_3^- + MVEG, and road dust + MVEG. For plaque inflammation, there were strong associations for whole MVE, $SO_4^=$, $SO_4^=$ + MVEG, and NO_3^- + MVEG and a smaller association with MVEG alone. For vasoconstriction, there was a strong association for $SO_4^=$, with less association for MVE and for $SO_4^=$ + MVEG. For oxidized LDL, there was a strong association for MVE, but none for

the other $PM_{2.5}$ mixtures. For MMP expression, the only significant association was for $SO_4^=$ +MVEG, while for vascular gelatinase activity, MVE had the only significant association. $SO_4^=$ alone had a very strong associations with vasoconstriction, while NO_3^- alone did not. $SO_4^=$ and NO_3^-, when combined with MVEG, had a strong association with plaque inflammation, but neither alone was associated with TBARS, but when mixed with MVEG, both mixtures were strongly associated with TBARS. These studies demonstrated that (1) subchronic exposure to vehicle-related mixed emissions resulted in statistically significant increases in lipid peroxidation, circulating oxLP, vascular MMP expression and activity, and enhanced vasoconstriction in $ApoE^{-/-}$ mice and (2) exposure to NO_3^-, $SO_4^=$, and road dust alone did not drive any of the statistically significant cardiovascular effects.

These various CAPs-related effects on cardiac function and the development of histological evidence of increased risk of clinically significant disease at the end of the exposures in animal models of atherosclerosis provide biological plausibility for the premature mortality associated with $PM_{2.5}$ exposure in human subjects and suggestive evidence for neurological disease as well. Thus, diseased and elderly rats respond to $PM_{2.5}$ inhalation with greater responses than healthy young animals and produce responses that appear relevant to excess mortality and morbidity in sensitive human populations.

9.14 NPACT SUBCHRONIC CAPS MOUSE INHALATION STUDIES

9.14.1 Subchronic Mouse CAPs Inhalation Exposures at Five Different U.S. Sites

As described by Chen and Lippmann (2013), the NYU NPACT subchronic ambient air CAPs inhalation study in $ApoE^{-/-}$ mice was conducted at five different U.S. sites having substantial $PM_{2.5}$ differences. The "cleaner" sites SEA at 61 $\mu g/m^3$ and EL at 68 $\mu g/m^3$ had half as much $PM_{2.5}$ as MS at 123 $\mu g/m^3$, IR at 138 $\mu g/m^3$, and SF at 136 $\mu g/m^3$. There were much greater component concentration differences within the MS: Fe from 0.30 $\mu g/m^3$ at EL to 1.88 at MS; Mn from 14.2 ng/m^3 at SF to 107 at MS; Zn from 51.3 ng/m^3 at EL to 760 at MS; Cu from 5.4 ng/m^3 at EL to 100.5 at IR; Se from 6.0 ng/m^3 at SEA to at IR; V from 17.1 ng/m^3 at SF to 45.7 at IR; and Ni from 6.6 ng/m^3 at EL to 69.9 at MS.

9.14.2 Cardiac Function Responses to CAPs in the NYU NPACT Study

For CAPs associations with 3 function indices (HR, SDNN, and MSRRD), 3 different lag days, and 4 different times of day, there were 56 statistically significant differences in cardiac function indices between CAPs- and FA-exposed mice at MS, 38 at SF, 6 at EL, 5 at IR, and 3 at SEA. The significant responses could be either positive (+) or negative (−) for day and time intervals, since functional cardiac change could be either accelerated or retarded depending on applied dose. Bidirectional physiological changes do not necessarily represent either "adverse" or "beneficial" effects *per se*. For example, H_2SO_4 aerosol produces accelerations in tracheobronchial mucociliary particle clearance at low levels of exposure and slowing of clearance at higher exposure levels (Leikauf et al., 1984; Lippmann and Schlesinger, 1984). Many, if not most, such changes may be adaptive and may only contribute to cumulative "adverse" effects upon long-term exposure.

There were many more functional changes at MS and SF than at the others, suggesting that components of the northeastern regional secondary aerosol, absent at IR, and SEA, and much lower at EL, were producing the short-term cardiac function responses, even though

the CAPs mass concentration at IR was twice that at EL and SEA. The changes in HR/HRV were expressed as change in beats/minute/mg/m^3.

The significant functional changes at the IR, EL, and SEA sites were mostly at the 2-day lag. In contrast, the much larger numbers of functional changes at the MS and SF sites were at 0- and 1-day lags, consistent with the hypothesis that higher levels of exposure result in significant differences in responses being seen at earlier times after exposure. There were nearly equal numbers of functional changes during the 4 time periods of the exposure day at all five sites, albeit with many fewer "hits" at EL, SEA, and IR than at MS and SF.

The number of components having statistically significant ($p < 0.05$) associations with functional responses was not in proportion to CAPs concentration. For example, MS with CAPs at 123 μg/m^3 had 56 significant functional responses (39+ and 17) between CAPs- and FA-exposed mice, while IR with CAPs at 138 μg/m^3 had just five such responses (2+ and 3−).

In terms of individual $PM_{2.5}$ components, there were many more "hits" at MS than at SF for BC, Al, Mg, Na, Ni, P, and V, while there were many more "hits" at SF than at MS for OC, Cr, Cu, K, Mn, Pb, and Zn.

There were highly correlated groupings of elements at MS and SF, the sites showing the most exposure–response relationships for cardiac function responses. Groupings included Al, Si, and Ti (soil) and Br, Se, P, and S (coal combustion). At MS, Ni was closely associated with V, S, and EC (residual oil combustion) (Peltier and Lippmann, 2010), while at SF, Ni was associated with Cr and Fe, which, along with Ni, have been associated with stack effluents from the distant upwind Ni smelter at Sudbury, ONT (Lippmann et al., 2006).

For 21 single component concentrations examined for 3 function indices for 3 lag days and 4 times of day, there were about 3 times as many significant differences in cardiac function indices between CAPS- and FA-exposed mice at MS and SF than at the EL and SEA sites and more than twice as many at the IR site.

The strongest regressions of the five-site regressions, in terms of HR, SDNN, and RSSMD, were for Ni ($r^2 = 0.96$), Al ($r^2 = 0.81$), EC ($r^2 = 0.79$), P ($r^2 = 0.77$), S ($r^2 = 0.65$), and V ($r^2 = 0.35$). Other elements had negative and less consistent exposure–response relationships; these were Se ($r^2 = 0.19$), K ($r^2 = 0.13$), and Zn ($r^2 = 0.27$). Some concentrations (Cu, Si, Fe, Mg, Mn, and Pb) showed less consistent and fewer exposure–response relationships with the cardiac function indices, with both positive and negative associations for different lag days and times of day.

There were strong associations of changes in HR, SDNN, and RSSMD for at least one source category at each of the five sites, but the most influential source category was differed at each site, with the strongest signals for soil and a moderate signal for residual oil combustion in SEA, strong signals for residual oil combustion and $SO_4^=$ at MS, strong signals for $SO_4^=$, and a moderate signal for a distant upwind Ni-refinery effluent at SF. The sources associated with Ni sources were at MS, SF, and SEA, which had the highest Ni concentrations and less influential at EL, with its much lower Ni concentration. Two source categories not generally considered as likely causal factors were among those associated with cardiac function changes, that is, soil in SEA and SF and salt in SEA.

9.14.3 Aortic Plaque Progression in the NYU NPACT Study

In terms of the effects of long-term CAPs exposure on plaque progression in the brachiocephalic artery (BA) as plaque volume, as measured by UBM, there was variation by site location, with substantial plaque volume progression at MS and SF in NY (with the

highest $SO_4^=$ and Ni concentrations). There was less but still statistically significant plaque volume progression at EL, but none in SEA and IR.

In supplemental measurements of plaque surface area by visual inspection in mice sacrificed after 2, 4, and 6 months of CAPs or FA exposure at IR, there was a progressive rise in plaque surface area over time. Comparable measurements were made for mice sacrificed after 6 months of exposure at EL, and the plaque surface areas attributable to CAPs exposure were greater than those for the IR mice. Unfortunately, such assays were not performed at the ends of earlier CAPs inhalation studies at MS, SF, and SEA.

9.14.4 PM Components Associated with Aortic Plaque Progression in Mice

In terms of aortic plaque progression in the mice exposed for 6 h/day, 5 days/week to CAPs at five sites for 6 months, there was significant progression at MS (NYC), SF (Tuxedo, NY), and EL (East Lansing, MI), but not at IR (Irvine, CA) and SEA (Seattle, WA). As a source of $PM_{2.5}$ mass concentration, traffic was substantial at MS, IR, and SEA but minor at SF and EL. In contrast, $SO_4^=$ was a substantial fraction of $PM_{2.5}$ at MS, SEA, and EL but minor at SEA and EL. These differences strongly suggested that coal and residual oil combustion, which accounts for most of the $SO_4^=$, are more influential in plaque progression than the traffic source. This interpretation is consistent with the different influences on plaque progression of SF CAPs, sidestream cigarette smoke, and diesel engine exhaust in prior NYU subchronic inhalation studies in $ApoE^{-/-}$ mice. As reported by Lippmann and Chen (2009), the CAPs produced more plaque progression with a $PM_{2.5}$ concentration of 110 μg/m^3 than did either sidestream smoke or DEP with $PM_{2.5}$ at 400 μg/m^3. Furthermore, the sidestream cigarette smoke and diesel engine exhaust contained OC and volatile organic vapors not present in the CAPs, while they lacked the transition and heavy metals present in the CAPs. To the extent that the traffic source did affect either short- or long-term health effects in the NYU NPACT studies, it was not at all clear whether the effects could be attributed to $PM_{2.5}$ constituents or gas-phase pollutants, although the results of a supplement to NYU's ACS cohort study did reduce the likelihood that the effects are attributable to gaseous criteria pollutants. To the extent that $PM_{2.5}$ constituent elements were causal, the analyses lacked the power to distinguish between tailpipe emissions of transition metals, brake wear emissions (e.g., Cu), and resuspended road dust containing metal oxides.

Traffic markers, such as EC and nitric oxide NO, can display significant variation on scales of 50–500 m within cities (Henderson et al., 2007), while elevated pollution sources (e.g., power plants with tall stacks) are more spatially homogeneous, potentially biasing the effect estimates of localized sources, such as traffic, toward the null. Another limitation is the use of $PM_{2.5}$ data from 2000 to 2004, which could introduce exposure misclassification if the $PM_{2.5}$ levels and constituents in the studied metropolitan areas have changed over time. However, previous work in this cohort has shown the $PM_{2.5}$ levels in different time periods to be correlated, with lower levels at the end of the follow-up period, but indicating that areas with the highest exposures in the 1980s are still the most highly exposed in the 2000 (Pope et al., 2002; Thurston et al., 2016a, 2016b).

9.15 CONSISTENCY, COHERENCE, AND IMPLICATIONS TO PUBLIC HEALTH

One basic challenge in digesting and integrating the voluminous literature on PM ambient air and its relation to human health impacts is the complexity of the data, especially in view of PM's range of particle size and chemical components. The temporal and spatial variations

of PM concentrations reflect variations in (1) direct PM emissions, (2) secondary PM formation in the atmosphere from gaseous pollutant emissions, and (3) further physicochemical transformations while airborne. People vary greatly in (1) inhalation rates, (2) particle deposition patterns within their respiratory tracts, (3) particle clearance from the airways, (4) translocation pathways to other organs, (5) dissolution and entry of soluble constituents in the bloodstream, (6) metabolism at critical organs, and (7) susceptibility to health-related short- and long-term biological effects.

A second basic challenge is that the biological response data comes from a variety of different kinds of research investigations, including (1) observational studies of human populations in natural settings and the associations with vital status, disease patterns, functional capacity, and lost times from school or work in relation to pollutant concentrations in the inhaled air, (2) laboratory-based short-term inhalation exposures of human volunteers to specific pollutants or mixtures of pollutants and frequent or continuous measurements of functional responses, and (3) laboratory-based short- or long-term inhalation exposures of laboratory animals to specific pollutants or mixtures of pollutants and continuous or intermittent measurements of functional responses, as well as pathological examinations of affected organs and tissues.

Each type of study has its advantages and limitations. Laboratory studies of human volunteers are generally limited to relatively small numbers of healthy adults and to short-term exposures and observations. Observational studies in large-scale human populations have limited measurements of ambient concentrations and uncertainties as to how concentrations vary among those inhaled by the numerous individuals in the populations. Interpretations of findings in inhalation exposure studies of laboratory animals are limited by the interspecies differences in particle deposition, retention, and metabolism.

A third challenge in the integration of the voluminous literature on PM ambient air and its relation to human health impacts is that the various studies seldom have the same effects measurements or temporal scales. In this context, a unique contribution was made by the NPACT studies that generated and compared the results of comparable exposures and effects measurements in both their observational epidemiology and animal inhalation substudies.

The discussion that follows examines the roles that the chemical components of the exposure atmospheres had in eliciting the biological responses, with special emphasis on the chronic effects, leading to lifespan shortening in human populations on the basis of their greater demonstrated public health impacts, as well as focusing on data coherence, as defined by Bates (1992), where evidence for one kind of health-related endpoint can be expected to be predictive of excesses in other endpoints.

9.16 MOST INFLUENTIAL $PM_{2.5}$ COMPONENTS AS CAUSAL FACTORS

In most of the NPACT short-term exposure studies, there were associations of daily hospital admission rates with exposure to traffic-related effluents (EC, OC, Pb, Cu, and road dust) and with one or more other transition elements (Fe, Ni, Zn, V, Cr). For short-term hospital admissions, the only significant associations were (1) the traffic factor and constituents such as EC, OC, Cu, and Pb and (2) resuspended road dust (Si and Fe). In identifying the constituents having the greatest influence on short-term health effects, the factors most closely influencing the CVD hospital admissions included residual oil combustion components (Ni, V, S) and seaport berth volume and traffic-related factors (Cu and road lengths). $SO_4^=$ had a nonsignificant negative association.

There was little evidence for an association of hospital admissions with markers for coal combustion effluents ($SO_4^=$, As, and Se). For daily mortality, $SO_4^=$ was the most influential constituent, and Se and As showed positive, albeit nonsignificant associations. Even for short-term peak exposures, the components associated with hospital admissions (OC and transition metals) differed from those associated with daily mortality (coal combustion effluents).

Bell et al. (2014) examined associations of $PM_{2.5}$ components (Ca, BC, V, and Zn) and sources (traffic, road dust, oil combustion, sea salt, and coal combustion) with hospital admissions for four counties in CT and MA for persons >65 years of age. For cardiovascular admissions, there were significant associations with road dust, BC, V, and Zn. For respiratory, there were significant associations with road dust, BC, Al, Ca, Si, Ti, Ni, and V, and the effect estimates were generally robust to adjustments for other components.

$SO_4^=$ and traffic (as indexed by OC and Cu) were more closely associated with chronic CVD responses than other source categories or individual $PM_{2.5}$ components in both NPACT human health effects studies, but they differed in terms of relative influence. For short-term CVD responses, which were measured by the NYU NPACT, Ni and V were influential. The specific associations for chronic effects are summarized below for each NPACT location.

9.17 DAILY MORBIDITY EFFECTS AND COHERENCE WITH EXCESS DAILY MORTALITY

To the extent that cardiopulmonary responses are attributable to elevated concentrations of PM, one may expect higher daily rates of emergency hospital admissions and visits to EDs and clinics have generally been significantly associated with excess daily emergency admissions to hospitals for either respiratory or cardiac diseases or both. For respiratory diseases, the association with summertime O_3 has generally been stronger than those with PM. In contrast, the mortality influence of PM was generally much stronger than that of O_3. For hospitalizations for cardiac diseases, the most influential criteria pollutants appeared to be PM and CO. Further discussion on pollutant interactions and joint effects is provided in the chapters on those toxicants.

Most of the recent epidemiological studies did not have the advantage of available $PM_{2.5}$, PM_{10}, and $PM_{10-2.5}$ component concentration data and were therefore limited to mass concentrations within those size ranges. There was coherence in terms of the RR ratings, with mortality risks increasing from total to cardiovascular to respiratory and with ED visits being more frequent than hospital admissions. However, there are, as yet, no generally accepted mechanistic bases to account for the epidemiological associations between ambient $PM_{2.5}$ and mortality, morbidity, and functional changes, while attempts to discredit the associations on the basis of the effects being due to other environmental variables that may covary with $PM_{2.5}$ have, to date, been unsuccessful, including the possible confounding influence of adjustments to models to account for weather variables, which has been found to be minimal (Pope and Kalkstein, 1996).

While mechanistic understanding of processes by which ambient air PM causes human health effects remains limited, the credibility of ambient $PM_{2.5}$ as a predictor of excess human mortality and morbidity has been enhanced by the growing number of inhalation studies in which human volunteers and laboratory animals have been exposed by inhalation to CAPs.

One of the most remarkable findings of the NYU NPACT epidemiologic studies was that the $PM_{2.5}$ components that were most closely associated with the short-term health effects were different from those associated with the long-term effects, with transition metals and traffic markers being more closely associated with daily mortality and hospital admissions and coal combustion components being more closely associated with annual rates of IHD and lung cancer mortality.

Another notable finding, in terms of short-term effects, was the differences in the $PM_{2.5}$ components more closely associated with total daily mortality and CVD hospital admissions. In time-series analyses, Cu, Ni, and V were more strongly associated with CVD hospital admissions than were $SO_4^=$, Se, As, OC. By contrast, total daily mortality was significantly associated with $SO_4^=$, with positive associations with Se and As, suggesting that the coal combustion source was responsible for excess short-term mortality as well as excess annual mortality.

For associations of excess risks of multiple $PM_{2.5}$ components present at relatively low mass concentrations, the magnitudes of the risks, per IQR, were usually similar to those of total $PM_{2.5}$, S, and EC, which were present at relatively large mass concentrations, possibly explaining why S and EC have often been useful markers of short-term public health risks.

Lack of associations of excess risks with both total $PM_{2.5}$ and multiple $PM_{2.5}$ components suggests that the concentrations of potentially causal $PM_{2.5}$ components were too low, at those times and places, to cause measurable effects. The epidemiological associations are often driven by responses to elevated concentrations on a relatively few days with little or no measurable response on a majority of days. The specific $PM_{2.5}$ components that were most often significantly associated with excess risks were markers of fossil fuel combustion, for example, EC from traffic, Se from coal combustion, V from residual oil combustion, and K from biomass combustion and a variety of other sources.

The largest single-day excess risks have typically been for hospital admissions associated with EC, and to a lesser extent with OC, at 0-day lag, with little or no excess at lag days 1 and 2, suggesting short-term responses to components or surface coatings of carbonaceous particles. The largest distributed-lag excess risks have typically for hospital admissions associated with the inorganic elements, with relatively little decrease in excess risk over several later lag days. These components appear to be more closely associated with chronic CVD responses. Excess CVD risks have mostly been attributed to IHD and heart failure, while excess respiratory disease risks have most often been attributed to COPD and/or acute respiratory failure. Characterizing risks in terms of total CVD or total respiratory categories tends to obscure the effects related to more specific disease subcategories.

The associations of IHD annual mortality rates with exposure to coal combustion effluents in the NYU NPACT study of the ACS cohort are broadly coherent with the associations of annual $PM_{2.5}$ exposure and CVD events with OC and $SO_4^=$ in the UW's WHI cohort. The association with $SO_4^=$ with annual mortality in the WHI cohort is most likely related to the strong correlations of $SO_4^=$ with Se and As (tracers of coal combustion). In two-pollutant models for OC and $SO_4^=$, there were significant and higher HRs for $SO_4^=$ than for OC for overall CVD events, as well as for the subcategories of CHD, MI, and coronary revascularization. In contrast, there were significant and higher HRs for OC than for $SO_4^=$, for the cerebrovascular disease and stroke subcategories, suggesting that OC exposure may be a more important risk factor for cerebrovascular effects than for CVD effects.

In the earlier literature, $SO_4^=$, most likely acting as a surrogate measure of exposure to coal combustion effluents, had significant associations with annual mortality (Lave and

Seskin, 1970; Ozkaynak and Thurston, 1987; Dockery et al., 1993; Pope et al., 1995a, 1995b, 2002; Ostro et al., 2007, 2010). The traffic source, as indexed by OC and Cu, was strongly associated with annual CVD mortality in the UW's WHI cohort but only marginally associated with annual IHD mortality in the NYU's ACS cohort. However, there were no consistent associations, in either the NYU or UW NPACT studies of annual mortality rates with exposures to other source-related factors, such as those associated with the soil or residual oil combustion sources of $PM_{2.5}$. As indicated in Table 9.4, associations of annual mortality rates with traffic were also found by Lipfert et al. (2006) and Ostro et al. (2010), while associations with residual oil components with long-term mortality were reported by Hedley et al. (2002) and Cahill et al. (2011) in studies in which there were much higher concentrations of residual oil combustion effluent concentrations than were present in the ACS and WHI cohorts in the years that their analyses covered.

In terms of hospital admissions, Kioumourtzoglou et al. (2016) investigated the impact of long-term $PM_{2.5}$ exposure on time to first hospital admission for dementia, Alzheimer's disease (AD) or Parkinson's disease in a Medicare population in 9.8 million elderly residents 50 cities across Northeastern United States. For a 1 $\mu g/m^3$ increment in annual average $PM_{2.5}$, there was an HR = 1.08 (1.05–1.11) for dementia, 1.15 (1.11–1.19) for AD, and 1.08 (1.04–1.12) for PD.

The UW NPACT study (Vedal et al., 2013) was limited to the cardiovascular effects of chronic exposures of ambient air $PM_{2.5}$ in two human cohorts: (1) the MESA, which was focused on subclinical measures (CIMT and CAC); and (2) and the Women's Health Initiative (WHI), which was focused on cardiovascular events (CVD mortality, hospital admissions, and other events, such as MI and coronary revascularization).

The MESA study was conducted in 6 U.S. cities, that is, NYC, Baltimore, Winston-Salem, Chicago, St. Paul, and Los Angeles, while the WHI was conducted in 45 U.S. cities (8 in the Northeast, 11 in the Southeast, 13 in the Midwest, 3 in the Southwest, 4 in California, and 6 in the Northwest). For both cohorts, the exposures at the individual cohort member level were estimated using a national spatial model using 2009 chemical speciation data for population centers and from the IMPROVE network for "background" sites. For the MESA cohort, a more complex spatiotemporal model utilized 2-week UW sampling measurements at 3–7 sites in each region, as well at 50 home sites in each of 2 seasons. The concentrations of EC, OC, S, and Si were chosen on the basis of them being major components of overall $PM_{2.5}$ mass and being representative of major $PM_{2.5}$ sources (EC representing primary emissions from fossil fuel and biomass combustion, OC representing secondary organic particles, S representing secondary inorganic particles, and Si representing soil-derived particles). The concentrations of $PM_{2.5}$ constituents (Ni, V, Cu) contributing much smaller amounts to $PM_{2.5}$ were considered to be of secondary interest and were investigated for their associations with measured indicators of health-related responses, along with $SO_4^=$, NO_3^-, SO_2, and NO_2.

For CIMT, there was a statistically significant association for $PM_{2.5}$ mass similar to those for EC and Si, with substantially larger effects associated with $SO_4^=$ and OC. The associations when using their national exposure model were essentially the same for $SO_4^=$, OC, and Si, and there was no association for EC. In associations of other measured $PM_{2.5}$ components on CIMT, the only component approaching significance was Cu, a marker for brake wear.

Based on the UW source apportionment, none of the four $PM_{2.5}$ components of primary interest in the epidemiologic analyses seemed to be influenced by the presence of MVE. Of these, EC [traditionally used as a marker of exposure to motor vehicle exhaust (MVE)] reflected a complex mix of sources. The diesel exhaust/brake wear-like feature contributed

to EC in each MESA city (ranging from 6 to 36%). Other potential markers of exposure to MVE were NO_2 and Cu. The source apportionment indicated that the diesel exhaust/brake wear-like feature also contributed to NO_2 to a greater extent than to EC, with contributions ranging from 1 to 46% across the MESA cities. Because NO_2 and NO_3^- were of secondary interest, their health analyses of NO_2 were completed only in the MESA cohort and provided little evidence that NO_x was associated with the effects endpoints. To the extent that exposure to MVE was reflected by either EC or NO_2, which are not particularly good markers of MVE exposure, they found little evidence to support a role for MVE. Cu, however, might have been a better marker of exposure to traffic-related pollutants than either EC or NO_2, with contributions from the source apportionment ranging from 32 to 57%. The UW epidemiologic findings for Cu, although limited in scope, did suggest that exposure to MVE could be important in the development of atherosclerosis. For CAC, there were no associations for any of the PM components for either exposure model, except for OC.

For CVD and CHD events in the WHI cohort, there were statistically significant and comparable HRs for $PM_{2.5}$ and $SO_4^=$, but none for EC, OC, and Si. For MIs, only $SO_4^=$ had a statistically significant HR. For coronary revascularization, $SO_4^=$ had a greater influence than $PM_{2.5}$, with no association for EC, OC, or Si. For stroke, there were statistically significant and comparable HRs for $PM_{2.5}$, OC, and $SO_4^=$, but none for EC or Si. Using two-pollutant models for OC and $SO_4^=$, there were higher HRs for $SO_4^=$ than for OC for CVD events, CHD, MI, and coronary revascularization. In contrast, there were significant and higher HRs for OC than for $SO_4^=$ for CVD and for stroke. For CVD deaths, there were statistically significant and comparable HRs only for OC. For atherosclerotic CVD deaths, there were statistically significant HRs for OC, EC, and $SO_4^=$ exposure, which were similar in HR magnitude to that for the nonsignificant HR for $PM_{2.5}$. There was no association for Si. For stroke deaths, the only significant HR was for OC.

9.18 EFFECTS OF $PM_{2.5}$ COMPONENTS IN TOXICOLOGICAL STUDIES

In the NYU NPACT study, $ApoE^{-/-}$ mice were exposed to CAPs for 6 h/day, 5 days/week for 6 months in NYC, Sterling Forest in Tuxedo, NY (SF), and in East Lansing, MI (EL), where coal combustion effluent accounts for significant fractions of the ambient air PM, had significant aortic plaque progression, while $ApoE^{-/-}$ mice exposed to CAPs for 6 h/day, 5 days/week for 6 months in Seattle, WA (SEA), and in Irvine, CA (IR), without coal effluent exposure, did not. While the number of sites was small, it is notable that the $PM_{2.5}$ mass concentrations in the exposure chambers were about twice as high at MS, SF, and IR as those in EL and SEA, yet IR mice had no plaque progression, while EL mice did.

In the LRRI part of the UW-LRRI NPACT study, $ApoE^{-/-}$ mice were exposed to laboratory-generated mixtures for 6 h/day for 50 days at $PM_{2.5}$ concentrations that were 3–6 times higher than those for the mice in the NYU NPACT study, but for fewer days of exposure. There was significant plaque growth for LRRI. Resuspended inorganic oxide dusts and reactive gases may differ with respect to stimulation of plaque progression from those of the combination of PM and vapors in the whole MVE exposures, $SO_4^=$, $SO_4^=$ + MVE, and NO_3^- + MVE. There were also significant associations of vasoconstriction with MVE, $SO_4^=$, and $SO_4^=$ + MVE.

It is noteworthy, in the LRRI mice, that $SO_4^=$, when mixed with MVE, was strongly associated with plaque inflammation and $SO_4^=$ (attributable of coal combustion), while MVE (attributable to traffic) was a large fraction of the NO_3^-. The strength of the association of $SO_4^=$ with IHD mortality in humans, and with aortic plaque progression in mice in the

NYU NPACT studies, could have been due to the coal combustion effluent mixture or to $SO_4^=$ itself. The LRRI mice plaque inflammation responses to $SO_4^=$ suggest that, even in the absence of a biological mechanism for such an effect, $SO_4^=$ may not only be a marker for coal combustion but also a causal factor by itself.

The limited coherence of the NYU and LRRI NPACT results may have been due to differences in the experimental protocols. The NYU mice ate NC, while the LRRI mice ate high-fat chow. While both used 6 h/day exposures, the NYU exposures extended over 6 months, while the LRRI exposures only extended over 50 days. There were relatively high concentrations of $SO_4^=$ in the CAPs at NYU's MS, SF, and EL sites, along with other components of fossil fuel combustion. In contrast, the $SO_4^=$ alone exposure at LRRI lacked other ambient air components. The freshly generated MVE and the $SO_4^=$ + MVE mixture in the LRRI study may have been more acidic due to the presence of organic monolayers on the ultrafine H_2SO_4 droplets, whose neutralization by NH_3 is retarded by organic surface layers. Strong acid may account for some of the vascular effects attributable to the exposures. The relatively high concentrations of OC in the MVE mixtures in the LRRI NPACT study were not present in the CAPs studies at NYU.

The LRRI study with $SO_4^=$ alone was the first to demonstrate that a subchronic inhalation exposure to pure $SO_4^=$ (at 300 $\mu g/m^3$) could cause significant effects on aortic contraction and that co-exposure to MVE could substantially enhance plaque progression. The association of $SO_4^=$ with cardiac function and plaque progression in the CAPs-exposed NYU mice could have been due to the copresence of $SO_4^=$ and other ambient air pollutants. The LRRI toxicological study showed effects of $SO_4^=$ both alone and in combination with the MVE-containing mixtures. Of all the LRRI pollutant atmospheres, an atmosphere of pure $SO_4^=$ caused the most substantial changes in aortic vasoreactivity that were also noted for $SO_4^=$ + MVE, although to a lesser extent. There was also a suggestion that pure $SO_4^=$ increased plaque area and plaque inflammation. Other than these effects, $SO_4^=$ had effects only when combined with mixtures containing MVE.

The $PM_{2.5}$ components that were significantly associated with excess risks in the mice also varied substantially between short- and long-term effects and with exposure site.

As for the epidemiological findings of the NYU NPACT study, the $PM_{2.5}$ components that that were most closely associated with the short-term health effects were different from those associated with the long-term effects, with transition metals and traffic markers being associated with daily changes in cardiac function and coal combustion components being associated with aortic plaque progression, suggesting that the coal combustion source may be responsible for a range of chronic health effects.

The specific $PM_{2.5}$ components that were most often significantly associated with excess short-term cardiac function changes were markers of fossil fuel combustion, for example, Ni and V from residual oil combustion, S from coal combustion, EC from traffic, and Al from soil. We cannot be sure if the effects were due to the elemental markers of the sources, to other source components, or to the combined effects of multiple components of the ambient air mixture.

9.19 THE ROLES OF $PM_{2.5}$ COMPONENTS ON HEALTH-RELATED RESPONSES

For both epidemiological and toxicological studies in NYU's NPACT, there were $PM_{2.5}$ components and source-related mixtures that were significantly associated with measured indices of health status that were stronger than those for $PM_{2.5}$ mass concentration. There

were also other component concentrations that were less strongly associated with these indices of health status or had negative associations. The positive associations could have been due to causality or perhaps to ambient air concentration correlations with a causal agent. The generally nonsignificant negative associations could have been due to health benefits arising from the exposures to those agents but were likely due to random influences, limited statistical power, analytical uncertainties, and exposure measurement errors. Thus, while these substantial studies could not, and did not, establish causality for specific $PM_{2.5}$ components, they were able to identify components that warrant further, more detailed, and better focused study as contributory factors affecting health-related outcomes and their underlying biological mechanisms.

9.20 COHERENCE OF NPACT TOXICOLOGICAL AND EPIDEMIOLOGICAL RESPONSES

For the NYU NPACT mouse inhalation study, the five-site regressions of mean component concentrations with the most significant responses in terms of indices of cardiac function in $ApoE^{-/-}$ mice (HR, SDNN, and RSSMD) were Ni ($r = 0.98$), S ($r = 0.88$), and V ($r = 0.66$), and these elements were among those most closely associated with ROS production and with mRNA levels in vascular endothelial and airway epithelial cells *in vitro*.

The observation that, among the CVD daily mortality and hospitalizations associated with human exposures to ambient air $PM_{2.5}$, the largest excess risks were for IHD is coherent with the observations of acute cardiac function changes in mice exposed 5 days each week by inhalation to $PM_{2.5}$. Transition metals were $PM_{2.5}$ constituents that were closely associated with the cardiac responses seen in the NYU NPACT human time-series studies, as well as in their studies of mice exposed to $PM_{2.5}$ *in vivo*.

The association of short-term variations of cardiac function in mice with the residual oil combustion source in Seattle is coherent with the findings of the NYU NPACT supplemental study that compared daily mortality and hospitalization rates in Seattle and Detroit. Zhou et al. (2011) described significant associations of excess daily mortality and hospital admissions with daily variations of $PM_{2.5}$ components for residents of Seattle that were not found for residents of Detroit, a considerably larger city with much higher annual average $PM_{2.5}$ concentrations (15.1 versus 9.1 $\mu g/m^3$ and one with much higher percentages of minority groups, lower income groups, and elderly residents, such population groupings being generally considered to be especially susceptible population subgroups for air pollution-related health effects.

9.21 COHERENCE OF NPACT STUDY OF CVD EFFECTS IN PEOPLE AND IN MICE

Any direct comparison can be challenged on the basis of (1) species difference and (2) severity of response, that is, modest functional response versus a clearly adverse health effect. On the other hand, we were able to detect functional responses in an animal model of atherosclerosis. Thus, an integrated consideration has some justification in terms of identifying $PM_{2.5}$ constituents responsible for short-term CVD responses. For other short-term human responses, such as hospital admissions for respiratory diseases, or for total mortality, there were no measurable clinical responses in the mice.

NYU's comparison showed that the $PM_{2.5}$ components most closely associated with cardiac function changes in the mice for five different sites were Ni, EC, S, and V. The $PM_{2.5}$ components that were most closely associated with hospital admissions in 64 U.S. MSAs lagged 3 days for IHD were EC, OC, V, and Ni. For the cold season in Seattle, there were significant risks for IHD hospital admissions for Al, Fe, Ni, S, Si, V, Zn, and EC. EC, Ni, and V were common to all three.

For short-term effects related to IHD, (1) effluents of residual oil combustion (Ni, V, S, and EC) were particularly influential; (2) tailpipes EC and S were also important; and (3) other $PM_{2.5}$ components may exacerbate the effects. In the prior study relating HR in COPD patients to $PM_{10—2.5}$ and $PM_{2.5}$ components in NYC and Seattle, the only component that had a significant association was Ni, but only in the $PM_{2.5}$ fraction in NYC, a city with notably high concentrations of Ni, V, S, and EC (Hsu et al., 2011).

9.22 COHERENCE: ANNUAL HUMAN ANNUAL MORTALITY WITH AORTIC PLAQUE PROGRESSION IN APOE$^{-/-}$ MICE

The NYU analyses of the ACS cohort data identified coal combustion and, to a lesser extent, traffic as the source categories that were most closely associated with excess annual IHD mortality. In addition, strong associations were also found for lung cancer mortality, consistent with past ACS analyses (Pope et al., 2002; Thurston et al., 2016b).

Long-term plaque volume progression in the brachiocephalic artery of CAPs-exposed ApoE$^{-/-}$ mice exposed to CAPs versus those exposed to FA varied by site location, with much more plaque at MS and SF than at EL where Ni concentrations were the lowest. There was no progression at all in SEA or at IR (where $PM_{2.5}$ was highest). Since the $PM_{2.5}$ concentration in EL was only about half of that in IR, and the contribution of the traffic source to local $PM_{2.5}$ was much lower in EL, thus, $PM_{2.5}$ attributable to coal combustion was more influential on plaque volume progression than traffic and residual oil combustion. Supplemental analysis of plaque surface area coverage, by visual inspection in mice after 2, 4, and 6 months of exposure at IR, provided evidence of plaque area progression, but the extent of progression at 6 months was less than for mice exposed at EL.

9.23 TRAFFIC AND $SO_4^=$ IN THE NPACT STUDIES

In the NYU NPACT study, the traffic source was significantly associated with daily mortality, as were two of its markers (Cu and OC). The traffic and salt sources were significantly associated with CVD hospital admissions. For daily CVD mortality, there were significant associations for $SO_4^=$ and V in the second-stage analysis, but not for EC, OC, and Cu, while traffic was more closely associated with hospital admissions for respiratory diseases than for either total CVD or IHD. Cu, Ni, V, NO_2, Fe, Na, and Zn were significantly associated with the second-stage daily CVD hospital admissions, but OC, EC, $SO_4^=$, As, and Se were not.

In the UW-LRRI NPACT study, MVE exposure caused the most consistent effects across all endpoints. Based on UW's source apportionment, none of the four $PM_{2.5}$ components of primary interest in the epidemiologic analyses seemed to be influenced largely by MVE. Of these components, EC, a traditional marker of exposure to MVE (specifically diesel exhaust), reflected a complex mix of sources. The diesel exhaust/brake wear-like

feature contributed to EC to some degree in every MESA city, with contributions ranging from 6 to 36%, depending on the city. The UW studies did not find much support for a role for MVE in atherosclerosis or in cardiovascular events. Predicted exposure to OC in the UW study was associated with CIMT and cardiovascular events, especially cardiovascular deaths. Their source apportionment indicated that a secondary aerosol-like contribution was prominent in all six MESA cities (with contributions ranging from 26 to 48%), a biomass-like contribution in four of the cities (contributions from 15 to 45%), and a diesel exhaust/brake wear-like contribution in five of the six cities (with contributions ranging from 3 to 23%).

The LRRI study did not include atmospheres of OC or biomass emissions. The diesel exhaust/brake wear-like feature from the UW source apportionment was a larger contributor to Cu than to the other components studied. For Cu, the contributions ranged from 32 to 57% across the MESA cities, and Cu was associated with both CIMT and the presence of CAC. Thus, the UW epidemiological studies provided, only limited support for the finding of the LRRI study on MVE. To the extent that exposure to MVE was reflected by either EC or NO_2, there was little evidence to support a role for MVE. Cu, however, might be a better marker of exposure to MVE than either EC or NO_2. The UW epidemiologic findings for Cu suggested that exposure to MVE could be important in the development of atherosclerosis. Predicted exposure to S in the MESA cohort study was associated with CIMT and with cardiovascular events, which might indicate that $SO_4^=$ was responsible for the observed cardiovascular associations, but UW was considered to be at least as likely that either $SO_4^=$ was exerting its effects in combination with other pollutants in the pollutant mix or that other pollutants in the mix were solely responsible for the effects.

The LRRI toxicological study findings, however, showed effects of $SO_4^=$ both alone and in combination with the MVE-containing mixtures. Of all the pollutant atmospheres, an atmosphere of pure $SO_4^=$ caused the most substantial changes in aortic vasoreactivity that were also noted for $SO_4^=$ + MVE, although to a lesser extent. Other than these effects, $SO_4^=$ had strong effects only when combined with mixtures containing MVE.

In terms of chronic effects in humans, the traffic source was less clearly associated with excess annual mortality than the coal combustion source. However, the traffic source was more closely associated with annual mortality than the $PM_{2.5}$ sources other than coal combustion. In the $ApoE^{-/-}$ mice, the traffic source category was considerably less closely associated with cardiac function in CAPs exposure than the residual oil combustion source, and EC was the only significantly associated individual component that was a traffic marker.

9.24 HOLISTIC PERSPECTIVES ON THE ROLE OF $PM_{2.5}$ IN CVD EFFECTS

The findings of both the NPACT programs supported the hypothesis that specific $PM_{2.5}$ components and sources are more closely associated with the chronic health effects that have been attributed to $PM_{2.5}$ mass concentrations than are other constituents and sources. This was particularly the case for IHD-related annual mortality, which accounts for a major part of the overall health impact and the major part of the tabulated benefits of ambient air pollution control. Furthermore, the clear association of coal combustion effluents with excess annual mortality in the NYU ACS cohort was coherent with aortic plaque progression in NYU's study of the mice exposed to $PM_{2.5}$ CAPs at three sites with coal combustion effluents in the ambient air, but not at two sites lacking such effluents. The UW WHI cohort

study did not perform source apportionment analyses, but the strong association of chronic effects with $SO_4^=$, which were demonstrated in the WHI and MESA cohorts, could have been due to coal combustion in Eastern and Midwestern regions of the United States. Significant associations between annual mortality rates and annual average $SO_4^=$ concentrations have been reported over the past half-century (e.g., Lave and Seskin, 1970; Dockery et al., 1993; Pope et al., 1995b, 2004b). There was also coherence of the results of the UW's CIMT findings in the MESA cohort with the findings of the biomarker assays in the LRRIs in mice exposed for 50 day to laboratory-generated atmospheres of $SO_4^=$ and other chemical components and component mixtures. However, it must be noted that the concentrations in the LRRI mouse exposures to $SO_4^=$ were much higher than those in ambient air and in the NYU 6-month CAPs exposures of mice. On the other hand, there was more H^+ associated with the ambient air $SO_4^=$ in the NYU NPACT study than $(NH_4)_2SO_4$ in the LRRI NPACT study.

In terms of cross-species coherence, the results from NYU's NPACT toxicological and epidemiological studies were coherent across species for both the short-term and long-term effects, but the putative causal components differed between short- and long-term effects. The chemical species most closely associated with short-term effects were EC, OC, and transition metals, with much weaker associations with the coal combustion source, a source that was so closely associated with the long-term effects in the ACS cohort and in the mice exposed subchronically to CAPs. The NYU NPACT subchronic CAPs exposure studies were at five sites, with none of them being in the southeastern, great plains, southwestern, or the western mountain states of the United States. On the epidemiological side, we do not yet have thorough evaluation of the short-term mortality and morbidity attributable to ambient air PM, which would require a more extensive speciation site network with continuous or, at least, daily monitoring at multiple sites within the larger communities.

On the basis that $PM_{2.5}$ exposures lead to excess CVD mortality and morbidity, with the greatest excess for IHD, the greatest financial benefits of $PM_{2.5}$ exposure controls are attributable to reductions in annual IHD mortality. The chronic IHD effects in both humans and animals are more closely associated with $SO_4^=$ than with $PM_{2.5}$ mass or with any of the other routinely measured $PM_{2.5}$ components. CVD effects coefficients were greater in the Northeastern United States than in other U.S. regions. The largest source of $SO_4^=$ in most of the Eastern United States is still coal combustion, a second $SO_4^=$ source in seaport cities is residual oil combustion in deepwater ships, and the largest $SO_4^=$ source in other U.S. cities is exhaust from motor vehicles. Residual oil combustion and exhaust from motor vehicles are also the largest sources of trace concentrations of Ni and V. $SO_4^=$ may itself have adverse IHD effects, based on the LRRI studies in $ApoE^{-/-}$ mice, and these effects are likely to be potentiated by simultaneous exposure to MVEG and vapors, based on the NYU studies in $ApoE^{-/-}$ mice, and by trace metals emitted during fossil fuel combustion. The source category with the second strongest association with CVD health effects is traffic.

The components of $PM_{2.5}$ attributable to traffic that are most likely to account for its association with CVD are EC and OC emitted from tailpipes and Cu from brake wear. The associations of adverse CVD effects with Cu, Ni, and V demonstrate that low concentrations of trace metals can be influential on health-related responses.

The $PM_{2.5}$ components most closely associated with acute responses differ from those most closely associated with chronic responses. Studies limited to speciation of $PM_{2.5}$ components making relatively large mass concentrations ($SO_4^=$, NO_3^-, OC, EC, Si, Al, and Fe) contribute less to our understanding than those including trace concentrations.

While we have gained an impressive amount of new knowledge on the associations of PM component exposures and human health effects, we still have important knowledge gaps, including (1) the roles of EC and OC exposures as causal and/or contributory factors in CVD effects, (2) whether effects associated with OC are mainly due to primary OC versus aged OC, (3) whether measurement technique differences for the thermal assays distinctions between EC and OC account for the differences in associations of EC and OC with health-related endpoints in the NYU and UW-LRRI NPACT studies, and (4) whether either EC or OC should be considered to be causal factors in CVD effects or are they really only serving as markers for other, more causal, components emitted by traffic sources.

9.25 SETTING OF NAAQS AND/OR CONTROL STRATEGIES FOR AMBIENT AIR PM

To the extent that coal combustion effluents indexed by $SO_4^=$ are driving the excess annual mortality and other chronic effects associated with exposures to ambient air $PM_{2.5}$, a uniform annual $PM_{2.5}$ NAAQS provides different degrees of public health protection in different regions, with less protection in the regions downwind of coal-fired power plants. This implication is of sufficient importance and magnitude to warrant further research to investigate its impact on health and the nature and urgency of further emission controls on specific source classes.

In the absence of a distinction based on the chemical composition of the $PM_{2.5}$, EPA's selections of 24 h as an averaging time or a short-term $PM_{2.5}$ NAAQS and an annual average for long-term $PM_{2.5}$ NAAQS were reasonable choices. However, the NYU NPACT study showed that the associations of $PM_{2.5}$ and source-related $PM_{2.5}$ mixtures and constituents with health-related effects differed between short- and long-term responses, with both short- and long-term responses being very much dependent on chemical composition. Recognition that the chronic health effects of $PM_{2.5}$ differ from the short-term health effects in the extent of their adversity and cost–benefit implications will make the future selections of short-term and annual NAAQS for $PM_{2.5}$ mass and/or their components a more complex task.

9.25.1 Short-Term NAAQS for $PM_{2.5}$

If the revision of the short-term NAAQS is to be limited to mass concentration, we should recognize the stronger and closer associations of short-term responses to ambient air $PM_{2.5}$ mass in the Northeastern United States than in other geographic regions in (1) NYU's time-series studies of daily IHD hospitalization and mortality in 64 U.S. SMSAs and (2) NYU's time-series analysis of cardiac function in $ApoE^{-/-}$ mice at 5 U.S. sites (2 in the northeast, 1 in the midwest, and 2 on the west coast); results consistent with the findings on regional variations in daily mortality attributable to $PM_{2.5}$ in the NMMAPS study (Dominici et al., 2007b). The geographic differences were most stark in terms of the cardiac function in the $ApoE^{-/-}$ mice, where the responses were much greater in the mice in both MS in NYC and Sterling Forest (SF) State Park in Tuxedo, NY (generally upwind of NYC), than they were in EL, SEA, and IR. There were some common $PM_{2.5}$ constituents at both NY sites in that both contain relatively high concentrations of long-range-transported regional aerosol, composed largely of secondary PM resulting from chemical reactions of gaseous precursors originating from fossil fuel combustion in upwind coal-fired power plants and from

traffic throughout the eastern megalopolis. Also, both MS and SF had much higher concentrations of Ni and S than any of the other locations, while MS had high concentrations of EC and V as well. In a previous study relating HR in COPD patients to coarse and $PM_{2.5}$ components in NYC and Seattle, the only constituent that had a significant association with HR was Ni, but only for the Ni $PM_{2.5}$ fraction in NYC (Hsu et al., 2011). NYU's 64 cities time-series studies in human populations showed that adverse acute health effects were occurring in cities that did not exceed, or barely exceeded, the current 24-h $PM_{2.5}$ NAAQS (35 $\mu g/m^3$) that was retained in December 2012 (Ito et al., 2013). It is still based on $PM_{2.5}$ mass concentration. Supplemental time-series study based on daily measurements in Seattle (Zhou et al., 2011) demonstrated that adverse short-term effects that were occurring in a city would meet even the more stringent concentration limit that was recently under consideration for a revised 24-h $PM_{2.5}$ NAAQS (25 $\mu g/m^3$). Therefore, consideration should be given to either replacing the current mass-based short-term primary (health-based) $PM_{2.5}$ NAAQS with a limit based on our increasing knowledge of exposure to constituents most closely associated with the short-term health risks, that is, those associated with the traffic and residual oil combustion sources, or shifting the focus on controls to more stringent emission limits on these sources.

If future $PM_{2.5}$ NAAQS are to recognize that chemical composition matters in terms of health-related responses, then the results of the NPACT studies and those of prior and contemporary studies having speciation data should greatly help in guiding future NAAQS selections. While the NYU NPACT research could not determine the extent to which individual $PM_{2.5}$ constituents causally contributed to adverse short-term health effects, they did show that some of them were much more closely associated with the measured effects than were others. Among the individual $PM_{2.5}$ constituents that were most closely associated with short-term IHD effects in both humans and mice were Ni, V, Cu, EC, and S, while total OC, Al, As, Ca, Fe, K, P, Se, Si, Ti, and Zn were less consistently associated with such effects and Pb was not associated with any of the tabulated effects. The NYU NPACT findings differed somewhat from those reported by Suh et al. (2011) for patients in Atlanta, GA, for CVD admissions, which were significantly associated with a group of transition metals (Cu, Mn, Zn, Ti, and Fe). Suh et al. (2011) showed that this group of metals was significantly associated with admissions for IHD, CHF, and AFib. In contrast, their microcrystalline oxide group (As, Br, Se, Pb, and Si) was associated with decreased CVD-related hospital admissions.

The differences between the NYU NPACT study findings and those of Suh et al. (2011) may be due to the fact that, for at least some constituents, associations are likely due to close correlation of the measured constituent's airborne concentration(s) with the concentrations of other constituents that are more causal, which come from the same source(s). Both these studies showed associations of CVD admissions for Medicare patients with Cu. Suh et al. (2011) did not show associations with Ni and V, which were not present at elevated concentrations in Atlanta. For both the NPACT study results, and for the Suh et al. study, there was a lack of consistently increased CVD effects associated with As, Se, Pb, OC, and Si, which have often served as markers of the coal combustion and traffic sources. S, which is present in ambient air as sulfate ion, is unlikely to have any inherent toxicity in isolation, but originates from the S content within coal, residual oil, and motor vehicle fuels, and may interact with other constituents (COMEAP, 2000). OC, which is inconsistently associated with effects, is a very broad category of organic compounds. As demonstrated by Delfino et al. (2009), OC includes some primary OC species that may be toxic, as well as many secondary OC species having little or no acute toxicity. Total OC, as measured in the CSN, has little predictive power with respect to short-term health effects.

9.25.2 Long-Term NAAQS for $PM_{2.5}$

If, as for a short-term NAAQS revision, the long-term NAAQS is to be limited to mass concentration, then it would be important to note that there were significant associations of annual IHD mortality rates with the long-term mean concentrations for ambient air $PM_{2.5}$ mass in the United States in NYU's ACS cohort that involved people living in 100 U.S. SMSAs that did not exceed the then current (as of November 2012) annual $PM_{2.5}$ NAAQS concentration limit of 15 $\mu g/m^3$, nor to the more stringent value of 12 $\mu g/m^3$ that was adopted in December 2012. If, however, future $PM_{2.5}$ NAAQS recognize that chemical composition matters in terms of health-related responses, then the results of the NPACT studies, and those of prior and contemporary studies having speciation data, should greatly help in guiding future NAAQS selections based on annual average $PM_{2.5}$ concentrations.

NYU's NPACT analyses showed that the risks of long-term exposures were primarily attributable to the $PM_{2.5}$ constituents arising from effluents of the coal combustion and, to a lesser extent, traffic sources. We have long known that the exposure to coal combustion-derived $PM_{2.5}$ is largely confined to the eastern half of the United States, while exposure to high concentrations of traffic-derived $PM_{2.5}$ is largely limited to residents of the largest cities and those living very close to major traffic arteries. Since little of the chronic effects excess was attributable to other nationwide $PM_{2.5}$ sources, the benefits of the more stringent annual mass-based $PM_{2.5}$ NAAQS adopted in December 2012 (12 $\mu g/m^3$) can be questioned. Controls focused on emissions from coal combustion and traffic sources, and in coastal regions impacted by marine transport sources, on emissions of $SO_4^=$, Ni, and V from residual oil combustion, may be more effective in reducing adverse chronic health effects.

While NYU's NPACT research could not determine the extent to which individual $PM_{2.5}$ constituents causally contributed to adverse long-term health effects, it did show that one $PM_{2.5}$ source, specifically coal combustion, appeared to be much more closely associated with the measured long-term effects (annual mortality in humans and aortic plaque progression in mice) than any of the others and appears to have a higher toxicity per unit mass than other components for these endpoints.

9.25.3 Consideration of a NAAQS for Coarse Thoracic PM ($PM_{10–2.5}$) and/or UFP

While the NPACT epidemiological studies and NYU's subchronic mouse inhalation study were limited to the effects of $PM_{2.5}$ exposures, some of NYU's short-term *in vitro* and *in vivo* results suggested that an NAAQS for $PM_{10–2.5}$, along with a program devoted to the acquisition of $PM_{10–2.5}$ speciation, could, if implemented, provide a more robust knowledge base for future rounds of reviews focused on a $PM_{10–2.5}$ NAAQS. Although the NYU NPACT studies did not compare the toxicity of urban versus rural coarse PM, an issue complicating previous consideration of a $PM_{10–2.5}$ NAAQS, their findings did demonstrate that different sources of coarse PM, as would occur at urban versus rural sites in the United States, produce different degrees of toxicity. Some of their *in vitro* and *in vivo* results also suggested that PM in sizes below 0.1–0.2 μm might also warrant a separate nationwide monitoring program, at least for initial research purposes.

With respect to an NAAQS for UFP, there is little likelihood that there will be any scientific basis for its establishment if it were to depend on either a particle number concentration or a total mass concentration of particles below an arbitrary maximum particle size. UFP number is dominated by nanoparticles of OC and H_2SO_4, which are

toxicants created in the atmosphere from gaseous precursors. They rapidly grow by coagulation into smaller numbers of larger-sized aggregates. The H_2SO_4 microdroplets then undergo neutralization by NH_3, while the OC droplets undergo some volatilization and oxidation. Furthermore, the number concentration varies with the instrument used, since the lower size detection limit varies from instrument to instrument. The total mass concentration of UFP ignores chemical composition, which is the major fault of the mass concentration limits in the other particle size ranges.

9.26 RESEARCH NEEDS

The design of future studies should also take into consideration the prior findings of the broad range of subchronic CAPs rodent inhalation studies performed by NYU and Ohio State University investigators that go beyond CVD effects. The results of these studies, showing chronic responses in the nervous system (Veronesi et al., 2005; Sama et al., 2007), liver (Tan et al., 2009; Laing et al., 2010; Zheng et al., 2013), atherosclerosis (Sun et al., 2008a), hypertension (Sun et al., 2008b), metabolic syndrome (Sun et al., 2009), insulin resistance and mitochondrial dysfunction (Xu et al., 2012), cardiac remodeling (Ying et al., 2009a, 2009b), oxidative stress and altered gene expression in adipose tissue (Xu et al., 2011a), obesity and diabetes (Xu et al., 2011b), and so on, can help to guide the design of future toxicological and epidemiological studies of the effects of peak exposures on short-term responses and long-term exposures and cumulative effects, especially for intervention studies of the health benefits of specific source controls, and especially if the epidemiological investigators had access to more chemical speciation data.

Future epidemiological studies will greatly benefit from a more complete PM speciation and gaseous pollutant database that has been available up to now if they are to succeed in identifying (1) the most causal constituents and (2) mortality and morbidity incidence for ICD codes other than CVD and respiratory disease. Ideally, they should include studies of communities having a large range of PM composition and multiple years of co-located PM speciation and gaseous pollutant data. Findings from NPACT studies, and from additional such studies, can also aid in the design of future controlled rodent inhalation studies that are focused on additional health endpoints as well as better definitions of the roles of specific PM constituents and their interactions of gaseous air pollutants and PM constituents that are added or subtracted from controlled exposures to PM mixtures. When more such information becomes available, it may become possible to build realistic multi-pollutant models that can account for the observed associations of exposures and effects in humans and animals. We currently lack adequate knowledge and understanding of the effects of complex mixtures and of the interactions among the constituents of the mixtures.

9.27 NEED FOR A MORE COMPREHENSIVE AIR QUALITY MONITORING PROGRAM

The NPACT research programs at NYU and UW-LRRI have clearly demonstrated that PM mass concentrations in ambient air provide relatively crude indices of health risks and that the risks vary considerably with particle size, chemical composition, and season. The NYU NPACT has also shown that the PM constituents that are most closely associated with acute

effects differ from those that are most closely associated with chronic effects. Furthermore, both NYU and UW have benefited from their access to the currently available CSN but found that these data are inadequate for definitive determinations of the roles of specific PM constituents as causal factors for the effects.

The CSN deficiencies include (1) too few monitoring sites for adequate characterization of spatial distributions of $PM_{2.5}$ constituent concentrations and of gaseous criteria pollutants; (2) too little $PM_{2.5}$ temporal and spatial resolution (limited currently to 24-h concentrations every third or sixth day at only one site in most cities); no discrimination for OC $PM_{2.5}$ constituents; (3) no measurements of biogenic components; (4) insufficient numbers of speciation sites with co-located gaseous air pollutant measurements, which limited the statistical power of the NYU time-series study of daily mortality and hospital admissions; and (5) no measurements of the chemical constituents of $PM_{10–2.5}$ or UFP.

A more robust monitoring network will make it possible to determine whether some of the associations of effects with PM constituents are causal or are likely to be due to other co-constituents whose concentrations are closely correlated with the monitored species. The data generated by such an enhanced CSN would enable EPA to establish better-targeted PM NAAQS and control strategies and therefore reduce the burden of adverse health effects.

9.28 CONCLUSIONS

Substantial increments of knowledge concerning ambient air PM exposures and their health effects have been provided by the HEI-sponsored NPACT projects and by other recent investigations of the roles of ambient air $PM_{2.5}$ constituents and/or their source-related mixtures on health-related biological responses. New insights were also gained by the NPACT studies on laboratory animals that have been exposed to CAPs or $PM_{2.5}$ components and complex mixtures of $PM_{2.5}$ components and engine exhaust vapors that complemented the epidemiological studies in order to (1) establish interspecies coherence of responses, (2) explore the underlying biological mechanisms for the responses, and (3) identify promising lines of inquiry for further toxicological and epidemiological studies.

One extremely important increment of knowledge coming out of the NPACT program is in the realm of the effects of long-term $PM_{2.5}$ exposure on chronic health effects, especially for the exposures that vary in multiple component concentrations on a national scale for population cohorts with extensive data on other personal risk factors (NYU's ACS-II cohort and UW's WHI and MESA cohorts). NYU's subchronic CAPs mouse inhalation studies at five sites provided data on substantial variations in aortic plaque progression by geographic region that was coherent with the regional variation in annual IHD mortality in the ACS-II cohort, with both the human and mouse responses being primarily attributable to the coal combustion source category.

While the UW cohort regressions of associations of CVD events and mortality in the WHI cohort and of CIMT and CAC progression in the MESA cohort were limited to the ambient air concentrations of OC, EC, Si, and $SO_4^=$ and did not extend to utilization of their source apportionment analyses, they did show that, of the 4 $PM_{2.5}$ components of UWs primary focus, there were statistically significant associations for only OC and $SO_4^=$, with $SO_4^=$ having the stronger associations with CVD-related human responses and OC having the stronger associations with cerebrovascular responses. The LRRI's mice had CVD-related biomarker responses to $SO_4^=$ that were exacerbated by co-exposure to MVEG. The

UW source apportionment description noted that the ambient air $SO_4^=$ concentrations were highly correlated with the concentrations of Se and As. Thus, it is reasonable to assume that, on a national scale, $SO_4^=$ can serve as a surrogate index of the coal combustion source and that the UW-LRRI findings are consistent with the NYU attribution of most of the chronic CVD effects to the coal combustion source.

Another extremely important increment of knowledge, coming out of the NYU NPACT program, is that the source-related component that is dominant for the chronic CVD effects (coal combustion) differs from the components that dominate acute health effects in both humans (hospital admissions) and mice (cardiac function). The components most closely associated with these acute effects are OC, EC, and Cu from the traffic source and Ni and V from the residual oil source. For daily mortality in humans, there appears to be a role for coal combustion, as indexed by $SO_4^=$, Se, and As. The major $PM_{2.5}$ components that appear to be least associated with health effects are soil-related minerals and NO_3^-, except insofar as such PM components serve as carriers of reactive vapors, enabling them to penetrate more deeply into respiratory tract airways. This was most clearly demonstrated in the LRRI subchronic inhalation studies in mice in which mixtures of MVEG and $SO_4^=$, NO_3^-, and road dust produced greater effects on CVD biomarkers than did $SO_4^=$, NO_3^-, and road dust alone. These new findings and insights provide further support for the hypothesis that some $PM_{2.5}$ components not only are considerably more toxic than others but also illustrate the remaining uncertainties concerning the adequacy of $PM_{2.5}$ mass as an index of the health risks of ambient air $PM_{2.5}$ pollution exposures and especially so for an annual average NAAQS where the variation in the contributions of coal combustion effluents to $PM_{2.5}$ mass is so extreme. In contrast, the contribution of the traffic source to $PM_{2.5}$ is far more uniform and more closely correlated with that of $PM_{2.5}$ mass.

While the recent research into the roles of $PM_{2.5}$ components on CVD has clearly filled some major knowledge gaps and helped to define remaining uncertainties, much more knowledge is needed if we are to identify and characterize the most effective and efficient means for reducing the still considerable adverse health impacts of ambient air PM. The recent research, discussed herein, being focused on CVD-related effects of $PM_{2.5}$ and its components, has done little to better define the risks in the respiratory tract, the nervous system, the liver, diabetes, and so on. Likewise, the risks associated with $PM_{10–2.5}$, which appears to be especially important in the respiratory tract, and those with UFP remain poorly defined. Advances in characterization of health risks associated these other organ systems will require more epidemiological studies, which in turn will require an expanded chemical speciation, network as described above, as well as more subchronic toxicological studies with comprehensive analysis of the exposure, biomarker variables, organ and tissue responses to specific $PM_{2.5}$ constituents and mixtures, and the underlying biological mechanisms.

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10

ARSENIC

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10.1 INTRODUCTION

10.1.1 Historical Overview of Arsenic (As) and Arsenical Exposures

Arsenic is a ubiquitous metalloid that is the twenty-first most abundant element in the Earth's crust. Its name derives from an ancient Persian word, Zarnikh, as translated to the Greek arsenikon, meaning "yellow orpiment," an arsenic sulfide. The toxicity of As and arsenicals has been recognized for millennia, as it has been known as the "poison of kings and the king of poisons." Odorless, colorless, and tasteless, as well as easily dissolved in common drinks, As is the perfect poison. The oldest documented environmental/occupational exposure to metals is that of the Tyrolean Neolithic mummy called Ötzi (c. 3359–3105 BC) with copper (Cu) and high As enrichment in his hair follicles (Bolt, 2012). Ötzi was exposed through the early production of bronze tools and weapons at the beginning of the Bronze Age when bronze was produced by combining molten copper ore that often contains As and other alloying metals and metalloids.

As presents an important paradox, as arsenicals have been used as therapeutic agents as well as poisons (Table 10.1). Inorganic As (iAs) and arsenicals were mainstays of Chinese, Indian, and Korean medicines for thousands of years (Wang et al., 2018). The ancient Greek philosopher Theophrastus of Eresos (370–287 BC) and the Roman Pliny the Elder (AD 23–79) discussed medicinal As. Fowler's solution (containing potassium arsenite) was introduced in 1765 as a health tonic and to treat skin conditions (e.g., psoriasis, eczema), as well as stomatitis. In the mid-nineteenth century, it was used to treat leukemias and cancers. However, when skin cancers resulted from treatment with Fowler's solution, As was almost completely eliminated from human medicine. In 1909, Paul Ehrlich designed the organoarsenical Salvarsan as the first effective cure for syphilis that was used until replaced by antibiotics after World War II (WWII). Lewisite was an organoarsenical that was produced as a chemical warfare agent for WWII and declared obsolete in 1950 as a

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TABLE 10.1 Chemical Names and Molecular Formulas for Inorganoarsenical and Organoarsenical Compounds

Chemical Name	CAS Reg. No.	Synonyms	Formula
Arsenic	744-38-2	Metallic arsenic	As
Arsenic(V) pentoxide	1303-28-2	Arsenic oxide	As_2O_5
Arsenic(III) sulfide	1303-3309	Arsenic sulfide, orpiment	As_2S_3
Arsenic(III) trichloride	7784-34-1	Arsenic chloride	$AsCl_3$
Arsenic(III) trioxide	1327-53-3	Arsenic trioxide	As_2O_3
Arsenobetaine	64436-13-1	Arsonium, (carboxymethyl) trimethyl-, hydroxide inner salt; 2-(trimethylarsonio)acetate	$C_5H_{11}AsO_2$
Arsine	7784-42-1	Arsenic hydride	AsH_3
Arsphenamine	139-93-5	Salvarsan, compound 606; 2-amino-4-(3-amino-4-hydroxyphenyl) arsanylidenearsanylphenol	$C_{12}H_{12}As_2N_2O_2$
Calcium arsenate	7778-44-1	Arsenic acid calcium salt	$(AsO_4)_2{\cdot}3Ca$
Dimethylarsinic acid	75-60-5	Cacodylic acid	$C_2H_7AsO_2$
Lead arsenate	7778-40-9	Arsenic acid, lead (2+) salt 1:1	$HAsO_4{\cdot}Pb$
Lewisite	541-25-3	2-Chloroethenylarsonous dichloride; 2-chloroethenyldichloroarsine	$C_2H_2AsCl_3$
Methanearsonic acid, disodium salt	144-21-8	Arsenic acid, methyl-, disodium salt	$H_4CAsO_3{\cdot}2Na$
Melarsoprol	494-79-1	2-[4-[(4,6-Diamino-1,3,5-triazin-2-yl)amino]phenyl]-1,3,2-dithiarsolane-4-methanol; melarsen oxide-BAL	$C_{12}H_{15}AsN_6OS_2$
Potassium arsenate	7784-41-0	Arsenic acid, monopotassium salt	$H_2AsO_4{\cdot}K$
Potassium arsenite	13464-35-2	Arsenous acid, potassium salt	$AsO_2{\cdot}K$
Realgar	12044-30-3	Realgar, ruby sulfur, ruby of arsenic	As_4S_4
Roxarsone	121-19-7	4-Hydroxy-3-nitrophenylarsonic acid; 3-nitro-4-hydroxyphenylarsonic acid	$C_6AsNH_6O_6$
Sodium arsenate	7631-89-2	Arsenic acid, monosodium salt	$H_2AsO_4{\cdot}Na$
Sodium arsenite	7784-46-5	Arsenous acid, sodium salt; sodium metaarsenite	$AsO_2{\cdot}Na$

result of the use of dimercaprol (British anti-lewisite) as an effective antidote. Melarsoprol, a complex organoarsenical, was introduced in 1949 as a frontline drug to treat sleeping sickness (African trypanosomiasis) and is on the WHO Model List of Essential Medicines. However, 1–5% of patients die from the drug, and many have side effects due to the strong inhibition of mitochondrial respiration that is expected to occur in the trypanosome before the patient. Arsenic trioxide (As_2O_3) has had a resurgence as a highly effective frontline therapeutic in treating acute promyelocytic leukemia (PML) (Breccia and Foa, 2019), and it is now used as an adjuvant with other cancer therapeutics.

The major environmental health concern with As is that it is a common element in the environment and hundreds of millions of individuals worldwide are exposed to unsafe levels of As in their food and water daily. Its insidious nature may result in a significant portion of what is often mislabeled as idiopathic disease. The challenge remains creating effective regulation, remediation, and interventions that reduce human disease burden from what is arguably the greatest environmental exposure.

10.1.2 Sources and Physical and Chemical Properties of Environmental As and Its Compounds

As is a common element found in soils and sediments, in the aquatic environment, in the atmosphere, and in most organisms. A comprehensive review by Cullen and Reimer (1989) details the biogeochemistry of As and As speciation in biological and geological materials. Numerous other reviews provide information on the global cycling of environmental As (Matschullat, 2000) and the pathways for human exposure through groundwater, food, and the atmosphere (Matschullat, 2000; Welch et al., 2000; Carey et al., 2012). By far, the majority of human exposure comes from drinking water where As is liberated from iron oxide or sulfide minerals (Welch et al., 2000; Poyla and Lawson, 2016). While this is a conventional generalization, a great amount of data on As chemistry gathered over the past two decades with modern scientific approaches and modeling techniques have greatly expanded the understanding of the location and extent of As hazards and exposures (Poyla and Lawson, 2016).

The distribution of environmental As is highly dependent on geological, biological, and anthropological process and is greatly influenced by local biogeochemistry, the chemical forms or species of the As, and the biogeochemical reactivity of the As species (Poyla and Lawson, 2016). Pure As is a gray-colored metalloid belonging to group V in the periodic table, with an atomic number of 33 and an atomic weight of 74.9. Thus, it is a metalloid that is often inappropriately referred to as a metal or, worse, a heavy metal. The most common oxidation numbers of As are 5, 3, 0, and −3. The different species have wide-ranging acid dissociation constants, allowing them to act as acids, bases, or amphoteric substances. Elemental As is very brittle and tarnishes in air. When heated, it rapidly oxidizes to arsenic trioxide (As_2O_3) that has a garlic-like odor (Table 10.1). As_2O_3 is a transparent crystal or white powder resembling table sugar that is slightly soluble in water, which made it a popular choice as a poison. In the environment, As is mostly found complexed with other elements, such as iron sulfides and sulfosalts: arsenopyrite (FeAsS), orpiment (As_2S_3), realgar (AsS), loellingite ($FeAs_2$), and tennantite ($Cu_{12}As_4S_{13}$) (Cullen and Reimer, 1989; Matschullat, 2000). Yellow orpiment and red realgar were two common medicinal sulfides that were often used in dyes and pigments. As pentoxide (As_2O_5) is a white amorphous solid that is very soluble in water, forming As acid. Arsine (AsH_3) is a gas more toxic than other As compounds and found in geothermal vents and sewage sludge and is used in the microelectronics industry (Hughes et al., 2011). Arsine is highly reactive with oxygen and thus not a common environmental contaminant. Additional gaseous As forms include methylated arsenicals found in geothermal vents (Planer-Friedrich et al., 2006) and $(CH_3)_3As$, as well as CH_3AsH_2 and $(CH_3)_2AsH$, found at low concentrations in bioreacting sewage sludge (Michalke et al., 2000).

As at 1–10 mg/kg is found in most rocks, but this varies greatly depending on the composition of crustal rocks and the degree of prior geological activity (Matschullat, 2000). Enrichment occurs in loess, glacial tills, peats, acid sulfate soils (up to 50 mg/kg), shales

(up to 200 mg/kg), and sulfide ore rock in mining regions (up to 10,000 mg/kg). Consequently, acid leachate from mines often produces very high As groundwater contamination (up to 150 mg/L) (Welch et al., 2000). In addition, As is often a major workplace and environmental contaminant in areas where gold (Au) and Cu are smelted (Enterline et al., 1995; Eisler, 2004). Weathering of rocks converts As sulfides to As_2O_3 and to the water-soluble oxyanion species arsenate and arsenite (Matschullat, 2000; Welch et al., 2000). Arsenite and arsenate are the most common forms of As in groundwater, with arsenite being predominant under reducing conditions and arsenate under oxidizing conditions (Zheng, 2017). The major processes controlling As species in the groundwater are inputs from geothermal fluids, desorption of mineral-bound As in oxidizing aquifers, microbial metabolism of arsenate in reducing aquifers, and to a lesser extent anthropogenic sources, such as mine leachate or agricultural wastes.

As in soils varies with climate-dependent weathering of surface rock, mineralogy and geology of crustal rock, and local anthropogenic sources. Similar mechanisms mobilize As from surface minerals and rocks, and As in soils can impact human health by contaminating surface waters and through uptake into foodstuffs, especially rice and grains (Punshon et al., 2017). Anthropogenic activities can increase soil concentrations through mining activity, fine particle dusts from mine tailings, land application of biosolids or manures from animals fed arsenicals, and use of arsenic containing pressure-treated lumber. In addition to the broad range of distribution of As in soils, the amount of As incorporated into food varies with differences between cultivars in transport mechanisms (Punshon et al., 2017). This creates challenges in predicting disease risk from eating a given type of food, for example, the variation in rice cultivars grown globally (Nachman et al., 2018).

The most important sources of airborne As are in particulate matter (PM). However, the amount of PM and volatile (see above) As in the air is low (between 1 and 15 ng/m^3) relative to the amounts found in water and soils. Atmospheric As becomes significant where fine particles ($PM_{2.5}$) from mining operations and mine tailings are incorporated into soils where crops are grown (Punshon et al., 2017). Road and urban dusts in highly industrialized areas and areas with excess coal combustion contain significant amounts of As (Poyla and Lawson, 2016). Combustion of coal and biosolids in indoor cooking was found to be a significant source of As inhalation linked to increased cancer and respiratory disease (Liu et al., 2002; Baoshan et al., 2005; Pal et al., 2007). In addition, sands in arid regions with high soil As levels disperse over large global areas causing significant human exposure (Morman, 2010).

10.1.3 Pathways and Extent of Human As Exposure

Chronic ingestion of low to moderate As is the most significant route of human As exposure and results in the greatest burden of disease caused by As. Ingestion of high, acutely lethal levels (70–180 mg) is rare, but still a concern for suicides and homicides. However, more than 200,000,000 individuals worldwide are exposed through their drinking water to potentially toxic levels of >10 µg/L As (the WHO and U.S. EPA drinking water standard) (Carlin et al., 2016). As is insidious as it is odorless, colorless, and tasteless even in high mg/L levels in water. Moreover, the amount of toxicant exposure per capita can be extremely high in densely populated countries, for example, Bangladesh (Bailey et al., 2016). A recent survey of Pakistan estimated that approximately 60 million individuals are exposed to >10 µg/L in their drinking water (Podgorski et al., 2017). It is quite conceivable that exposure in this region was associated with As-promoted disease over centuries, if not millennia.

There are few countries in the world where As in drinking water poses no health problems. Naturally elevated drinking water As levels are found in Taiwan, Vietnam, China, Mongolia, Japan, Argentina, Chile, Bolivia, Mexico, Germany (Bavaria), Hungary, Romania, Spain, Greece, Ghana, and Canada (NRC, 2014). Globally, the majority of groundwater wells are below 10 μg/L, but local geological hot spots produce water in the mg/L range. For instance, a misconception is that groundwater As concentrations are low in the United States. This is true for approximately 90–95% of wells sampled (Welch et al., 2000). However, one study of 30,000 wells sampled across the United States found 10% exceeded 10 μg/L (Welch et al., 2000), and others find 39–60% of wells in specific regions or townships are above 10 μg/L (Spencer, 2000; Nielsen et al., 2010). A Washington State Environmental Biomonitoring Survey conducted with 172 participants and tap water samples from 82 households found that urine As levels were above the CDC's reporting level (50 μg/L) among 28% of the participants and 54% of the water samples were above 10 μg/L (ATSDR, 2016). While the geology in the Western United States favors As leaching from iron oxides and sulfides, levels as high as 3000 μg/L have been found in township drinking water wells in Maine (Nielsen et al., 2010). With modern sampling and modeling, the magnitude of global human As exposures from drinking water is now viewed as ubiquitous and arguably the most significant environmental health concern.

Drinking water may, as already indicated, be contaminated with As from mining, agricultural runoff, or improperly disposed chemicals. Countries with documented drinking water contaminated by anthropogenic sources include India, Mexico, Chile, Brazil, Nicaragua, and Thailand (IARC, 2012), as well as the United States (Welch et al., 2000). The EPA reduced the drinking water standard to 10 μg/L from 50 μg/L in 2006. However, certain states, such as New Jersey, have a lower standard of 5 μg/L and others are contemplating reductions to reduce noncancer disease risks. While the European standard and WHO guidelines are at 10 μg/L, individual countries, such as Denmark, have reduced their standard to 5 μg/L. However, many other countries in the developing world struggle to reduce levels to 50 μg/L.

Ingestion of As in food is the second largest source of human exposure and becomes more significant as As levels in drinking water are reduced (Kile et al., 2007; Nachman et al., 2018). Vegetables, grains, meats, and fish are the primary food sources that naturally contain significant levels of As (Schoof et al., 1999; Kile et al., 2007; Davis et al., 2012; Nachman et al., 2018). A large market basket survey of food consumed in the United States estimated that individuals consumed approximately 16–19 μg/day, with seafood and rice being the most significant dietary sources (Schoof et al., 1999). However, the forms of As in seafood, predominantly arsenobetaine, are viewed as inert and the actual amount of toxic As from food may be much less.

As in food comes from uptake from the soil and irrigation water (Meharg et al., 2009; Punshon et al., 2017; Nachman et al., 2018). Soil can be impacted by both anthropogenic and natural geological factors causing the content of As in a given food to vary greatly (Carey et al., 2012; Punshon et al., 2017). However, genetics for plant uptake mechanisms also vary greatly, making generalized risk assessment a challenge (Punshon et al., 2017; Nachman et al., 2018). In addition, genetically modifying plants to reduce As uptake is now a viable strategy to decrease human disease burden (Punshon et al., 2017).

Assessing the disease risk from As in food is more complicated than assessing risk from As in drinking water, since there are many more mitigating factors in food than in water. Ingestion of folate, B vitamins, niacin, and selenium (Se) in foods reduces the toxic effects of As (Chen et al., 2007a, 2007b; Hall and Gamble, 2012; Nachman et al., 2018).

Further, the bioavailability of As in drinking water is greater than 95%, while bioavailability in food can range from 50 to 100% (JECFA, 2011).

Use of organoarsenical antibiotics, such as roxarsone and 3-nitro, was extensive in the poultry industry and to a lesser extent pork industry until 2016, when chemical suppliers voluntarily withdrew the compounds from commercial sale. This was after U.S. Food and Drug Administration (FDA) studies and USGS survey studies found that the organoarsenicals left trace amount of As in meat and released large amounts of As into groundwater after land application of animal wastes (Garbarino et al., 2003; Rutherford et al., 2003; Fisher et al., 2015). Another common misconception was that these organoarsenicals were nontoxic, as roxarsone and similar structures were the racemic mixture of Paul Ehrlich's Salvarsan. However, the LD_{50} in rats for roxarsone is less than an order of magnitude above As (MSDS). Despite FDA recommendations for ending use before time of harvest, significant levels of the arsenicals and As remained in poultry tissues (Silbergeld and Nachman, 2008), and public pressure resulted in the total ban (Fisher et al., 2015).

Preparation (e.g., making bread or pasta) and cooking (e.g., boiling rice) of food with As-contaminated water can also increase the As content of food (Kile et al., 2007; Signes et al., 2008). Additional sources of As contamination in food come from use of As-contaminated coal in food preparation or as a cooking fuel source. Studies in Guizhou province, China, where As-rich coal is used to dry peppers and other food stuffs found increased rates of hepatic cancers associated with As in the kitchen air (150–760 $\mu g/m^3$) and in dried hot peppers (52.2–1090 mg/kg) (Liu et al., 2002; An et al., 2007). Raising awareness in the province of the association between the coal burning and arsenicosis and diseases, as well as providing advanced ventilated stoves, effectively reduced As levels and disease burden in thousands (An et al., 2007). Another dietary source is ingestion of As-containing clays and soils, such as in the deliberate practice of geophagia (Al-Rmalli et al., 2010; Ngole-Jeme et al., 2018), especially in pregnant women (Nyanza et al., 2014) as well as pica in children. The bioaccessibilities of As from mineral complexes in the soil are variable, but significant (Ngole-Jeme et al., 2018).

Occupational exposures to As are widespread, although mostly limited to agricultural operations or mining and smelting. In agriculture, As and toxic organoarsenicals used in pesticides, herbicides, and animal promote disease in workers with direct contact to the arsenicals, as well as environmental contamination. However, as noted above, arsenical use has declined in recent years, including the example of roxarsone in large commercial agricultural feeding operations. Chromated arsenicals such as chromated copper arsenate (CCA) are a widely used pesticide that protects wood against termites, fungi, and other pests that degrade wooden structure integrity. CCA poses a health risk to workers using specialized high-pressure equipment in wood treatment facilities, as well as construction workers creating dusts when cutting the treated wood. Manufacturers discontinued use of CCA in homeowner products in 2003. However, it is still widely used in industrial settings. Cacodylic acid [$(CH_3)_2AsO_2H$] and other organoarsenicals were extensively used in cotton fields, sod farms, golf courses, and highway right of ways as a defoliant. Cacodylic acid was a primary component of the Agent Blue defoliant broadly spread in the Vietnam War. The EPA and EU have banned all of this type of organoarsenicals, although environmental contamination persists.

As mentioned, metal ore mining and smelting are significant occupational and environmental sources of As exposure. Cu smelting operations created the largest Superfund sites in the United States (e.g., Iron Mountain Mine, Redding, CA, and Anaconda Co.

Smelter, Anaconda, MT) and are associated with As-promoted lung cancers in workers (Enterline et al., 1995). Gold mining operations, especially in sulfide-rich ores, pose both occupational exposures and risks to workers health, as well as significant exposures to surrounding communities (Eisler, 2004; Ng et al., 2019). Review of occupational exposures in the semiconductor manufacturing industry with production of gallium arsenide wafers and chips found that maintenance workers had significant As exposure from dusts (Park et al., 2010).

10.2 KINETICS OF AS UPTAKE, DISTRIBUTION, AND ELIMINATION

Arsenic (As) is well absorbed (80–90%) from the gastrointestinal tract, distributed throughout the body, metabolized by methylation, and then excreted primarily in urine (Hughes et al., 2011; ATSDR, 2016). Trivalent As and trivalent methylated metabolite absorption from the gastrointestinal track, as well as movement into and out of cells throughout the body, occurs through aquaporins (aka aquaglyceroporins) 3, 7, 9, and 10 (AQP3, AQP7, and Aqp9) (Liu, 2010). Human AQP9 is a more effective As(III) transporter than AQP7, with much less transport by AQP3 or AQP10 (Mukhopadhyay et al., 2014). These channels are primarily water, and glycerol channels allowing trivalent arsenicals to freely distribute throughout the body with the volume of distribution of water. Pentavalent inorganic and methylated As species resemble phosphate and must compete with mM amounts of phosphate for cell entry (McDermott et al., 2010). However, the pentavalent species are also substrates for human AQP9, important for export of methylated species from hepatocytes and elimination through the circulation (McDermott et al., 2010). Transport of trivalent and pentavalent arsenicals through AQP9 differs in kinetics and inhibitor sensitivity (McDermott et al., 2010). In addition a glucose transporter, solute carrier family 2 member 1 (SLC2A) (aka GLUT1), also transports As_2O_3 and methylarsonous acid (CH_5AsO_2) (Liu et al., 2006).

Skin is a potential route of exposure to industrial As, and systemic toxicity has been reported in persons having dermal contact with solutions of As (Hostynek et al., 1993), but the relevance of this to modern exposures is limited. Airborne As is largely trivalent arsenic oxide, and deposition in airways and absorption of arsenicals from lungs are dependent on particle size and chemical form (ATSDR, 2016). Excretion of absorbed As is mainly via the urine. The whole-body biological half-life of ingested As is about 10 h, and 50–80% is excreted into the urine over 3 days (Kenyon et al., 2005; ATSDR, 2016). The biological half-life of methylated arsenicals is in the range of 30 h (Hughes et al., 2005). Arsenicals preferentially accumulate in skin, where they are excreted by desquamation of skin, and in sweat, particularly during periods of profuse sweating. Another elimination pathway is complexing with keratins in forming fingernails and hair. High As exposure produces characteristic transverse white bands across fingernails (Mees' line) that appear about 6–8 weeks after the onset of symptoms of As toxicity (Patel et al., 2011). As in the fingernails and hair is as a biomarker for subchronic and chronic past exposure, while blood and urinary arsenicals are good indicators of current exposure.

Enzymatic methylation of As species is protective and enhances excretion. However, this process can create methylarsenite (MMA) and dimethyl arsenite (DMA) that are as or more potent in the cell than As (Hughes et al., 2011; Li et al., 2017). High proportions of MMA and low proportions of DMA in urine are associated with increased risks of cancer

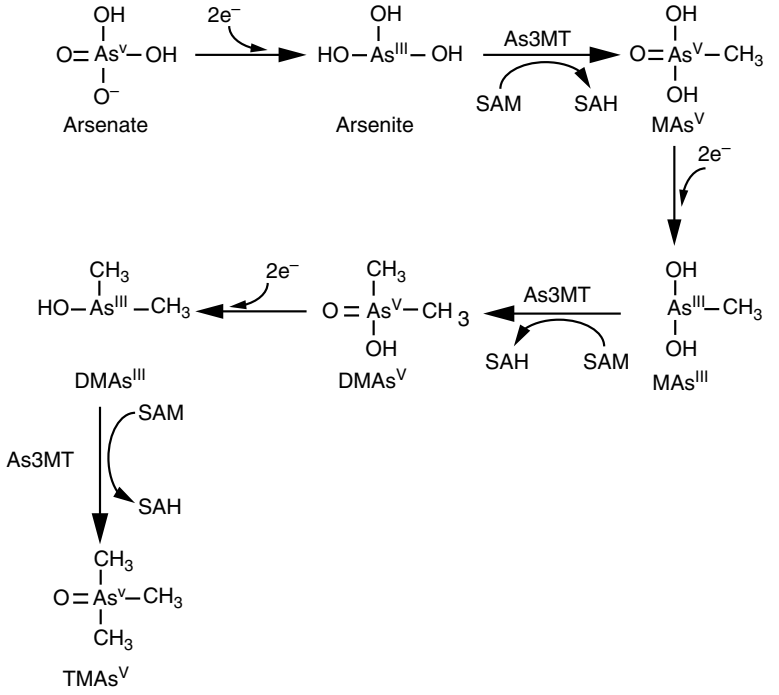

FIGURE 10.1 Metabolism of As in humans. Arsenate and metabolites undergo a series of two electron reductions and methylation reactions. Arsenic (+3 oxidation state) methyltransferase (AS3MT) catalyzes the oxidative methylation using *s*-adenosylmethionine (SAM) as the methyl donor and forming *s*-adenosylhomocysteine (SAH) in the reaction. DMA is the predominant metabolite found in human urine.

and cardiovascular disease, whereas low levels of MMA and high DMA are linked to adiposity and the incidence of diabetes (Kuo et al., 2017). Figure 10.1 illustrates As biotransformation. Arsenate (As^{5+}) is rapidly reduced to arsenite (As^{3+}) by arsenate reductase (presumably purine nucleoside phosphorylase). This and subsequent reductive steps in the pathway occur by two-electron reduction and thus, unlike cationic metals that undergo one-electron reduction, do not generate radical species capable of generating reactive oxygen through redox cycling. Arsenite is then sequentially methylated to form monomethylarsonic acid and dimethylarsinic acid (DMA^{5+}) by arsenite methyltransferase (AS3MT) using *s*-adenosylmethionine (SAM) as a methyl group donor (Hughes et al., 2011, Li et al., 2017).

In humans, urinary arsenicals are composed of 10–30% As, 10–20% MMA, and 55–76% DMA (IARC, 2012; Cosselman et al., 2015). However, large variations in As methylation occur due to factors such as age and sex. Genetic polymorphisms can influence As metabolism (Engstrom et al., 2013; Li et al., 2017), and the role of these variants in promoting disease is currently under investigation. As metabolism also changes through the course of pregnancy, reflected in higher urinary excretion of DMA and lower urinary levels of As and MMA, which may have toxicological impact on the developing fetus (Hopenhayn et al., 2003).

10.3 TOXICITY AND MECHANISMS OF TOXICITY

10.3.1 Mechanisms of Toxicity

The trivalent arsenicals are thiol reactive and therefore activate or inhibit enzymes or alter protein structure by reacting with proteinaceous thiol groups. Arsenite and the mono- and dimethyl trivalent As metabolites prefer binding to dithiols, while man-made organoarsenicals with ring structures or withdrawing groups, such as phenylarsine oxide, preferentially bind monothiols. The trivalent arsenicals can bind to cysteines in zinc (Zn) coordination pockets of DNA binding proteins to replace the Zn, inhibit protein activity, and promote genomic instability (Hartwig et al., 2002; Zhou et al., 2011; IARC, 2012). Binding to critical Zn fingers in the PML protein is a primary mechanism for arsenic trioxide efficacy in treating leukemia (Zhang et al., 2010). Pentavalent arsenate mimics phosphate and can inhibit phosphotransfer reactions, such as mitochondrial oxidative phosphorylation, when present in concentrations that are stoichiometrically competitive with phosphate. Arsine gas is a potent hemolytic agent (ATSDR, 2007, 2016).

In addition to these basic modes of action, several mechanisms have been proposed for As toxicity and carcinogenicity. As and its metabolites stimulate oxidant production through activation of NADPH oxidases and interference with mitochondrial respiration. Transition between trivalent and pentavalent oxidation states occurs through two-electron transfer, and thus metabolism of As is not capable of directly generating reactive oxygen species (ROS). Instead, reaction with critical cysteines in receptors and signaling enzymes stimulates NADPH oxidase and other sources of ROS generation (Samikkannu et al., 2003; Straub et al., 2008, 2009; ATSDR, 2016). The oxidants generated are second messengers in signal transduction cascades leading to activation of tyrosine and serine/threonine kinase pathways for enhanced proliferation, cell turnover, and potential transformation (Simeonova and Luster, 2002; Samikkannu et al., 2003; Andrew et al., 2009; NRC, 2014; ATSDR, 2016). Signaling through oxidant activation of the nuclear factor, erythroid 2 like 2 (NFE2L2) (aka Nrf2) transcriptional program produces adaptation to As-promoted stress with broad expression of antioxidant capacity and phase II metabolism genes (Lau et al., 2013; Sinha et al., 2013).

Chronic As exposures produce longer-term phenotypic change through a range of epigenetic effects including altered DNA methylation, histone modification, altered miRNA expression, and genomic instability (Rossman and Klein, 2011; ATSDR, 2016; Bailey et al., 2016; Howe and Gamble, 2016; Martin et al., 2017; Cardoso et al., 2018). Some mechanisms, however, may be cell type or organ specific, as emerging evidence suggests that As impacts target tissue stem cells in various ways to facilitate oncogenic change or impair tissue metabolism and regeneration (Bailey et al., 2016; Zhang et al., 2016; Ngalame et al., 2018).

The carcinogenic potential of As was recognized over 110 years ago by Hutchinson, who observed an unusual number of skin cancers occurring in patients treated for various diseases with medicinal arsenicals (IARC, 2012). IARC classified As as a known human carcinogen, most associated with tumors of the skin, lung, and urinary bladder and possibly kidney, liver, and prostate (Straif et al., 2009; IARC, 2012). Because elemental As and As species share the same metabolic pathway (arsenate → arsenite → MMA → DMA), different As species should be considered as carcinogenic, independent of the mechanisms of the carcinogenic action, and independent of which of the metabolites is the actual ultimate carcinogen.

IARC has concluded that sufficient evidence in humans exists for the carcinogenicity of mixed exposure to As compounds, including As_2O_3, arsenite, and arsenate. As compounds cause cancer of the lung, urinary bladder, and skin. Also, a positive association has been observed between exposure to and As compounds and cancer of the kidney, liver, and prostate. In addition, sufficient evidence exists in experimental animals for the carcinogenicity of dimethylarsinic acid, calcium arsenate, and sodium arsenite. There is limited evidence in experimental animals for the carcinogenicity of sodium arsenate, gallium arsenide, arsenic trioxide, and trimethylarsine oxide, and there is inadequate evidence in experimental animals for the carcinogenicity of monomethylarsonic acid and arsenic trisulfide. In view of the overall findings in animals, IARC concluded that there is sufficient evidence in experimental animals for the carcinogenicity of As compounds. Thus, these compounds are carcinogenic to humans (group 1). DMA and MMA are possibly carcinogenic to humans (group 2B). Arsenobetaine and other organic As compounds not metabolized in humans are not classifiable as to their carcinogenicity to humans (group 3).

The mode of action for As-induced cancers remains unresolved and is likely to be multifactorial. Resolution of a mode of action has been complicated by the difficulty in confirming the carcinogenicity of As in experimental animals (States et al., 2011; Tokar et al., 2011c). Unlike many carcinogens, As is not mutagenic in bacteria and acts weakly in mammalian cells, but can induce chromosomal abnormalities, aneuploidy, and micronuclei formation (IARC, 2012; ATSDR, 2016). As can also act as a co-mutagen and/or co-carcinogen (Rossman et al., 2002; IARC, 2012; ATSDR, 2016).

It is clear that As and its metabolites are not directly genotoxic and As does not react directly with DNA. Instead, reaction with thiols in critical proteins produces transcriptional and epigenetic changes that promote cancers and tumor growth (Cohen et al., 2013; NRC, 2014; Cardoso et al., 2018). Interference with cysteine coordination pockets in the Zn fingers of DNA binding protein inhibits DNA repair mechanisms and promotes genomic instability (Hartwig et al., 2002; Zhou et al., 2011; IARC, 2012; Cardoso et al., 2018). Signaling for increased ROS generation and ROS modification of critical cysteines accounts for a large portion of As promotion of cell proliferation at lower concentrations and DNA damage at higher concentrations that interfere with mitochondrial respiration (IARC, 2012; NRC, 2014; ATSDR, 2016). As inhibits a number of DNA repair mechanisms (Hartwig et al., 2002; Kitchin and Wallace, 2008; Zhou et al., 2014) and decreases expression of DNA repair transcripts (Andrew et al., 2003; IARC, 2012). Genomic instability and transformation may also result from epigenetic regulation and changes in miRNA processing that promote the cancer cell phenotype [reviewed in Cardoso et al. (2018)]. Inhibition of the tumor suppressor p53 and promotion of maladaptive antioxidant and anti-apoptotic responses may underlie the co-carcinogenic properties of arsenicals (IARC, 2012; NRC, 2014).

Acute poisoning from ingesting large doses (70–180 mg) of As can be fatal. Symptoms of acute intoxication include fever, anorexia, hepatomegaly, melanosis, cardiac arrhythmia, and, in fatal cases, terminal cardiac failure. Acute high-level ingestion damages mucous membranes of the gastrointestinal tract, causing irritation, gastritis, vesicle formation, and even sloughing. Sensory loss in the peripheral nervous system is the most common neurological effect, appearing at 1–2 weeks after large doses and consisting of Wallerian degeneration of axons, a condition that is reversible if exposure is stopped. Anemia and leukopenia, particularly granulocytopenia, occur a few days following high-dose As exposure and are reversible.

Intravenous As infusion at clinical doses in the treatment of acute PML may be significantly or even fatally toxic in susceptible patients. Approximately 30% of patients undergoing As chemotherapy experience life-threatening reentrant cardiac arrhythmias caused by As impairment of inwardly rectifying potassium channels (Ficker et al., 2004; Cubeddu, 2016). As cancer therapies are also associated with noncardiogenic pulmonary edema and severe pulmonary leukocytosis (Camacho et al., 2000; Briasoulis and Pavlidis, 2001). Acute exposure to a single high dose can produce encephalopathy, with signs and symptoms of headache, lethargy, mental confusion, hallucination, seizures, and even coma (ATSDR, 2007).

Arsine gas, generated by electrolytic or metallic reduction of As in nonferrous metal production, is a potent hemolytic agent, producing acute symptoms of nausea, vomiting, shortness of breath, and headache accompanying the hemolytic reaction. Exposure to arsine is fatal in up to 25% of the reported human cases and may be accompanied by hemoglobinuria, renal failure, jaundice, and anemia in nonfatal cases when exposure persists (Pullen-James and Woods, 2006).

10.4 EVIDENCE OF HUMAN DISEASES CAUSED BY ARSENIC

Chronic exposure to As in the environment is associated with a number of noncancer disabilities and diseases, as well as several cancers. As is unique among environmental contaminants because the ubiquitous exposure affecting hundreds of millions of individuals globally has resulted in a wealth of human epidemiological data that support dose-dependent assessment of disease risk and disease burden. There is sufficient human data to infer mechanisms for many morbidities and mortalities. There are also sufficient human data to confirm the effects of whole-life exposure on risk to human health and that *in utero* exposures associate with greater morbidity and mortality in later life. The following summarizes pertinent portions of this large amount of data recognizing that modern techniques of systematic review and meta-analyses are continuing to be applied to fully appreciate disease risk and inform interventions to mitigate disease risk.

The skin is a major target organ in chronic As exposure and skin lesions are often diagnostic of high-level As exposure. In humans, chronic As exposure induces a series of characteristic changes in skin epithelium, perhaps due to preferential uptake into skin structures. Diffuse or spotted hyperpigmentation and, alternatively, hypopigmentation can first appear between 6 months and 3 years with chronic As exposure (ATSDR, 2007). The characteristic hyperpigmented spots or palmar-plantar hyperkeratosis usually follows the initial appearance of As-induced pigmentation changes within a period of years (ATSDR, 2007). Susceptibility to skin lesions increases with deficiencies in folic acid and vitamin B due to impaired methylation of ingested As (Gamble et al., 2006; Ahsan et al., 2007). Skin cancer that presents as *in situ* squamous carcinoma that is often indistinguishable from Bowen's disease is common with protracted high-level arsenical exposure (ATSDR, 2007; IARC, 2012).

As-induced skin cancers include basal cell and squamous cell carcinomas, both arising in areas of As-induced hyperkeratosis (Davis et al., 2000; IARC, 2012). The basal cell cancers are usually only locally invasive, but squamous cell carcinomas, resembling Bowen's disease, may have distant metastases. In humans, the skin cancers often occur on areas of the body not exposed to sunlight (e.g., on palms of hands and soles of feet) and often occur as multiple primary malignant lesions. In experimental animals, As acts as a rodent skin

tumor co-promoter with 12-*O*-teradecanoyl phorbol-13-acetate in v-Ha-*ras* mutant Tg.AC mice (Germolec et al., 1998) or as a co-carcinogen with UV irradiation in hairless mice (Rossman et al., 2004).

Cardiovascular disease and coronary artery disease in particular are the noncancer diseases most strongly associated with environmental As exposures (NRC, 2014; Nigra et al., 2016; Moon et al., 2017). Systematic review and meta-analysis of recent highly powered prospective epidemiological studies of the dose-dependent association of As with coronary artery disease morbidity and mortality confirmed significant risk of disease even at the current EPA maximum contaminant level (MCL) of 10 μg/L (Moon et al., 2017). The dominant form of disease stems from the atherogenic potential of As and its propensity to enhance both vessel disease and prolongation of the cardiac Q-T interval (Wu et al., 2014; Nigra et al., 2016).

Mechanistically, As-stimulated ROS and inflammatory cytokine expression promote endothelial cell dysfunction, proatherogenic monocyte and macrophage phenotypes, smooth muscle expansion, and vessel wall stiffening (Lemaire et al., 2014, 2015; Wu et al., 2014). The vascular endothelium is highly sensitive to As exposures, with low-level exposure promoting proliferation, angiogenesis, vessel remodeling, and loss of vasodilator response (Soucy et al., 2003, 2005; Tseng et al., 2005; Straub et al., 2008). In contrast, high-level As_2O_3 exposure in cancer therapies is antiangiogenic and causes endothelial cell death with loss of vessels (Roboz et al., 2000; Soucy et al., 2003, 2005).

Atherosclerotic mouse models show increased atherosclerosis following even moderate As exposures with smooth muscle cell remodeling and aberrant macrophage lipid metabolism as the prime pathogenic targets (Lemaire et al., 2011, 2015). In addition, mouse models have also demonstrated that low to moderate levels (<100 μg/L) of exposure cause perivascular fibrosis and loss of perivascular matrix integrity, especially in the heart (Soucy et al., 2005; Hays et al., 2008). Blackfoot disease is a severe occlusive peripheral vascular disease associated with chronic, high-level As exposure in drinking water, especially in endemic regions in Taiwan (Tseng, 2005; Wang et al., 2007). It is an arteriosclerosis manifested by acrocyanosis and Raynaud's phenomenon that may progress to endarteritis and gangrene of the lower extremities.

Chronic exposure to As is associated with a range of nonmalignant respiratory symptoms, chronic obstructive pulmonary disease (COPD), and respiratory disease mortality (Parvez et al., 2013; Sanchez et al., 2016, 2018). Susceptibility to the respiratory effects of As is enhanced by *in utero* exposure (Rahman et al., 2011; Ramsey et al., 2013b; Sanchez et al., 2016; Steinmaus et al., 2016) and co-exposure to tobacco smoke (Parvez et al., 2013). In addition, As exposure is associated with increased respiratory tract infections (Rahman et al., 2011; Farzan et al., 2016) and chronic lung infections, including pulmonary tuberculosis (Smith et al., 2011). Prospective epidemiologic studies indicate that As causes chronic depletion of lung defenses, such as secretion of CC16 protein from airway cells (Parvez et al., 2010). Rodent studies suggest that low to moderate levels of *in utero* As exposure decrease immune gene expression and promote inflammatory protein expression (Kozul et al., 2009b; Ramsey et al., 2013a) that may make mice more susceptible to airway infections (Kozul et al., 2009a). In keeping with reduced lung defenses, as in the heart, As compromises lung matrix, wound repair, and barrier function (Hays et al., 2008; Lantz et al., 2009; Petrick et al., 2009; Sherwood et al., 2013).

Lung cancer accounts for the majority of As-related cancer deaths, with *in utero* exposures increasing the risk of lung cancer later in life (Smith et al., 2006; Tokar et al., 2011b; IARC, 2012; ATSDR, 2016; Bailey et al., 2016; Steinmaus et al., 2016). Based upon a

meta-analysis, Begum et al. (2012) estimated about 4.51 additional lung cancer cases per 100,000 people for a maximum contamination level of 10 μg/L of As in drinking water and enhanced risk with co-exposure to tobacco smoke (Ferreccio et al., 2013). It is likely that As is not the primary carcinogen, but synergizes with the carcinogenic potential of tobacco smoke constituents. As is unique among the metals and chemical carcinogens, since As-promoted lung cancer is independent of route of exposure, with oral ingestion and inhalation giving equivalent increases in risk of disease (Smith et al., 2009). Metabolism of As is also an important component to lung cancer risk as higher percentages of MMA are associated with higher risk (Kuo et al., 2017).

Chronic As exposures, especially higher-level exposures, are associated with metabolic diseases. As is predominantly metabolized in the liver, and chronic exposure is associated with liver disease. As-associated liver disease manifests initially as jaundice, abdominal pain, and hepatomegaly (Mazumder and Dasgupta, 2011). Liver injury may progress to cirrhosis and ascites, as well as to hepatocellular carcinoma (Liu and Waalkes, 2008; Straif et al., 2009; IARC, 2012). However, liver cancer is poorly associated with As exposure (IARC, 2012). As exposure and As metabolites are associated with diabetes (Kuo et al., 2015, 2017; Grau-Perez et al., 2017). It is not clear, however, whether low-level As is directly diabetogenic or enhances diabetes in diabetic individuals (Maull et al., 2012; Grau-Perez et al., 2017). It is interesting that a lower percentage of MMA is associated with the diabetogenic effects of As (Grau-Perez et al., 2017), and again *in utero* exposure may enhance disease in later life (Young et al., 2018). In cell and animal studies, As directly impairs pancreatic beta cells and insulin release (Fu et al., 2010; Martin et al., 2017).

The unique kinetics of As and metabolites greatly increase risk for transitional cell carcinoma of the bladder (Tokar et al., 2011a; IARC, 2012; Cohen et al., 2013). The metabolites are readily excreted into the urine, and enhanced concentration promotes cyclic epithelial cell death and regeneration that ultimately results in cellular transformation (Cohen et al., 2013). However, metabolism of As is not required to produce carcinogenic responses in the bladder epithelium, as hyperplasia and transformation is increased in mice lacking As3MT, relative to wild-type controls (Chen et al., 2011). As also forms intracellular cytoplasmic granules in the bladder epithelium (Yokohira et al., 2011). Bladder cancer risk is up to sixfold higher in As-exposed females relative to males, and the association of As exposure with bladder cancer risk is greatly enhanced in smokers or ever-smokers (Ferreccio et al., 2013). It appears that most renal cancers associated with As exposure are transitional cell carcinomas and are thought to arise from the bladder urothelial cells (IARC, 2012).

As, especially with chronic early-life exposure, is immunotoxic and potentially increases risk of infections and inflammatory-like diseases during childhood and in adulthood (ATSDR, 2016; Ferrario et al., 2016). This immunosuppressive potential is prevalent in respiratory tract infections (Kozul et al., 2009a; Rahman et al., 2011; Ramsey et al., 2013a; Steinmaus et al., 2016), and enhancement of a global inflammatory state may underlie the etiology of As-promoted neural, cardiovascular, metabolic, and cancer disease. For example, As exposures alter transcriptional programing in circulating macrophages to enhance atherogenesis that underlies As-promoted cardiovascular disease (Lemaire et al., 2014). In addition to increasing inflammation, As may increase allergy and autoimmune diseases (Ferrario et al., 2016). Further impacts on the circulation include hematologic consequences of chronic exposure to arsenic that interferes with heme synthesis and increases urinary porphyrin excretion, which is a biomarker for As exposure (Ng et al., 2005).

The neurotoxicity of As is well recognized, especially in the clinical signs of severe polyneuropathy from poisonings (ATSDR, 2007). Occupational exposures, as in copper

smelters, or repeated exposures to high levels of As produce peripheral neuropathy (ATSDR, 2007; Sinczuk-Walczak et al., 2010). This neuropathy usually begins with sensory changes, such as numbness in the hands and feet, but later may develop into a painful "pins and needles" sensation. Both sensory and motor nerves can be affected, and muscle tenderness often develops, followed by weakness, progressing from proximal to distal muscle groups. Sensorimotor dysfunction is pronounced in exposed children (Parvez et al., 2011), suggesting that *in utero* or early development exposures increase susceptibility.

As exposures, especially *in utero* and during early developmental exposures, also impair central cognition and memory behaviors (Wasserman et al., 2011, 2014; Tyler and Allan, 2014). A large cross-sectional study in Maine, USA, found that drinking water levels greater than 5 μg/L were associated with cognitive deficits in children (Wasserman et al., 2014). This suggests that the 10 μg/L U.S. drinking water standard may not be protective for all health effects of As. Studies in mice confirm that As exposure during development impairs neural stem cell function, as well as epigenetically reduces cognition and enhances depression (Cronican et al., 2013, Tyler and Allan, 2014). Interestingly, studies in mice suggest that the impact of low (50 μg As/L) *in utero* and perinatal exposures on cognition may be less in females that respond to As with greater antioxidant adaptation (Allan et al., 2015).

10.5 CONCLUSIONS

Given the extent of human exposure to environmental As, it could be argued that As is the most significant environmental toxicant for causing human disease. As is ubiquitous in the environment and the main cause for concern is As ingested in food and water. It is clear that As biogeochemistry is complex, resulting in difficulties in predicting exposures and distribution of As into food and drinking water. It is also apparent that human activities and industries affect this biogeochemistry to produce large-scale environmental contamination and human exposure. However, the majority of disease-promoting As exposures result from placing drinking water wells in As-rich aquifers or growing crops in As-rich soils. As such, human exposures are decreasing with increased awareness of the need for water and soil testing to reduce exposures. The minimum contaminant levels in water and food needed to protect health continue to be debated, especially with realization that the current MCLs that are based on cancer risk may not be protective for cardiovascular or respiratory disease risks, as well as cognitive impairment and immune suppression. In addition, the insidious nature of environmental As often makes it a generational toxicant, and it is well established that *in utero* or perinatal exposures greatly enhance disease risks later in life. A challenge is to balance the practicality of reducing exposures in susceptible population with identifying dietary and therapeutic interventions that protect human health from this pervasive environmental contaminant.

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11

ASBESTOS AND OTHER MINERAL AND VITREOUS FIBERS

Morton Lippmann

11.1 INTRODUCTION

Durable mineral fibers, synthetic vitreous fibers (SVFs), and more recently carbon fibers have many important commercial uses in high tensile structural composite applications and as insulators. Asbestos is a family of silicate minerals whose elongated crystals can be found in natural deposits with very high aspect (length-to-width) ratios. These fibers are stable at very high temperatures, making them ideal for thermal insulation. Unfortunately, thin asbestos fibers that are dispersed into workroom or ambient air are readily inhaled and have caused lung and pleural fibrosis, lung cancer, and mesothelioma, a cancer of the pleura or peritoneum. This recognition led to the use of less toxic fibers for most of the products that formerly used asbestos. The most widely used substitutes include SVFs such as rock wool, slag wool, ceramic, and fibrous glass and crystalline carbon microfibers and microtubes.

11.1.1 Important Special Properties Of Mineral And Vitreous Fibers

11.1.1.1 Asbestos Case et al. (2011) discussed definitions of "asbestos" provided by various research scientists, technical advisory groups, and regulators in considerable detail. They noted that the definitions advocated by mineralogists differed from those preferred by health scientists, and both types have evolved over time. For the purpose of this discussion that is focused on the public health implications of the inhalation of asbestos fibers, I will use asbestos as a generic term that covers a family of crystalline SiO_3 minerals varying in crystal structure and metals content as described by Langer et al. (1990) and Meeker et al. (2003). The mineralogy and cation composition of asbestos fibers vary within and between the members of the family and are summarized in Table 11.1. It is the fibrous habit or "asbestiform" nature of these minerals, as well as their occurrence as millimeter-long fibers, which distinguish them from other silicate minerals and gave them commercial importance.

Environmental Toxicants: Human Exposures and Their Health Effects, Fourth Edition.
Edited by Morton Lippmann and George D. Leikauf.

All asbestiform asbestos varieties are good thermal and acoustic insulators, and their high tensile strengths and flexibility made them useful in many commercial applications. Asbestos varieties low in iron content are good electrical insulators, and some varieties are stabile in alkaline and in acidic environments.

The asbestiform nature is due to the crystallographic properties of the fibers. The sheet silicate structure of the serpentine mineral known as chrysotile asbestos has a dimensional mismatch between its tetrahedral coordinate Si_2O_5 layer and the octahedral coordinated MgOH (Brucite) layer, resulting in a structural deformation that causes the sheets to form cylindrical or "tubular" minifibers (fibrils) (Whittaker and Zussman, 1956). Weak interatomic bonds hold together many hundreds of these individual fibrils to form a fiber (Yada, 1967). All of the other asbestos minerals are in a class known as amphiboles and have double chain silicate structures. Crystal structure and defects facilitate the release of the ultimate amphibole asbestos fibers (Veblen, 1980; Chisholm, 1983), which are all more rodlike and less curly than chrysotile fibers.

The amphibole asbestos minerals tremolite, actinolite, and anthophyllite have the same name for both their asbestos and their common rock-forming variety, while amosite and crocidolite, two other amphiboles, refer only to the fibrous form. Thus, there has been some confusion in regard to the presence of the asbestos fibers in some products and environments. The asbestos minerals may occur in greater than trace amounts as both asbestiform fibers or as mixtures containing mineral cleavage fragments that are more compact particles. When the asbestiform fiber content exceeds 1% by mass, it becomes an asbestos-containing material (ACM) under the current EPA definition.

The formation, geological origin, and crystal structure of chrysotile inhibit cation substitution and the content of Fe and Mg depletion from weathering are the main variations. By contrast, the amphibole fibers have a crystal structure more favorable to cation substitution. Most commercial amphibole deposits can be reliably identified as individual fibers by analytical transmission electron microscopy (TEM). The chemistry, structure, and properties

TABLE 11.1 Asbestos Fiber Types

Commercial Name	Mineral Name	Mineral Group	Major Cations
Chrysotile	Chrysotile	Serpentine	Mg, Fe
Crocidolite	Riebeckite	Amphibole	Na, Mg, Fe
Anthophyllite	Anthophyllite	Amphibole	Mg, Fe
Amosite	Grunerite	Amphibole	Fe, Mg, Mn
Actinolite	Actinolite	Amphibole	Ca, Fe, Mg
Tremolite	Tremolite	Amphibole	Ca, Mg, Fe
	Winchite	Amphibole	Ca, Mg, Fe
	Richterite	Amphibole	Ca, Mg, Fe

Chrysotile, actinolite, anthophyllite, and tremolite are generally encountered as asbestiform minerals and are grayish white in color.
Actinolite asbestos is found as a contaminant of amosite from South Africa.
Amosite is the asbestiform version of grunerite and is brown in color.
Anthophyllite asbestos was commercially worked in Finland.
Crocidolite is the asbestiform version of riebeckite and is blue in color.
Tremolite asbestos is exploited commercially in Korea and is a contaminant in some chrysotile ores, as well as in vermiculite ore mined in Libby, Montana.
Winchite and richterite are major asbestiform contaminants of Libby vermiculite ore.

of asbestos have been reviewed many times (e.g., Walton, 1982; Chisholm, 1983; Langer et al., 1990; Meeker et al., 2003).

Each of the asbestos fiber types has its own size range in airborne and tissue evaluations (Pooley and Clark, 1980; Burdett, 1985). TEM-based fiber size distributions of airborne asbestos were reviewed by Berman and Chatfield (1989). Their principal conclusion was that some 9% (range: 1–50%) of chrysotile, 4% (range: 1–18%) of crocidolite, and 25% (range: 8–43%) of amosite meet the conventional industrial hygiene definition of hazardous asbestos fibers [i.e., fibers >5 μm long, >0.25 μm wide, and aspect (length/width) ratio >3:1]. Measurements in U.K. textile and friction product plants (Rood and Scott, 1989) found that only about 4% of chrysotile fibers fell into this category. However, a study by Dement and Wallingford (1990), of the same industries in the United States, reported some 20% of fibers that would be counted by the industrial hygiene definition. Evaluations by TEM reveal that (1) crocidolite may form very fine fibers that range between 0.04 and 0.15 μm in diameter; (2) amosite fibers are found to be greater in diameter, that is, between 0.06 and 0.35 μm; (3) chrysotile's fibrils are smallest of all, some 0.02–0.05 μm in diameter. As discussed later in this chapter, specific properties that affect the biological activity of asbestos fibers include fiber type, length, diameter, and their durability within the lungs and at other sites in the body.

11.1.1.2 Synthetic Vitreous Fibers As hazards from naturally occurring mineral fibers became evident, they were replaced in commerce by SVFs, such as continuous glass filaments, glass, slag, and mineral wools, as well as refractory ceramic fibers (RCFs). In recent years, new high-technology synthetic fibers, such as carbon nanotube (CNTs) and nanowires, have also found their way into commercial products.

SVFs, also known in the literature as man-made mineral fibers (MMMFs), and man-made vitreous fibers (MMVFs) are all generic names that have been applied to fibers that are generally made by spraying or extruding molten glass, rock, or furnace slag. Their production technology and historical development were summarized by Konzen (1984). Both fiber lengths and diameters are polydisperse, especially for sprayed fibers. Subsequent fabrication of fiber mats for commercial products such as insulation, filters, and fiber-reinforced composites results in the breakage of fibers into shorter length segments. Konzen (1984) reported that the average fiber diameters decreased, by design, from 10–12 μm in the 1940s to ~7.5 μm in present-day products and that the percentage less than 1 μm decreased from 2.8 to 1.7% over the same period. Even smaller diameter fibers (aka microfibers) can be fabricated, but require much more energy to produce, and are much more expensive. The superior properties of microfibers for insulation and tensile strength lead to their use in special low volume applications where the greater costs can be justified, such as insulation in space shuttle cabins, earplugs, and so on. The chemical compositions of SVF vary with the source materials, and their mechanical properties and durability, both in the products and in the body after inhalation and deposition, can vary greatly with their composition.

Rock and slag wools are terms used for vitreous products made from the precursor materials that are melted, drawn, centrifugally spun and steam- or air-jet blown. These processes produce individual SVF fibers. Feldspar, kaolinite, and Indiana limestone are used to make rock wool. Slags, by-products from many sources including iron and steel-making and from base metal and copper smelting, have been used in slag-wool production. The bulk and trace metal chemistry of these products vary greatly. The principal RCF in commerce in the United States is of alumina-silica composition. These RFF fibers have great chemical stability and withstand greater temperatures than other SVFs.

The focus on SVFs here is on human exposures to commercial fibrous glass and the health risks associated with such exposures. The assessment is based on (1) epidemiological data on occupational exposures and their health effects; (2) biological responses to airborne fiber suspensions inhaled by laboratory animals or injected into their lungs or pleural or peritoneal spaces; and (3) the aerodynamics, deposition, and clearance of airborne fibers within the respiratory tract.

Most of the biological effects of SVFs are essentially the same as those produced by asbestos fibers, but with substantially less biological potency. All varieties of asbestos, as well as some varieties of SVFs, can cause the same diseases in laboratory animals. Glass fibers have been associated with dermatitis and eye irritation in industrial workers, but these non-respiratory health effects will not be reviewed further in this chapter, since they are not likely to be relevant to the much lower levels of nonoccupational exposures.

Different cationic metals in silica melts may coordinate oxygen differently, due to their cation charge (Z) and ionic radius (r). The immiscibility regions in glass melts is compositionally dependent, a function of Z/r^2 (the determining factor in influencing cation field strength).

Because of their different chemistries, SVFs behave differently in biological hosts. The aluminum-silicon ceramic fibers are stable (similar Z/r^2 values) and are therefore durable *in vivo*. Slag wools, rich in trace metals, are usually neither stable nor durable in biological hosts, and high-soda glasses are most unstable. The issue of fiber durability is of extreme biological importance. Because most SVFs are generally less stable *in vivo* than asbestos, they carry less risk of producing disease (Lippmann, 1990).

Most commercially available SVFs that are used in insulation, fire retardant, and acoustical applications have median diameters between 4 and 6 μm. These values are consistent with aerodynamic diameters in the range of 12–18 μm (Timbrell, 1972). Thus, commercial SVFs have aerodynamic diameters too large for much penetration into the thorax during inhalation, providing one important basis for the conclusion that SVFs are less biologically hazardous than asbestos fibers (Lippmann, 1990).

Insulating glasses may be coated with binders, for example, phenol-formaldehyde resins, or with mineral oil lubricants in a range of concentrations. The biological significance of these coating materials for long-term, chronic, diseases is unknown.

11.1.1.3 Other Inorganic Mineral Fibers Wollastonite, a naturally occurring calcium silicate ($CaSiO_3$), is available in a fibrous form. However it is not biopersistent and is therefore not considered to be hazardous (Maxim and McConnell, 2005).

Erionite, a naturally occurring zeolite mineral also exists in a fibrous form, has been implicated as a potent cause of mesothelioma in humans and animals (Baris et al., 1981, 1987; Wagner et al., 1985; Baris and Grandjean, 2006; Carbone et al., 2007; Ryan et al., 2011).

11.1.1.4 Other Inorganic Fibers The rapid development of nanotechnology has made it possible to fabricate carbon lattices forming multi-walled tubes, metallic nanowires, and silicon carbide and alumina whiskers with extremely high aspect ratios and lengths greatly exceeding 5 μm. Products incorporating CNTs, nanowires, and whiskers combine high tensile strength with durability while being light in weight. For CNTs, some commercial formulations were less durable than others in Gamble's solution *in vitro* (Osmond-McLeod et al., 2011), who measured the durability and inflammogenic impact of CNTs after incubation for up to 24 weeks in Gamble's solution. Three single-walled and multi-walled CNT types showed no or minimal losses of mass, change in fiber length, or morphology, while a fourth type of long

CNT lost 30% of its mass within 3 weeks and lost pathogenicity when injected into the peritoneal cavities of mice. Schinwald et al. (2012a) indicated that silver nanowires injected into mice IP retained *in vivo* structural integrity for 1 day, but not for 1 week.

Durable SVFs can also be produced from pure chemicals. For example, DuPont developed Fybex (potassium octatitinate) and Kevlar, an aramid, as fibrous asbestos substitutes. They found that Fybex produced mesotheliomas in hamsters (Lee et al., 1981) and did not pursue commercial application. By contrast, Kevlar has been shown to have biological effects similar to those associated with nuisance dusts (Lee et al., 1983). These SVFs will not be discussed in any detail here except insofar as toxicity data obtained using them sheds light on the physical properties of fibers affecting *in vivo* toxicity.

11.1.2 Major Commercial Uses of Inorganic Fibers

There are many asbestos-containing products found in buildings, including thermal system insulation, structural fireproofing, acoustical and decorative finishes, sheet products, floor and ceiling tiles, asbestos-containing felts, and so on (Sawyer, 1989). A number of other construction products have, in the past, contained asbestos fibers as well, for example, spackling, patching, and plastering compounds used in drywall construction and interior repair. In addition to natural mineral fiber, many of these formulations contained SVFs in combination with asbestos, and, over recent decades, SVFs have replaced asbestos in most applications. The major asbestos-containing items to be found in buildings were outlined by Spengler et al. (1989).

11.2 INHALATION EXPOSURES TO FIBERS

11.2.1 Exposure Indices

There has never been a fully satisfactory method for measuring airborne fiber exposures relevant to health effects, and confusion has arisen because the various methods that have been used cannot be reliably interconverted. Three different types of concentration indices have been used for airborne asbestos. In the first half of the twentieth century, the most widely used index for occupational exposures was the number of all airborne particles per unit volume of air, expressed in millions of particles per cubic foot (MPPCF) and determined from liquid impinger dust samples analyzed by the now obsolete U.S. Public Health Service standard optical microscopic dust-counting technique. It used a 10× objective lens to count the number of visible particles that settled to the bottom of a dust-counting cell filled with a liquid suspension of particles from the impinger flask. Since there was no discrimination between fibrous and nonfibrous particles, and since fibers were a very variable fraction of the total dust in most cases, dust counting for occupational fiber exposure evaluations was replaced by a technique that counts fibers only.

When the fiber-counting technique was first adopted (in the United Kingdom), it was already clear that long thin fibers were of most concern in terms of inhalation hazard. This, combined with the practical limitation that fibers shorter than ~5 μm could not be reliably identified by light microscopy, led to the adoption of a counting procedure that uses a 45× phase-contrast objective lens to count the fibers collected on the surface of a membrane filter, provided that they had a length >5 μm and an aspect ratio >3. This phase-contrast optical method (PCOM) is specified in the Occupational Safety and Health Administration (OSHA) occupational health standard for asbestos. Table 11.2 summarizes

recommended occupational exposure limits and standards used in the United States over the last 60 years. Detailed guidance on the PCOM method was provided by the World Health Organization (WHO, 1997).

The third type of concentration index that was used in inhalation exposure studies in animals for health risk assessments is based on the mass concentration of asbestos or on the mass concentration passing a pre-collector meeting the British Medical Research Council (BMRC) or American Conference of Governmental Industrial Hygienists (ACGIH) sampler acceptance criteria for "respirable" dust.

Environmental exposures have been measured either in terms of fiber count or fiber mass. Fiber counts have been made using both PCOM and TEM. The reported concentrations have differed according to the size distributions of the fibers, the resolving power of the microscope, and whether there was any discrimination in the analyses according to fiber type.

A fiber mass concentration index was developed by Selikoff et al. (1972), in which the fibers were identified and measured by TEM. It specified that fiber bundles in the sample were to be mechanically reduced to individual fibers and fibrils, prior to their identification and measurement. Mass concentrations in nanograms per cubic meter were calculated from the numbers of fibers and fibrils and their dimensions and densities.

The use of these various exposure indices has sometimes led to the development of a site- or industry-specific exposure–response relationship for one or more of the asbestos-related diseases, but it has not been possible to develop any generic relationships.

11.2.2 Airborne Exposure Levels

Esmen and Erdal (1990) reviewed published data on human occupational and nonoccupational fiber exposures. They concluded that for the traditional PCOM defined asbestos fibers, that is, fibers >5 μm long, large amounts of the available data suffered from the diversity of sample collection and analysis methods. Simple generalizations suggest that occupational exposures are several orders of magnitude higher than environmental exposures; and currently extant data and the current routine measurement practices present significant difficulties in terms of consistent

TABLE 11.2 Recommended Air Concentration Limits and Standards for Asbestos

Group	Year	Limit
ACGIH	1946	5×10^6 particles/ft^3
ACGIH	1968[a]	12 fibers/ml or 2×10^6 particles/ft^3
ACGIH	1970[a], 1974[b]	5 fibers/ml
OSHA	1972[c]	5 fibers/ml
NIOSH	1976	0.1 fiber/ml
ACGIH	1978[a], 1980[b]	0.2 fiber/ml for crocidolite
		0.5 fiber/ml for amosite
		2.0 fiber/ml for chrysotile and other forms
ACGIH	1997[a]	0.1 fiber/ml for all forms
OSHA	1976[c]	2.0 fiber/ml
OSHA	1986[c]	0.2 fiber/ml
OSHA	1992[c]	0.1 fiber/ml

[a]Notice of intent.
[b]Adopted as threshold limit value (TLV).
[c]Adopted as a permissible exposure limit (PEL).

interpretation of the data with respect to health effects. The human exposure data to many non-asbestos minerals that exist in fibrous habit are very scanty, and in view of the variations of their biological activity, this lack of relevant data limits interpretability.

With respect to airborne asbestos fiber exposures within occupied buildings, the Literature Review Committee of Health Effects Institute-Asbestos Research (HEI-AR, 1991) grouped building occupants into five main categories with respect to ACMs: C1, bystanders or nonoccupationally exposed building occupants, for example, office workers, visitors, students and teachers; C2, housekeeping or custodial employees who may disturb materials in the course of routine cleaning and service functions; C3, maintenance or skilled workers that may disturb ACM in the course of making repairs, installing new equipment, or during minor renovation activity; C4, abatement workers or others involved in the removal or renovation of structures with ACM; and C5, firefighters and other emergency personnel who may be present during or after the fabric of the building has been extensively damaged by fire, wind, water or earthquake.

Building employees and contractor employees may disturb ACM during the course of their normal work assignments, especially during maintenance and custodial activities. Exposures of such workers can be high and warrant special concern. By far the most numerous are the C1 building occupants. Persons in categories C2–C5 fall under OSHA regulations for personal monitoring if their exposures exceed the permissible exposure limit (PEL) of 0.1 fiber/mL (f/mL) >5 μm in length as an 8-h time-weighted average or the OSHA excursion limit of 1 f/mL in a half-hour, both as determined by PCOM. Persons in category C1 are not covered by any federal exposure limits.

There are relatively few published data on the concentrations of airborne fibers in public buildings. The HEI-AR Panel compiled the available data, both published and such unpublished data as it could assemble (HEI-AR, 1991). They concluded that (1) a large number of buildings in the United States and other countries were examined for airborne asbestos fibers within the prior 20 years, but few building environments were characterized in sufficient detail or sampled with sufficient analytical sensitivity to describe adequately the exposures of general building (C1) occupants; (2) available data on the dimensions most relevant to human health (i.e., fibers >5 μm long) generally showed average concentrations on the order of 0.00001 f/mL; (3) outdoor urban average concentrations above 0.0001 f/mL were attributable to local sources, for example, downwind from, or close to, frequent vehicle braking or activities involving the demolition or spray application of asbestos products; and (4) ambient indoor levels of asbestos from direct TEM measurements, when averaged for each of a number of individual buildings, ranged from 0.00004 to 0.00063 f/mL. Grouped by building category, the mean concentrations are 0.00051, 0.00019, and 0.00021 f/mL in schools, residences, and public and commercial buildings, respectively, with upper 90th percentiles of 0.0016, 0.0005, and 0.0004, respectively.

11.3 FIBER DEPOSITION IN THE RESPIRATORY TRACT

There are five mechanisms that are important with respect to the deposition of airborne fibers in respiratory tract airways. These are impaction, sedimentation, interception, electrostatic precipitation, and diffusion (see Fig. 11.1).

Impaction and sedimentation probabilities are governed by the aerodynamic diameter of the fibers, which, for long mineral fibers, are close to three times their physical

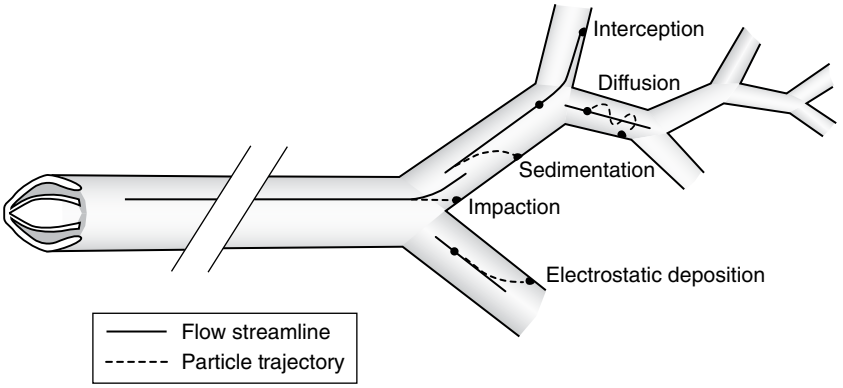

FIGURE 11.1 Particle deposition mechanisms in human tracheobronchial airways during inspiratory flow. *Source*: From CRT-2014. Informa Healthcare USA, Inc. doi: 10.3109/10408444.2014.928266 (page 662).

diameters (Stöber et al., 1970; Timbrell, 1972). Most impaction occurs downstream of air jets in the larger airways, where the flow velocities are high and the momentum of the fiber propels it out of the bending flow streamlines and onto relatively small portions of the epithelial surfaces. Sedimentation, on the other hand, is favored by low flow velocity, long residence times, and small airway size and is more uniformly distributed along the lower surface of the airway.

Electrostatic precipitation occurs primarily by image forces, in which charged particles induce opposite changes on airway surfaces. It is dependent on the ratio of electrical charge to aerodynamic drag. Jones et al. (1983) reported that asbestos fiber processing operations do generate fibrous aerosols with relatively high charge levels and that these charge levels are sufficient to cause an enhancement of fiber deposition in the lungs. Fiber deposition enhancement, for chrysotile asbestos in rats exposed by inhalation, was demonstrated by Davis (1976).

Interception increases with fiber length. The greater the length, the more likely it is that the position of a fiber end will cause it to touch a surface that the center of mass would have missed. Once an end touches, the fiber is drawn to the airway surface.

Diffusional displacement results from collisions between air molecules and airborne fibers. For compact particles, diffusion is important for diameters smaller than about 0.5 μm. Fibers of similar diameter would be more massive and therefore be displaced less by a single molecular impact. Long fibers may have nearly simultaneous impacts from several gas molecules, and their random trajectories may tend to damp the net displacement. On the other hand, a single collision near a fiber end may rotate the fiber sufficiently to alter its interception probability. Gentry et al. (1983) measured the diffusion coefficients of chrysotile and crocidolite asbestos fibers and found good agreement with theoretical predictions for chrysotile (0.4 μm mean diameter) but poor agreement with the more rodlike crocidolite (0.3 μm mean diameter).

The conductive airway region of the human lung consists of a series of bifurcating airways. Single symmetrical fibers suspended in a laminar flow stream should tend to become aligned with the flow axis as they move through a lung airway. On the other hand, fiber agglomerates would have more random orientations that would depend on their distributions of masses and drag forces. A fiber whose flow orientation differs from axial alignment would have an enhanced probability of deposition by interception.

A fiber's alignment is radically altered as it enters a daughter airway, and this loss of alignment with the flow at the entry contributes to its deposition by interception at or near the carinal edge. To the extent that a fiber is entrained in the secondary flow streams that form at bifurcations, its deposition probability by interception is further enhanced.

Sussman et al. (1991a) performed an experimental study of fiber deposition within the larger tracheobronchial airways of the human lung using replicate hollow airway casts. For crocidolite fibers with diameters primarily in the 0.5–0.8 μm range, interception increased deposition for fibers >10 μm in length. Sussman et al. (1991b) also found that the deposition patterns of fibers in the larger lung airways are similar to those for particles of more compact shapes, that is, the added deposition due to interception increased the deposition efficiencies without changing the pattern of deposition.

Morgan and Holmes (1984) and Morgan et al. (1980) exposed rats for several hours by inhalation (nose only) to glass fibers 1.5 μm in diameter and 5, 10, 30, or 60 μm long. For fibers longer than 10 μm, essentially all of the fibers were deposited, mostly in the head. The penetrability of airborne fibers into the rat lung dropped sharply with aerodynamic diameter above 2 μm.

Concern about sites of enhanced surface deposition density is stimulated by the observation that the larger bronchial airway bifurcations, which are favored sites for deposition, are also the sites most frequently reported as primary sites for bronchial cancer (Schlesinger and Lippmann, 1978).

Deposition patterns within the non-ciliated airways distal to the terminal bronchioles may also be quite nonuniform. Brody et al. (1981) studied the deposition of chrysotile asbestos fibers in lung peripheral airways of rats exposed for 1 h to 4.3 mg/m^3. The animals' airways at 0, 5, and 24 h and at 4 and 8 days after the end of the exposure were examined by scanning electron microscopy (SEM) of lung sections that revealed terminal bronchiolar surfaces and adjacent airspaces. Immediately after exposure, asbestos fibers were rarely seen in alveolar spaces or on alveolar duct surfaces except at alveolar duct bifurcations, with relatively high concentrations on bifurcations nearest the terminal bronchioles and lesser concentrations on more distal duct bifurcations. After 5 h, the patterns were similar, but the concentrations were reduced. Subsequent studies have shown that crocidolite asbestos (Roggli et al., 1987), Kevlar aramid synthetic fibers (Lee et al., 1983), RCF fibers (Lentz et al., 2003), and particles of more compact shape (Brody and Roe, 1983) also deposit in similar patterns and that the deposition patterns seen in the rat also occur in mice, hamsters, and guinea pigs (Warheit and Hartsky, 1990).

The sudden enlargement in air path cross section at the junction of the terminal bronchiole and alveolar duct may play a role in the relatively high deposition efficiency at the first alveolar duct bifurcation. Little has previously been known about the flow profiles in this region of the lung. Briant (1988) showed that a net axial core flow in a distal direction and a corresponding net annular flow in a proximal direction take place during steady-state cyclic flow in tracheobronchial airways and that this could account for such concentrated deposition on the bifurcations of distal lung airways.

11.4 FIBER RETENTION, TRANSLOCATION, DISINTEGRATION, AND DISSOLUTION

The fate of fibers deposited on surfaces within the lungs depends on both the sites of deposition and the characteristics of the fibers. Within the first day, most fibers deposited on the tracheobronchial airways are carried proximally on the surface of the mucus to the larynx,

to be swallowed and passed into the gastrointestinal tract. Fibers deposited in the nonciliated airspaces beyond the terminal bronchioles are more slowly cleared from their deposition sites by a variety of mechanisms and pathways. These can be classified into two broad categories, that is, translocation and disintegration.

11.4.1 Translocation

Translocation refers to the movement of the intact fiber (1) along the epithelial surface to dust foci at the respiratory bronchioles, (2) onto the ciliated epithelium at the terminal bronchioles, or (3) into and through the epithelium, with subsequent migration to interstitial storage sites within the lung, along lymphatic drainage pathways and, for very thin fibers, access via capillary blood to distant sites, as suggested by Monchaux et al. (1981). Boutin et al. (1996) suggested that thin fibers longer than 5 or 10 μm migrate toward the parietal pleura via the lymphatic pathway, where they accumulate preferentially in anthracotic "black spots" of the parietal pleura.

Dodson et al. (1990) compared the fiber content of tissues from chronically exposed shipyard workers and reported that while 10% of amphibole fibers in pleural plaque samples were longer than 5 μm and 8% were longer than 10 μm, the corresponding figures for chrysotile fibers were 3.1 and 0%. In lymph nodes, the corresponding figures for >10 μm and >5 μm lengths were 6.0 and 2.5% for amphiboles and 0 and 0% for chrysotile. In lung tissue, they were 41.0 and 20.0% for amphiboles and 14.0 and 4.0% for chrysotile.

Boutin et al. (1996) noted that the black spots that concentrate longer fibers were in close contact with early pleural plaques. Translocation may also occur after ingestion of the fibers by alveolar macrophages if the fibers are short enough to be fully ingested by the macrophages. Holt (1982) proposed that fibers phagocytosed by alveolar macrophages are carried by them toward the lung periphery by passing through alveolar walls and that some of these cells aggregate in alveoli near larger bronchioles and then penetrate the bronchiolar wall. Once in the bronchiolar lumen, they can be cleared by mucociliary transport.

11.4.2 Biopersistence

The biopersistence of chrysotiles from other locations was studied by Bernstein et al. (2004) following inhalation exposures to aerosols with large number concentrations of fibers >20 μm in length. For chrysotile from the Cana Brava mine in central Brazil, the clearance half-times of the fibers >20, those 5–20, and those <5 μm in length were 1.3, 2.4, and 23 days, respectively. For chrysotile from Coalinga mine in California (Calidria RG144), the clearance half-times of the fibers >20, those 5–20, and those <5 μm in length were 7 h, 7 days, and 64 days, respectively, while for tremolite asbestos, there was no clearance from the rat lungs over the 1-year period of observation (Bernstein et al., 2005a). The tremolite exposures produced lung inflammation, granulomas, and lung fibrosis, while the chrysotile, despite involving a much higher long fiber concentration, did not produce any measurable response. For chrysotile from the Eastern Townships of Quebec, the clearance half-time for fibers >20 μm in length was 11.4 days, which was similar to that for glass and stone wools previously studied (Bernstein et al., 2005b).

Similar differential retention has been found in humans (Churg 1994) for analyses of lung tissue for 94 chrysotile asbestos miners and millers from the Thetford region of Quebec, Canada. The exposure atmosphere contained a very small percentage of tremolite, yet the lungs contained more tremolite than chrysotile, and the tremolite content increased

rapidly with the duration of exposure. While most of the inhaled chrysotile was rapidly cleared from the lungs, a small fraction was retained indefinitely. After exposure ended, there was little or no clearance of either chrysotile or tremolite from the lungs.

Albin et al. (1994) studied retention patterns in lung tissues from 69 Swedish asbestos-cement workers and 96 controls. The chrysotile had a relatively rapid turnover in human lungs, whereas amphiboles (tremolite and crocidolite) had a slower turnover. They also noted that asbestos retention may be (1) dependent on dose rate, (2) increased by smoking, and (3) increased by the presence of lung fibrosis.

The most direct evidence for the effect of altered dust clearance rates on the retention of inhaled fibers in humans comes from studies of the fiber content of the lungs of asbestos workers in various countries. Timbrell (1982) developed a model for fiber deposition and clearance in human lungs based on his analysis of the bivariate diameter and length distributions found in air and lung samples collected at an anthophyllite mine at Paakkila in Finland. The length and diameter distributions of the airborne dust at this particular mine were exceptionally broad, and historic exposures were very high. For workers with the highest exposure and most severe lung fibrosis (Ashcroft et al., 1988), the fiber distributions in some tissue segments approached those of the airborne fibers. Adjacent tissue, analyzed for extent of fibrosis, showed severe fibrotic lesions. He concluded that long-term retention was essentially equal to deposition in such segments. Figure 11.2 shows a series of retention curves for different degrees of lung fibrosis. These curves were determined by comparing the anthophyllite fiber size distributions in other tissue samples from the same lung with the distribution in the sample for which all fibers deposited were retained. Lung fibrosis was associated with increased fiber retention, and fiber retention was clearly associated with fiber length and diameter. The critical fiber length for mechanical clearance from the lungs was ~17 μm. More precise descriptions of the effect of fiber loading in the lung on fibrosis need to be based on the use of the most appropriate index of fiber loading.

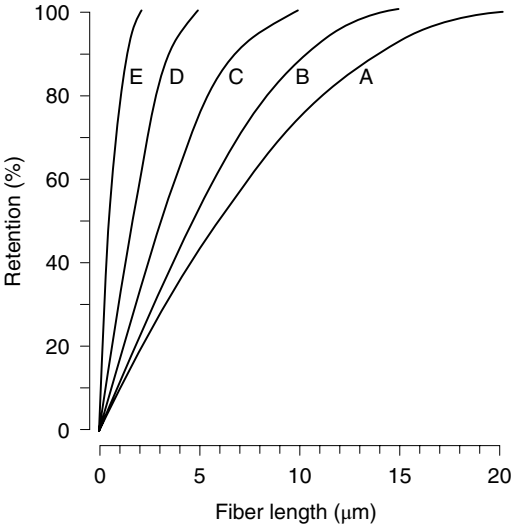

FIGURE 11.2 Effect of lung fibrosis on fiber retention in human lungs as a function of fiber length. *Source*: From CRT-2014. Informa Healthcare USA, Inc. doi: 10.3109/10408444.2014.928266 (page 664).

Morgan et al. (1982) and Morgan and Holmes (1984) studied the retention of 1.5 μm diameter glass fibers administered to rat lungs by intratracheal (IT) instillation. Retention at 1 year for 5 μm long fibers was 10%, whereas for 10 μm long fibers, it was 20%. For the fibers that were 30 or 60 μm long, there was no measurable clearance during the first 9 months. Further retention measurements were not made for these long fibers because of evidence that they were disintegrating and dissolving. This macrophage-mediated mechanical clearance was less effective for 10 μm fibers than for 5 μm fibers and was ineffective for fibers 30 μm long and longer. For the glass fibers, there was much less dissolution of the 5 and 10 μm fibers than of the 30 and 60 μm fibers. The dissolution of the long 1.5 μm diameter fibers was very nonuniform. Some were little changed in dimension, whereas others were reduced in diameter to 0.2 μm. On the other hand, for rock-wool fibers >20 μm in length, there was no observable change in fiber dimensions after 6 months. Morgan and Holmes (1984) attributed the dependence of dissolution on fiber length to the differences and intra- and extracellular pH. The shorter fibers within macrophages are exposed to a pH of 7.2, whereas those outside were exposed to the extracellular pH of 7.4.

Bernstein et al. (1984) and Hammad (1984) also found evidence of substantial *in vivo* dissolution of glass fibers. Insight on the solubility of fibers *in vivo* has also been obtained from *in vitro* solubility tests. Griffis et al. (1981) found that glass fibers suspended either in buffered saline or serum simulant at 37°C for 60 days exhibited some solubility and that the sodium content of the residual fiber was reduced. Forster (1984) used Gamble's solution for tests on samples of 18 different SVFs at temperatures of 20 and 37°C and for exposure times ranging from 1 h to 180 days using static tests, tests with once-daily shaking, tests with continuous shaking, and tests with single fibers in an open bath. There was some solubility for all fibers. Klingholz and Steinkopf (1982, 1984) studied dissolution of mineral wool, glass wool, rock wool, and basalt wool at 37°C in water and in a Gamble's solution modified by omission of the organic constituents. Most of the tests used a continuous-flow system in which the pH was 7.5–8. There was relatively little dissolution in distilled water in comparison to that produced by the modified Gamble's solution. The surfaces developed a gel layer that, for the smaller diameters, extended throughout the fiber cross section. Thus, the fibers can become both smaller in outline and more plastic to deformation.

Scholze and Conradt (1987) performed a comparative *in vitro* study of the chemical durability of SVFs in a simulated extracellular fluid under flow conditions. Seven SVFs, three RCFs, and three natural fibers were involved. Silicon concentrations in the leachates were used to roughly classify the fibers according to their chemical durability. SVFs exhibited relatively poor durability (with dissolution rates ranging from 3.5 to 0.2 nm/day for a glass wool and an E-glass fiber, respectively), whereas natural fibers were very persistent against the attack of the biological fluid (e.g., <0.01 nm/day for crocidolite).

Johnson et al. (1984) exposed rats to SVF aerosols at 10 mg/m^3 for 7 h/day, 5 days/week for 1 year. The percentage of glass fibers with diameters less than 0.3 μm recovered from the lungs was consistently less than that in the original fiber suspension, and the reduction was more marked in the animals sacrificed following a period of recovery from the exposures than from those sacrificed at the end of the exposure. The degree of fiber etching increased with residence times in the lungs. Glass wool was also etched, but to a lesser extent, and the etching of the rock-wool fibers was even less.

Bellmann et al. (1986) instilled reference suspensions of UICC crocidolite and chrysotile A as well as suspensions of glass fibers into rat lungs and examined the residual fibers after 1 day and 1, 6, 12, and 15 months. Crocidolite fibers longer than 5 μm did not decrease in number for over 1 year. The number of chrysotile fibers >5 μm doubled, presumably as

a result of longitudinal splitting, while the number of glass fibers >5 μm was reduced with a half-time of 55 days by dissolution. All fibers <5 μm in length were cleared with half-times of 100–150 days. When the crocidolite fibers were pretreated in acid, there was no change in retention. On the other hand, acid-treated chrysotile and glass fibers had much more rapid clearance, with half-times of 2 and 14 days, respectively. Bellmann et al. (1987) reported the persistence of some SVFs, crocidolite and chrysotile in the rat lung for 2 years after IT instillation. Experiments were based on the assumption that thin, long, and durable fibers are of special importance for the carcinogenic potency of these types of substances. For a special type of glass microfiber and for ceramic wool, which both had low alkaline earth contents, the half-times of lung clearance were similar to that for crocidolite. Another type of glass microfiber, with a very low *in vivo* half-time, had a high alkaline earth content and a median diameter of about 0.1 μm. The glass and rock wools studied, which were thicker than the other fibers, had intermediate half-times.

Collier et al. (1994) studied two experimental continuous-filament glass fibers of 2 μm diameter and 50 μm length following intraperitoneal (IP) injections of 5 mg in rats. They had *in vitro* dissolution rates of 150 and 600 ng/cm^2/h. In the lung, the diameters of the long fibers (>20 μm) declined at a rate consistent with their exposure to a neutral pH environment, while the diameters of shorter fibers declined much more slowly, consistent with exposure to the more acidic environment found in the phagolysosomes of alveolar macrophages. In the peritoneal cavity, all fibers, regardless of length, dissolved at the same rate as short fibers in the lung. The effect of dose on the distribution of fibers in the peritoneal cavity was investigated using experimental glass fibers and a powder made from ground fibers. At doses up to 1.5 mg, both fibers and powder were taken up by the peritoneum in proportion to their surface area, and uptake was complete 1–2 days after injection. At higher doses, the majority of the material in excess of 1.5 mg formed clumps of fibers (nodules) that were either free in the peritoneal cavity or loosely bound to peritoneal organs. These nodules displayed classic foreign body reactions, with an associated granulomatous inflammatory response. Collier et al. (1997) reported on the clearance of two rock-wool fibers administered to rats by IT, one a conventional product (MMVF21) and the other an experimental, more soluble fiber (HTN). Unlike glass wool, rock wool is more soluble at the acid pH in macrophages than in the more neutral lung tissue. They found that MMVF21 had relatively slow clearance, with somewhat faster clearance for short fibers. The clearance of HTN was much faster.

Eastes and Hadley (1995) administered suspensions of fibers to rats by IT, and the numbers, lengths, and diameters of fibers recovered from the lungs were measured by PCOM at intervals up to 1 year. Five different glass fibers had dissolution rates ranging from 2 to 600 ng/cm^2/h measured *in vitro* in simulated lung fluid at pH 7.4. For fibers longer than 20 μm, the peak diameter decreased steadily with time after instillation, at the same rate measured for each fiber *in vitro*, until it approached zero. Measurements of the total number of fibers remaining in the rats' lungs at times up to 1 year after instillation suggested that few of the administered fibers were being cleared by macrophage-mediated transport via the conducting airways. They concluded that glass fibers longer than 20 μm are removed from the lung by dissolution at the same rate measured *in vitro*.

In a study of dissolution of inhaled fibers by Eastes and Hadley (1995), rats were exposed for 5 days to four types of airborne, respirable-sized SVF and to crocidolite fibers. The SVFs included two glass wools and one each of rock and slag wools. After exposure, animals were sacrificed at intervals up to 18 months, and the numbers, lengths, and diameters of a representative sample of fibers in their lungs were measured. Long fibers (>20 μm) were eliminated from the rats' lungs at a rate predicted from the dissolution rate measured

in vitro. The long SVFs were nearly completely eliminated in several months, whereas the long crocidolite asbestos fibers remained in significant numbers at the end of the study. The number, length, and diameter distributions of fibers remaining in the rats' lungs agreed well with a computer simulation of fiber clearance that assumed that the long fibers dissolved at the rate measured for each fiber *in vitro* and that the short fibers of every type were removed at the same rate as short crocidolite asbestos. Thus, long SVFs were cleared by complete dissolution at the rate measured *in vitro*, and short fibers did not dissolve and were cleared by macrophage-mediated physical removal.

In an inhalation study, using nine fiber types, Bernstein et al. (1996) exposed rats to an aerosol (mean diameter of ~1 μm) at a concentration of 30 mg/m^3, 6 h/day for 5 days with post-exposure sacrifices at 1 h, 1 day, 5 days, 4 weeks, 13 weeks, and 26 weeks. At 1 h following the last exposure, the nine types of fibers were found to have lung burdens ranging from 7.4 to 33×10^6 fibers/lung with geometric mean diameters (GMD) of 0.40–0.54 μm, reflecting the different bivariate distributions in the exposure aerosols. The fibers cleared from the lungs following exposure with weighted half-lives ranging from 11 to 54 days. The clearance was found to closely reflect the clearance of fibers in the 5–20 μm length range. An important difference in removal was seen between the long fiber ($L > 20$ μm), shorter fiber (L between 5 and 20 μm, and $L < 5$ μm) fractions, depending upon composition. For all glass wools and the rock wools, the longer fibers were removed faster than the shorter fibers. It was found that the time for complete fiber dissolution based on the acellular *in vitro* dissolution rate at pH 7.4 was highly correlated ($r = 0.97$, $p < 0.01$) with the clearance half-times of fibers >20 μm in length. No such correlations were found with any of the length fractions using the acellular *in vitro* dissolution rate at pH 4.5. Examination of the fiber length distributions and particles in the lung from 1 h through 5 days of exposure indicated that, especially for those fibers that form leached layers, fiber breakage may have occurred during this early period. These results demonstrate that, for fibers with high acellular solubility at pH 7.4, the clearance of long fibers can be very rapid.

Eastes and Hadley (1995) fitted data cited above into a mathematical model of fiber carcinogenicity and fibrosis. Their model predicted the incidence of tumors and fibrosis in rats exposed to various types of rapidly dissolving fibers in an inhalation study or in an IP injection experiment. It took into account the fiber diameter and the dissolution rate of fibers longer than 20 μm in the lung and predicted the measured tumor and fibrosis incidence to within approximately the precision of the measurements. The underlying concept for the model was that a rapidly dissolving long fiber has the same response in an animal bioassay as a much smaller dose of a durable fiber. Long, durable fibers have special significance, since there is no effective mechanism by which these fibers may be removed. In particular, they hypothesized that the effective dose of a dissolving long fiber scales as the residence time of that fiber in the extracellular fluid. The residence time of a fiber is estimated directly from the average fiber diameter, its density, and the fiber dissolution rate as measured in simulated lung fluid at neutral pH.

The incidence of fibrosis in chronic inhalation tests, the observed lung tumor rates, and the incidence of mesothelioma in the IP model were all well predicted by the model. The model allows one to predict, for an inhalation or IP experiment, what residence time and dissolution rate are required for an acceptably small tumorigenic or fibrotic response to a given fiber dose. For an inhalation test in rats at the maximum tolerated dose (MTD), the model suggests that less than 10% incidence of fibrosis would be obtained at the maximum tolerated dose of 1 μm diameter fibers if the dissolution rate were greater than 80 ng/cm^2/h.

The dissolution rate that would give no detectable lung tumors in such an inhalation test in rats is much smaller. Thus, a fiber with a dissolution rate of 100 ng/cm^2/h has an insignificant chance of producing either fibrosis or tumors by inhalation in rats, even at the maximum tolerated dose. This model provides manufacturers of SVFs with design criteria for fibrous products that minimize, if not eliminate, their potential for producing adverse health effects.

Support for the use of biopersistence data for the prediction of fibrosis and tumor responses in rats from both IP injection studies and chronic inhalation studies for fibers >5 and >20 μm in length was provided by Bernstein et al. (2001a, 2001b). For the inhalation studies they used collagen deposition at the bronchoalveolar junction as a predictor of interstitial fibrosis on the basis that it has been shown to be associated with tumor response in previous studies.

11.5 FIBER-RELATED DISEASES/PROCESSES

Fiber dimensions, chemical composition, and surface properties are important factors in biological reactivity of mineral fibers. As discussed previously, fiber length influences the deposition, clearance, and translocation of fibers in the lungs. Moalli et al. (1987) concluded that fiber length determines clearance from the pleural and peritoneal spaces, with fibers longer than 8 μm being trapped at the mesothelial lining because the opening of lymphatic channels draining these spaces is 8–12 μm in diameter. This provides an anatomic basis for the Stanton hypothesis (Stanton et al., 1977) that long fibers, regardless of their chemical composition, are more effective in producing mesotheliomas than shorter fibers, after direct intrapleural or IP injection into rodents.

Cations within the crystal lattice may affect the toxicity of asbestos fibers. Mg^{2+} ions on the surface of chrysotile asbestos are important in cytotoxicity and carcinogenicity; acid-leached fibers are less active than native fibers (Monchaux et al., 1981). The Fe^{2+} and Fe^{3+} content of amphibole fibers may be important because these cations can catalyze the Fenton or Haber–Weiss reactions, generating highly toxic and potentially mutagenic ROS (Weitzman and Graceffa, 1984; Zalma et al., 1987).

Macrophages ingest inhaled particles that are deposited in the terminal airways and alveolar spaces. Phagocytosis of mineral fibers by macrophages leads to generation of reactive oxygen species (ROS) and release of lysosomal enzymes, arachidonic acid metabolites, neutral proteases, chemotactic factors, and growth factors (Adamson, 1997). The interactions between mediators released from macrophages and other inflammatory cells and from the target cell populations, can initiate a sequence of events culminating in fibrosis of the lungs and pleura, bronchogenic carcinoma, and malignant mesothelioma. However, for 2-week exposures to chrysotile, the early fibrotic lesions in rats are gradually resolved over the course of the following year (Pinkerton et al., 1997).

Diffuse, bilateral interstitial fibrosis of the lungs characterizes asbestosis, a disease that usually develops in humans after prolonged exposure to high concentrations of asbestos fibers. Progressive scarring of the alveolar walls due to increased proliferation of fibroblasts and deposition of collagen produces radiographic evidence of disease and interferes with gas exchange, leading to disability and premature death. The sequence of events that lead to the development of asbestosis includes: (1) asbestos fiber phagocytosis by alveolar and/or interstitial macrophages; (2) release of ROS from alveolar macrophages, causing

acute injury to the alveolar epithelial lining (the importance of hydrogen peroxide in the pathogenesis of asbestosis was demonstrated by the protection against asbestos-induced pulmonary fibrosis provided by catalase conjugated to polyethylene glycol) (Mossman et al., 1990); and (3) phagocytosis of asbestos fibers by alveolar or interstitial macrophages also triggers increased synthesis and release of growth factors for fibroblasts. Growth factors are released from macrophages exposed to asbestos fibers *in vitro* or *in vivo*: a homolog of platelet-derived growth factor (PDGF) (Bauman et al., 1990) and transforming growth factor-β (TGF-β) (Kane and McDonald, 1993). These growth factors cause chemotaxis of inflammatory cells and fibroblasts, stimulation of collagen synthesis, and inhibition of collagen degradation.

Another reaction to asbestos exposure is the development of acellular fibrous scars, called pleural plaques, on the parietal pleural lining and diaphragm. Asbestos exposure may also lead to pleural effusions or diffuse fibrosis of the visceral pleura, but these reactions cause little disability. The most important reaction of the pleural and peritoneal linings to asbestos fibers is development of diffuse malignant mesothelioma, a rare neoplasm that is most closely associated with occupational or environmental exposure to amphibole forms of asbestos after a long latent period (up to 30–60 years). There is no increased incidence in cigarette smokers or in workers with asbestosis. This malignant neoplasm has a variable histology, ranging from epithelial to fibroblastic or mixed patterns.

Malignant mesothelioma has usually spread diffusely when first diagnosed and responds poorly to radiation or chemotherapy (Craighead, 1987). The following sequence of events can lead to the development of diffuse malignant mesothelioma: (1) phagocytosis of fibers that reach the pleura or peritoneal lining by macrophages; (2) release of ROS, causing acute injury to the mesothelial cell monolayer lining the pleural or peritoneal spaces (such injury can be prevented by coating the administered fibers with the iron chelator deferoxamine or by exogenous superoxide dismutase or catalase) (Goodglick and Kane, 1990; Kane and McDonald, 1993); and (3) acute injury to the mesothelial lining, which is repaired by proliferation of adjacent, uninjured mesothelial cells. Growth factors released from macrophages, following phagocytosis of asbestos fibers, may modulate mesothelial cell regeneration (Kane and McDonald, 1993); (4) direct interaction of asbestos fibers with the regenerating mesothelial cell population, which may cause chromosomal aberrations and aneuploidy. Additional DNA damage may be produced by ROS, especially the hydroxyl radical produced by the iron-catalyzed Haber–Weiss reaction (Barrett et al., 1989; Floyd, 1990); (5) repeated episodes of mesothelial cell injury and regeneration may lead to the emergence of a subpopulation of autonomously proliferating cells; and (6) neoplastic mesothelial cells may produce growth factors that promote growth of an invasive tumor.

People exposed to asbestos fibers have an increased risk of developing bronchogenic carcinoma, and workers who also smoke cigarettes have a substantially greater risk. Cancers can arise from the epithelial lining of the large airways or terminal bronchioles. Bronchogenic carcinomas have a variety of histologic appearances: adenocarcinoma, squamous cell carcinoma (presumably arising in areas of squamous metaplasia of the respiratory epithelium), large cell carcinoma, and small cell (oat cell) carcinoma. These are the same histologic types of cancer associated with cigarette smoking in the absence of asbestos exposure (Mossman and Craighead, 1987). Lung cancer and mesothelioma occur in people without radiographic evidence of lung fibrosis. de Klerk et al. (1997) demonstrated that the level of radiographic fibrosis conferred additional risk for lung

cancer beyond that associated with level of exposure, but that asbestosis was not a prerequisite for asbestos-associated cancer.

11.6 BIOLOGICAL EFFECTS OF SIZE-CLASSIFIED FIBERS IN LABORATORY ANIMALS AND HUMANS

The pathological effects produced by fibers depend upon both the characteristics of the fibers and their persistence at sensitive sites. A number of carefully designed studies have been performed in which the size distributions of fiber suspensions have been well characterized as well as their persistence and/or effects.

11.6.1 *In Vivo* IT Instillation Exposures into Animal Lung Airways

King et al. (1946) instilled 100 mg of Rhodesian chrysotile into rabbit lungs at monthly intervals. One group received fibers microtomed to a length of 15 μm, and another group received fibers cut to 2.5 μm in length. At this huge dosage level, both groups showed foreign body reactions in the lungs. The long fiber produced a nodular reticulinosis, whereas the short fiber produced a diffuse interstitial reticulinosis.

Wright and Kuschner (1977) used short and long asbestos and SVFs in IT instillation studies in guinea pigs. With suspensions containing an appreciable number of fibers longer than ~10 μm, all of the materials produced lung fibrosis, although the yields varied with the materials used. However, with equal masses of short fibers of equivalent fiber diameters, none produced any fibrosis. The yields were lower for the long glass fibers than for the long asbestos, and this was attributed to their lesser durability within the lungs.

11.6.2 *In Vivo* Exposures in Animals via Intraperitoneal Injection

For fibers injected IP (Davis, 1976; Pott et al., 1976; Wagner et al., 1976), or for fibers placed in a pledget against the lung pleura, a similar kind of fiber size and composition dependence was observed (Stanton and Wrench, 1972). The yield of mesotheliomas varied with fiber diameter and length, and with dose, with very little response when long, thin fibers were not included. Asbestos fibers were more effective than glass fibers in these studies also. At a dose of 2 mg of chrysotile, crocidolite, or glass fiber, Pott et al. (1976) found only slight degrees of fibrosis, but tumor yields of from 16 to 38% in rats. When the chrysotile was milled to the extent that 99.8% of the fibers were shorter than 5 μm, the dose required to produce a comparable tumor yield (32%) was 50 times greater.

Various hypotheses have been proposed to account for the pathological effects produced by asbestos. One was the contamination of the surface by trace metal and/or organic carcinogens. However, the studies of Stanton and Wrench (1972) found that surface contaminants played no role in mesothelioma yield and concluded that the carcinogenicity of asbestos and fibrous glass was primarily related to the structural shape of these fibrous materials rather than their surface properties.

Miller et al. (1999a) reviewed the collective outcomes of nine rat IP injection studies involving fibers of amosite, silicon carbide, four SVFs (100/475, MMVF10, MMVF21, MMVF22), and three RCFs (RCF1, RCF2, RCF4) on mesothelioma. They reported a link between the numbers of injected fibers >20 μm in length and to the biopersistence in the rat lung of fibers >5μm in length.

11.6.3 *In Vivo* Exposures of Animals to Fibers by Inhalation

The relative potencies of the various mineral forms of asbestos after inhalation exposure are still not firmly established. Crocidolite is generally considered the most hazardous because of its association with significant numbers of human mesotheliomas. The relative toxicity of various asbestos minerals has been compared in a variety of experimental inhalation studies on small animals, but the results are in apparent conflict. Wagner (1963) reported more asbestosis with amosite than with chrysotile in guinea pigs, rats, and monkeys. However, in studies involving inhalation exposures of rats for 1 day to 2 years, Wagner et al. (1974) found that amosite and crocidolite were the least fibrogenic of five types of UICC asbestos, the others being Canadian chrysotile, Rhodesian (Zimbabwe) chrysotile, and anthophyllite. Davis et al. (1978) used UICC chrysotile A, amosite, and crocidolite in 12-month rat exposures at respirable mass concentrations comparable with those used by Wagner et al. (1974) and found a similar pattern; that is, chrysotile was the most fibrogenic, and amosite and crocidolite the least. Hiett (1978) exposed guinea pigs by inhalation for 9 and 18 days and also found that chrysotile was more fibrogenic than amosite. Davis et al. (1986b) subsequently repeated the protocol with amosite with fiber length both shorter and longer than UICC amosite. The short amosite produced virtually no fibrosis, whereas the long amosite was more fibrogenic than chrysotile. The most fibrogenic asbestos was tremolite.

Berman et al. (1995) analyzed the lung tumor and mesothelioma responses from 13 of the chronic inhalation studies in rats performed by Davis and colleagues at the Institute of Environmental Medicine in Edinburgh in relation to new measurements of the fiber distributions on archived chamber atmosphere sampling filters. The measure most highly correlated with tumor incidence was the concentration of fibers >20 μm in length.

Miller et al. (1999b) reviewed the collective outcomes of 18 rat inhalation studies involving fibers of amosite, silicon carbide, four SVFs (100/475, MMVF10, MMVF21, MMVF22), and three RCFs (RCF1, RCF2, RCF4). The primary influences on biological responses was the number of fibers <1.0 μm in diameter and >20 μm in length and the dissolution rate of the fibers. Another important observation was that *in vivo* and *in vitro* cell responses did not significantly predict the risk of cancer following inhalation.

McConnell et al. (1999) exposed hamsters for 78 weeks to amosite and two different fibrous glasses (MMVF10a and MMVF33) at 250–300 f/ml for fibers >5 μm in length and to amosite at 125 and 25 f/mL as well. MMVF10a produced only mild inflammation, while MMVF33 produced more severe inflammation and mild interstitial and pleural fibrosis, as well as one mesothelioma. Amosite produced severe pulmonary fibrosis and many mesotheliomas (3.6, 25.9, and 19.5% at the low, medium, and high doses). The effects were most closely related to the retained fibers >20 μm in length and inversely paralleled the *in vitro* dissolution rates.

Hesterberg and Hart (2001) reviewed the results of chronic inhalation studies in rats of seven SFVs (RCF_1, thin E-glass, 475-glass, 901-glass, CT-glass, slag wool, rock wool), as well as amosite and crocidolite. The aerosols contained ~100 f/mL >20 μm in length. Rats were exposed for 6 h/day, 5 days/week for 2 years and hamsters for 1.5 years. They also had biopersistence data for 5-day inhalations with retention measurements extending over 1 year. The more biopersistent fibers were fibrogenic (rock wool) or fibrogenic and carcinogenic (amosite, crocidolite, RCF_1, 475-glass, and thin E-glass).

Cullen et al. (2000) compared the pathogenicity of amosite to that of two special-purpose glass microfibers with low dissolution rates (104E and 100/475) in a study in which rats were exposed for 7 h/day, 5 days/week for 12 months to 1000 f/ml longer than 5 μm. In terms of mesothelioma and lung cancers produced after the exposures and 12 months

without further exposure, 104E and amosite fibers were considerably more potent than 100/475 fibers. They attributed the lower pathogenicity of 100/475 to the greater leaching of its component elements while in the lungs.

11.6.4 Fibers Retained in the Lungs of Occupationally Exposed Humans

It is generally believed that amphibole fibers account for much of the mesothelioma incidence among exposed workers, even when they are predominantly exposed to chrysotile, since amphibole fibers are more biopersistent. Pooley (1976) examined postmortem lung tissue from 20 workers with asbestosis in the Canadian chrysotile mining industry and found that amphibole and other fibers were present in 16 cases. In seven of these, they were more numerous than chrysotile. In a later study of lung asbestos in chrysotile workers with mesothelioma, Churg et al. (1984) reported that the concentration ratio between cases and controls was 9.3 for tremolite but only 2.8 for chrysotile. In a Norwegian plant using 91.7% chrysotile, 3.1% amosite, 4.1% crocidolite, and 1.1% anthophyllite, Gylseth et al. (1983) reported that the percentage of chrysotile in lung tissue ranged between 0 and 9%, while the corresponding numbers for the amphiboles were 76–99%.

Case et al. (2000) examined the relationships between asbestos fiber type and length in the lungs of chrysotile textile workers as well as miners and millers in Quebec. Despite the lower cancer risk for the Quebec workers, the chrysotile, tremolite, total amphibole, and total count of fibers longer than 18 μm were all highest in the Quebec workers. They concluded that the textile workers' experience should not be used to assess the cancer risks in other cohorts.

In their review of the assessment of mineral fibers from human lung tissue, Davis et al. (1986a) attributed the high amphibole/chrysotile ratios to the dissolution of chrysotile within lung tissue and the generally poor correlation between dust counts and mesothelioma as likely to be due to the differences among the various asbestos types in the fraction that reaches the pleural surface. Amosite fibers need to be longer to produce pulmonary fibrosis and pulmonary tumors in experimental animals than to produce mesotheliomas after injection (Davis et al. 1986b). Davis (1987) noted that both chrysotile and amphibole asbestos fibers inhaled by rats are plentiful in the most peripheral alveoli bordering on the pleura but penetration of the external elastic lamina of the lung appears to be a rare event. On the other hand, erionite, a natural zeolite fiber, causes a very high incidence of mesotheliomas in humans exposed to low environmental concentrations (Baris et al., 1987) and 100% incidence in rats exposed by inhalation (Wagner et al., 1985). Davis (1987) reported that the erionite used by Wagner et al. (1985) had a general appearance and fiber size distribution very close to that of UICC crocidolite, which produces a much smaller mesothelioma yield in rats exposed by inhalation. He attributed the difference to the enhanced ability of erionite to cross the pleural membrane.

Lockey et al. (2012) demonstrated that RCFs longer than 5 μm are biopersistent in human lungs for at least 20 years.

11.7 CRITICAL FIBER PARAMETERS AFFECTING DISEASE PATHOGENESIS

11.7.1 Critical Fiber Dimensions for Asbestosis

Asbestosis has been caused by exposure to high concentrations of respirable fibers of all of the commercially exploited kinds of asbestos. Within the respirable fraction, the fibers often differ in diameter and length distributions, as well as in retention times.

Timbrell et al. (1987) explored and described the influence of these variables on fibrosis in the human lung through analyses of both retained fibers and fibrosis in lung samples from exposed workers. He analyzed the fiber distributions in 0.5 g lung samples from several hundred workers by a technique that he called magnetic alignment and light scattering (MALS) (Timbrell, 1982). He measured the degree of fibrosis, by optical microscopy, in paraffin sections prepared from adjacent samples of the same specimens. There wide intra- and inter-subject variations were observed in fiber concentration and fibrosis score. He described the quantitative relationships for the main sources of amosite (Transvaal, South Africa), anthophyllite (Paakkila, Finland), and crocidolite (NW Cape and Transvaal, South Africa, and Wittenoom, Australia). As illustrated in Fig. 11.2, the fibrosis-producing ability of the fibers was independent of amphibole type when normalized by the total surface area of long resident fibers per unit weight of lung tissue, presumably because the surface area was the main determinant of the magnitude of the fiber–tissue interface. The wide range of the concentrations of retained fiber required to produce the same degree of fibrosis in the groups of mineworkers when fiber quantity is expressed as number or total mass stemmed from the large differences between the distributions in diameter and length of the airborne fibers.

Although the main focus of the Timbrell et al. (1987) study was on amphibole asbestos, they also reported results for three Wittenoom workers whose dominant exposure was to chrysotile asbestos. For these workers, chrysotile produced a similar degree of fibrosis to Wittenoom crocidolite for equal fiber mass concentrations in the lungs. Long residence in the tissue had almost completely dispersed the chrysotile fibers into fibrils, to give them a ratio of total surface area to mass resembling that of the particularly fine Wittenoom crocidolite fibers. The result indicates that the fibrogenicity of the retained chrysotile per unit of surface area within the lungs was similar to that of the amphiboles.

Timbrell et al. (1987) also reported that amphibole mineworkers with a given fiber mass concentration in their lungs showed much higher degrees of fibrosis than gold miners with roughly the same mass concentration of retained quartz grains. The amphibole and quartz produced about the same fibrogenicity per unit of surface area, but the smaller diameters and higher area/mass ratios of the amphibole fibers endowed them with the greater surface area and therefore the superior fibrosis-producing capability.

Knowledge of the interrelationships between retained fibers and fibrosis is critical in understanding the pathogenesis of the disease but is inadequate, by itself, in evaluating exposures to airborne fibers. Timbrell (1983, 1984) developed a mathematical model relating fiber deposition and retention based on analyses of lung samples. Specifically, he used samples from a woman at Paakkila who worked at a job that gave her exposure to an amphibole (anthophyllite) at high concentrations of fibers with a range of diameters and lengths sufficiently wide to encompass the size limits of respirable fibers. Her lungs contained 1.3 mg fiber per gram of dry tissue, and she had asbestosis. One lung sample contained a fiber distribution matching the expected deposition. Timbrell speculated that severe fibrosis in the tissue in this sample had blocked the macrophage-mediated clearance. Another sample from the same lung yielded a retention pattern more closely matching those found in other Paakkila workers, with small fiber burdens and virtually no short fibers. He assumed that the latter represents long-term retention in the normal lung.

From the differences in retention, Timbrell developed a model for the retention of fibers as a function of length and diameter. Fiber retention rose rapidly with fiber lengths between 2 and 5 μm and peaks at ~10 μm. Fiber retention also rose rapidly with fiber diameters between 0.15 and 0.3 μm, peaks at ~0.5 μm, and dropped rapidly between 0.8 and 2 μm.

The utility of the model was demonstrated by applying it to predict the lung retention of Cape crocidolite and Transvaal amosite workers on the basis of the measured length and diameter distributions of airborne fibers. The predicted lung distributions did, in fact, closely match those measured in lung samples from a Cape worker (Timbrell, 1984) and, as shown in Fig. 11.3, from a Transvaal worker (Timbrell, 1983). Thus, fibrosis was most closely related to the surface area of fibers with diameters between 0.15 and 2 μm and lengths greater than ~2 μm. The work of King et al. (1946) showing that chrysotile with length of 2.5 μm produced interstitial fibrosis in rabbits following multiple IT instillations is consistent with the retention shown in Fig. 11.3 and a critical fiber length of ~2 μm.

Churg et al. (2000) examined putative biological mechanisms involved in fibrogenesis and conclude that (1) fiber length, biopersistence, and dose, but that it was uncertain whether alveolar macrophages, were central to fibrosis or whether fibers penetrating tissue were the real effector agents; (2) short fibers, readily degraded fibers, and small numbers of any fibers are non-fibrogenic; and (3) the ability of macrophages to clear fibers is probably crucial to preventing fibrosis.

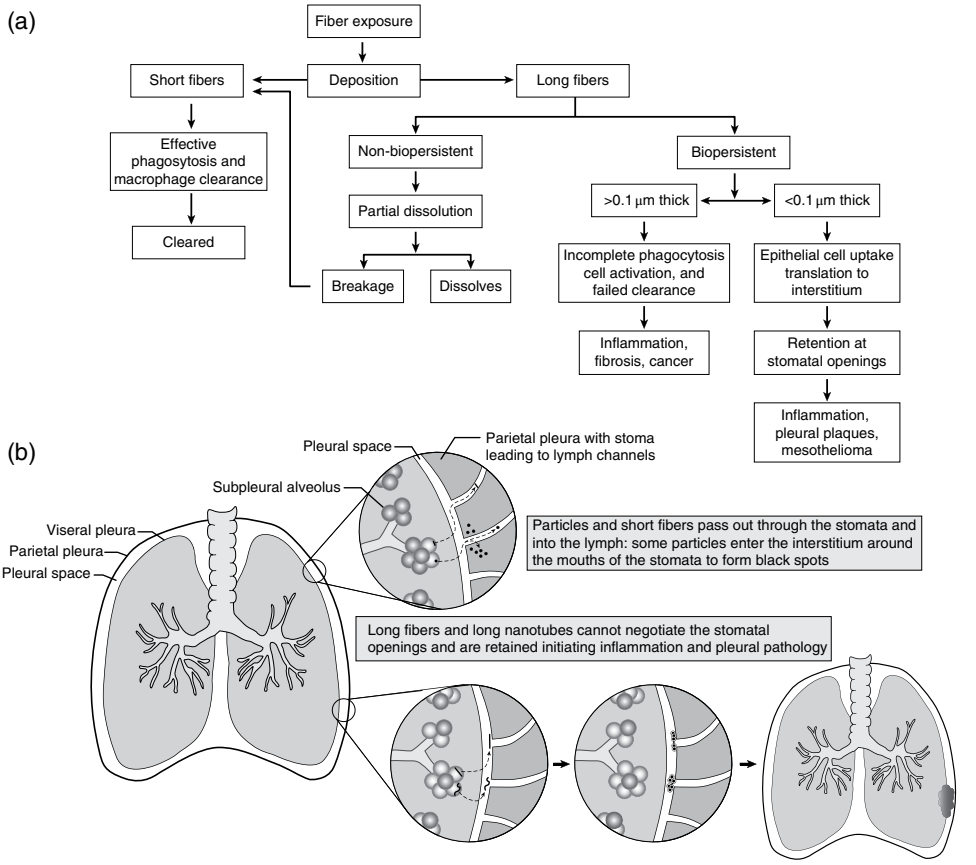

FIGURE 11.3 (a) Paradigm for the roles of fiber deposition, clearance, translocation, retention, and biopersistence in pathological effects Source: Adapted, in part, from Donaldson et al. (2006). (b) Hypothesized sequence of events leading to pleural responses as a consequence of long fiber retention at the parietal pleura stomatal openings. *Source*: From Donaldson et al. (2010).

11.7.2 Critical Fiber Dimensions for Mesothelioma

A National Research Council study (NRC, 1984) summarized mortality data for mesothelioma and lung cancer in asbestos-exposed occupational cohorts. In 20 studies in which there was an excess in respiratory cancer and/or mesothelioma, the percentage of the excess that was mesothelioma varied from 0 to 100%, with a mean (±SD) of 38 ± 29%. A study with 0% was that of Meurman et al. (1974, 1979), who reported 44 observed lung cancers (vs. 22 expected) in a population of 1045 workers exposed to anthophyllite in Finland. Anthophyllite is an amphibole with larger fiber diameters than other forms of asbestos. By contrast, in several occupational cohorts the mesotheliomas accounted for more than 70% of the total. These included (1) the study of Newhouse et al. (1982) of 7474 British workers exposed to mixed asbestos, among whom there were 8 mesotheliomas and only 3 more than the 140 expected lung cancers; (2) the study of Rossiter and Coles (1980) of 6076 British shipyard workers exposed to mixed asbestos, among whom 3 were 31 mesotheliomas and 13 fewer lung cancers than the expected number of 101; (3) the study of Jones et al. (1980) of 578 British female workers exposed to crocidolite, among whom there were 17 mesotheliomas and 6 lung cancers more than the 6 expected; and (4) the study of Newhouse et al. (1982) of 3708 British female workers exposed to mixed asbestos, among whom there were 2 mesotheliomas and 5 fewer lung cancers that the 11 expected.

Timbrell (1983), Timbrell et al. (1987), and Harington (1981) noted that animal inoculation experiments have been interpreted as suggesting a fairly high value of diameter, for example, 1.5 μm (Stanton et al., 1977), 1 μm (Pott et al., 1976), and 0.25 μm (Wagner and Pooley, 1986), below which a fibrous material, so long as it is durable in lung fluids, can produce mesothelioma. In their view, these diameter limits are too high for human fiber-induced mesothelioma. If fibers with diameters >0.5 μm produced mesothelioma, then Paakkila, where the dust clouds contained on the order of 50 fibers/mL (PCOM) and a high proportion of fibers in the 0.5–3 μm diameter range, should have produced many mesotheliomas, as well as excesses in fibrosis and lung cancer. As noted earlier, an average of 38% of the excess lung cancer plus mesothelioma in working populations exposed to asbestos was expressed as mesothelioma. Despite the very high exposures of the Paakkila population, few mesotheliomas were observed.

Timbrell's (1983) examination of the size distributions and mesothelioma incidence at Paakkila and other asbestos mines worldwide led him to conclude that a good correlation was obtained if the threshold diameter was reduced to 0.1 μm. The mesotheliomas that Paakkila fiber has produced in animals were, most likely, caused by the use of excessive doses, 10,000 times that observed in man. Paakkila asbestos contains only 1% of fibers with diameters below 0.1 μm, but with such a large dose this represents an enormous absolute number. Harington (1981) noted that the data for the northwest Cape in South Africa, where numerous mesotheliomas have been reported, and for the northeastern Transvaal, where mesotheliomas are rare, are consistent with a low-fiber-diameter limit. In the northwest Cape, about 60% of the fibers have diameters <0.1 μm, whereas for the Transvaal, only about 1% have diameters <0.1 μm, comparable with Paakkila.

Timbrell (1983) also noted that the length distributions at Paakkila and the northwest Cape point to a need to reduce the 10 μm length threshold in Stanton's criteria with respect to mesothelioma. Paakkila had a high percentage of fibers longer than 10 μm, whereas the northwest Cape had virtually none. And yet, the northwest Cape has been the major source of mesothelioma. Attributing potential carcinogenicity to shorter fibers by lowering the length threshold brings the estimated levels of significant fibers into closer line with the observed mesothelioma rates.

In reviewing the literature on mesothelioma induction in rats exposed by inhalation to fibrous aerosols, I concluded that, for mesothelioma, the relatively low tumor yields seemed to be highly dependent upon fiber type. Combining the data from various studies by fiber type, the percentage of mesotheliomas was 0.6% for Zimbabwe (Rhodesian) chrysotile, 2.5% for the various amphiboles as a group, and 4.7% for Quebec (Canadian) chrysotile. This difference, together with the fact that Zimbabwe chrysotile had 2–3 orders of magnitude less tremolite than Quebec chrysotile, provides support for the hypothesis that the mesotheliomas that have occurred among chrysotile miners and millers could be largely due to their exposures to the more biopersistent tremolite fibers. The chrysotile fibers are insufficiently biopersistent because of dissolution during translocation from the sites of deposition to sites where more durable fibers can influence the transformation or progression to mesothelioma.

Combining the findings of Timbrell with the results of rat inhalation experiments reported by Davis et al. (1986a) for studies with length-classified fibers leads to the conclusion that the critical fibers for mesothelioma induction have lengths between 5 and 10 μm. Davis et al. reported that IP injections of short amosite (1.7% >5 μm) produced only one mesothelioma among 24 rats (after 837 days), whereas UICC amosite (11% >5 μm, 2.5% >10 μm) produced 30 mesotheliomas among 32 rats and long amosite (30% >5 μm, 10% >10 μm) produced 20 mesotheliomas among 21 rats. Thus, fibers shorter than 5 μm appear to be ineffective, and an appreciable fraction longer than 10 μm appears to be unnecessary.

11.7.3 Critical Fiber Dimension for Lung Cancer

Excess incidence of lung cancer has been reported for workers exposed to amphiboles (amosite, anthophyllite, crocidolite, and tremolite), to chrysotile, and to mixtures of these fibers (NRC, 1984), but these studies have been uninformative with respect to the fiber parameters affecting the incidence. The series of rat inhalation studies performed by Davis et al. (1978), which have also produced lung cancers, have provided the most relevant evidence on the importance of fiber length on carcinogenicity in the lung.

The Wagner et al. (1974) study found that the yield of squamous cell carcinoma and adenocarcinoma was greatest with Rhodesian chrysotile, with decreasing yields for Canadian chrysotile, crocidolite, anthophyllite, and amosite, respectively. As shown in Table 11.3, Davis et al. (1978) reported 2 squamous cell carcinomas, 6 adenocarcinomas, and 7 adenomas in 40 rats exposed to 10 mg/m^3 of respirable chrysotile. In 42 rats exposed to 2 mg/m^3 of chrysotile, there were 6 adenomas, 1 adenocarcinoma, and 1 squamous cell carcinoma. There were also adenomas in the groups exposed to amosite at 10 mg/m^3 (two) and to crocidolite (one at 10 mg/m^3, two at 5 mg/m^3). Davis et al. (1978) examined the influence of fiber number concentration in relation to mass concentration in their inhalation studies. Their five exposure groups included three at the same respirable mass concentration of 10 mg/m^3, one each with chrysotile, crocidolite, and amosite. Of these, the amosite produced the lowest number concentration of fibers >5 μm in length. This fiber count was then matched with crocidolite (5 mg/m^3 respirable mass) and chrysotile (2 mg/m^3 respirable mass). In attempting to explain the greater fibrogenic and carcinogenic responses in the chrysotile-exposed animals than the crocidolite- or amosite-exposed groups, this results from the greater number of >20 μm long fibers in the chrysotile aerosol. The ratio of >20 to >5 μm long fibers in the chrysotile was 0.185 compared with 0.040 for crocidolite and 0.011 for amosite. The diameter distributions of all three types of asbestos were similar, with a median diameter of ~0.4 μm.

TABLE 11.3 Lung Deposition, Biopersistence, and *In Vitro* Dissolution of SVFs Correlated with Lung Pathogenicity

		Lung Deposition[a]		Lung Clearance	*In Vitro* Dissolution		Pathogenicity		
		$F/L \times 10^6$ + St. Dev.		$F>20\,\mu m$	pH 7	pH 4.5	Chronic Inhalation		
Fiber	Type	$F/L>5\,\mu m$	$F/L>20\,\mu m$	$WT_{1/2}$[b] (d)	K_{dis}[c]	K_{leach}[d]	Fibrosis Tumors		References
Amosite	Asbestos	10.9+1.0	1.6+0.3	418	<1	nd	+	+	McConnell et al. (1994)
Crocidolite	Asbestos	29.8+7.1	1.0+1.0	817	<1	nd	+	+	McConnell et al. (1994)
MMVF32	E-glass	5.7+1.3	1.3+0.3	79	9	7	+	+	Davis et al. (1996)
RCF1[e]	Refractory	8.3+2.0	1.5+0.2	55	3	nd	+	+	Mast et al. (1995)
MMVF33	475-glass	7.1+0.6	1.4+0.3	49	12	13	+	+	McConnell et al. (1999)
MMVF21	Rock wool	7.7+1.0	1.1+0.1	67	20	72	+	+/–	McConnell et al. (1994)
MMVF10	Insul. glass wool	8.6+1.6	1.0+0.2	15	300	329	–	–	Hesterberg et al. (1998a, 1998b)
X607[d]	Hybrid SVF	3.6	nd	10	990	nd	–	–	Hesterberg et al. (1993)
MMVF11	Insul. glass wool	5.6+1.2	1.0+0.2	9	100	25	–	–	Hesterberg et al. (1993)
MMVF22	Slag wool	3.4+0.6	0.4+0.1	9	400	459	–	–	McConnell et al. (1994)
MMVF34	Stone wool	9.1+1.7	1.5+0.4	6	59	1010	–	–	Kamstrup et al. (1998)

Source: Reproduced, with permission, from Hesterberg et al. (2012).

[a]Details of fiber classification are contained in the papers referenced in footnote *e*.

[b]$WT_{1/2}$, weighted clearance half-time in days (d).

[c]K_{dis} (dissolution rate, ng/cm^2·h) values for MMVF34 from Kamstrup et al. (1998); others from Eastes and Hadley (1995). K_{dis} values may differ from those published elsewhere due to varying methodologies.

[d]K_{leach} dissolution rate constant of leaching elements represented by Ca and Mg at pH 4.5 (rounded up to whole numbers).

[e]Tables from Hesterberg and Hart (2001) and Hesterberg et al. (1998b).

The importance of fiber length to toxicity was further demonstrated by Davis et al. (1986a) using amosite fibers both shorter and longer than the UICC amosite studied earlier. Both aerosols had median diameters between 0.3 and 0.4 μm. The short-fiber amosite (1.7% >5 μm in length) produced no malignant cancers in 42 rats, while long-fiber amosite (30% >5 μm, 10% >10 μm) produced 3 adenocarcinomas, 4 squamous carcinomas, and 1 undifferentiated carcinoma in 40 rats. In terms of adenomas, the frequencies were 3/40, 2/43, 0/42, and 1/81 for the long, UICC, short, and control groups, respectively. Davis et al. (1985) also studied tremolite asbestos using the same protocols with length distribution similar to those of the chrysotile in the 1978 study and the long amosite in the 1986 study (i.e., 28% >5 μm, 7% >10 μm), but with a lower median diameter (0.25 μm). There were 2 adenomas, 8 adenocarcinomas, and 8 squamous carcinomas in 39 rats. Davis (1987) reported on a study comparing the carcinogenic effects of "long" and "short" chrysotile at 10 mg/m^3. Unfortunately, the discrimination between "long" and "short" fibers was less successful than that achieved for amosite. PCOM fiber counts for the fibers >10 μm in length for the "long" and "short" chrysotile were 1930 and 330 f/mL, whereas for the amosite they were 1110 and 12 f/mL, respectively. Despite the much more rapid clearance of the chrysotile from the lungs, the tumor yields were higher. For the "long" fiber, there were 22 tumors for the chrysotile vs. 13 for the amosite. For the "short" fiber, there were seven vs. none. Davis (1987) concluded that fibers <5 μm in length may be innocuous, since the tumors produced by the "short" chrysotile are explicable by the presence of 330 f/mL longer than 10 μm.

Wagner et al. (1985) exposed rats by inhalation to 10 mg/m^3 of respirable dust composed of either UICC crocidolite (52.7% >5 μm, 11.6% >10 μm, median diameter 0.30 μm) or Oregon erionite (44% >5 μm, 7.4% >10 μm, median diameter of 0.22 μm). The UICC crocidolite produced one squamous carcinoma in 28 rats (but no mesotheliomas), whereas the erionite produced no carcinomas in 28 rats but did produce 27 mesotheliomas.

In summary, 10 mg/m^3 of short amosite, UICC crocidolite, and Oregon erionite failed to produce malignant lung cancers, whereas 10 mg/m^3 of UICC chrysotile, long amosite, and tremolite (all with ≥10% >10 μm) produced malignant lung tumors. Thus, carcinogenesis incidence increases with both fiber length and diameter. Since Timbrell (1983) showed that fiber retention in the lungs peaks between 0.3 and 0.8 μm diameter, it is likely that the thinner fibers, which are more readily translocated to the pleura and peritoneum, play relatively little role in lung carcinogenesis. Therefore, it appears that the risk of lung cancer is associated with long fibers, especially those with diameters between ~0.3 and 0.8 μm, and that substantial numbers of fibers >10 μm in length are needed.

In my own review of the literature on the chronic rat inhalation studies with amosite, brucite, chrysotile, crocidolite, erionite, and tremolite (Lippmann, 1994), I found that, for lung cancer, the percentage of lung tumors (y) could be described by a relation of the form $y = a + bf + cf^2$, where f is the number concentration of fibers and a, b, and c are fitted constants. The correlation coefficients for the fitted curves were 0.76 for >5 μm f/mL, 0.84 for >10 μm f/mL, and 0.85 for >20 μm f/mL and seemed to be independent of fiber type. This supported the hypothesis that the critical length for lung cancer induction is in the 10–20 μm range. In terms of the critical sites within the lungs for lung cancer induction, brief inhalation exposures to chrysotile fiber produced highly concentrated fiber deposits on bifurcations of alveolar ducts, and many of these fibers are phagocytosed by the underlying type II epithelial cells within a few hours. Churg (1994) showed that both chrysotile and amphibole fibers retained in the lungs of former miners and millers do not clear much with years since last exposure. Thus, lung tumors may be caused by that small fraction of

the inhaled long fibers that are retained in the interstitium below small airway bifurcations, where clearance processes are ineffective.

One reason for short fibers being less damaging is that they can be fully ingested by macrophages (Beck et al., 1971) and therefore more rapidly cleared from the lung. The fibrogenic response to long fibers could result from the release of tissue-digesting enzymes from alveolar macrophages whose membranes are pierced by the fibers they are attempting to engulf (Allison, 1977). The fibers may also cause direct physical injury to the alveolar membrane. There was a positive association of asbestosis with lung tumors (Wagner et al. (1974). The induction of fibrosis impaired clearance of deposited fibers, increasing the persistence of fibers in the lung.

The preceding implies that short fibers will have a low order of toxicity within the lung, comparable to that of nonfibrous silicate minerals. Within this concept, the critical fiber length would most likely be on the order of the diameter of an alveolar macrophage, that is, about 15 μm. This line of reasoning leads to the same conclusion reached on the basis of the incidence of lung cancer in rats exposed to fibrous aerosols, that is, that the hazard is related to the number of fibers longer than ~10 μm deposited and retained in the lungs. The Timbrell (1983) model predicts alveolar retention of deposited fibers approaching 100% for 10 μm long fibers in the 0.3 to 0.8 μm diameter range. Airborne fibers longer than ~100 μm may be much less hazardous than those in the 10–100 μm range because they do not penetrate deeply into the airways, since interception deposition increases with fiber length.

11.7.4 Summary of Critical Fiber Parameters

The various hazards associated with the inhalation of mineral fibers, that is, asbestosis, mesothelioma, and lung cancer, are all associated with fibers with lengths that exceed critical values. It appears that the critical length is different for each disease, that is, 2 μm for asbestosis, 5 μm for mesothelioma, and ~15 μm for lung cancer. There are also different critical values of fiber diameter for the different diseases. For asbestosis and lung cancer, which are related to fibers retained in the lungs, only fibers with diameters > ~0.15 μm need to be considered. On the other hand, for mesothelioma, which is initiated by fibers that migrate from the lungs to the pleura and peritoneum, the hazard has been related to fibers with smaller diameters and lengths.

Dufresne et al. (1996) studied that the fibers in lungs of Quebec miners and millers with and without asbestosis supported the critical influence of long fibers on fibrosis and cancer incidence. The mean concentrations were higher in cases than in the controls for chrysotile fibers 5–10 μm long in patients with asbestosis with or without lung cancer; tremolite fibers 5–10 μm long in all patients; crocidolite, talc, or anthophyllite fibers 5–10 μm long in patients with mesothelioma; chrysotile and tremolite fibers ≥10 μm long in patients with asbestosis; and crocidolite, talc, or anthophyllite fibers ≥10 μm long in patients with mesothelioma. The cumulative smoking index (pack-years) was higher in the group with asbestosis and lung cancer, but was not statistically different from the two other disease groups.

Although all durable fibers of sufficient length can produce fibrosis and cancer, as documented in various animal studies, it appears that factors other than fiber size can influence the extent of the response. For example, inhaled erionite appears to be much more potent for mesothelioma in both humans and animals because of its greater ability to penetrate the pleural surface. On the other hand, the animal and human data differ on the

ability of inhaled chrysotile to induce mesothelioma. Animal data indicate that chrysotile produces as much or more mesothelioma than the amphiboles, whereas human data more often implicate amphiboles, even when the predominant exposures are to chrysotile. However, examination of the fiber content of the lungs of asbestos workers and animals exposed by inhalation showed that chrysotile is cleared much more rapidly than the amphiboles. It breaks down within the lungs both by disaggregation into fibrils and by dissolution. The differences between the responses in animals and humans may be in relative persistence, that is, time of persistence of the long fibers in the lung relative to the time interval between exposure and the expression of the disease. The long fibers are retained in the lung for a greater fraction of the lifespan in the rat.

Although all durable fibers in the right size range can cause the asbestos-related diseases, they may have different potencies and need different concentration limits. The remainder of this discussion addresses the indices of exposure but not the concentration limits for the fibers that fall within the indices. The concentration limits warrant separate and further discussion later in this chapter.

11.7.5 Implications of Critical Fiber Parameters to Health Relevant Indices of Exposure

Although the current occupational exposure indices, which were based on PCOM assays of fibers with an aspect ratio >3 and a length >5 μm, was a reasonable choice, when it was made, for occupational exposures involving specific known fiber types, it cannot provide a scientifically adequate index for any of the differing hazards resulting from exposures to chrysotile, the various amphibole fibers, other mineral fibers, and the various SVFs. Its most important inadequacies for occupational exposure evaluations in mining, milling, and manufacturing industry include (1) thin fibers of health relevance, especially for mesothelioma, that is, those with widths <~0.25 μm, cannot be seen by PCOM and (2) the PCOM protocol identifies the presence, but not the composition or the distribution of lengths and widths for the >5 μm long fibers that are visualized. It has even greater limitations for occupational exposures resulting from demolition, building renovation, asbestos remediation projects, emergency response to steam pipe explosions, building collapses, and so on, where most of the dust collected on the sampling filter is nonfibrous. The background dust makes the counting of fibers difficult, if not impossible, and the fibers that are seen have a variety of compositions and toxicities.

Most of these limitations can be overcome by using analytical TEM and X-ray diffraction analysis for fiber counting and analysis of mineral type. These measurement methods enable visualization of fiber widths down to ~0.1 μm and compositional analysis of each individual fiber in the field of view. Unfortunately, such analyses are considerably more expensive than PCOM for routine analyses, and the standard laboratory protocols have not utilized the available capabilities to generate fiber size distribution data that recognize the rapidly increasing lung cancer hazard with fiber length as length increases beyond 5 μm.

There is an additional major limitation of the TEM methodology. The TEM methodology has traditionally been used in EPA-endorsed protocols to enumerate the count of all fibers greater than 0.5 μm in length, which ends up in insufficient filter area scanning to get a statistically valid sample of the health-relevant fibers longer than 5, 10, or 20 μm.

EPA considered the adoption of new dimensional criteria for hazardous fibers based on a proposal by Berman and Crump (2001) and sponsored a peer consultation workshop

(ERG, 2003a) on the topic. The Berman and Crump index is based in large measure on modeling of the results of the chronic inhalation studies in rats for which detailed fiber length and width data were available (Berman et al., 1995). They assigned zero risk to fibers <5 μm in length and some small risk to fibers between 5 and 10 μm in length, and the bulk of the risk was assigned to longer fibers. The Report of an Expert Panel on the Health Effects of Asbestos and Synthetic Vitreous Fibers to the Agency for Toxic Substances and Disease Registry (ATSDR) came to similar conclusions, agreeing that there was a strong weight of evidence that asbestos and SVFs shorter than 5 μm are unlikely to cause cancer in humans (ERG, 2003b). However, since EPA did not endorse any new dimensional criteria for hazardous airborne fibers. It would therefore be desirable for risk assessors to use analytical protocols that permit the measurement of the lengths and diameters of all fibers longer than 5 μm, so that the results can be tabulated according to the conventional criteria as well as others that will be adopted in future years.

11.8 EXPOSURE–RESPONSE RELATIONSHIPS FOR ASBESTOS-RELATED LUNG DISEASE: HUMAN EXPERIENCE

11.8.1 Asbestosis

The first disease to be associated with the inhalation of airborne inorganic fibers was asbestosis. The first record of a case of asbestosis in the United Kingdom was attributed to Montague Murray in 1900 (see Sayers and Dreessen, 1939). Hoffman (1918) was the first to call attention to the magnitude of the asbestosis problem in the United States, reporting 13 deaths from asbestosis among asbestos textile workers, and provided the first complete description of the disease and of the "curious bodies," subsequently named asbestos bodies. Asbestos bodies contain asbestos fibers were seen in lung tissue and sputum in two cases in the United Kingdom by Cooke (1927). Ellman (1933) provided a more complete overall description of the disease and its progression. He noted the slow development of a characteristic type of fibrosis (pulmonary asbestosis); it produced insidious lung changes, but one where the patient may be comparatively free from symptoms for 5–15 years. Ellman also noted the length of time that may elapse between exposure to the dust and a fatal termination is only one-half of that in silicosis. The timing is dependent largely upon the nature and concentration of the dust. In a larger study of asbestosis in the United Kingdom, Merewether and Price (1930) estimated that there were about 2200 persons in the United Kingdom exposed in their daily work to the inhalation of asbestos dust, either alone or admixed with a small proportion of cotton. This figure did not include the considerable number of workers exposed to the influence of mixed dusts containing asbestos. They selected 363 workers exposed to chrysotile, crocidolite, or amosite, who were longest employed (75.5% more than 8 years, and 36.7% for more than 10 years). Of those, 95 were found to have a diffuse fibrosis of the lungs.

Studies of occupational exposures to asbestos in the 1930s in the United States included exposure measurements based on total dust counts in terms of MPPCF. Lanza et al. (1935) studied five U.S. plants with most of the maximum dust counts below 10 MPPCF. However, dust counts reached 44.5 in the Felt Department in one plant, 43 in the Preparation Department at another plant, and 82 at the Preparation Department at a third plant. They concluded that the following: 1) prolonged exposure to asbestos dust caused a pulmonary fibrosis (asbestosis) demonstrable on X-ray films, (2) cardiac enlargement

associated with asbestosis, (3) no predisposition to tuberculosis due to asbestos dust, and (4) the amount of dust in the air in asbestos plants studied can be substantially reduced.

Sayers and Dreessen (1939) studied 541 textile mill employees in North Carolina exposed to Canadian chrysotile asbestos, which may have included some tremolite. Pulmonary asbestosis was the principal effect found, with the most serious form of the disease in carders, spinners, weavers, twisters, willowers, and pickers. The exposures, reported in terms of dust counts, ranged from 0.1 to 76 MPPCF. Definite clinical and X-ray evidence of pulmonary asbestosis was seen in persons after 5–10 years of work to exposures exceeding 5 MPPCF.

11.8.2 Cancer Risks Associated with Asbestos

The majority of published dose-specific estimates of the cancer risk caused by asbestos exposure in the literature have been based on models that assume (1) the increase in relative risk for lung cancer is proportional to cumulative asbestos exposure, and the effects of asbestos and cigarette smoking multiply each other, and (2) the increase in mesothelioma incidence caused by each brief period of exposure is proportional to the amount of that incremental exposure (exposure level × duration) and to a power of time since it occurred, independent of age or smoking. The power of time is approximately 2 or 3. However, the validity of these previously used models remains questionable in light of the findings described above and those that follow below.

11.8.3 Human Asbestos Exposure–Cancer Response

11.8.3.1 Lung Cancer Extensive epidemiological literature has established that (1) workers exposed to asbestos fibers have an increased risk of developing bronchogenic carcinoma, (2) workers who also smoke cigarettes have a lung cancer risk greater than those of nonsmokers with equivalent asbestos fiber exposures, and (3) exposed workers also exposed to cigarette smoke have a lung cancer risk greater than additive (Markowitz et al., 2013).

Cancers can arise from the epithelial lining of the large airways or terminal bronchioles. Bronchogenic carcinomas have a variety of histologic appearances: adenocarcinoma, squamous cell carcinoma (presumably arising in areas of squamous metaplasia of the respiratory epithelium), large cell carcinoma, and small cell (oat cell) carcinoma. These are the same histologic types of cancer associated with cigarette smoking in the absence of asbestos exposure (Mossman and Craighead, 1987). In the United Kingdom, where most of the asbestos was imported from Quebec, Lynch and Smith (1935) made the first report of lung cancer in persons with asbestosis. However, it only involved two cancer cases. Also, in the United Kingdom, Gloyne (1951) reported lung cancer in 17 out of 21 necropsies of persons with asbestosis, but only in 55 of 796 (6.9%) of necropsies of persons with silicosis. Doll (1955) reported on all of the 105 necropsies performed on persons employed in a large asbestos works. There were 18 lung cancers, with 15 of them having asbestosis. Of the 113 men employed for at least 20 years, there were 11 lung cancer cases versus 0.8 expected. Doll did not indicate the type of asbestos being processed in the mill.

The cancer experience of 7279 Quebec chrysotile miners and millers in the 1891–1920 birth cohort by Liddell and Armstrong (2002) indicated that the risks of chrysotile exposure and smoking were not multiplicative, but rather independent, casting doubt on the validity of the previously adopted predictive models.

One study produced a relatively higher than average risk coefficient for chrysotile. Dement et al. (1982, 1994) confirmed the relatively high rate of lung cancer in this population and attributed it to the high proportions of long fibers. Further modeling of the available data on this cohort by Stayner et al. (1996) used a model designed to evaluate evidence of a threshold response. Lifetime risks of lung cancer and asbestosis were estimated with an actuarial approach that accounted for competing causes of death. There was a highly significant exposure–response relation for both lung cancer and asbestosis. The relation for lung cancer was linear on a multiplicative scale, consistent with previous analyses of lung cancer and exposure to asbestos. By contrast, the exposure–response relation for asbestosis seemed to be nonlinear on a multiplicative scale. There was no significant evidence for a threshold in models of either the lung cancer or asbestosis. The excess lifetime risk for white men exposed for 45 years at the current OSHA standard of 0.1 fiber/mL was predicted to be about 5/1000 for lung cancer and 2/1000 for asbestosis.

11.8.3.2 Mesotheliomas Many early reports of the association of mesothelioma with asbestos exposure lacked an adequate analysis of the mortality data. The death rate rises sharply with time since exposure, yet only a few data sets have been analyzed by time since first exposure, and few of these reports also provided estimates of average exposure level. Furthermore, there were serious weaknesses in those few, particularly for assessing the individual effects of the amphibole fibers and the chrysotile fibers. The exposure data and results for the cement factory workers' study reported by Finkelstein (1983) were of doubtful reliability for quantitative risk assessment, and there are no contemporary exposure data for the insulation workers (Selikoff et al., 1979) or the amosite textile workers (Seidman et al., 1979).

A study of vermiculate miners exposed to tremolite fibers in Libby, Montana, but not to chrysotile asbestos, has provided an opportunity to examine the role of amphibole contamination of commercially important chrysotile products. The miners had significantly elevated risks for lung cancer, nonmalignant respiratory disease, and mesothelioma. They concluded that amphibole fibers, and tremolite in particular, are likely to be disproportionately responsible for cancer mortality in persons exposed to commercial chrysotile.

In a South African crocidolite mining area of the North Western Cape Province, Wagner et al. (1960) reported that inhaled asbestos was significantly associated with diffuse pleural mesothelioma in 33 histologically proven cases, while there were no cases in other parts of South Africa. Some of these cases were heavily exposed workers, and asbestosis was seen in most of them, while other cases were in residents who had lesser exposures and who did not have evidence of asbestosis. In the United States, Selikoff et al. (1964) studied 632 building trade insulation workers exposed intermittently to amosite asbestos in NY and NJ, of whom 45 died from lung (42) or pleural cancers (3). There was also one peritoneal mesothelioma. Of the 255 deaths in this cohort, 12 died from asbestosis, and 29 died of cancer of the stomach, colon, or rectum.

Lung cancer and mesothelioma can also occur in people without radiographic evidence of lung fibrosis. de Klerk et al. (1997) demonstrated that the level of radiographic fibrosis conferred additional risk of lung cancer beyond that associated with level of exposure but that asbestosis was not a prerequisite for the diagnosis of asbestos-associated cancer. On the other hand, a number of pulmonologists have argued that lung cancer is not observed without the histologic presence of fibrosis. In the series of animal inhalation studies conducted in the 1990s, neither lung cancer nor mesothelioma was observed in rats or hamsters without the presence of fibrosis, which usually occurred as early as 3 months after the exposure was initiated (Hesterberg and Hart, 2001; Bernstein et al., 2005a, 2005b).

The clearest difference between the effects of different fiber types is in the proportion of mesotheliomas that are present in the peritoneum. Almost all cases among chrysotile workers (usually with some exposure to crocidolite and/or tremolite) or among crocidolite miners are pleural, whereas workers with some amosite exposure have suffered similar and sometimes higher risks of peritoneal than pleural mesothelioma (Levin et al., 1998). The only exception appears to be female gas mask workers exposed mainly to crocidolite, for whom several mesotheliomas were peritoneal. The possibility that some amosite exposure occurred in these workers was, however, not discussed.

Direct comparison of workers employed for similar duration to different forms of asbestos (e.g., in mining or gas mask manufacture) indicates a much higher mesothelioma risk for amphiboles than for chrysotile. Chrysotile friction product workers in the United Kingdom suffered no detectable increase in lung cancer, and 11 of the 13 mesotheliomas in this cohort occurred in the subgroup of workers who were exposed to crocidolite (Berry and Newhouse, 1983; Newhouse and Sullivan, 1989). U.K. chrysotile textile workers had a high risk of mesothelioma in contrast to those in South Carolina, although there was a substantial lung cancer risk in both cohorts. The only marked difference between these two textile plants was the use of some crocidolite (less than 5% of the fiber processed) in the U.K. plant.

11.8.4 Mesothelioma and the Amphibole Hypothesis

As discussed previously, there have been marked differences between cohorts in the ratio of excess lung cancer to mesothelioma. Peritoneal mesotheliomas can usually be attributed to amosite exposure, but even when only pleural tumors are considered, the cancer ratio varies remarkably. U.K. shipyard workers with mixed exposure, including a substantial amount of crocidolite, suffered a high mesothelioma risk but no excess of lung cancer (Rossiter and Coles, 1980), whereas among workers at a South Carolina chrysotile textile plant, there was a marked excess of lung cancer and a low incidence of pleural mesothelioma (McDonald et al., 1984; Dement et al., 1982, 1994). These data have been almost universally accepted as indicating that amphiboles, particularly crocidolite, cause a disproportionate mesothelioma risk.

Based on a pooling various cohorts, Doll and Peto (1985) and Nicholson (1986) concluded that among men the ratio of excess lung cancer to pleural mesothelioma is about three times greater for chrysotile than crocidolite, varying from at least four for chrysotile to between one and two for crocidolite, with substantially lower ratios for women. However, such pooling of generally inconsistent data conceals the most extreme inconsistencies, most notably the marked excess of mesothelioma in the absence of any detectable excess of lung cancer observed among shipyard workers by Rossiter and Coles (1980) and in the subgroup of friction product workers with crocidolite exposure studied by Berry and Newhouse (1983).

Other investigators believe that virtually all mesotheliomas are due to amphibole exposure and that inhaled chrysotile fibers pose a negligible mesothelioma risk. The only strong evidence against the inference that mesothelioma is seldom, if ever caused by chrysotile fibers alone, is the observation of substantial numbers of cases among Quebec chrysotile miners and millers. However, these mesotheliomas are related to the presence of fibrous tremolite in this material (Mossman et al., 1990). Tremolite constituted less than 1% of the fiber extracted but more than half of the long (>5 μm) fibers found in the lung tissue of the workers, apparently because chrysotile is cleared much more rapidly (Sebastien et al., 1988). Similarly, high levels of crocidolite were found in lung tissue from U.K.

textile workers who were exposed mainly to chrysotile but suffered a high incidence of mesothelioma (Wagner et al., 1982).

The early evidence that chrysotile fibers rarely cause pleural mesothelioma was consistent, but not conclusive. There have been only two cohorts of heavily exposed asbestos workers who worked only with chrysotile (in both cases exposed to chrysotile from Quebec that is often contaminated with tremolite). There was an initial absence of pleural mesothelioma in the South Carolina plant in spite of the substantial risk of lung cancer (59 observed, 29.6 expected; Dement et al., 1982; McDonald et al., 1984). The Quebec chrysotile miners and millers suffered 230 lung cancers compared with 184.0 expected and 10 mesotheliomas, and lung burden studies show no marked differences between these cohorts in the type, size, or amount of either chrysotile or tremolite fibers (Sebastien et al., 1988).

In follow-up study of the South Carolina cohort by Dement et al. (1994) of 15 years of additional experience, there were two deaths attributable to mesothelioma, and the number of lung cancers had risen to 126 and was expressed as an increase in relative risk of 2.3% for each year of cumulative chrysotile exposure.

Begin et al. (1992) brought the experience in Quebec up to 1990 and concluded that the incidence of pleural mesothelioma in chrysotile miners and millers, while less than that for crocidolite workers, was well above the North American male rate.

In a further examination of the Quebec asbestos cohort of ~ 11,000 chrysotile workers by McDonald and McDonald (1997), of whom 80% had already died, they addressed the amphibole hypothesis by analyzing the deaths among the ~4000 miners employed by the largest company in the Thetford region. The number of cancer deaths, by type, was mesothelioma (21), lung (262), larynx (15), stomach (99), and colon and rectum (76). Risks, in relation to case referents, were analyzed by logistic regression separately for those working in the 5 chrysotile mines located centrally and for the 10 mines located more peripherally, on the basis that tremolite concentrations were four times higher in the central region. Odds ratios were significantly and substantially elevated for workers at the centrally located chrysotile mines for mesothelioma and lung cancer, but not for gastric, intestinal, or laryngeal cancers, while for the workers at the more peripherally located chrysotile mines, there was little or no elevation in odds ratio for any of the cancer groups. Airborne dust concentrations of the two groups were similar, and lung tissue analyses showed that the concentration of tremolite fibers was much higher in the lungs from the central area in comparison with those from workers at the peripheral mines (McDonald et al., 1997).

In an earlier study of Thetford mine workers by Gibbs (1979), pleural calcifications were also much more common among miners who had worked in the centrally located mines than those who worked in the peripherally located mines, suggesting that tremolite accounted for much of both pleural calcification and cancers of the lung and pleura. Furthermore, the studies cited earlier by Dodson et al. (1990) and Boutin et al. (1996) suggest that some of this difference in potency was due to the greater retention of longer amphibole fibers in the lung and pleural lymph nodes than is the case for chrysotile. Additionally, the experimental animal inhalation studies, summarized in Table 11.3 and discussed earlier, support the critical role of amphibole fibers in the causation of mesothelioma.

Thus, despite the publication of contrary views (Smith and Wright, 1996; Stayner et al., 1996; Frank et al., 1997), the hypothesis that mesothelioma is largely, if not exclusively caused by amphibole fibers, remains consistent with published evidence in humans and animals.

No other epidemiological studies have addressed the mesothelioma-inducing potency of chrysotile directly. The substantial incidence of pleural mesothelioma in various cohorts exposed to both chrysotile contaminated by tremolite and by mixtures of chrysotile and other amphiboles is consistent with the hypothesis that these tumors were caused by amphibole exposure. However, it constitutes weak evidence that chrysotile *per se* does not cause mesothelioma. A prudent conclusion is that a very substantial proportion of the mesotheliomas in such cohorts are caused by amphibole exposure.

11.8.5 Pleural Plaques and Pleural Thickening

Pleural plaques in U.K. asbestos workers were first described by Gloyne (1930). Such pleural plaques were observed by Wagner et al. (1960) in South African asbestos workers exposed to crocidolite and amosite asbestos. Further evidence has accumulated that inhalation exposure to airborne asbestos fibers is associated with pleural plaques that are detectable by examination of X-ray images.

11.9 EXPOSURE–RESPONSE RELATIONSHIPS FOR SVF-RELATED DISEASE: HUMAN EXPERIENCE

11.9.1 Lung Cancer

The epidemiological literature on SVFs has focused almost exclusively on lung cancer. Many of the studies of SVF production workers have reported excess lung cancer among some cohorts, primarily those heavily exposed to slag wool and rock wool in earlier years, when exposure levels were largely uncontrolled (Enterline et al., 1983, 1987; Saracci et al., 1984; Shannon et al., 1984, 1987; Simonato et al., 1986).

In the large multinational study in Europe involving approximately 22,000 SVF production workers at 13 plants in 7 countries, the overall lung cancer SMR was 125, and the SMR for the subcohort with more than 30 years was 170. Adjustment for regional variations in mortality substantially reduced the excess lung cancer incidence for those workers exposed only to glass wool, but not for those exposed to rock wool/slag wool. Within this group, most of the excess was accounted for by those 20–29 years since first exposure (SMR of 270). The SMR was 244 for those with 30 or more years, who worked before dust controls were installed.

On the basis of measurements of airborne fiber concentrations made in the period 1977–1980 in the same European plants, Cherrie et al. (1986) reported that the average combined occupational group concentrations in the rock- and glass-wool plants were generally low (<0.1 fibers/mL). In the glass continuous-filament factories, the airborne fiber concentrations were very low (<0.01 fibers/mL). The average plant median for fiber length ranged from 10 to 20 μm, and the corresponding median diameters ranged from 0.7 to 2 μm. In general the glass-wool fibers were thinner than the rock-wool fibers. Higher levels (between 0.1 and 1.0 fibers/mL) were found in some insulation wool production, secondary production, and user industries. The highest levels (>1.0 fibers/mL) occurred in very fine glass-fiber production and in other specialty insulation wool usage.

In a follow-up study of the subcohort of rock- and slag-wool workers in Scandanavia and Germany, for whom smoking and other occupational exposure data were available, Kjaerheim et al. (2002) reported that, for a smoking-adjusted model with a 15-year lag, the

lung cancer odds ratio for the second, third, and fourth cumulative exposure quartiles were 1.3, 1.0, and 0.7, suggesting that the fiber exposures were not causal.

For the 17 U.S. plants producing and using ordinary fibrous glass insulation products that were studied by Enterline et al. (1983, 1987), Esmen (1984) reported that 35% of the airborne fibers were >5 μm in length and that only 3.9% were less than 1.0 μm in diameter. The average exposure concentrations, as determined by phase-contrast optical microscopy, were between 0.01 and 0.05 fibers/mL in 13 plants handling ordinary glass fibers. For a slag-wool plant, the average was ~0.07 fibers/mL, while for a rock-wool plant and a glass microfiber plant, the averages were ~0.25 fibers/mL.

Enterline et al. (1987) reported the 1946–1982 mortality experience of 16,661 SVF workers employed 6 months or more during 1940–1963 at one or more of 17 U.S. manufacturing plants. Using local death rates to estimate expected deaths, there was a statistically significant excess in lung cancer 20 or more years after first employment. The excess was greatest for rock-wool workers and workers ever exposed in the production of small diameter fibers. These two groups of workers are believed to have had mean exposures to respirable fibers of around 0.3 fibers/mL. For glass-wool workers and glass filament workers, SMRs for respiratory cancer were much lower. For these workers, exposures were estimated to be about 1/10 the level for rock-wool workers. There were few positive relationships between respiratory cancer SMRs and duration of exposure, time since first exposure, or measures of fiber exposure. Marsh et al. (2001a) introduced and summarized the results of the 1986–1992 update (Marsh et al., 2001b; Stone et al., 2001). The only outcome with a statistically significant 6% ($p = 0.05$) excess risk was respiratory cancer. However, the duration of fiber and other exposures, the cumulative exposures, and the time since first exposure were not associated with the cancer risk. The smoking habit data (Buchanich et al., 2001) indicated more smoking in the exposure cohort than in the referent population, suggesting that at least some of the cancer excess was due to smoking.

Engholm et al. (1987) studied the incidence of respiratory cancer in 135,000 male Swedish construction workers in relation to exposure to SVFs. The men were all examined at regular health checkups in 1971–1974, and the cohort was followed for mortality through 1983 and for cancer incidence through 1982 by linkage to various national registries. A case–control study within the cohort was carried out on 518 cases diagnosed as having respiratory cancer. The subjects were classified into categories based on self-reported exposure and on estimates of average intensity of exposure in occupations concerned. Smoking habits and density of population were included as potential confounders. Overall, there was an excess of mortality from industrial accidents and an excess incidence of mesothelioma, but in other respects the mortality and the cancer incidence in this population compared favorably with those of the general Swedish population. For lung cancer the overall incidence was below that expected, but there was a risk related to high asbestos exposure. The risk fell close to unity for SVFs when both exposures were fitted simultaneously.

The human experience, based on long-term follow-up on SVF workers in the United States, Canada, and Europe, is encouraging. Many of these workers were exposed to very high concentrations of fibers in the 1940s and 1050s. As summarized by Doll (1987), the evidence of excess lung cancer among these workers appears confined to those subcohorts exposed to slag or rock wool or to those exposed to small diameter biopersistent glass fibers as well as to conventional fibrous glass. When Doll excluded short-term and office workers and compared the numbers of deaths with those that would have been expected had the workers experienced national mortality rates (or provincial rates in the Canadian series),

he found that the mortality from lung cancer (SMR = 121) was raised but that the mortality from other cancers (SMR = 101), other respiratory disease (SMR = 103), and all other causes of death (SMR = 100) was close to that expected.

Division of the workers by type of product and time since first exposure showed that the mortality from lung cancer was highest in the rock- or slag-wool sector of the industry (SMR = 128), intermediate in the glass-wool sector (SMR = 110), and lowest in the continuous-filament glass sector (SMR = 93) and that within the first two groups, mortality rose with time since first exposure to a maximum after 30 or more years (rock or slag wool, SMR = 141; glass wool, SMR = 119). Within the U.S. glass-wool industry, the mortality from lung cancer was higher in those men who had ever been exposed to small diameter fibers (SMR = 124) than in others (SMR = 108). No relationship was observed with duration of employment or with cumulative fiber dose. In a case–control study, however, a weak relationship with cumulative fiber dose was observed in the rock- and slag-wool sectors of the industry after differences in smoking habits had been taken into account. Doll concluded that an occupational hazard of lung cancer has been demonstrated in the rock- and slag-wool section of the industry and possibly in the glass-wool section. Uncertainty about fiber counts in the early years of the industry and about the extent to which other carcinogens were present in the atmosphere of the plants precludes an estimate of the quantitative effects of exposure to current fiber levels, except that it is unlikely to be measurable.

An epidemiologic study of SVF workers in the United States (Wong and Musselman, 1994) included four plants previously studied and five additional plants. This was a nested case–control study based on 55 lung cancers. They analyzed lung cancer risk in relation to cumulative fiber exposure (concentration and duration) and smoking history and controlled for other co-exposures such as asbestos contamination. No increased lung cancer risk with exposure to slag-wool fibers was found.

11.9.2 Respiratory Morbidity

Quantitative data on exposure–response relationships in occupational groups exposed to SVFs for effects other than lung cancer are sparse. The best documented effects are for workers exposed to RCFs. LeMasters et al. (1998) reported on an industry-wide RCF cohort that was characterized as either production or non-production activities and duration of production employment. Both male and female production workers had significantly more respiratory symptoms. For male production workers, there was a significant decline, over 10 years, in FVC for both smokers and nonsmokers, while only the male smokers had a significantly greater decline in FEV_1. Female nonsmokers had a greater decline in FVC than their male counterparts. In an earlier report on this population, LeMasters and Lockey (1994) reported a correlation between pleural changes and RCF exposures. In an analysis of the mortality experience of this cohort through 2000, LeMasters et al. (2003) reported no excess for all deaths, all cancers, mesotheliomas, or respiratory diseases, but there was an excess for urinary organ cancer. Lockey et al. (2005, 2012) reported that the RCF exposures for this cohort were significantly associated with pleural plaques detected radiographic chest exams, but there was no significant increase in interstitial changes.

By contrast, studies of a comparable industry-wide cohort in Europe by Trethowan et al. (1995) found no excess in illness or chest X-ray abnormalities related to fiber exposures. Cowie et al. (2001) found that pleural changes in this RCF cohort were related to age and exposure to asbestos, consistent with time since first RCF exposure. Among men,

FEV_1 and FVC were inversely related to fiber exposure, but only in smokers, and chronic bronchitis showed some association with recent fiber exposure.

For symptoms, lung function changes related to exposures to other SVFs, the epidemiologic evidence had been largely negative. In terms of X-ray abnormalities, the only positive findings reported by Hughes et al. (1993) were for a population cohort exposed to very thin glass fibers.

For symptoms, lung function changes related to exposures to SVFs, the epidemiologic evidence had been largely negative (Hook et al., 1970; Utidjian and deTreville, 1970; Malmberg et al., 1984; Hughes et al., 1993). In terms of X-ray abnormalities, the only positive findings reported by Hughes et al. (1993) were for a population cohort exposed to very thin glass fibers. The best documented respiratory morbidity effects are for SVF workers exposed to RCFs. As noted above, LeMasters et al. (1998) reported on an industry-wide RCF cohort that was characterized as either production or non-production activities and duration of production employment. Both male and female production workers had significantly more respiratory symptoms. For male production workers, there was a significant decline, over 10 years, in FVC for both smokers and non-smokers, while only the male smokers had a significantly greater decline in FEV_1. Female nonsmokers had a greater decline in FVC than their male counterparts. In a follow-up study by McKay et al. (2011) that extended the observations for up to 17 years, there was no consistent decline in lung function associated with RCF exposure.

In an early report on RCF workers, LeMasters and Lockey (1994) reported a correlation between pleural changes and RCF exposures. In a more recent report, Lockey et al. (2012) reported on lung tissue fiber burden on 10 RCF workers with no asbestos exposure and a 20-year longitudinal radiographic study on 1323 RCF workers. They concluded that RCF was biopersistent for up to 20 years and that they may contribute to a significant association between cumulative fiber exposure and radiographic pleural changes. Studies of an industry-wide cohort in Europe by Trethowan et al. (1995) found no excess in illness or chest X-ray abnormalities related to fiber exposures [for further information, consult the IARC Monograph on Man-Made Vitreous Fibres (IARC, 2002) and Boffetta (2014)].

11.10 SUMMARY OF HUMAN RESPONSES TO LONG-TERM FIBER INHALATION EXPOSURES

One common theme in the epidemiologic literature on both workers and community residents is that amphibole (especially crocidolite and tremolite) and erionite fibers are most often more commonly associated with mesothelioma and pleural plaques than are chrysotile fibers, while chrysotile fibers are more often more commonly associated with lung cancer and asbestosis than are amphibole fibers. Furthermore, in those studies of workers exposed to chrysotile who developed mesothelioma, there was tremolite in the chrysotile source. The greater association of chrysotile fiber exposure with lung cancer than with mesothelioma is likely related to the large historical usage of chrysotile and to the longer length of chrysotile fibers in most cases. Hodgson and Darnton (2000) concluded that the risk of lung cancer is 10 times higher for amosite and 50 times higher for crocidolite in comparison with chrysotile, which is likely due to the greater biopersistence of the amphiboles.

A second theme comes from the few studies that addressed the issue of critical fiber lengths. Loomis (2010) demonstrated that the high lung cancer incidence in the

South Carolina textile workers cohort was most closely associated with chrysotile fiber longer than 20 μm, while the Rodelsperger and Bruckel (2006) mesothelioma case–control study did not find a significant odds ratio for chrysotile, but reported a significant exposure–response relationship for mesothelioma for amphibole fibers longer than 5 μm. The studies of fibers retained in human lung tissues of Timbrell for crocidolite, amosite, anthophyllite, and chrysotile miners (described in Lippmann, 1988) by Dufresne et al. (1996) for Quebec chrysotile miners and by Dodson et al. (1990) for shipyard workers are consistent with these different critical lengths for lung and pleural diseases.

For SVF, there is little evidence for any health hazard associated with conventional fibrous glasses, but there are greater risk potentials for the more biopersistent SVFs among specialty glasses, glass and slag wools, and RCFs. However, the risks for the more biopersistent SVFs are clearly much lower than those for chrysotile asbestos fibers, which, in turn, are lower than those for amphibole fibers and erionite fibers.

11.10.1 Fibrogenesis Responses

In studies involving inhalation exposures of rats to UICC asbestos for 1 day to 2 years, Wagner et al. (1974) found that amosite and crocidolite were the least fibrogenic of the five types of UICC asbestos, the others being Canadian chrysotile, Rhodesian (Zimbabwe) chrysotile, and anthophyllite. Davis et al. (1978) used UICC chrysotile A, amosite, and crocidolite in 12-month rat exposures at respirable mass concentrations comparable with those used by Wagner et al. (1974) and found a similar pattern; that is, chrysotile was the most fibrogenic, and amosite and crocidolite the least. Hiett (1978) exposed guinea pigs by inhalation for 9 and 18 days and also found that chrysotile was more fibrogenic than amosite. Davis et al. (1986b) subsequently repeated the protocol with amosite for fiber lengths both shorter and longer than that of UICC amosite. The short amosite produced virtually no fibrosis, whereas the long amosite was more fibrogenic than chrysotile. The most fibrogenic asbestos was tremolite.

11.10.2 Carcinogenesis Responses

Some of the studies that reported carcinogenic responses also noted histological evidence of fibrogenic responses as well. Davis et al. (1991) studied the carcinogenicity of six different tremolite asbestos minerals. Three of them were considered to be asbestiform, while the three others were considered to be cleavage fragments. The carcinogenicity was high for all three asbestiform materials and was very low for two of the cleavage fragment dusts. Carcinogenicity was at an intermediate level for the third cleavage fragment material in rats, which contained more fragments in the size range that met the WHO counting criteria and would be counted as fibers.

The series of rat inhalation studies performed by Davis and colleagues, at the Institute of Environmental Medicine in Edinburgh that have also produced numerous lung cancers, have provided the most relevant evidence on the importance of fiber length on carcinogenicity in the lung. Davis et al. (1978), in attempting to examine the influence of fiber number concentration in their inhalation studies, had five exposure groups that included three at respirable mass concentrations of 10 mg/m^3, one each with chrysotile, crocidolite, and amosite. Of these, the amosite produced the lowest number concentration of fibers >5 μm in length. This fiber count was then matched with crocidolite (5 mg/m^3 respirable mass) and chrysotile (2 mg/m^3 respirable mass). In explaining the greater fibrogenic and

carcinogenic responses in the chrysotile-exposed animals than the crocidolite- or amosite-exposed groups, they emphasized the greater number of >20 μm long fibers in the chrysotile aerosol. The ratio of >20 to >5 μm long fibers in the chrysotile was 0.185 compared with 0.040 for crocidolite and 0.011 for amosite. The diameter distributions of all three types of asbestos were similar, with a median diameter of ~0.4 μm.

The importance of fiber length to lung cancer was further investigated by Davis et al. (1986b) in inhalation studies with amosite aerosols that were both shorter and longer than the UICC amosite studied earlier with the same protocols. Both aerosols had median diameters between 0.3 and 0.4 μm. The short-fiber amosite (1.7% >5 μm in length) produced no malignant cancers in 42 rats, whereas the long-fiber amosite (30% >5 μm, 10% >10 μm) produced 3 adenocarcinomas, 4 squamous carcinomas, and 1 undifferentiated carcinoma in 40 rats. In terms of adenomas, the frequencies were 3/40, 2/43, 0/42, and 1/81 for the long, UICC, short, and control groups, respectively. Davis et al. (1985) also studied tremolite asbestos using the same protocols. Its length distribution was similar to those of the chrysotile in the 1978 study and of the long amosite in the 1986 study (i.e., 28% >5 μm, 7% >10 μm), but its median diameter was lower, that is, 0.25 μm. It produced 2 adenomas, 8 adenocarcinomas, and 8 squamous carcinomas in 39 rats. Davis and Jones (1988) compared the carcinogenic effects of "long" and "short" chrysotile at 10 mg/m^3. Unfortunately, the discrimination between "long" and "short" fibers was less successful than that achieved for amosite. PCOM fiber counts for the fibers >10 μm in length for the "long" and "short" chrysotile were 1930 and 330 f/mL, whereas for the amosite they were 1110 and 12 f/mL, respectively. Despite the much more rapid clearance of the chrysotile from the lungs, the tumor yields were higher. For the "long" fiber, there were 22 tumors for the chrysotile vs. 13 for the amosite. For the "short" fiber, there were 7 vs. 0. Davis (1987) concluded that fibers >5 μm in length may be innocuous, since the tumors produced by the "short" chrysotile are explicable by the presence of 330 f/mL longer than 10 μm. The comparisons in tumor yields for the "long" and the "short" amosite and chrysotile are complicated by the differences in biopersistence between the two fiber types and the unknown number counts of fibers in length categories other than >5 and >10 μm. Furthermore, even for the >5 and >10 μm categories, there were differences in the fiber counts by factors of 2–3, and the ratios would likely be higher for longer length categories. Thus, while the precise characterization of the effects of long fibers on tumor yields in rats cannot be made on the basis of Davis et al. (1986b) and Davis and Jones (1988), it is clear, at least to me, that the implications of Davis (1987) conclusions are clear, that is, that asbestos fibers >5 μm in length are likely innocuous and that the significant yields of lung tumors in rats and human populations exposed to chrysotile are almost certainly attributable to its relatively high content of long fibers.

11.11 SUMMARY OF PULMONARY AND PLEURAL RESPONSES IN ANIMALS

The most common theme from the epidemiologic literature, that is, that amphibole (especially crocidolite and tremolite) and erionite fibers are most often more closely associated with mesothelioma and pleural plaques than are chrysotile fibers, could not be tested adequately in rat inhalation studies because rats developed very few mesotheliomas over their ~2-year lifespans. However, it is possible that the mesothelioma yield may have been influenced by the high mass concentrations used in most of the chronic inhalation studies

in rats, which may have introduced lung overload. In any case, when rats were exposed to crocidolite or erionite, the crocidolite produced 1 lung cancer in 28 rats (but no mesotheliomas), whereas the erionite produced no lung cancers in 28 rats but did produce 27 mesotheliomas.

In terms of the second theme that chrysotile fibers are more often closely associated with lung cancer and asbestosis than are amphibole fibers, the best evidence comes from the long series of studies by Davis and colleagues who exposed rats to equal number concentrations of fibers >5 μ m in length of chrysotile, crocidolite, and amosite, all with median diameters of ~ 0.4 μm. The concentration ratios of >20 to >5 μm were 0.185, 0.040, and 0.011, respectively. There were greater fibrogenic and carcinogenic responses in the chrysotile exposures than in the crocidolite or amosite exposures. When they subsequently used amosite with fiber lengths both shorter and longer than UICC amosite, the short amosite produced virtually no fibrosis, whereas the long amosite was more fibrogenic than chrysotile. For a comparable study with short, UICC, and long chrysotile, the highest lung tumor yield was for the long fiber chrysotile (Davis and Jones, 1988). When Berman et al. (1995) analyzed the lung tumor and mesothelioma responses from 13 of the studies by Davis and colleagues in relation to new TEM measurements of the fiber distributions on archived chamber filters, the measure most highly correlated with tumor incidence was the concentration of fibers >20 μm in length.

In my own review of the chronic rat inhalation studies with amosite, brucite, chrysotile, crocidolite, erionite, and tremolite (Lippmann, 1994), I found that, for lung cancer, the percentage of lung tumors correlated best with fibers >20 μm in length and seemed to be independent of fiber type. Similar conclusions, concerning the influence of fiber length, were drawn by Miller et al. (1999b) for 18 rat inhalation studies involving fibers of amosite, silicon carbide (SiC) whiskers, various SVFs, and various RCFs. In their analysis, the primary influence on biological responses was the number of fibers >1.0 μm in diameter and >20 μm in length, along with the dissolution rate of the fibers. Another important observation was that *in vivo* and *in vitro* cell responses did not significantly predict the risk of cancer following inhalation. Likewise, McConnell et al. (1999) exposed hamsters for 78 weeks to amosite and two different fibrous glasses. One of the glasses produced only mild inflammation, while the other one produced more severe inflammation and mild interstitial and pleural fibrosis, as well as one mesothelioma. Amosite produced severe pulmonary fibrosis and many mesotheliomas. The effects were most closely related to the retained fibers >20 μm in length and were consistent with the *in vitro* dissolution rates. Cullen et al. (2000) also compared the pathogenicity of amosite with that of glass microfibers having low dissolution rates (104E and 100/475) in a study in rats. In terms of mesothelioma and lung cancers produced, a biopersistent glass and amosite fibers were considerably more potent than the more soluble glass fibers.

For asbestos fibers and SVFs instilled IT in guinea pigs, Wright and Kuschner (1977) found that fibers longer than >10 μm were needed to produce lung fibrosis. Variation in yields was attributed to their varying *in vivo* durability.

For Libby amphibole (LA), asbestos instilled into WKY, SH, and SHHF rats, Shannahan et al. (2012) reported that all of the rats developed concentration- and time-dependent interstitial fibrosis. For mice, injected IT with 120-nm-thick silver nanowires with mean lengths of 3, 5, 10, 14, and 28 μm, Schinwald et al. (2012b) reported a length-dependent response in the lung, with a threshold length of 14 μm. For impaired motility, there was a length threshold at 5 μm. For mice exposed IT to CNTs in three length ranges, 1–2, 1–5, and 84% longer than 15 μm, Murphy et al. (2013) reported that the long

nanotubes, but not the two shorter samples, produced an inflammatory response at 1 week post-exposure in the BALF, as well as a progressive thickening of the alveolar septa. They also reported that only the long nanotubes also produced an inflammatory response and pulmonary lesions along the chest wall and diaphragm at 6 weeks post-exposure, but not at 1 week.

Miller et al. (1999a) reviewed the collective outcomes of nine rat IP injection studies involving fibers of amosite, SiC, four SVFs, and three RCFs on mesothelioma and reported links between the numbers of injected fibers >20 μm in length and the biopersistence in the rat lung of fibers >5 μm in length. Schinwald et al. (2012a) injected mice IP with 120-nm-thick silver nanowires with mean lengths of 3, 5, 10, 14, and 28 μm, along with short and long nickel nanowires (4 and 20 μm long), CNTs (13 and 36 μm long), and two amosite asbestos dusts (long fibers with 100% longer than 5 μm, 50% >15 μm, and 35% >20 μm and short with 3% >5 μm). They reported an acute pleural response with a threshold fiber length of 4 μm. They suggested that fibers that are 5 μm or longer that are translocated to the pleural space, both free and in macrophages, are retained because they cannot negotiate the stomata in the parietal stomata, where they can elicit inflammation. These relatively recent studies utilizing IP injection studies in rats with precisely sized carbon and metallic fibers have provided impressive and important new insights on the critical roles played by fiber diameter and length on both the pathways traveled and the site-specific biological responses to such fibers, as summarized in Fig. 11.4.

11.11.1 Coherence of the Human and Animal Response Data with Known Characteristics of Fiber Type and Dimensional Distributions

Knowing that both fiber type and dimensional distributions affect the type and potency of asbestos fibers, and that dimensional distributions vary with fiber type, we should expect that crocidolite, with the greatest proportion of thin fibers than the other amphiboles, would be more potent in terms of causing pleural plaques and mesothelioma in humans than the other amphiboles. Since amosite, with thicker fibers, is intermediate in its association with mesothelioma, and anthophyllite, with the thickest fibers, is least associated with mesothelioma in humans, the weight of the evidence supports the supposition that thin amphibole fibers are most potent for causing the pleural diseases. The fact that chrysotile, which has thin fibers, causes relatively little pleural disease in humans, and that little may be due to the tremolite within the fiber mix, is most likely due to the much greater *in vivo* solubility of chrysotile fibers, especially of the thinnest fibers, than of the amphiboles. Thin chrysotile fibers >5 μm in length may dissolve before they can even reach the pleura, and if they do, they may dissolve before they produce any chronic effect. Pulmonary fibrosis and lung cancer have been most closely related to asbestos fibers >~20 μm of all of the asbestos types and have been more closely associated with chrysotile fiber exposures than with amphibole fiber exposures. These factors may be related to the much greater usage and exposures to chrysotile. Also, there is evidence that at least some long chrysotile fibers, as well as tremolite fibers that accompany some chrysotiles, are retained within the lungs for long time periods. The lengths of fibers >10 μm in the exposure chambers of the chronic rat inhalation studies of Davis et al. (1985) and Davis (1987) of short and long fibers of amosite and chrysotile by PCOM were 1930 and 330 f/mL for chrysotile, whereas for the amosite they were 1110 and 12 f/mL. Despite the much more rapid clearance of the chrysotile from the lungs, the tumor yields for chrysotile were higher. For the "long" fiber, there were 22 tumors for the chrysotile vs. 13 for the amosite. For the "short" fiber, there were 7 vs. 0.

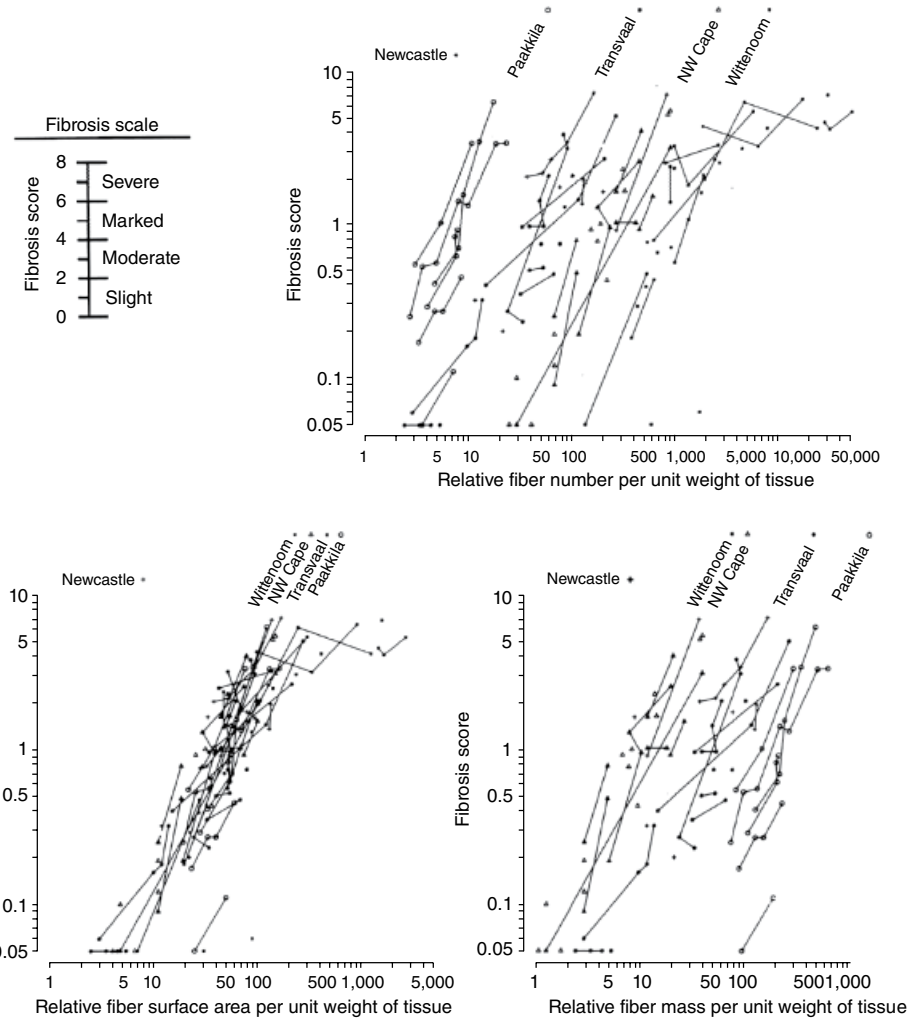

FIGURE 11.4 Relationships between lung fibrosis scale and relative concentrations of fibers per unit weight of dry lung tissues. The lines connect data points from the same subject. The relative fiber surface area normalizes the data better than either the relative fiber number concentration or the fiber mass concentration. *Source*: From Lippmann (1988).

Thus, there were substantial exposures to long fibers in the short chrysotile exposure (330 f/mL longer than 10 μm). Since chrysotile fibers are the thinnest among the asbestos types, and the PCOM counts could not include fibers <0.25 μm in width, the exposures to long, thin chrysotile fibers in both human and chronic animal inhalation studies were much larger than those based on PCOM counts.

For the exposures to the less biopersistent SVFs, there has been little evidence for either pleural or pulmonary diseases for uncomplicated exposures. Since SVFs, with the exception of the relatively small volumes of glass microfibers, have very small fractions of fibers less than even 1 μm, we should not have expected any pleural responses. Considering their relatively rapid *in vivo* dissolution, we should not have expected any pulmonary

responses to the fibers >∼20 μm in length. The limited evidence of some responses for some more biopersistent RCFs and slag wools is consistent with what we should have expected.

In summary, when we take what we now know about the influences of fiber type, dimensional distributions, and biopersistence into account, the nature and extent of pleural and pulmonary responses to the chronic inhalation of airborne fibers of chrysotile, various amphiboles, and SVFs are coherent, even when we have had to rely on inadequate methods of exposure assessment.

11.12 OVERALL SUMMARY OF *IN VIVO* BIOLOGICAL RESPONSES TO VARIOUS DURABLE FIBERS

1. Durable fibers thinner than ∼0.1 μm in diameter are translocated from the lung parenchyma to pleural sites where they can initiate pleural inflammation, plaque formation, and mesothelioma.
2. Durable fibers thicker than ∼0.1 μm in diameter that remain on or in cells at or near the lung parenchyma can initiate lung inflammation, pulmonary fibrosis, and lung cancer.
3. Pleural responses require that the fibers have lengths 5 μm.
4. Pulmonary responses to fibers require fiber lengths greater than ∼20 μm.
5. All varieties of amphibole asbestos and erionite fibers are sufficiently durable *in vivo* to be considered to be highly toxic, and differences in their abilities to produce lung and pleural responses depend primarily on their fiber length and diameter distributions.
6. Chrysotile asbestos fibers are less toxic than amphibole fibers because their greater *in vivo* solubility leads to their dissolution and breakage by length. However, airborne chrysotile fibers are often present in longer lengths than those of other asbestos fibers, and lengths >20 μm are not rapidly cleared from the lungs.
7. SVFs, RCFs, CNTs, and other inorganic fibers are generally less toxic than amphibole fibers in humans and in the rat due to *in vivo* dissolution but can have compositions that make them biopersistent.

11.13 RISK ASSESSMENT ISSUES

11.13.1 Dose Response for Mesothelioma

The form of the mesothelioma dose response for asbestos is not known. Hodgson and Darnton (2000) concluded that for comparable high-level occupational fiber exposures to chrysotile, amosite, and crocidolite, the mesothelioma risks were 1:100:500, respectively. Rodelsperger and Bruckel (2006), in a mesothelioma case–control study, examined fiber burdens in the lungs of 66 cases and controls. They did not find a significant odds ratio for chrysotile but reported a significant exposure–response relationship for amphibole fibers longer than 5 μm.

For eight environmental exposures that involved exposures judged to be high (but without extensive exposure measurements), Bourdes et al. (2000) concluded that the

relative mesothelioma risk of household exposures was 8.1 and that for neighborhood risk was 7.0. The belief that the mesothelioma risk is anomalously high following very low exposure is not supported by observation. In particular, the mesothelioma risk following short exposure to chrysotile may, if anything, be less than that predicted. Peto et al. (1985) studied approximately 18,000 men with no previous asbestos exposure employed in 1933 or later in a chrysotile textile plant. The incidence of mesothelioma was high among men with 20 or more years' exposure, but only two cases were observed among more than 16,000 men with under 10 years' exposure, and one of these seems certain not to have been caused by his employment, as the man was employed for only 4 months and died 4 years later. The current model for mesothelioma may thus overestimate the risk for brief (under 10 years) exposure, at least for chrysotile.

11.13.2 Dose Response for Lung Cancer

The observation that excess lung cancer risk is roughly proportional to cumulative dose at high concentrations does not constitute very strong evidence of a linear relationship with fiber level, particularly at very low levels. This prediction is even more difficult to test directly for lung cancer than for mesothelioma, as lung cancer is so common in the general population, affecting more than 1 smoker in 10 and about 1 nonsmoker in 200, that even quite large increases in risk are difficult to estimate reliably. Prolonged low exposure to chrysotile in friction products, asbestos cement, and chrysotile mining has produced no detectable excess of lung cancer (Paustenbach et al. 2004). Even in chrysotile textile production, the sector in which the highest dose-specific risks for chrysotile have been observed, over 10 years' exposure at low average levels (about 5 f/mL) produced little increase in risk in the U.K. study reported by Peto et al. (1985), although workers employed for less than 10 years in the South Carolina plant studied by Dement et al. (1983), who were more heavily exposed, suffered an increased risk (SMR = 1.9). Moreover, there is evidence that the relative risk for lung cancer in chrysotile-exposed workers eventually falls after exposure to chrysotile has ceased (Walker, 1984; Peto et al., 1985). The risk is very likely to differ between chrysotile and the amphiboles, with Hodgson and Darnton (2000) putting the risk differential between 1:10 and 1:50. In the absence of evidence that the model used for lung cancer underestimates the long-term risk for brief or low exposure, and in view of the previously cited reassuring observations, the resulting predictions may, if anything, be too high for nonoccupational exposure. Camus et al. (1998) examined the risk of developing lung cancer among nonoccupationally exposed women living in the vicinity of the Quebec chrysotile mines and mills. While the relative risk predicted by EPA's model was 2.1, the measured risk was 1.0.

11.13.3 Dose Response for Amphiboles

No extensive measurements of historical exposure levels are available for the cohorts exposed predominantly to crocidolite or amosite. Estimated levels have been published for the crocidolite miners of Western Australia (Armstrong et al., 1988) and varied from 20 to 100 f/mL. Most were employed for less than a year, and more than half had estimated cumulative exposures under 10 f/mL-years, although only 5% exceeded 100 f/mL-years. In a subsequent publication de Klerk et al. (1989) reported a case–control analysis indicating a significantly elevated lung cancer risk only in the minority of workers (about 3%) exposed for over 5 years, among whom the relative risk was 2.2, based on 11 deaths. No other study

provides any useful exposure data for pure crocidolite, however, and this study alone is inadequate as a basis for a firm conclusion.

The situation for amosite is also unsatisfactory. The only study of amosite workers for which dose estimates have been provided (Seidman et al., 1979; see Nicholson, 1986, for updated lung cancer data) is a cohort of men manufacturing amosite insulation in Paterson, NJ, at the beginning of World War II. The dose estimates were based on very limited measurements taken more than 25 years later in two different factories using similar materials and equipment. There was a marked increase in lung cancer SMR, even in men employed for less than 2 months (SMR = 264, based on 15 deaths), and possible estimates of K_L vary from 0.01 (using the lung cancer rate in short-term workers as the baseline) to 0.04 (by regression on the SMR, based on local rates) (Nicholson, 1986). The SMR for men exposed for over 2 years was 650, and there were 14 mesotheliomas (7 pleural, 7 peritoneal). There are three major difficulties in interpreting this study: the lack of any direct exposure data, the anomalous pattern of SMR in relation to duration of exposure, and the uncertainties related to extrapolation from brief very high exposure to prolonged low exposure.

Both amosite and crocidolite have caused high risks of mesothelioma after brief exposure, which has not been observed for chrysotile. Brief amosite exposure can also cause a high lung cancer rate. Moreover, there is consistent evidence that the ratio of mesothelioma to excess lung cancer is higher for amosite, and higher still for crocidolite, than for chrysotile alone.

One interesting inconsistency relates to the groups of workers exposed to some crocidolite who suffered a substantial risk of mesothelioma but no detectable excess of lung cancer, in contrast to the more heavily exposed crocidolite miners, who appear to have suffered a larger excess of lung cancer than of mesothelioma. Perhaps the most plausible interpretation of these (and many other) differences is that different fiber sizes have different effects, either in their ability to reach the bronchus or to reach the lung and penetrate the pleura or in their biological activity in different tissues. Unfortunately, however, our understanding of these processes is at present too limited to justify more specific conclusions.

11.13.4 Risks Associated with Nonoccupational Exposures

Mesothelioma among people not occupationally exposed to asbestos has been reported among people living near asbestos mining and processing areas, including members of households containing asbestos workers, as well as for those without. Presumably, exposures to fibers were higher in homes with workers bringing home dust on their work clothing and shoes, but quantitative exposure data are lacking.

Wagner et al. (1960) reported that one-third of the mesothelioma cases reported in his South African population were not occupationally exposed to amphibole asbestos. Also, three studies from Europe and one from the United States reported excess neighborhood cases around factories processing South African amphiboles (Newhouse and Thompson, 1965; Hain et al., 1974; Hammond et al., 1979; Magnani et al., 1995). In the United States, there was very heavy and visible community exposure to chrysotile asbestos in Manville, NJ (Borow et al., 1973). Berry (1997) reported on the environmental, nonoccupational component of mesothelioma incidence among persons living in Manville. Prior to removal of occupational exposure cases, residents of Manville had an average annual (1979–1990) mesothelioma rate of 636 male cases and 96 female cases per million, about 25 times higher than average NJ State rates. Cases were removed from the analysis when their

"usual employment" was reported as being at the asbestos plant, as evidenced through union lists or occupational information from either the Cancer Registry or mortality records. Standardized incidence ratios (SIRs) were computed for residents of Manville and Somerset County (less the Manville population) by sex. NJ mesothelioma rates less than Somerset County, 1979–1990, were used to generate the expected number of cases. The SIRs for Manville males and females were, respectively, 10.1 [95% confidence interval (CI): 5.8–16.4] and 22.4 (95% CI: 9.7–44.2). Male and female Somerset County mesothelioma incidence rates were 1.9 (95% CI: 1.4–2.5) and 2.0 (95% CI: 1.0–3.6). Some of these excesses were due to household exposures, but clearly the generally community exposures caused some of the excess.

Populations not occupationally exposed to mineral fiber may have very high incidences of mesothelioma. The most extreme case is the study of Baris et al. (1987) of people living in four villages in Central Cappadocia in Turkey. Three villages (Karain, Sarihidir, and Tuzkoy) were exposed to erionite, a fibrous zeolite, and a fourth village (Karlik) lacked this exposure and served as a control. There were 141 deaths during the study period in the four villages, including 33 mesotheliomas, 17 lung cancers, 1 cancer of the larynx, 8 cancers of other sites, and 13 cancers not specified. Thus, there were 72 cancers out of 141 deaths, with at least 33 of them due to mesothelioma. The age- and sex-specific mortality rates per 1000 person-years from mesothelioma and respiratory cancer for the four villages were 20.2, 13.5, 5.2, and 0 for males from Karain, Sarihidir, Tuzkoy, and Karlik, respectively. The corresponding rates for females were 10.9, 3.9, 4.9, and 0. Sebastien et al. (1984) examined ferruginous bodies in the sputum of residents of Karain, Tuzkoy, and Karlik. They found that the content of ferruginous bodies increased with age in Karain and Tuzkoy, but only one of 19 specimens from Karlik had any.

Mesotheliomas among nonoccupationally exposed people living near crocidolite mining and milling regions in South Africa and Western Australia have been known to occur for some time (Wagner and Pooley, 1986; Reid et al., 1990). For a population living near the Wittenoom crocidolite mine in Western Australia, Hansen et al. (1997) were able to show a significant exposure–response relationship based on proximity and duration of exposure. Mesothelioma cases among residents of Cyprus, who had no occupational exposures to fibers, were attributed to environmental tremolite fibers (McConnochie et al., 1987). In California, the incidence of mesothelioma was associated with distance of homes from natural outcrops containing asbestos (Pan et al., 2005)

For modeling purposes, however, risk extrapolations for community residents have had to rely on the quite considerable cancer risks associated with past occupational exposures. Within the range of observation, the models are consistent with the conservative assumption of a linear, non-threshold response. Thus, one can predict risks at the much lower exposure levels observed in schools and in commercial and public buildings. These predictions are likely to overestimate them. Based on the lung cancer risk model of Doll and Peto (1985), the increase in cancer risk associated with 20 years of exposure to daily 8-h exposures in commercial buildings, public buildings, and schools at average concentrations of fibers >5 μm in length in such buildings of 0.0002 f/mL corresponds to a lifetime risk of about 2×10^{-6}. However, it should be noted that concentrations in buildings are seldom much higher than concentrations in the air outside the buildings, and therefore much of this small risk is related to the entry of outdoor fibers into the building with the ventilation air.

In contrast to risk estimations based on human experience in occupational populations and logical extrapolations to background concentration levels, as in the model of Doll and

Peto (1985) described above, Larsen (2003) applied the EPA Proposed Guidelines for Carcinogen Risk Assessment (EPA, 1996) to a set of lung cancer mortality data to obtain a "safe" fiber concentration based on a default linear extrapolation to one excess death per one million people, as specified for carcinogenic hazardous air pollutants by the Clean Air Act. He found that the "safe" concentration was 1/1,000 of ambient air background concentrations of asbestos fibers. Because the calculated "safe" level cannot be achieved, Larsen suggested that his risk assessment techniques be used only for airborne carcinogens that have only anthropogenic sources. Perhaps because he was an EPA employee, he did not question the reliability of the EPA Proposed Guidelines, with their numerous conservative defaults (Lippmann, 2003).

Our current inability to (1) reliably measure the concentrations of health-relevant fibers at concentrations near background levels and (2) reliably quantitate the risks, if any, of exposures at such levels has often led to confusion, alarm, and misguided acts of risk avoidance. For example, removal of in-place asbestos insulation in schools and public buildings has often increased rather than decreased exposures to asbestos fibers for workers doing the remediation and for building occupants after the remediation. Another example was the inappropriate focus, following the collapse of the World Trade Center buildings, on asbestos fibers in air and residual dust as indices of health risk for rescue workers and volunteers, workers removing debris, and neighborhood residents, office workers, and service workers. The measured airborne fiber levels neither warranted that level of concern nor could be related to the health effects that were documented (Lippmann, 2014).

11.14 RISK ASSESSMENT ISSUES—SVFs

Although there has been a significant advance in our knowledge about the deposition and elimination of SVFs and other fibers in recent years, as well as some new knowledge about exposure response in controlled animal inhalation studies, some further concern about lung cancer among heavily exposed workers in industry, and some new insight into the critical fiber dimensions affecting disease pathogenesis, there are also many important questions that remain to be addressed. In some cases, the behavior and risks of airborne SVFs can be inferred from those of either compact particles or asbestos fibers. On the other hand, the validity of such inferences depends on some critical assumptions about the aerodynamic properties of the various fibers and about the responses of lung and mesothelial cells to such fibers. The differences may be critical, and more *in vivo* studies with SVFs should be performed in order to further clarify these issues. In the interim, we already know a great deal about the nature and extent of fiber toxicity and the factors that modify its expression. This knowledge provides a good basis for a fairly definitive risk assessment for SVFs.

SVFs differ from asbestos fibers in several critical ways and tend to produce less lung deposition and more rapid elimination of those fibers that do deposit in the lungs. One difference is in diameter distribution. Except for glass microfiber, SVFs tend to have relatively small mass fractions in diameters small enough to penetrate through the upper respiratory tract. Asbestos, on the other hand, usually contains much more "respirable" fiber. Furthermore, once deposited, chrysotile asbestos fibers may split into a larger number of long thin fibers within the lungs. SVFs rarely split but are more likely to break into shorter length segments.

There are also differences in solubility among the fibers that affect their toxic potential, among both the asbestos types and the SVFs. Conventional glass fibers appear

to dissolve much more rapidly than other SVFs and asbestos. Dissolution of glass fibers takes place both by surface attack and by leaching within the structure. The diameters are reduced and the structure is weakened, favoring break up into shorter segments. Since the smallest diameter fibers have the greatest surface-to-volume ratio, they dissolve most rapidly. Thus, the relatively small fraction of the airborne glass fibers having diameters small enough to penetrate into the lungs are the most rapidly dissolved within the lungs.

The more durable and less soluble SVFs, that is, slag and rock wool, some specialty glasses, and ceramic fibers, require a higher degree of concern because of their longer retention within the lungs. *In vitro* studies and studies of dissolution in simulated lung fluids can be very useful in preliminary evaluations of the toxic potential of the various SVFs. On the other hand, the dissolution of SVFs *in vivo* depends on many additional factors that cannot readily be simulated in model systems. For example, the differences in solubility *in vivo* of long and short fibers noted by Morgan and Holmes (1984) were attributed to small difference in intracellular and extracellular pH. The mechanical stress on fibers *in vivo* may also contribute to their disintegration and cannot readily be simulated in model systems. Thus, hazard evaluations of specialty product SVFs made for limited and specific applications should include detailed *in vivo* studies in which animals are exposed to appropriate sizes and concentrations of the fibers of interest.

In the case of conventional fibrous glasses, we have sufficient information to conclude that the occupational health risks associated with the inhalation of fibers dispersed during their manufacture, installation, use, maintenance, and disposal are not measurable (Doll, 1987) and hence of an extremely low order. The health risk from casual and infrequent indoor air exposure of building occupants to relatively low concentrations of fibrous glass is therefore essentially nil. These judgments are based on a series of interacting factors, each of which individually leads to a far lower order of risk for conventional glass fibers than asbestos. Specifically, (1) conventional glass fibers are less readily aerosolized than asbestos during comparable operations, as demonstrated by the much lower fiber counts measured at various industrial operations (Esmen, 1984; Cherrie et al., 1986). (2) A much smaller fraction of conventional glass fibers than asbestos fibers have small enough aerodynamic diameters to penetrate into lung airways (i.e., fibers with diameters below ~3 μm) (Konzen, 1984). 3) Glass fibers that can penetrate into the lungs are much less durable within the lung than asbestos. They tend to break up into shorter segments, so that fewer fibers longer than the critical length limits are retained at critical sites. They also tend to dissolve, further reducing their retention (Bernstein et al., 1984). (4) The inherent toxicity of conventional glass fibers is much lower than that of asbestos fibers of similar dimension, as shown by studies in which fiber suspensions are applied directly to target tissues by IT instillation (Wright and Kuschner, 1977) or application of a fiber mat to the lung pleura (Stanton and Wrench, 1972).

In consideration of these factors, the risk for lung fibrosis is virtually nil unless there is continuous exposure at concentrations high enough to maintain a high level of lung burden for this relatively rapidly cleared type of particle. The risk of lung cancer is also virtually nil unless there is continuous exposure to long fibers at high concentrations because of the relatively rapid breakup of long fibers into short fiber segments within the lungs. Finally, the risk of mesothelioma from inhaled conventional glass fibers is virtually nil under almost any circumstance. There are hardly any glass fibers thin enough to cause mesothelioma in the aerosols, and the very few that may be present would dissolve rapidly within the lungs.

11.15 RECAPITULATION AND SYNTHESIS: FACTORS AFFECTING FIBER DOSIMETRY AND TOXICITY

11.15.1 Critical Fiber Properties Affecting Toxicity

Review of the *in vitro* studies clearly indicates that fiber length, diameter, and composition are critical determinants of biopersistence, cytotoxicity, and cell transformation. A review of the *in vivo* animal studies, both by inhalation and injection, shows that fiber dimensions and composition are important factors affecting pathological measures such as fibrosis and cancer yields. Review of human exposure response shows that the proportions of the different diseases caused by asbestos, that is, asbestosis, lung cancer, and mesothelioma, vary greatly among occupational cohorts and that the mesothelioma/lung cancer ratio tends to increase with decreasing fiber diameter for the amphibole forms of asbestos.

11.15.2 Influence of Fiber Diameter

Fiber diameter affects airborne fiber penetration into and along the lung airways and therefore the initial deposition patterns. The aerodynamic diameters of mineral fibers are about three times their physical diameters (Stöber et al., 1970; Timbrell, 1972). Thus, fibers with diameters larger than ~3 μm will not penetrate in the lungs (Lippmann, 1990). Fibers with diameters ≤0.1 μm are less well retained in the lungs than larger fibers (Lippmann and Timbrell, 1990). Their large surface-to-volume ratio favors dissolution (Lippmann, 1990). Those sufficiently durable not to dissolve can readily penetrate the epithelial surface and be translocated to the lung interstitium and pleural surfaces. The fibers that remain in the lungs can cause fibrosis and lung cancer, and those durable fibers that are translocated to pleural surfaces can cause mesothelioma. Thus, for asbestosis and lung cancer, the upper fiber diameter limit is on the order of 3 μm. For mesothelioma, the upper fiber diameter limit is likely to be much less for two reasons. First, the thinner fibers penetrate to the gas exchange region to a greater extent. Second, fibers thinner than 0.5 μm are translocated from the deposition sites to postnodal lymphatic channels more than the thicker fibers and thus reach any organ of the body (Oberdörster et al., 1988).

11.15.3 Influence of Fiber Length

Fiber length can also affect fiber penetration into and along the airways. As the length increases beyond ~10 μm, the interception mechanism begins to significantly enhance deposition (Sussman et al., 1991a, 1991b). Thus, longer fibers have proportionately more airway deposition and less deposition in the gas exchange region. Lung retention also increases markedly with increasing fiber length above 10 μm for biopersistent fibers, both on theoretical grounds (Yu et al., 1990) and on the basis of analysis of residual lung dust in humans (Churg and Wiggs, 1987; Timbrell et al., 1987; Pooley and Wagner, 1988) and animals (Morgan, 1979). Furthermore, fibers shorter than about 6 μm in length can readily penetrate through tracheobronchial lymph nodes and be translocated to more distant organs (Oberdörster et al., 1988).

Exact specification of the critical lengths for the different diseases remains difficult, since the experimental studies generally have had, of practical necessity, to use imperfectly classified fiber suspensions. Also, the experimental studies have used very large concentrations, and apportioning attribution of the cytotoxicity and pathology produced to the effects

of fiber size vs. dust overload phenomena is difficult. In other words, the results described in the *in vivo* section of this review would be consistent either with short fibers having a much smaller effect than long fibers or with their contributing to the growth of fibrotic lesions caused by the relatively few long fibers in the tail of the fiber length distribution. In any case, the fibers shorter than ~5 μm have very much less toxicity, whereas cytotoxicity and disease increase with fiber length for fibers longer than 5 μm.

11.15.4 Influence of Fiber Composition

Comparative retention and toxicity studies with various kinds of asbestos and other fibrous minerals, ceramics, and glasses indicate that properties other than fiber dimensions affect fiber retention and toxicity. Among these are solubility, specific surface area, surface electrical charges that may contribute to redox reactions generating ROS, and so on. Thus dimensional characteristics alone, although important, are insufficient indicators of fiber toxicity. A major research need is a systemic exploration of the surface properties and factors affecting solubility of fibers in lung fluids and cells, so that due considerations can be given to fiber composition in hazard assessment.

11.15.5 Risk Assessment for Inorganic Fibers

For asbestos, there is general agreement that occupational exposures to all fibrous forms have caused asbestosis and contributed to excesses of lung cancers. For mesothelioma, it is accepted that inhalation of amphibole and erionite fibers in workers and the general population has been causal. It is also generally agreed that occupational exposure to chrysotile asbestos has been associated with cases of mesothelioma, but many believe that these cases were more likely due to the contamination of most commercial chrysotile with amphibole fibers, and if chrysotile fibers do cause mesothelioma, they are considerably less potent in that regard than amphibole fibers. More definitive conclusions will require studies having better descriptions of the fiber sizes and compositions.

For SVFs, the International Agency for Research on Cancer (IARC, 2002) has, on the basis of their own review of the data on carcinogenic risk, concluded that (1) for humans, there is *inadequate evidence* for the carcinogenicity of glass wool, continuous glass filament, rock (stone) wool/slag wool, and RCFs; (2) for experimental animals, there is *inadequate evidence* for the carcinogenicity of continuous glass filament; (3) for certain newly developed, less biopersistent fibers (X-607 and HT wools and A, C, F, and G), there is *limited evidence* for insulation glass wool, rock (stone) wool, slag wool, and more biopersistent fibers such as fiber H; and (4) there is *sufficient evidence* for the carcinogenicity of special-purpose glass fibers, as well as for RCFs.

For all inorganic fibers, as for other airborne toxicants, the dose makes the poison. However, for these fibrous toxicants, the physical form and properties can be as important, or more important, than the chemical form. The aerodynamic diameters of the fibers, and therefore their deposition patterns and efficiencies within the lung airways, are determined largely by fiber width. For fibers >10 μm in length, interception enhances airway deposition, but even more importantly, these longer fibers elicit cellular responses that shorter fibers do not, and they also are subject to different clearance pathways and rates. Another physical property, their solubility within lung fluids, then becomes a major determinant of their toxicity. While these special determinants of risk are being increasingly recognized, they are not yet reflected in standards or guidelines for exposure assessment,

an essential tool in risk assessment. Thus, there is an urgent need for new and improved occupational and ambient air quality limits for specific fiber compositions that recognize fiber length and diameter as critical risk factors. Dissolution rate *in vivo* is the other main dimension in the risk equation. Fortunately, the SVF manufacturing industry in the United States and the European Community has recognized the critical importance of fiber biopersistence to risk and has revised many product formulations so that they have higher dissolution constants.

The bottom line is that (1) as long as exposure assessment remains a weak link in the performance of risk assessments, our ability to perform credible quantitative risk assessments will remain severely limited and (2) to be able to perform credible exposure assessments, we will first need to reach an expert consensus on (a) the fiber parameters that should be measured, (b) appropriate methodologies for air sampling and laboratory analyses, and (c) procedures and repositories for data management, retention, and access.

11.16 DISCUSSION

This review has shown the following: (1) Fiber dimensions and types are critical determinants of biological responses, with different critical fiber lengths and diameters for lung fibrosis, pleural plaques, mesothelioma, and lung cancer. (2) Short fibers that are effectively cleared from epithelial cells by phagocytic cells and mucociliary clearance can be considered to be nuisance dusts. (3) Biopersistent fibers longer than $\sim 20\,\mu m$ can cause lung cancer. (4) Very thin fibers penetrate lung epithelia and translocate via lymphatic channels to pleural surfaces where biopersistent fibers longer than $\sim 5\,\mu m$ can cause pleural plaques and mesothelioma. (5) All long amphibole fiber types are sufficiently durable *in vivo* to be considered to be biopersistent and, except for the substantial influence of their differing dimensions, to be equally toxic. (6) Chrysotile fibers, except for the long fibers that remain outside of phagocytic lung cells, are less biopersistent than amphibole fibers. (7) Mesotheliomas associated with exposures to the processing of chrysotile fibers are most likely caused by the co-presence of amphibole fibers (e.g., tremolite) in some chrysotile ore bodies. (8) Conventional fibrous glass, especially glass products recently reformulated for greater biosolubility, dissolves too quickly *in vivo* to cause lung fibrosis or cancer. (9) Durable fibers can continue to expose cells *in vivo* for years after they are inhaled, and chronic inhalation exposure is not needed for the initiation and progression of the chronic diseases associated with fiber exposures. (10) Incidental and low-level chronic exposures to durable fibers are unlikely to lead to disability due to lung fibrosis because of the large reserve capacity in our lungs. However, such exposures do present significant cancer risks in the general population; and (11) the quite different responses to chronic inhalation exposures of humans and rats among chrysotile fibers, the various amphibole fibers, and SVFs, in terms of pleural and pulmonary diseases, become coherent when current knowledge of the influences of fiber type, dimensional distributions, and biopersistence is taken into account.

Based on the limitations of the available literature, uncertainty remains with respect to (1) the critical coefficients for *in vivo* fiber dissolution that determine residual human health risks, (2) the roles of fiber surface area or other surface properties on health risks, and (3) the extent to which the results of laboratory-based toxicology studies on CNTs and metallic nanowires can be considered to be reliable surrogates for the prediction of adverse effects associated with inhalation exposures to mineral fibers and SVFs.

There are also remaining uncertainties associated with the characterization of exposures to durable airborne fibers. These include (1) determination of the factors that make rats more susceptible to lung cancer and hamsters more susceptible to mesothelioma in long-term fiber inhalation assays and (2) determination of the extent to which fiber dimensions, dissolution rates, and surface properties account for the toxicity. In order to address these uncertainties, we will need to perform long-term fiber inhalation studies in rats with size-classified fibers having comparable rat-respirable diameters and length distributions for (a) an amphibole; (b) a pure chrysotile; (c) a chrysotile with a small percentage of tremolite; (d) SVFs of graded dissolution coefficients, including conventional fibrous glass, rock wool, RCF; and of various kinds of CNTs.

11.16.1 Controlling Ambient Air Exposures to Airborne Inorganic Fibers

In the absence of credible exposure assessment methods, it would be prudent, at this time, to focus our efforts in protecting general populations from exposures and on further efforts to specify the usage of fibers that are not biopersistent in new applications. The strong dependence of effects on fiber type, diameter, length, and biopersistence makes reliable quantitative exposure and risk assessment impractical in some cases, since it would require TEM examination of representative filter samples for statistically significant numbers of fibers longer than both 5 and 20 μm and thinner than 0.1 μm, by fiber types using X-ray analysis of the individual fibers. If such data cannot be generated, an alternative approach, used in the United States for hazardous air pollutants, is to designate National Emissions Standards that can be applied to industrial operations involving the handling and/or processing of materials containing durable inorganic fibers.

11.16.2 Strategies to Control Exposures to Ambient Air Fibers

Prevention of excessive exposures will need to be based on proven means of emission control strategies for hazardous fibers that can be dispersed during industrial operations involving the handling and/or processing of materials containing durable inorganic fibers and during facility renovations and demolitions. There will also be a need for continuing monitoring of the effectiveness of the designated source controls.

11.17 CONCLUSIONS

The following conclusions are based on current knowledge provided by (1) epidemiologic studies that specified the origin of the fibers by type, and especially those that identified their fiber length and diameter distributions, (2) laboratory-based toxicological studies involving fiber size characterization and dissolution rates and long-term observation of biological responses, and (3) the largely coherent findings of the epidemiology and the toxicology studies:

1. Airborne fibers, if they are sufficiently biopersistent, can cause chronic pleural diseases not caused primarily by their chemical components, as well as pulmonary fibrosis and excess lung cancers.
2. Mesothelioma and pleural plaques are caused by biopersistent fibers thinner than ~0.1 μm and longer than ~5 μm.

3. Excess lung cancer and pulmonary fibrosis are caused by biopersistent fibers that are longer than ~20 μm.
4. While biopersistence varies with fiber type, all amphibole and erionite fibers are sufficiently biopersistent to be highly toxic.
5. The greater *in vivo* solubility of chrysotile fibers makes them less toxic for the lungs and much less toxic for the pleural diseases.
6. The greater of lengths of airborne chrysotile fibers~20 μm in length in human exposures and in the chronic animal inhalation studies made them potent agents for pulmonary fibrosis and lung cancer.
7. Most SVFs are more soluble *in vivo* than chrysotile and pose little, if any, health pulmonary or pleural health risk.
8. Some specialty glass, mineral wool, and RCFs are sufficiently biopersistent to be toxic.
9. Extrapolations of exposure–response relationships from the results of some early epidemiological and chronic animal inhalation studies need to be interpreted cautiously, since the effects observed may have been influenced by overloading of clearance capacity.

In addition the strong dependence of effects on fiber diameter, length, and biopersistence makes reliable quantitative exposure and risk assessment impractical in some cases, since it would require TEM examination of representative membrane filter samples for sufficient numbers of fibers longer than 5 and 20 μm, and thinner than 0.1 μm, by fiber types. Prevention of excessive exposures will need to be based on proven means of source control and continuing monitoring of their effectiveness.

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ACRONYMS

ACGIH	American Conference of Governmental Industrial Hygienists
ACM	Asbestos-containing material
AM	Alveolar macrophage
BMRC	British Medical Research Council
HEI-AR	Health Effects Institute-Asbestos Research
ip	Intraperitoneal, a dose delivery technique for fibers
MALS	Magnetic alignment and light scattering
MMMF	Man-made mineral fiber, an alternate name for SVF
MMVF	Man-made vitreous fiber, an alternate name for SVF
MPPCF	Millions of particles per cubic foot
MTD	Maximum tolerated dose
OSHA	Occupational Safety and Health Administration

ACGIH	American Conference of Governmental Industrial Hygienists
PCOM	Phase-contrast optical method
PEL	Permissible exposure limit
PMN	Polymorphonuclear leukocytes
RCF	Refractory ceramic fiber
ROS	Reactive oxygen species
SIR	Standardized incidence ratio
SMR	Standardized mortality ratio
SVF	Synthetic vitreous fiber
TEM	Transmission electron microscopy
TLV	Threshold limit value
UICC	International Union Against Cancer (English translation of name of organization in French)

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12

CARBON MONOXIDE

Michael T. Kleinman

12.1 INTRODUCTION

Carbon monoxide (CO) poisoning is the second most common cause of reported non-medicinal poisonings deaths in the United States. During the 13-year period between 1999 and 2012, more than 6100 CO poisoning fatalities were reported in Taiwan (Sircar et al., 2015). The installation of CO alarm systems in homes could significantly increase the number of times excessive exposures are detected and therefore reduce CO-related mortality and morbidity (Wheeler-Martin et al., 2015). Total CO poisoning deaths decreased from 1967 in 1999 to 1319 in 2014 ($p<0.001$), and accidental poisoning accounted for 13% fewer deaths per year in 2014 than in 1999 ($p<0.001$), which could have been due to increased voluntary use of CO alarms. However, the study did not show a difference in death rate declines between 19 states that required residential CO alarms by 2010 and 31 states that did not (Hampson, 2016). Ambient air CO exposure in the United States is regulated under Clean Air Act and the primary National Ambient Air Quality Standard (NAAQS) for CO, with a 35 ppm maximum for 1 h exposures and a 9 ppm maximum for 8 h, with both limits not to be exceeded more than once in a year (https://www.epa.gov/co-pollution/table-historical-carbon-monoxide-co-national-ambient-air-quality-standards-naaqs). This NAAQS was initially established in 1971 and was not changed after its last review in 2011.

CO is emitted from virtually all sources of incomplete combustion, including internal combustion engines (e.g., automobiles, trucks, and small engines) (Renner, 1988; Ott et al., 1994; Utell et al., 1994; Mott et al., 2002; Duci et al., 2003; El-Fadel and El-Hougeiri, 2003); fires, both natural and man-made; improperly adjusted gas and oil appliances, e.g., space heaters (Setiani, 1994; Pennanen et al., 1997), water heaters (Huguenin et al., 1957; Thomsen and Kardel, 1988; Chong et al., 1997; Howell et al., 1997; Breindl and Pollak, 1989; Bizovi et al., 1998; Sedda and Rossi, 2006), stoves (Samet et al., 1987; Guggisberg

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et al., 2003), and ovens (Angle, 1988; Pennanen et al., 1997); and tobacco smoking (Gourgoulianis et al., 2002; Murray et al., 2002; Calafat et al., 2004; Viegi et al., 2004).

Waterpipe (hookah) smoking usage is rapidly increasing, especially among younger smokers, and clinically significant CO poisoning with symptoms ranging from none to unconsciousness has been reported (Eichhorn et al., 2018). Owners, employees, and consumers within active hookah lounges (shisha premises) are exposed to concentrations of CO and $PM_{2.5}$ at levels considered hazardous to human health (Gurung et al., 2016). Daher et al. (2010) reported that "a single waterpipe use session emits, in the sidestream smoke, approximately 4 times the carcinogenic PAH, 4 times the volatile aldehydes, and 30 times the CO of a single cigarette." Because of the large number and the ubiquity of CO sources with significant source strengths (e.g., tobacco smoke contains about 1% CO by volume, or 10,000 ppm CO), ambient CO concentrations show large temporal and spatial variations.

The exposure of individuals to CO is, therefore, also quite variable, depending upon the types of activities in which a person is engaged, how long they are engaged (time–activity profiles), where the activity takes place (microenvironments, e.g., indoors, at a shopping mall, outdoors, in a vehicle, at work or school, in a parking garage, or even in a skating rink) (Viala, 1994; Jovanovic et al., 1999; Horner, 2000; Levesque et al., 2000, 2005), and the proximity to CO sources (Ott, 1990; Ott et al., 1992; Linn and Gong, 1999; Campbell et al., 2005). Spectators and participants can be exposed to high levels of CO and nitrogen oxide gases at ice hockey games, indoor monster truck rallies, and car demolition shows (Lee et al., 1994; Levesque et al., 2000). Maximum time-weighted average concentrations measured during some monster truck rallies and car demolition exhibitions were 100 ppm, with several peaks exceeding 200 ppm (maximum value: 1600 ppm) (Levesque et al., 2000). CO concentrations were examined in various public transit vehicles and were higher in LPG bus cabins than in other vehicles, suggesting possible self-pollution issues along with penetration of on-road pollutants inside cabins during bus transit (Yang et al., 2015).

Various methods have been used to document potential or actual exposures, including the use of air quality data from fixed-site ambient monitors, the use of microenvironmental exposure assessment models, personal exposure monitoring methods, and biological monitoring methods (Ott et al., 1992).

Controls on motor vehicle exhaust and the use of catalytic converters on vehicles sold in the United States have been very effective in reducing ambient CO emissions and commuter exposures (Hysell et al., 1975; Hinkle, 1980; Mott et al., 2002; Hutchinson and Pearson, 2004). However, while the increased use of oxidizing catalysts resulted in decreased emissions of CO, they also increased emissions of noble metals and ultrafine particles, which may also contribute to health effects (Brubaker et al., 1975; Finklea et al., 1975). Reductions in automotive emissions in the United States brought about by the Clean Air Act have reduced traffic CO exposures and traffic-related CO concentrations well below those measured prior to the year 2000 (U.S. EPA, 1999).

12.2 CO EXPOSURE AND DOSIMETRY

CO competes with oxygen (O_2) for binding sites on the heme portion of the hemoglobin (Hb) molecules in red blood cells to form carboxyhemoglobin (COHb). The affinity of Hb for CO is about 240–250 times that for O_2 (Roughton, 1970). The formation of COHb by

the binding of CO to circulating Hb reduces the O_2-carrying capacity of blood. In addition, binding of CO to one of the four hemoglobin binding sites increases the O_2 affinity of the remaining binding sites, thus interfering with the release of O_2 at the tissue level. When O_2 content of blood (mL O_2/mL blood) is plotted vs. O_2 partial pressure (mmHg) in blood, the increased O_2 affinity is seen as the so-called leftward shift in the curve for blood partially loaded with CO (Okada et al., 1976; Zwart et al., 1984). CO-induced tissue hypoxia is therefore a joint effect of the reduction in O_2-carrying capacity and the reduction of O_2 release at the tissue level. The brain and heart, under normal conditions, utilize larger fractions of the arterially delivered O_2 (about 75%) than peripheral tissues and other organs (Ayres et al., 1970) and are therefore the most sensitive targets for hypoxic effects following CO exposures. The potential for adverse health effects is increased under conditions of stress, such as exercise, which increases O_2 demands at the tissue level to sustain metabolism.

The measure of biological dose that best relates to observed biological responses and to deleterious health effects is the concentration of COHb expressed as a percentage of available, active Hb. Thus, it represents the percent of potential saturation of Hb. COHb can be measured directly in blood, or it can be estimated from the CO content of expired breath (Rea et al., 1973; Vogt et al., 1977; Vreman et al., 1996; Groman et al., 1998; Wickramatillake, 1999; Laranjeira et al., 2000; From Attebring et al., 2001; Berny et al., 2002). It is currently accepted that the most accurate and reliable method for measuring COHb concentration is by gas chromatographic analysis (Vreman et al., 1984; Vreman et al., 1994; Berny et al., 2002). Spectrophotometric measures and instruments have been widely used in both clinical and occupational health settings (Boumba and Vougiouklakis, 2005). However, spectrophotometric instruments may have limited accuracy and precision at COHb concentrations below 5% (Allred et al., 1989a; Johansson and Wollmer, 1989; Chaitman et al., 1992).

When used for research studies, measurements should verified by a "gold standard," such as gas chromatography (Wigfield et al., 1981; Johansson and Wollmer, 1989; Widdop, 2002). When direct measurements cannot be made, COHb can be estimated from ambient air CO concentrations (Ott et al., 1988), indoor air CO concentrations, and personal CO monitoring data (Ott and Mage, 1978, 1979) using pharmacokinetic and other models (Coburn et al., 1965; Peterson and Stewart, 1975; Joumard et al., 1981; Hauck and Neuberger, 1984; Tikuisis et al., 1987; Chung, 1988; Benignus et al., 1994; Bruce and Bruce, 2003) that link the concentration of inhaled CO, breathing rate and volume, blood volume, metabolic production of endogenous CO, and rate of removal of CO. The Coburn–Forster–Kane (CFK) model (Coburn et al., 1965) has been widely used for this purpose. In the process of establishing ambient air quality guidelines, the CFK model has been the basis for associating ambient and workplace air CO concentrations with concentrations of COHb that could be hazardous for sensitive exposed individuals. The CFK model has been experimentally verified for exposures at 25–5000 ppm, during rest and exercise (Peterson and Stewart, 1975; Tikuisis et al., 1987). Sensitivity analyses of CFK can be used to identify those model input parameters for which errors in the parameter values used will have the greatest errors in predicted COHb concentrations (McCartney, 1990). While validation of the CFK model under well-controlled conditions at environmental concentrations with larger numbers of subjects would be useful, Kleinman et al. (1998) used the CFK model to successfully predict blood COHb concentrations in 17 human volunteers exposed to 100 ppm CO, suggesting that the CFK model is valid under such circumstances.

12.3 MECHANISMS OF CO TOXICITY

CO affects health indirectly by interfering with the transport of O_2 to tissues (especially the heart and other muscles and brain tissue) (McGrath, 2000). The resulting impairment of O_2 delivery causes tissue hypoxia and interferes with cellular respiration. CO taken up by cells can complex with Fe^{2+} in hemoproteins such as myoglobin (McGrath, 2000), cytochrome oxidase, and cytochrome P450 (so-named because the Fe^{2+}–CO complex absorbs light with a maximum absorption at 450 nm) (Williams, 1992) and thus interfere with electron transport processes and energy production at the cellular level. Thus, in addition to observed physiological effects and cardiovascular effects, CO can modify electron transport in nerve cells, resulting in behavioral, neurological, and developmental toxicological consequences.

The possible role of CO as an etiologic factor in development of atherosclerosis is suggested by effects of tobacco smoke exposure (Hart, 1993; Leone, 1995) and mobile source emissions (Utell et al., 1994), but long-term exposures to 200 ppm CO in a sensitive animal model failed to show an effect of CO (Penn, 1993). The role of CO as a causative factor in cardiac arrhythmias, sudden cardiac arrest, and myocardial infarctions remains an area of active research activity. The hemodynamic responses to CO have been reviewed for both animal models and humans (Kanten et al., 1983; Penney, 1988). Chronic CO exposures, usually at COHb concentrations greater than 10%, produce changes that may be adaptive responses to induced hypoxia, such as increases in numbers of red blood cells (polycythemia), increased blood volume, and increased heart size (cardiomegaly). In addition, heart rate, stroke volume, and systolic blood pressure may be increased. Some of these effects have been seen in smokers. Other environmental factors such as effects of other pollutants (both from conventional air pollution sources and from environmental tobacco smoke), interactions with drugs and medications, health and related factors (e.g., cardiovascular and respiratory diseases, anemia, or pregnancy), and exposures at high altitude are possible risk modifiers for the health effects of CO.

Exposures to high concentrations of CO due, for example, to fires and emissions from faulty appliances result in over 2000 deaths per year in the United States and other countries (Sadovnikoff et al., 1992; Abu-al Ragheb and Battah, 1999; Raub et al., 2000), as well as in illness sufficient to cause upwards of 15,000 individuals to seek medical attention or to miss one or more days of work in the United States (Centers for Disease Control and Prevention, 2014). The available data may substantially underestimate the total numbers of such cases, especially those related to unsuspected CO exposure in the home because some CO-related symptoms are similar to those of flu (headache and dizziness) and possible to those of certain seizure disorders (Heckerling, 1987; Kirkpatrick, 1987; Leikin et al., 1988; Heckerling et al., 1990a, 1990b). Therefore, many cases may be misdiagnosed and missed as being related to CO exposure. As a consequence, patients may not receive the proper treatment, and their cohabitants may go untreated if they did not independently seek medical help (Heckerling et al., 1990a; Kao and Nanagas, 2004). Many of the inadvertent incidents of morbidity and mortality are preventable through the use of CO detectors, which are available at a moderate cost (Yoon et al., 1998). Blood tests for COHb concentrations or breath analyses for CO improve the accuracy of the diagnoses. While many improvements have been made with respect to exposure to ambient CO, this remains as an important issue. The remainder of this chapter deals with ambient environmental exposures and focuses on the recent findings of CO-related health effects.

12.4 POPULATIONS AT RISK OF HEALTH EFFECTS DUE TO CO EXPOSURE

12.4.1 People with Cardiovascular Diseases

Daily variations in CO have been strongly associated with hospital admissions among persons with ischemic heart disease (IHD) conditions, even after controlling for potential effects of ozone (O_3), nitrogen dioxide (NO_2), or particulate matter (PM) less than or equal to 10 micrometers (μm) in aerodynamic diameter (PM_{10}). A 1-ppm increase in 8-h average CO was associated with a 3.60% increase in same-day IHD admissions (Mann et al., 2002). A short-term interquartile range increase of 0.2 ppm CO was associated with a 5.4% increased risk of an IHD emergency department visit (Szyszkowicz, 2007). In a study of 1837 hospital admissions for IHD, CO exposure was significantly associated with increased relative risk (RR = 1.08–1.10) in male and in female patients (Tuan et al., 2016). IHD, also categorized as coronary artery disease, is a leading cause of disability and death in industrialized nations and may be associated with chronic elevation of COHb (Mall et al., 1985).

IHD is a clinical disorder of the heart resulting from an imbalance between O_2 demand of myocardial tissue and O_2 delivery via the bloodstream. The ability of the heart to adjust to increases in myocardial O_2 demands resulting from increased activity or to reductions in O_2 delivery by arterial blood due, for example, to COHb or reduced partial pressure in O_2 in inspired air, by increasing O_2 extraction, is limited, because the extraction rate in myocardial tissue is already high. Normally, coronary circulation responds to increased O_2 demands by increasing blood flow. In coronary artery disease, the coronary artery is occluded by lipid deposits, which can impede augmentation of local coronary blood flow in response to increased O_2 demands. Under these conditions, the myocardium is forced to extract more O_2, resulting in reduced coronary venous and tissue O_2 tensions, which can produce myocardial ischemia. Severe myocardial ischemia can induce a myocardial infarction (heart attack) or can alter cardiac rhythms, that is, cause arrhythmias. The association of acute CO exposure to heart attacks has been described (Scharf et al., 1974; Marius-Nunez, 1990; Tan et al., 1993; Martys, 1994; Koskela et al., 2000).

Individuals with obstructed peripheral arteries may experience intermittent claudication, which is severe pain, usually in their legs, during walking or other relatively mild activities. CO exposure, for example, from cigarette smoking, can exacerbate the imbalance between O_2 demand by exercising peripheral muscular tissue and O_2 delivery in individuals with diseased peripheral arteries (Wald et al., 1977).

12.4.2 People with Anemia and Other Blood Disorders

Individuals with reduced blood hemoglobin concentrations, or with abnormal hemoglobin, will have reduced O_2-carrying capacity in blood. In addition, disease processes that result in increased destruction of red blood cells (hemolysis) and accelerated breakdown of hemoproteins accelerate endogenous production of CO (Solanki et al., 1988; Sannolo et al., 1992; Sears et al., 2001), resulting in higher COHb concentrations than in normal individuals. For example, patients with hemolytic anemia have COHb concentrations 2–3 times those seen in normal individuals (Coburn et al., 1966). Endogenously produced CO, from the breakdown of hemoglobin by heme oxygenase, was originally thought to be a superfluous by-product of heme catabolism. However, CO is now known to play a central role in blood pressure regulation, maintenance of organ-specific vascular

tone, neurotransmission, stress response, platelet activation, and smooth muscle relaxation (Morse and Sethi, 2002; Wu and Wang, 2005). Thus, CO may be an important and beneficial mediator at normal physiological levels, but is toxic at elevated levels.

12.4.3 People with Chronic Lung Disease

Chronic lung diseases such as chronic bronchitis, emphysema, and chronic obstructive pulmonary disease (COPD) are characterized by impairment of the lung's ability to transfer O_2 to the bloodstream because diseased regions of the lung are poorly ventilated and blood circulating through these regions will therefore receive less O_2 and accumulate carbon dioxide (CO_2) (so-called ventilation–perfusion mismatch) (West, 1971, 1978; Wagner et al., 1977). Exertional stress often produces a perception of difficulty in breathing, or breathlessness (dyspnea) in these individuals. Although exercise, and the metabolic acidosis associated with exercise in COPD patients, increases ventilatory drive, they have limited ventilatory capacity with which to respond (Sue et al., 1988). Reduction of blood O_2 delivery capacity due to formation of COHb could exacerbate symptoms and further reduce exercise tolerance in these individuals.

12.5 POTENTIAL RISKS FOR PREGNANT WOMEN, FETUSES, AND NEWBORN CHILDREN

A CO-induced leftward shift in the O_2Hb saturation curve may be significant for fetuses because the O_2 tension in their arterial blood is low (20–30 mmHg) compared with adult values (~100 mmHg) and because fetal Hb has a higher O_2 affinity than maternal Hb (Longo, 1976, 1977). Fetal blood also has higher Hb concentrations than maternal blood (Heilmann et al., 2005), which may compensate for the higher O_2 affinity to some extent. In pregnant women, O_2 consumption is increased 15–25%, and Hb concentration may be simultaneously reduced, lowering the O_2-carrying capacity of their blood (Sady and Carpenter, 1989). Epidemiological studies show that odds ratios for cardiac ventricular septal defects increased in a dose-responsive fashion with increasing CO exposure, a 1 ppm increase in mean CO exposure during the first trimester of pregnancy is associated with a reduction of 23 g in birth weight, and first-trimester CO exposures were associated with 20% increased risk of intrauterine growth retardation (Newill, 1974; Ritz et al., 2002; Gouveia et al., 2004; Salam et al., 2005).

12.6 HISTORICAL REGULATORY BACKGROUND

The NAAQS for CO in the United States were first promulgated by the Environmental Protection Agency (EPA) in 1971 on the scientific basis of a study that indicated that subjects exposed to low levels of CO resulting in COHb concentrations of 2–3% of saturation exhibited neurobehavioral effects (Beard and Wertheim, 1967). A reexamination of the scientific evidence, as reported in a revised CO criteria document (U.S. EPA, 1979), concluded that it was unlikely that significant, and repeatable, neurobehavioral effects occurred at COHb concentrations below 5%. Medical evidence, accumulated during the intervening years, however, indicated that aggravation of angina pectoris, and other symptoms of myocardial ischemia, occurred in men with chronic cardiovascular disease (CVD), exposed to low levels of CO resulting in COHb concentrations of about 2.7% (Aronow et al., 1972; Anderson et al., 1973; Aronow and Isbell, 1973; Aronow, 1974, 1979, 1981; Goldsmith and

Aronow, 1975). EPA proposed, in 1980, based in part on the above studies, to retain the 8 h 9 ppm primary standard level, to reduce the 1 h primary standard from 35 to 25 ppm, and to revoke the secondary CO standards (because no adverse welfare effects had been reported at near ambient levels). However, an EPA investigation found flaws in some of Aronow's studies from which data were used as part of the basis for the proposed reduction in the 1 h standard (Budiansky, 1983); EPA later decided to keep the 1 h standard at 35 ppm.

In l984, EPA published an addendum to the 1979 CO criteria document that reevaluated the CO health effects data previously reviewed and took into account research that had been published in the interim (U.S. EPA, 1984). The document reviewed four effects associated with low-level CO exposure: cardiovascular, neurobehavioral, fibrinolytic, and perinatal. Dose–response data provided by controlled human studies allowed the following conclusions to be drawn:

1. Cardiovascular effects. Among those with chronic CVD, a shortening of time to onset of angina was observed at COHb concentrations of 2.9–4.5%. A decrement in maximum aerobic capacity was observed in healthy adults at COHb concentrations at and above 5%. Patients with chronic lung disease demonstrated a decrease in walking distance when COHb concentrations were increased from 1.1–5.4% to 9.6–14/9%.
2. Neurobehavioral effects. Decrements in vigilance, visual perception, manual dexterity, and performance of complex sensorimotor tasks were observed at, and above, 5% COHb.
3. Effects on fibrinolysis. Although evidence existed linking CO exposure to fibrinolytic mechanisms, controlled human studies did not demonstrate consistent effects of CO exposure on coagulation parameters.
4. Perinatal effects. While there were some epidemiological associations between CO exposure and perinatal effects, such as low birth weight, slowed postnatal development, and incidences of sudden infant death syndrome (SIDS), the available data were not sufficient to establish causal relationships.

In September 1985, EPA issued a final notice that announced the retention of the existing 8 h 9 ppm and 1 h 35 ppm primary NAAQS for CO and the rescinding of the secondary NAAQS for CO.

The EPA reviewed health-related data in 1991 and completed the most recent CO criteria document in 1999 (U.S. EPA, 1999). In that temporal interval, several controlled human studies, population-based studies, and inhalation studies using laboratory animal models were added to the available database. These studies provided insights into the possible mechanisms of toxic action of CO, in addition to those related to hypoxia, and illuminated effects that had not been identified in human studies, such as perinatal and developmental effects. Following review of the 1991 and the 1999 CDs, the existing NAAQS for CO were retained and remained the current CO NAAQS.

12.7 HEALTH EFFECTS OF CO

12.7.1 Population-Based Studies

12.7.1.1 Acute Exposures and Their Effects Most of the population-based studies in the literature relating to the health effects of CO in humans have been concerned with exposures to combustion and pyrolysis products from combustion sources such as tobacco

smoking, fires, motor vehicle exhaust, home appliances fueled with wood (Pierson et al., 1989; Ellegard, 1996), gas or kerosene (Cooper and Alberti, 1984; Amitai et al., 1998), and small engines (Baldauf et al., 2006). The individuals in these studies were therefore exposed to variable, and usually unmeasured, CO concentrations and also to high concentrations of other combustion products. Exposures to CO in occupational settings represent another substantial exposure classification, but such exposures are also often accompanied by exposures to other air contaminants as well.

The symptoms of CO poisoning are often nonspecific or masked by an exacerbation of an underlying illness, such as congestive heart failure. The effects can range from mild, annoying symptoms that resolve after removal of the source to severe morbidity with profound central nervous system dysfunction and acute complications. Acute CO intoxication can result in neurologic and/or myocardial injury; many patients who survive CO poisoning suffer from long-term neurological and affective sequelae that do not necessarily correlate with blood CO levels and might therefore result from direct effects of CO on cellular mitochondrial respiration, cellular energy utilization, inflammation, and free radical generation, especially in the brain and heart (Rose et al., 2017).

Long-term neurocognitive deficits occur in 15–40% of patients, whereas approximately one-third of moderate to severely poisoned patients exhibit cardiac dysfunction, including arrhythmia, left ventricular systolic dysfunction, and myocardial infarction. Studies have reported that 2% to approximately 10% of patients display delayed neurological sequelae (Choi, 1983; Mathieu et al., 1985; Thom and Keim, 1989; Raub et al., 2000). Hyperbaric O_2 treatment accelerates the rate of COHb elimination, and some evidence suggests that it modulates inflammatory sequelae instigated by CO poisoning, and in some cases might be a superior treatment modality compared with treatment with normobaric O_2 (Weaver, 2014). Patients with a history of CO poisoning have a higher risk of developing dementia than individuals with no CO poisoning history (Wong et al., 2016).

Estimates suggest that about one-third of nonfatal cases of CO poisoning go undetected and undiagnosed (Heckerling, 1987; Heckerling et al., 1987; Abelsohn et al., 2002). CO poisoning, even when treated with supplemental O_2 (Mathieu and Mathieu-Nolf, 2005), can lead to permanent neurocognitive or affective deficits; thus increased awareness and prevention of CO poisoning is imperative (Weaver, 1999). The mechanism involved in delayed neurological damage after CO exposure was studied in rats. There were significant increases in glutamate release and •OH generation during and immediately after CO hypoxia, and CO-exposed rats showed learning and memory deficits that were associated with cell loss in the cortex, globus pallidus, and cerebellum. Both neuronal necrosis and apoptosis were observed, indicating that both necrosis and apoptosis contribute to brain cell death after acute CO poisoning (Piantadosi et al., 1997). This lends some mechanistic support to findings that Parkinsonism, which is an outcome of lesions or losses of dopaminergic neurons, may be associated with exposures to CO (Bleecker, 1988; Choi and Cheon, 1999; Choi, 2002).

Necrosis of muscle tissue (myonecrosis) has been reported as a possible but fairly unusual sequela to CO exposure (16–20 cases have been reported in the English-language literature) (Herman et al., 1988; Shapiro et al., 1989; Wolff, 1994; Waisman et al., 1998). Some of the cases involved firefighters, and it is not clear that CO alone is a causal factor. Cyanide, which is a frequent co-contaminant in fires, has been suggested as a contributor to myonecrosis (Shapiro et al., 1989). Marius-Nunez (1990) reported a case of an individual who suffered an acute myocardial infarction (shown by ECG and serum enzyme findings)

after an acute CO exposure. This case was of interest because the patient's medical profile was negative for coronary heart disease risk factors and because a coronary angiogram performed 1 week after admission failed to show coronary obstructive lesions. A similar case was reported (Ebisuno et al., 1986), and the circumstances of both cases suggested that contributing factors to the CO-induced reduction in O_2 supply to the myocardium might include induction of coronary artery spasm, inadequate myocardial perfusion, and a direct toxic effect on myocardial mitochondria. Leikin and Vogel (1986) reported that patients admitted to intensive care units with proven myocardial infarctions had higher COHb levels than a control group, but these differences could have been accounted for by smoking alone and a relationship to ambient urban CO could not be established.

Sokal and Kralkowska (1985) examined 39 cases of acute CO poisoning in Poland who were poisoned at home by emissions from household gas or coal stoves. The duration of CO exposure and the degree of metabolic acidosis, indicated by lactate concentrations in the blood, were better predictors of the clinical severity of symptoms than the COHb concentration in blood at the time of admission to the hospital. The importance of exposure duration was suggested in earlier evaluations of CO toxicity and is consistent with the possible involvement of myoglobin in CO poisoning, particularly in those who develop delayed sequelae after their initial recovery (Hwang and Park, 1996; Kelafant, 1996; Scheinkestel et al., 1999; Deschamps et al., 2003; Shahbaz Hassan et al., 2003; Webber, 2003; Ersanli et al., 2004; Kanazawa and Yoshikawa, 2004; Lam et al., 2004; Gupta et al., 2005). For example, Lee and Marsden (1994) followed 31 patients with CO poisoning sequelae for a year (Lee and Marsden, 1994). Eight had a progressive course and four of the eight died. Twenty-three had a delayed relapse after an initial recovery period of approximately 20 days. Nine of these developed a parkinsonian state with behavioral and cognitive impairment, but 14 of the cases progressed further and were bed bound; the deterioration to either condition occurred rapidly over a few days to a week, and 3 died. The mean initial CO hemoglobin level was not different in the two groups. Brain computed tomography (CT) scans were obtained at the onset of sequelae in both groups. Ten patients had a normal CT scan, 13 had white matter low-density lesions, and 4 had low-density lesions in the globus pallidus, a structure in the basal ganglia of the brain involved with the regulation of voluntary movement. The mechanisms for these sequelae may involve ischemia–reperfusion injury (Mathieu et al., 1996; Wattel et al., 1996) or cerebral biochemical and metabolic changes (Pall, 2001; Thom et al., 2004).

12.7.1.2 Chronic Exposures and Their Effects

Cardiovasculr Kristensen (1989) examined the relationship between CVD and exposures in the work environment and concluded that CO exposure increases the acute risk of CVD, but that there was no lasting atherosclerotic effect. Stern et al. (1988) performed a retrospective study of heart disease mortality in 5529 bridge and tunnel officers. The socioeconomic and smoking characteristics of the two groups were well matched, and the populations were limited to individuals who were assigned their positions and did not transfer between groups. The bridge officers experienced significantly lower CO exposures than the tunnel officers. Significantly elevated risk of coronary artery disease was found in the tunnel officers relative to the bridge officers (61 deaths observed vs. 45 deaths expected); however the risk declined after cessation of exposure, dissipating substantially after 5 years. Although convincing evidence from animal studies is lacking, CO may elevate plasma cholesterol and does appear to enhance atherosclerosis when serum cholesterol is greatly elevated by diet (Penney and Howley, 1991).

Lung Function In addition to cardiovascular effects, individuals exposed to relatively high CO concentrations of CO in both indoor and outdoor environments may also be at risk of lung function decreases. It should be noted, however, that in addition to CO, these individuals were also exposed to high concentrations of other products of combustion and pyrolysis, and it is difficult to separate the effects of CO from those of these other compounds, many of which are known to be respiratory system irritants. Firefighters exhibit losses of lung function associated with acute and chronic smoke and CO exposure. Bronchoalveolar lavage (BAL) fluid from firefighters after smoke exposure shows evidence of inflammation (Bergstrom et al., 1997), and decrements in function (days in which fires were fought compared with routine work shifts without fires) lasted for up to 18 h in some individuals (Sheppard et al., 1986). However, Slaughter et al. (2004) did not find a significant association between CO exposure and pulmonary function deficits in firefighters after exposures during controlled burns. In a study of matched populations of tunnel and bridge officers (Evans et al., 1988), whose primary job was to collect tolls, tunnel officers consistently had greater concentrations of COHb, compared with a population of bridge officers with a similar demographic profile that performed essentially similar work, but the differences were small. Lung function measures of forced vital capacity (FVC) and forced expiration volume in 1 second ($FEV_{1.0}$) were slightly reduced in tunnel vs. bridge officers.

No changes in FVC or $FEV_{1.0}$ were observed in loggers who complained of dyspnea and eye, nose, and throat irritation after felling trees and cutting logs using chain saws (Hagberg et al., 1985). Exposures to typical ambient concentrations of CO, both outdoors and indoors, have not been significantly associated with pulmonary diseases or lung function decrements (Lebowitz et al., 1987), although other components of ambient pollution do show some significant associations (O_3, PM), as do the use of gas stoves and tobacco smoking.

Pregnancy Outcomes Alderman et al. (1987) performed a case–control study of the association between low-birth-weight infants and maternal CO exposures in approximately 1000 cases in Denver. CO exposures were assigned to residential locations using fixed-site outdoor monitor data. After controlling for race and education (a surrogate for smoking behavior), no relationship was detected between the assigned CO exposure during the last 3 months of pregnancy and lower birth weights. The investigators suggested that failure to directly account for unmeasured sources of CO exposure, such as smoking, emissions from gas appliances, and exposures to vehicular exhaust, was a limitation of the study design. They also noted that the use of personal monitors for CO would have permitted a more direct evaluation of the potential relationship (exposure evaluations could be made after cases were identified, the relationship of personal to fixed-site assignments could be established, and then applied to the retrospective fixed-site data, author's note). More recent studies have borne out the association between CO exposure and low birth weight. A 1 ppm average CO exposure during the first trimester of pregnancy was associated with a 23-g decrease in infant birth weight (Gouveia et al., 2004). Similar results have been obtained in Los Angeles, CA, and Sydney, Australia (Ritz and Yu, 1999; Mannes et al., 2005; Salam et al., 2005).

Fetotoxicity has been demonstrated in laboratory animal studies at elevated (125 ppm) CO levels (Singh and Scott, 1984). Moderate CO exposure can alter neuron development and modify neurochemical signaling in rats (Fechter, 1987). Prenatal exposure can adversely alter responses of dopaminergic neurons (Cagiano et al., 1998) and alter development of serotonergic and adrenergic neurons (Storm and Fechter, 1985; Fechter et al., 1986) that can lead to behavioral changes later in life (Fechter and Annau, 1980).

12.8 EXPOSURE AND RELATIONSHIP TO COHB CONCENTRATIONS

A study of over 1500 nonsmoking people sampled as part of the second National Health and Nutrition Examination Survey (NHANES II) demonstrated that CO concentrations measured at fixed-site monitors only accounted for 3% of the variance in blood COHb concentrations using Spearman rank order correlations between the fixed-site monitor readings and sampled blood COHb concentrations (Wallace and Ziegenfus, 1985). They found that the correlations were not significant ($p < 0.05$) for 24 of the 36 sampling stations in 20 U.S. cities surveyed. The failure of 8-h average concentrations to correlate strongly with measured COHb concentrations indicates that outdoor monitoring data does not adequately reflect personal CO exposure.

Using data from personal monitors worn by a probability sample of over 1500 residents of Denver and Washington, DC, Akland et al. (1985) found that over 10% of Denver residents and 4% of Washington residents were exposed during the wintertime to CO concentrations in excess of 9 ppm for 8 h or longer.

12.8.1 Controlled Human Studies

Several clinically based studies have provided a relatively coherent picture of the effects of CO on the cardiopulmonary system. Some of the key studies cited in the 1991 and 1999 CO criteria documents (U.S. EPA, 1991, 1999), as well as those published since then, are described below.

12.8.1.1 Cardiovascular Effects Individuals with IHD have limited ability to compensate for increased myocardial O_2 demands during exercise, and exercise testing is often used as a means for evaluating the severity of an individual's cardiovascular impairment. Four useful parameters of ischemia that are measurable during exercise testing are ST-segment depression (at least 1 mV of horizontal or downsloping depression of the ST segment of an electrocardiographic tracing persisting for 70 ms in three successive complexes), exercise-induced angina (chest pain during exercise, which is increased with effort and then resolves with rest; some individuals may experience pain in the jaw, neck, or shoulder areas), impaired work capacity (maximum work levels expressed as a percentage of normographically predicted, normal values) (Bruce, 1971, 1974, 1994), and an inadequate blood pressure response to exercise [blood pressure that falls on exercise (*test would be discontinued*) or fails to rise more than 15 mmHg at a work level of at least 40% of the predicted norm]. There are some individuals who exhibit one or more of these responses during exercise who do not have abnormal coronary arteries, as determined by measuring luminal narrowing using angiographic methods; however these parameters, taken in combination, can identify 85–90% of people with coronary artery disease (Allison et al., 1996). However, exercise testing alone has limitations with respect to its ability to predict future cardiac events (Fubini et al., 1992). Since CO exposure impairs myocardial O_2 delivery, CO exposure would be expected to worsen symptoms of ischemia in individuals with coronary artery disease. Therefore, exercise tests of such individuals have been an important means of providing quantitative and dose-related estimates of the potential impact of CO on health.

Sheps et al. (1987) exposed 30 subjects with IHD, aged 38–75 years, to CO (100 ppm) or air, during a 3-day, randomized, double-blind protocol, to achieve an average post-exposure COHb concentration of 3.8% on the CO exposure day (COHb on the air exposure

day averaged 1.5%). After exposure to either CO or air, subjects performed an exercise stress test. All exercise tests were performed with the subjects in a supine position using a cycle ergometer at the same time of day, with the subjects in a fasting state. The workload was set at 0 for the first min, was then increased to 200 kp-m for the next 4 min, and was increased in 50–100 kp-m increments at 4 min intervals until a maximal level was achieved. Exercise was continued until anginal pain required cessation of exercise, fatigue precluded further exercise, or blood pressure plateaued or decreased, despite the increase in workload. All of the subjects were nonsmokers and had documented evidence of IHD, defined by either exercise-induced ST-segment depression (1 mV or more), exercise-induced angina, or abnormal left ventricular ejection fraction response to exercise (failure to increase ≥5 units from rest). Not all of the subjects in the study, which included both men and women, reported exercise-induced angina, and the CO exposure produced only small, and nonsignificant, decreases in time to onset of angina (1.9%) and maximal exercise time (1.3%) compared with air exposures (Sheps et al., 1987). Times to significant ST decreases, double product (DP) (heart rate × systolic blood pressure) at significant ST depression and maximal DP were similar for both air and CO exposure conditions. DP, in the absence of arterial obstructions, can be used to estimate myocardial O_2 consumption during dynamic exercise (Sim and Neill, 1974). The change in ejection fraction (rest to maximal) was slightly lower for CO exposures (air = 3.5%, CO = 2%; $p = 0.049$). The authors concluded that there were no clinically significant effects of low-level CO exposures at COHb concentrations of 3.8%.

Adams et al. (1988) subsequently extended the above study to an average post-exposure COHb concentration of 5.9%, during exercise, using an identical protocol and 30 subjects (22 men, 8 women; mean age 58 years). Not all of the subjects in this study experienced exercise-induced angina, and only 21 subjects reported angina on both exposure days. The time to onset of angina in these 21 subjects was slightly, but not significantly, decreased after CO exposure (10.3%) compared with air exposure. An actuarial analysis of the data from all subjects reporting angina indicated that subjects were likely to experience angina earlier during stress on the CO day ($p \leq 0.05$). The left ventricular ejection fractions at rest were the same after both air and CO exposures; however the level of submaximal ejection fraction was significantly higher after air, when compared with the CO exposure (3.3%; $p \leq 0.05$), and the change in ejection fraction, from rest to submaximal exercise, was significantly lower after CO exposure, compared with air exposure (air = 1.6% and CO = −1.2%; $p \leq 0.05$). No statistically significant exposure-related differences were seen for either maximal ST-segment depression, time to onset of significant ST-segment depression, or maximal DP. The authors concluded that exposures to CO resulting in COHb concentrations of about 6% significantly impaired exercise performance in subjects with IHD.

Kleinman et al. (1989) exposed 24 nonsmoking male subjects with stable angina and positive exercise tests to 100 ppm CO or air to achieve an average COHb concentration of 2.9%, during exercise, on the CO exposure day. Subjects ranged in age from 51 to 66 years, with a mean age of 59 years. All but one of the subjects had additional confirmation of IHD, such as previous myocardial infarction, coronary artery bypass surgery, positive thallium isotope exercise test, or positive angiogram or cardiac catheterization. Subjects were exposed to CO or to clean air in a randomized, double-blind protocol. Subjects performed an incremental exercise test on a cycle ergometer until the point at which they could detect the onset of their typical anginal pain and then stopped exercising. Workload was set at 50 W initially and was increased in 25-W increments at 3-min intervals. Blood pressure was measured at the end of each 3 min of exercise, ECG tracings were taken at the end of

each minute, and respiratory gas exchange was measured at 15-s intervals and averaged for each minute. Data were analyzed statistically using a 2-factor analysis of variance and 1-tailed tests of significance. The time to onset of angina was decreased after CO exposure (5.9%; $p = 0.046$) relative to air exposure. The duration of angina was longer after CO exposure compared with air exposure (8.3%), but this change was not statistically significant. Oxygen uptake at the angina point was slightly reduced after CO exposure compared with air exposure (2.2%; $p \leq 0.04$), but the increase in O_2 uptake with increasing workload was similar on both exposure days. A subgroup of 11 subjects who, in addition to angina, exhibited arrhythmias or ST-segment depressions during exercise showed a greater reduction in time to angina after CO exposure, compared with air exposure (10.6%; $p \leq 0.016$), than the overall group. The time to significant ST-segment depression was significantly reduced for the eight subjects with this characteristic after CO exposure, compared with air exposure (19.1%; $p \leq 0.044$). The number of subjects exhibiting exercise-induced ST-segment depression identified in this study was small; however those subjects in whom angina preceded detection of ST-segment changes would not have been identified in the protocol used because exercise was stopped at the point of onset of angina.

A large multicenter CO exposure study was conducted in three different cities (Allred et al., 1989a, 1989b, 1991). Sixty-three men with documented coronary artery disease underwent exposure to air, 117 ppm CO, or 253 ppm CO, on 3 separate days in a randomized, double-blind protocol, followed by an incremental treadmill exercise test. Average COHb concentrations of 2.2 and 4.3%, during exercise, were achieved on the two CO exposure days (2.0 and 3.9%, respectively, at the end of exercise). All of the subjects were males, aged 41–75 years (mean age of 62 years), with stable exertional angina and a positive exercise stress test with ST-segment changes indicative of ischemia. In addition, all had objective evidence of coronary artery disease indicated by at least one of the following: (1) angiographic evidence of at least 70% obstruction in one or more coronary arteries, (2) previous myocardial infarction, and (3) a positive thallium stress test.

On each of the exposure days, the subject performed a symptom-limited treadmill exercise test, was exposed to one of the three test atmospheres (clean air, 117 ppm CO, or 253 ppm CO), and then performed a second exercise test. The subjects exercised until the subjects (1) were too fatigued to continue, (2) experienced severe dyspnea, (3) experienced grade 3 angina (on a subjective scale where grade 1 indicated the first perception of angina and grade 4 represented the worst angina the subject had ever experienced), (4) exhibited ECG changes (ST depression ≥3 mV or important arrhythmias), (5) exhibited high systolic (≥240 mmHg) or diastolic (≥130 mmHg) blood pressure, (6) exhibited a 20 mmHg drop in systolic blood pressure, or (7) made a request.

The time to onset of angina and the time to significant ST depression were determined for each test, and the percent changes (pre-exposure vs. post-exposure) for the two CO exposure days were compared to the same subject's response to the randomized clean air exposure. The time to onset of angina was significantly reduced by CO exposure in a dose-dependent manner (4.2% at 2% COHb, $p = 0.054$; 7.1% at 4% COHb, $p = 0.004$). Linear regressions of time to angina vs. COHb concentrations for each subject indicated that time to angina decreased $1.9 \pm 0.8\%$ for every 1% increase in COHb ($p \leq 0.01$). The time to onset of 1-mV ST-segment depression was also reduced by CO in a dose-dependent manner (5.1% at 2% COHb, $p = 0.02$; 12.1% at 4% COHb, $p \leq 0.0001$) compared with the clean air exposure. There was a decrease of approximately $3.9 \pm 0.6\%$ in time to ST depression for every 1% increase in COHb ($p \leq 0.0001$). There was a significant correlation between the percent change in the time to onset of angina and the time to onset of ST depression ≥1 mV ($p \leq 0.0001$).

There is some evidence that acute hypoxia that can result in myocardial ischemia and reversible angina can also lead to arrhythmias (Jacobs and Nabarro, 1970; Farber et al., 1990; Dahms et al., 1993). Hinderliter et al. (1989) exposed 10 subjects, with IHD and no ventricular ectopy at baseline, to air, 100 ppm CO, and 200 ppm CO; COHb averaged 4 and 6% on the two respective CO exposure days. The exposures were randomized and double blinded. Following exposure, each subject performed a symptom-limited supine exercise test; ambulatory electrocardiograms were obtained prior to exposure, during exposure, during exercise, and over a 5-h post-exercise period. The ECGs were analyzed for the frequency and severity of arrhythmias. Eight of the 10 subjects demonstrated evidence of ischemia on one or more of the exposure days (angina, 1-mV ST-segment depression, or abnormal ejection fraction response). There were no CO-related increases in the frequency of premature ventricular beats and no multiple arrhythmias occurred, and it was concluded that low-level CO exposure (4–6% COHB) was not arrhythmogenic in patients with coronary artery disease and no ventricular ectopy at baseline. However, researchers from this same team (Sheps et al., 1990) reported that for a larger study population (41 subjects) with some evidence of ventricular ectopy, exposed to air, 100 ppm CO, and 200 ppm CO in a similar protocol, the frequency of single ventricular premature depolarizations (VPDs) per hour increased ($p \leq 0.03$) from 127 ± 28 (mean $\pm$ SD) after the air exposure to 168 ± 38 after exposure to achieve a COHb concentration of 6%.

The frequency of multiple VPDs per hour increased approximately threefold during exercise at 6% COHb, compared with air exposure ($p \leq 0.02$). No significant differences in these parameters occurred after exposures that achieved COHb concentrations of 4%, compared with air exposures. The subjects who exhibited single VPDs with increased frequency after CO exposure were significantly older than the subjects who had no increased arrhythmias. The subjects who exhibited increased frequencies of multiple VPDs were older, exercised for longer durations, and had higher peak workloads during exercise than those who did not have complex arrhythmias. Leaf and Kleinman (1996) reported evidence of effects of CO exposure on cardiac rhythm after relatively low CO exposures (3% COHb) in a small group of volunteers with coronary artery disease that exhibited abnormal rhythms on one or more exercise tests.

In all of the above clinical studies of CO-related effects, subjects with coronary artery disease were maintained on individualized regimens of medications, some of which might interact with CO-induced responses, increasing the apparent variations in observed responses. Specifically, blockade of β-adrenergic receptors (Melinyshyn et al., 1988) and α-adrenergic receptors (Villeneuve et al., 1986) was shown to modify hemodynamic responses to CO in animal studies. Examination of the potential influence of medications on observed responses to CO could provide additional insights on the possible mechanisms of action of CO in individuals with coronary artery disease.

12.8.1.2 Cardiopulmonary Effects (Lung Function and Exercise Tolerance) Normal Individuals Reduction of O_2 delivery could reduce the ability to perform work in healthy individuals. Studies of the cardiopulmonary effects of CO have demonstrated that maximal O_2 uptake during exercise (V_{O_2} max) decreases linearly with increasing COHb concentrations ranging from 2.3 to 35% COHb in healthy volunteers (Horvath, 1981; Shephard, 1984; Horvath et al., 1988b). The linear relationship can be expressed as percent decrease in V_{O_2} max = 0.91 [%COHb] + 2.2. Changes in V_{O_2} max are significant because they represent changes in an individual's maximal aerobic exercise (or work) capacity (Ekblom et al., 1975). Klausen and colleagues exposed 16 male smokers to CO (5.26%

COHb) and compared the effects on maximal exercise performance to performance after 8 h without smoking and performance after smoking three cigarettes (4.51% COHb) (Klausen et al., 1983). Both exposures reduced V_{O_2} max by about 7%, but exercise time was decreased more after cigarette smoking than after CO exposure, suggesting that other components of smoke may contribute to the observed effects (Ekblom and Huot, 1972; Klausen et al., 1983).

In a controlled laboratory test, Horvath et al. (1988a) exposed 23 subjects (11 males, 12 females) to 0, 50, 100, and 150 ppm CO at four different simulated altitudes (55, 1524, 2134, and 3048 m); each exposure was followed by an incremental exercise test. COHb concentrations ranged from $0.5 \pm 0.2\%$ to $5.6 \pm 0.4\%$ of saturation after sea-level exposures. There was a significant effect of increased altitude on decreased work performance and V_{O_2} max. While CO exposure tended to slightly decrease these parameters at all altitudes, the statistical analyses did not demonstrate a CO × altitude interaction, suggesting that these factors acted independently, and perhaps additively, but not synergistically. The female subjects appeared to be more resistant to the hypoxic effects of altitude than the males. The rate of CO uptake (i.e., formation of COHb) decreased with increasing altitude, in part due to the reduced driving pressure of CO at altitude. In this study, significant fractions of CO were moved to extravascular spaces during exercise, probably in temporary combination with myoglobin, when exercise levels exceeded 80% of V_{O_2} max. (i.e., COHb concentrations increased 5-min post-exercise compared with concentrations measured at the point of maximum workload). While this might suggest a mechanism for CO acting, in part, by directly affecting cardiac myoglobin, evidence for direct cardiotoxicity of CO is still lacking.

Horvath and Bedi (1989) demonstrated that longer-term, low-level (9 ppm for 8 h) exposures at 2134 m result in lower COHb concentrations than the same exposure at 55 m, again suggesting slower CO uptake during altitude exposure. However endogenous CO production is increased in rats chronically maintained at high altitudes (1000–6000 m) (McGrath, 1989, 1992), suggesting that high-altitude residents have higher initial COHb concentrations and might therefore achieve 2% or greater COHb levels (the COHb level associated with the CO NAAQS) more quickly than sea-level residents. It has been reported that unacclimated workers exposed to about 25 ppm CO at an altitude of 2.3 km above sea level exhibited significantly increased symptoms of headache, vertigo, fatigue, weakness, memory impairment, insomnia, and heart palpitations compared with local residents (Song, 1993). The subjects in these human clinical studies of exercise tolerance have been relatively young, and all were in good health. There is not sufficient information available to determine if relationships between CO exposure, altitude, and COHb concentrations would be similar for individuals with coronary artery disease, chronic lung diseases, or anemia or in pregnant women.

Kleinman et al. (1998) and Leaf and Kleinman (1996) demonstrated that hypoxia due to high altitude and CO exposure may cause additive effects on exercise tolerance, hemodynamic changes, and cardiologic parameters. The subjects were older men with confirmed coronary artery disease.

12.8.1.3 Individuals with Chronic Obstructive Pulmonary Disease (COPD) Individuals with COPD usually have limited exercise tolerance because of their low ventilatory capacity that can result in desaturation of arterial blood and hypoxemia (a relative deficiency of O_2 in the blood) and hypoxia (a relative deficiency of O_2 in some tissue) during exercise. Exercise performance in such individuals can be improved by providing supplemental O_2

(Kramer et al., 1999; Knower et al., 2001). Reduced O_2-carrying capacity of blood due to formation of COHb could exacerbate this limitation; hence individuals with COPD could represent a potentially sensitive group. Aronow et al. (1977) exposed 10 men with COPD, aged 53–67 years, to 100 ppm CO for 1 h, achieving increases in COHb from baseline concentrations of 1.4% to post-exposure concentrations of 4.1%. Mean exercise time was reduced by 33%. Calverley et al. (1981) exposed six smokers (who stopped smoking 12 h prior to testing) and nine nonsmokers to 200 ppm CO for 20–30 min (increasing COHb concentrations to between 8 and 12% COHb above baseline COHb) and measured the distance each subject walked in a 12-min period. All of the subjects had severe bronchitis and emphysema. Significant decreases in walking distance were seen in individuals with 12.3% COHb or greater (levels that are seen in smokers with COPD).

Individuals with severe COPD, even without clinically apparent coronary artery disease, may exhibit exercise-related cardiac arrhythmias. The exercise-induced arrhythmias were associated with arrhythmias at rest, but were not related to the severity of pulmonary disease, O_2Hb desaturation, or ECG evidence of chronic lung disease (Cheong et al., 1990). Sheps et al. (1990, 1991) studied exercise-related arrhythmias in CO-exposed subjects with coronary artery disease. Overall, the information available on individuals with COPD are consistent with the hypothesis that they represent a population potentially at risk of CO-related health effects during submaximal exercise, as may occur during normal daily activities. The available data are however based on population group sizes that are too small and too diverse with respect to disease characteristics to draw firm conclusions.

12.9 NEUROTOXICOLOGICAL AND BEHAVIORAL EFFECTS

The neurologic effects of relatively high-level acute CO exposures have been well documented (Gilbert and Glaser, 1959; Remick and Miles, 1977; Lacey, 1981). Subtle neurotoxic effects associated with lower-level CO exposures may be underreported or not associated with CO exposure because the symptoms, which resemble those of a flu-like viral illness, may be misdiagnosed (Balzan et al., 1996; Foster et al., 1999; Raub et al., 2000; Ares et al., 2001). Population-based studies on the potential neurotoxicological and behavioral effects of chronic CO exposure at ambient concentrations have not been reported. However, several clinical studies of CO-related sensory effects that evaluated several different parameters under controlled laboratory conditions showed little or no effect at COHb levels up to 17%. Hudnell and Benignus (1989) demonstrated, in a double-blind study, that visual function in healthy, young adult males, as defined by measurements of contrast threshold, luminance threshold, and time of cone/rod break, was not affected by COHb concentrations maintained at 17% for over 2 h. Von Restorff and Hebisch (1988) reported no changes in time to dark adaptation and sensitivity after adaptation, at COHb concentrations ranging from 9 to 17%. One earlier study had demonstrated CO-induced visual threshold effects, that is, a slowing of dark adaptation (McFarland, 1973). However, the number of subjects tested was small, and documentation of the study was scant. Recent studies of temporal resolution of the visual system associated with elevated COHb levels have been reported; however those exposures involved cigarette smoking. The normal capacity for increased blood flow velocity in the central retinal artery in darkness was markedly reduced in smokers, which might explain the reduced dark vision after recent smoking, reflecting the combined effects of an increased blood viscosity and the

vasoconstrictive action of nicotine, in addition to the reduced capacity of the blood to transport O_2 due to COHb (Havelius and Hansen, 2005).

In general, neurotoxicity at COHb levels near 5% has not been convincingly demonstrated in normal healthy adults (Benignus et al., 1987), even though several early studies had suggested possible effects on critical flicker fusion at COHb levels at or below 9% (Vonpost-Lingen, 1964; Weber et al., 1975; Seppanen et al., 1977). Benignus et al. (1987) exposed 24 healthy nonsmoking males to 0 or 100 ppm CO for 4 h (mean COHb = 8%). They measured the subject's ability to perform fast and slow tracking tasks (maintaining the position of a moving point of light on a computer screen using a joystick) and monitoring tasks (judging the brightness of two red spots on a computer screen) once per hour during exposure. CO exposure increased tracking errors, but did not interfere in the monitoring task. An earlier study demonstrated significant decrements in both tracking and monitoring tasks at a COHb concentration of 4.6%, but not at 3.5% (Putz et al., 1979). A large number of studies have investigated the effects of CO on several other behavioral parameters; however effects in general are only seen at COHb concentrations above 5%, and there are inconsistencies between the study results. Other studies, published in 1984 and later, showed interactive effects of exercise and CO exposure (>7% COHb) on cognitive tasks (Bunnell and Horvath, 1988), but no changes in visually evoked response potentials in young (23 years) and older (69 years) subjects were observed at 5.3% COHb (Harbin et al., 1988).

12.10 FETAL DEVELOPMENTAL AND PERINATAL EFFECTS

There are both theoretical reasons and supporting experimental data that indicate the fetus being more susceptible to the effects of CO than the mother. Fetal Hb has greater affinities for CO and O_2 than maternal Hb. The partial pressure of O_2 in fetal blood is about 20–30% of that in maternal blood because of the greater O_2 affinity of fetal Hb. In addition, COHb shifts the O_2Hb dissociation curve to the left in maternal blood, reducing the transfer of O_2 across the placenta from maternal to fetal circulation. As in adults, the nervous and cardiovascular systems of the fetus are the most sensitive to the effects of CO. For humans, information is available for women who smoked during pregnancy or was acutely exposed to CO; however most of the available reports did not characterize the relevant CO exposure levels and cannot, in general, rule out toxic effects of co-contaminants. Acute CO exposure may play a role in fetal death (Caravati et al., 1988), and environmental exposures, as well as maternal smoking, have been linked to SIDS in some (Hoppenbrouwers et al., 1981; Hutter and Blair, 1996), but not all (Variend and Forrest, 1987). Prenatal exposure to CO affects cholinergic and catecholaminergic pathways in the medulla of the guinea pig fetus, particularly in cardiorespiratory centers, regions thought to be compromised in SIDS (Tolcos et al., 2000). Additional animal studies suggest that high-level maternal CO exposures can have other significant neurotoxicological consequences for the fetus, including disruption of neuronal proliferation and possible disruption of markers of neurochemical transmission (Fechter, 1987). Neonatal mortality and low birth weights are more prevalent in children born in high-altitude regions (Moore, 1987; Yip, 1987; Unger et al., 1988), suggesting high-altitude hypoxia interuterine growth and further suggesting that low birth weights in children born to women who smoke during pregnancy could possibly be a result of CO-induced hypoxia. Immune system changes have also been noted in rats exposed to CO prenatally; however the changes may be reversible (Giustino et al., 1993).

12.11 CO AS A RISK FACTOR IN CARDIOVASCULAR DISEASE DEVELOPMENT

Evidence from population-based studies indicates that workers exposed to CO in combination with other combustion products from automobile exhaust (Stern et al., 1988) and other workers as well (Kristensen, 1989) have increased risk of development of atherosclerotic heart disease. Also, individuals hospitalized for myocardial infarction frequently exhibit higher COHb concentrations than individuals hospitalized for other reasons (Leikin and Vogel, 1986). Central to the development of atheromatous plaques is the deposition and retention of fibrinogen and lipids within the arterial wall. It is known that cigarette smoke increases the permeability of the arterial wall to fibrinogen. Allen and colleagues (Allen et al., 1989; Allen and Browse, 1990) demonstrated in a canine model that both CO and nicotine in cigarette smoke might produce an atherogenic effect, but they act via different mechanisms. CO increases arterial wall permeability and nicotine reduces clearance of deposited fibrinogen. Activation and dysfunction of blood platelets is associated with production of chemokines that elicit the migration of smooth muscle and inflammatory cells into the vascular intima, which is a major factor in the process of atherogenesis (Munro and Cotran, 1988; Nomoto et al., 1988; Weber, 2005) and in cardiac-related sudden deaths due to the platelets' role in the initiation of thrombosis (Harker and Ritchie, 1980; Meade, 1992). There is a significant association of CO poisoning and incidents of deep vein thrombosis, a condition that can be life threatening (Chung et al., 2015).

Studies have reported biochemical evidence that cigarette smoking induced both platelet and vascular dysfunctions in apparently healthy individuals (Folts et al., 1990; Krupski, 1991). Production of platelet-derived growth factor (PDGF) by endothelial cells is upregulated in response to hypoxia and is a major growth factor for vascular smooth muscle cells and a powerful vasoconstrictor (Kourembanas et al., 1990; Humar et al., 2002). Platelet dysfunction may also be a contributory cause of thrombosis during pregnancy and may increase fetal mortality and morbidity among women who smoke (Davis et al., 1987). Abnormalities in platelet aggregation occur after CO exposure (Mansouri and Perry, 1982) and may be linked to guanylate cyclase activation (Brune and Ullrich, 1987). When 10 healthy nonsmokers were exposed passively to cigarette smoke (in hospital corridors), resulting in a small increase in COHb concentration from $0.9 \pm 0.3\%$ to $1.3 \pm 0.6\%$, before and after passive exposure, respectively (Davis et al., 1989), they showed evidence of changes in platelet aggregation and endothelial cell damage. The changes in endothelial cell counts (pre- to post-exposure) were significantly correlated to changes in COHb concentrations from before to after exposure, but plasma nicotine levels were not. The contribution of CO relative to other components of tobacco smoke in causing platelet dysfunction is not established.

12.12 SUMMARY AND CONCLUSIONS

The current U.S. CO NAAQS was designed to protect susceptible individuals from exposures that would result in COHb concentrations of 2% and above. Occupational standards are designed to protect workers from concentrations of 5% COHb. Studies of individuals with coronary artery disease and residents of New York, NY, Denver, CO, Washington, DC, and Los Angeles, CA, suggest that susceptible individuals frequently exceed 2% COHb in cities that frequently exceed NAAQS. Control of exposures is difficult because the sources of CO are widespread, the distribution of ambient CO is very nonuniform, and emissions

from unregulated sources, especially indoors, probably contribute substantially to individual CO doses. The distributions of COHb concentrations in workers, also very nonuniform, may also often reach the 5% level.

The contribution of CO to the aggravation of symptoms of myocardial ischemia is reasonably well defined for a selected subset of people with existing coronary artery disease. The individuals comprising the populations tested in the various studies on which this conclusion was drawn were carefully selected to have sufficiently pronounced disease such that effects would be measurable, but they were also sufficiently healthy so that they could perform moderate levels of exercise with minimal risk. Thus, more impaired individuals, who might presumably be at equal or greater risk of detrimental CO-induced health effects, and relatively asymptomatic individuals, so-called silent ischemics, have not been well characterized. Incorporation of broader, possibly more representative, subject populations into the clinical studies of Sheps et al. (1990) and Adams et al. (1988) significantly increased the variance in subject responses and increased the difficulty of attributing statistical significance to observed findings.

Convincing documentation for effects of CO on other potentially susceptible individuals at ambient exposure levels is becoming available. The most extensive body of evidence of CO effects on pregnant women, fetuses, and neonates comes from the literature on smoking and from acute, high-level accidental CO exposures. In most cases actual CO exposures are poorly, if at all, documented, and the contribution of co-pollutants to the observed effects cannot be assessed. However, animal studies demonstrating developmental changes and associations between environmental CO and SIDS indicate that risks to pregnant women, fetuses, and neonates may be important.

The importance of occult CO exposures leading to clinically significant symptoms and effects is well appreciated. The large number of such incidents suggests the potential that there may be many undetected incidents that lead to subclinical manifestations but are ignored if they are not serious enough to prevent relatively normal daily activities. The home environment is very poorly characterized with respect to indoor pollutant levels, and given the apparently large potential for CO-related health effects, the home indoor environment should be the focus of significant new study initiatives. The potential downstream risk of serious long-term effects, for example, the increased risk of developing dementia following CO poisoning (Wong et al., 2016), is less well appreciated, and requires further research, especially as to the possible effectiveness of hyperbaric O_2 treatment in alleviating neurological sequelae of CO poisoning (Chan et al., 2016; Lai et al., 2016).

Indoor air quality guidelines developed by the World Health Organization and Health Canada, among others, are set to prevent exposures that would increase COHb levels above 2.0% (Barn et al., 2018), a target based on experimental evidence on toxicodynamic relationships between COHb and cardiac performance among persons with CVD. However, exposure conditions (e.g., exercise or other forms of exertion) and physiological conditions that adversely alter CO uptake and CO elimination can increase the severity of outcomes.

It would seem, from this review, that both occupational and ambient standards are placed near the limits at which significant effects can be seen, albeit in sensitive individuals, thus affording a narrow if any margin of safety. The number of studies suggesting roles for CO in the development of CVD and in infant mortality is increasing, but not yet conclusive. Additional studies under well-controlled conditions with accurate estimates of CO exposure history should be a priority. Accidental CO poisoning is still an important cause of sickness and death. The effectiveness of CO alarm systems in the home as well as the workplace should be further assessed, emphasized, and disseminated.

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13

CHROMIUM

HONG SUN AND MITCHELL D. COHEN

13.1 INTRODUCTION

Chromium (Cr) is one of the more abundant elements on Earth, ranging from 0.1 to 250 ppm (Langard and Costa, 2015). First discovered by the French chemist Nicolas Louis Vauquelin in 1779, Cr was named after the Greek word "chroma"—meaning color—as it forms a variety of colorful compounds (Jacobs and Testa, 2005). Cr occurs naturally in chromite ore, a major source of mined Cr metal, as well as in various Cr compounds. Due to its high resistance to heat and chemicals, Cr metal is widely used in the stainless steel and alloy industries, as a superalloy in jet engines, and in other metallic alloys. Several Cr compounds are commonly used in leather/pelt tanning and electroplating or as additives in the production of pigments, catalysts, corrosion inhibitors, and wood preservatives (Jacobs and Testa, 2005; Langard and Costa, 2015).

Cr exists primarily in two major valence states, that is, trivalent [Cr(III)] and hexavalent [Cr(VI)]. Of the two, Cr(III) is the most stable and exists naturally in chromite ore. Cr(VI) rarely occurs in nature and is mainly a by-product of industrial activities (Zhitkovich, 2011). While Cr(VI) and Cr(III) not only differ in oxidation states, they also diverge in chemical properties and toxicities. Cr(VI) is highly toxic, and exposure leads to a wide variety of cellular injuries, including DNA damage and chromosomal aberrations (O'Brien, 2003; Nickens et al., 2010). In contrast, Cr(III) is known to increase insulin sensitivity and elicits beneficial effects on glucose and lipid metabolism (Schwarz and Mertz, 1959; Vincent, 2017). While Cr(III) compounds are generally insoluble in water [with the exceptions of Cr(III) acetate, Cr(III) nitrate, and Cr(III) chloride hexahydrate], Cr(VI) compounds can be either water soluble or insoluble (ATSDR, 2012). It is worth noting that the beneficial roles of Cr(III) have been challenged (ATSDR, 2012; Vincent, 2017). In 2014, the Panel on Dietetic Products, Nutrition and Allergies (NDA) re-evaluate Cr(III) as an essential element for humans, concluding there was no evidence of beneficial effects associated with Cr intake in healthy subjects (EFSA, 2014).

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Exposure to Cr can occur via three major routes: inhalation of airborne Cr-containing particles, oral ingestion of Cr-rich or Cr-contaminated foods or water, and direct dermal contact with Cr-containing products (ATSDR, 2012; IARC, 2012). The primary source of Cr exposure in non-occupational (environmental) settings comes from ingestion of foods and drinking water; occupational exposures to Cr involve all three major routes (ATSDR, 2012; Langard and Costa, 2015). Symptoms of acute toxicity include allergic contact dermatitis (ACD), skin ulcers, nasal membrane inflammation, and nasal ulceration. Chronic occupational exposure can result in nasal septum perforations, rhinitis, liver damage, pulmonary congestion, edema, and nephritis (ATSDR, 2012; Langard and Costa, 2015). Epidemiological studies have reported high incidences of lung cancers among Cr workers chronically-exposed to Cr(VI) through inhalation (Cohen et al., 1993; Gibb et al., 2000a; Park et al., 2004; Costa and Klein, 2006). An early epidemiological study showed that 21.8% of deaths among Cr-exposed workers were due to respiratory cancers, while only 1.4% of deaths were attributed to respiratory cancers in unexposed reference populations (ATSDR, 2000). As a result of these types of findings, the International Agency for Research on Cancer (IARC) classified Cr(VI) as a Group I carcinogen, that is, carcinogenic to humans (IARC, 1990).

13.2 EXPOSURE

13.2.1 Chromium Sources

13.2.1.1 Soil and Water The presence of Cr in soil and/or water can originate from natural sources, including the weathering of rocks, precipitation, diagenetic reactions, and volcanic eruption (Langard and Costa, 2015). Background levels of Cr in soil vary with location, and content may be as high as 3000 ppm in certain areas of the world (Shahid et al., 2017). The soils in the United States contain on average 54 ppm Cr (Burt et al., 2003). The levels of Cr in water (EPA, 1984, 1998) vary and are dependent on salinity. Average Cr concentrations in uncontaminated water are very low, about 1–30 ppb in rivers and lakes, 0.2–1 ppb in rainwater, and an average concentration of 0.3 ppb in seawater (ATSDR, 2012; Langard and Costa, 2015).

Industrial applications, however, lead to large amounts of Cr being released to soils, groundwater, and other water systems. Industrial wastewater contains levels of total Cr in the range of 0.005–525 ppm, with concentrations of Cr(VI) averaging 0.004–335 ppm (EPA, 1996). In 2009, estimated releases of Cr compounds into surface waters from domestic manufacturing and processing facilities were 486,063 pounds (TRI09, 2011). While the extent of such releases has long been known, a wider public understanding of Cr(VI) contamination of drinking water was achieved as a result of the 2000 film *Erin Brockovich* that depicted a situation that occurred in Hinkley, California. In fact, elevated levels of Cr(VI) in drinking water were reported in many U.S. cities (Sutton, 2010). Average Cr(VI) level measured in 35 U.S. cities was about 0.18 ppb, a level higher than California's proposed safe limit (0.06 ppb).Thus, this issue poses an important question regarding health effects from exposures to Cr(VI) from drinking water and demonstrates the urgency of developing effective means to control Cr release at point sources. Some controls against Cr waste entering water system could be taken by acting on release/effluents from chrome plating and metal finishing industries, as well as from textile and tanning plants (EPA, 2012).

13.2.1.2 Ambient Air Typical atmospheric concentrations of Cr are, on average, < 10 ng/m^3 in rural and semirural areas and 0–30 ng/m^3 in urban areas (ATSDR, 2012). In the remote continental areas, such as the Arctic and Antarctic regions, atmospheric Cr concentrations range from 0.005 to 2.6 ng/m^3. The vast majority of Cr present in ambient air originates primarily from industrial sources. In 2009, the estimated releases of Cr to air from facilities that produce, process, or use Cr metal of Cr compounds were 781,733 pounds (TRI09, 2011). The remainder of atmospheric Cr may come from residential fuel combustion of natural gas, oil, or coal (Seigneur and Constantinous, 1995; Kimbrough et al., 1999; Pacyna and Pacyna, 2001). The Cr content in coals and crude oils varies from 1 to 100 ppb and 0.005 to 0.7 ppb, respectively (Pacyna, 1986). Airborne particulate matter (PM) from coal-fired power plants has been shown to contain Cr in a range of 2.3–31 ppm Cr; however, these levels were reduced to 0.19–6.60 ppm Cr by fly ash collection processes (Goyer, 1986). Overall, the distribution of Cr(III) to Cr(VI) is ≈2:1 in atmospheric Cr emissions; this arises as a result of the fact that most Cr(VI) that enters air is reduced by the action of many common environmental constituents and other ambient pollutants, including acidic aerosols and dissolved sulfides (Sheehan et al., 1991; ATSDR, 2000).

13.2.1.3 Foodstuffs Levels of Cr found in foodstuffs range from < 10 to 1300 ppb, with the highest amounts in meats, fish, fruits, and vegetables (MAFF, 1985). Most foods, with the exception of herbs and condiments, probably contain < 100 ppb Cr (Guthrie, 1982; Kiovistoinen, 1982). Higher Cr levels have been measured in some beverages, with typical values in spirits, beer, and wine being 175, 300, and 450 ppb, respectively (Jenning and Howard, 1980). Recently, using improved sample preparation and analytical techniques, studies have reevaluated the amounts of Cr(VI) in a variety of foodstuffs (Mathebula et al., 2017; Hamilton et al., 2018). According to these studies, the levels of Cr(VI) were < 10 ppb in the majority of tested foodstuffs, with the exceptions of breakfast cereals that contained ≈41–470 ppb of Cr(VI).

There are several factors regulating whether certain foodstuffs, primarily vegetables, can be a major source for Cr. Apart from the fact the vegetables/fruits *a priori* must have been grown in a Cr-containing site, the levels of Cr in the soil must be below the range that would retard plant growth, and the Cr taken up from the soil must localize to those portions that are edible. Plants growing on soil with low Cr content have been estimated to contain 0.02 ppm Cr (wet weight) (Hertel, 1986). Even in soils with higher Cr concentrations, plants usually contain low levels of Cr, although in many cases higher Cr content is often found in the roots. This is most likely related to the fact that only chelated Cr compounds (and not soluble Cr molecules) are absorbed from the soil by plants (Kabata-Pendias and Pendias, 1984).

13.2.2 Environmental Exposure

Exposure to Cr for general populations comes from inhalation of ambient air (polluted), eating/drinking Cr-containing foods and water, and contact (skin) with Cr-bearing products. Environmental exposure under certain standards or limits is not considered a major risk. Nevertheless, some exposure scenarios can present significant hazards to health to general populations.

13.2.2.1 Children Children, particularly those who live near Cr manufacturing and/or processing plants or waste disposal sites, may ingest Cr-containing soils via hand-to-mouth activity. Among children, average soil/dirt consumption has been found to range from

10 to 90 mg/day; an average of 10 mg/day has been calculated for those >6 years of age (Paustenbach, 1987). Using these consumption rates for each age group, and the factor of amount of time available for possible soil ingestion, average daily uptakes/intakes of Cr [primarily as Cr(III)] have been calculated to be 0.07–0.2 μg Cr/kg/day for children and ≈4–9 ng Cr/kg/day for adults using parameters of the most likely-exposed individual (MLEI) or maximally-exposed individual (MEI), respectively, during data analysis within each age group (Sheehan et al., 1991). A study measured the total Cr levels on the hands of children that played in a playground containing chromate copper arsenate (CCA)-treated wood (Hamula et al., 2006). There was a significantly higher level of total Cr on the hands of children played in CCA playgrounds (1112 [±1089] ng, $n = 63$) compared with those who played in non-CCA playgrounds (652 [±586] ng, $n = 64$) ($p < 0.01$). A recent study analyzed blood Cr levels of 711 children from Guiyu, one of most heavily Cr-polluted areas in China (Xu et al., 2015). Guiyu children had significantly higher blood Cr levels compared with those living in Chendian (control area) in the same period (2004–2008). It is worth noting that both the Hamula and Xu studies measured total Cr levels, without separating the species of Cr(III) and Cr(VI).

13.2.2.2 Household Dust Inhalation of suspended dust/soil PM-containing adsorbed Cr is a likely route of exposure for general populations. The amount of exposure to any Cr-bearing soil particles in residential environs is expected to be low, with outside soil particles representing no more than 10% (on average) of the total composition of home-associated dusts (Sheehan et al., 1991). In general, following inhalation, the Cr-bearing soil/dust particles are expected to undergo redistribution, with 25% being exhaled, 50% landing in the upper airways (and subsequently swallowed and excreted in feces), and the remainder being deposited in the lungs (Cowherd et al., 1985). Using this redistribution profile, the maximal lung deposition of Cr [as Cr(III)] at the Cr level of 10^{-9} g/m^3 has been estimated to be 5 or 3.5×10^{-12} g/kg/day for an exposed child or adult, respectively. In addition, mucociliary clearance leads to removal of a significant amount of the Cr-bearing material that can reach the lungs. Since the levels of Cr(VI) alone in the airborne PM would be much lower in this model (i.e., 3×10^{-12} g/m^3), thus daily deposition levels would be on the levels of 10^{-15} g/kg.

13.2.2.3 Cigarette Smoking Cigarettes contain ≈0.62 g tobacco, on average. Tobacco grown in the United States contained 0.24–6.30 ppm Cr (IARC, 1990); tobaccos in other nations had Cr levels that were highly variable (Chen, 2003; Grant et al., 2004; Gendreau and Vitaro, 2005). A study analyzed the concentration of toxic metals in cigarettes consumed by smokers in the United States reported an estimated median daily exposure to Cr of ≈0.26 μg; this value was based on self-reported cigarettes smoked/day, metal concentration/cigarette, and cigarette tobacco weight (Caruso et al., 2014). This amount was much higher than 0.001 μg/day, the "no significant risk level" defined by the state of California (OEHHA, 2017).

13.2.2.4 Contact Exposure Dermal exposure to Cr occurs when skin contacts Cr-containing products, such as wood preservatives, cement, cleaning materials, textiles, and/or leather tanned with Cr agents (WHO, 1988). Both Cr(III) and Cr(VI) penetrate the skin, but Cr(VI) can pass more readily through the epidermal barrier than Cr(III) (Samitz and Katz, 1964; Robson, 2003). Significant amounts of skin exposure can arise from daily contact with many household materials and clothing (Paustenbach et al., 1992). In cleaning

items such as bleaches and detergents, chromate has been included as both a stabilizing and a coloring agent. Exposure to these Cr-containing cleaning agents has been associated with a condition known as "housewive's eczema." With clothing, particularly tanned leathers, sweat is the primary vehicle for both liberating Cr from the material and for providing a vehicle to concentrate Cr onto the skin during evaporation. Other less frequently reported sources for dermal exposure to Cr include military uniforms, match heads, magnetic tapes, and green felt used on gaming tables. It is worth noting that many orthopedic implants contain Cr as the major component of the metallic alloy. Cr ions may be released from orthopedic implants and subsequently distributed in body fluid and tissue compartments (Afolaranmi et al., 2008; Bradberry et al., 2014). Urine Cr levels were elevated in a 54-year-old man about 2 years after his prosthetic knee implants (Price, 2011). Green (2017) reported the neurocognitive and depressive deficits in patients with cobalt (Co) and Cr metallosis following metal-on-metal hip implants.

13.2.3 Occupational and Industrial Exposure

Workers in Cr-related industries experience occupational Cr exposures. The primary route of occupational Cr exposure is inhalation of airborne Cr-containing PM, while dermal exposure of Cr-containing products and ingestion of Cr-contaminated food or drinks are also fairly common in industrial settings. The industries with potential Cr exposure include chromate production, Cr plating, ferrochrome alloys and chrome pigment production, stainless steel production, welding, and tanning industries (Ashley et al., 2003). Cr exposures in occupational settings include both Cr(III) and Cr(VI) that are present as both soluble and insoluble fractions. The largest exposures have been found in stainless steel wielding, chromate pigment, and chromate production. Typical concentration ranges of airborne Cr(VI) to which workers in these industries were exposed during an average of 5–20 years of employment were as follows: chromate production, 100–150 $\mu g/m^3$; chromium plating, 5–25 $\mu g/m^3$; ferrochromium alloy, 10–140 $\mu g/m^3$; chrome pigment, 60–600 $\mu g/m^3$; and stainless steel welding, 50–400 $\mu g/m^3$ (Stern, 1982). Thus, workers in these industries may be exposed to Cr at a concentrations that were about 100–1000 times higher than those of general populations exposed to environmental Cr(VI).

There are large numbers of workers potentially exposed to Cr in occupational settings. Based on the National Occupational Hazard Survey (NOHS) conducted by National Institute for Occupational Safety and Health (NIOSH) from 1972 to 1974, an estimated 2.5 million workers were potentially exposed to Cr and its compounds, with about 175,000 workers potentially exposed to Cr(VI) compounds (NIOSH, 1974). In 1981–1983, NOHS estimated that 196,725 workers were potentially exposed to Cr(VI) compounds (NIOSH, 1983). In 2006, the U.S. Occupational Safety and Health Administration (OSHA) estimated that >558,000 U.S. workers were exposed to Cr(VI) compounds (Shaw Environmental, 2006; NIOSH, 2013). Numerous epidemiological studies have reported a variety of health problems associated with such occupational Cr exposures (discussed in more detail in Section 13.4).

13.2.4 Exposure Guidelines

Accumulating evidence from both epidemiologic and animal exposure studies indicated the strong carcinogenic potential of Cr(VI) compounds. To reduce the lung cancer risk in workers associated with occupational Cr(VI) exposure, NIOSH has revised the recommended

exposure limits (RELs) for airborne Cr(VI) compounds to $<0.2\,\mu g\,Cr(VI)/m^3$ for an 8-h time-weighted average (TWA) exposure during a 40-h workweek (NIOSH, 2013). The revised REL was based on the quantitative risk assessment of lung cancer deaths in the Baltimore MD cohort (Gibb et al., 2000a; Park et al., 2004). According to this assessment, the risk of lung cancer among Cr(VI) workers is extremely high (0.1%). Any risk >0.1% is considered significant and requires OSHA intervention. It is worth noting that the revised REL applies to all Cr(VI) compounds, both soluble and insoluble forms. IARC (2012) announced that "the term 'Cr(VI)' includes this ionic state of the metal, in any form or chemical compound in which it occurs, in any solution or other mixture, and even if encapsulated by another or several other substances. The term also includes Cr(VI) when created by an industrial process, such as when welding of stainless steel generates Cr(VI) fume." Other than cancer risk, the revised REL is expected to reduce the nonmalignant respiratory effects of Cr(VI) compounds, including irritated, ulcered, or perforated nasal septa and other potential adverse health effects.

13.3 CHROMIUM UPTAKE AND METABOLISM

13.3.1 Absorption

Absorption of Cr is largely dependent on its oxidation state and physical characteristics of the given compound. As occurs with structurally similar sulfate and phosphate anions, Cr(VI) ions can enter cells via nonspecific anion transporters (Zhitkovich, 2005). In contrast, Cr(III) agents cannot enter cells by any similar transport mechanism (Costa, 1997, Salnikow and Zhitkovich, 2008) and so must be taken in by phagocytic/pinocytic processes.

When inhaled, Cr is presented to a wide variety of cell types in the respiratory system. Most of the Cr(VI) entrained is readily reduced by epithelial lining fluids secreted by lung/bronchial epithelial cells. Soluble Cr(VI) is readily absorbed by cells lining the respiratory tract; Cr(III) is absorbed to a much lesser degree. However, when present as insoluble particles, Cr in either valence state can be phagocytized by epithelial cells. Under normal conditions (i.e., atmospheric Cr), absorption from the respiratory tract has been estimated to be $<1\,\mu g\,Cr$/day (Hertel, 1986); occupationally-exposed individuals may inhale several μg/day. Absorption from the lung depends on the Cr-containing particle characteristics, including size, shape, hygroscopicity, and overall electric charge (Stern et al., 1984; Hertel, 1986). Other factors that may influence absorption include temperature, solubility in body fluid, and reactions with other airborne agents.

Following oral ingestion, Cr(VI) can be reduced to Cr(III) by saliva and gastric juices in the gastrointestinal (GI) tract. The estimated reducing capacity to convert Cr(VI) to Cr(III) is ≈80 mg/day (DeFlora et al., 1997). Cr(III) is poorly absorbed in the GI system (<1% of ingested amount) (Mertz, 1969), while Cr(VI) is readily absorbed by cells lining the GI tract. The reduction of Cr(VI) in the GI system is considered a major detoxification process (DeFlora et al., 1997; DeFlora, 2000; Proctor et al., 2002; Paustenbach et al., 2003). It was hypothesized that the reduction of Cr(VI) to Cr(III) outside of cells and absorption of Cr(VI) by cells via nonspecific anion transporters occur almost simultaneously and that both processes compete with each other for substrate (Stern, 2010; Zhitkovich, 2011). This competition, combined with other factors including gastric emptying time, food content, and interspecies differences in reduction capacity, results in some of the ingested Cr(VI) (10–20%) escaping gastric detoxification and being absorbed into

cells of target tissues, even at very low concentrations (Stern, 1982; Zhitkovich, 2011). This possibility has been well documented by tissue distribution analyses of ingested Cr(VI) in both humans and animal models (Davidson et al., 2004; Uddin et al., 2007; Collins et al., 2010).

Compared with Cr(III) compounds, Cr(VI) agents can penetrate the skin more readily; this uptake is further enhanced with increases in the pH of the Cr-containing substances (Nriagu and Nieboer, 1988). While, under normal conditions, absorption of Cr through the skin is limited due to ongoing chemical reduction, these processes can be circumvented. Specifically, absorption of Cr(VI) may be increased by a presence of broken skin, as occurs frequently with workers with Cr-induced skin ulcerations. There have been documented cases in which extensive absorption of Cr(VI) also occurred following a chromic acid burn and that this patient developed significant damage to many tissues (i.e., kidneys) at distal sites in the body (WHO, 1988).

13.3.2 Transportation and Excretion

Absorbed Cr is widely distributed in nearly all tissues. Tissues with higher concentration of Cr are hair (200–2000 ppb), lung (700 ppb), liver (270 ppb), and kidney (90 ppb). Blood levels of Cr are quite low (< 0.14 ppb). The oxidation state of Cr is the determining factor for its transport within the bloodstream. Cr(III) is mainly transported in the serum, bound to iron-binding transferrin and the β-globulin fraction of serum proteins; at high levels, Cr(III) binds to serum albumin or α_1- or α_2-globulins (Gray and Sterling, 1950; Harris, 1977). In contrast to Cr(III), Cr(VI) can readily cross the erythrocyte membrane and bind to the globulin portion of hemoglobin following oxidation of the heme group (Gray and Sterling, 1950; Saner, 1980; Nieboer and Jusy, 1988). Human studies, where volunteers ingested Cr(VI) in drinking water, showed that a substantial amount enters red blood cells, indicating that not all Cr(VI) was reduced to Cr(III) in the GI tract (Kuykendall et al., 1996). Consequently, the degradation products of erythrocytes may explain, in part, the high concentration of Cr found in the spleen and the slow excretion of Cr from the body.

Excretion of Cr occurs primarily via urine and, to a lesser degree, the feces. A minor route of excretion is through the skin via hair, fingernails, and sweat (ATSDR, 2012). Humans exposed to Cr(III) or Cr(VI) by inhalation had elevated urinary Cr(III) levels. Lack of urinary Cr(VI) in exposed workers suggested the rapid reduction of Cr(VI) to Cr(III) before excretion (Cavalleri and Minoia, 1985; Minoia and Cavalleri, 1988). Humans exposed to Cr(III) or Cr(VI) by oral ingestion had most of this Cr excreted in feces, with a small fraction excreted in the urine, an outcome consistent with low absorption by oral ingestion. A study showed that the average urinary excretion half-life of Cr(VI) administrated via drinking water (0.05 ppm) was 39 h (Kerger et al., 1997).

13.3.3 Reduction

Once it has entered a cell, Cr(VI) undergoes a series of metabolic reductions and forms intermediate Cr species, Cr(V) or Cr(IV), and is finally reduced to Cr(III) (Zhitkovich, 2011). At physiological pH, intracellular reduction of Cr(VI) is facilitated by non-enzymatic antioxidants including ascorbate (Asc), reduced glutathione (GSH), and cysteine (Cys), as well as a number of enzymatic antioxidants such as cytochrome P_{450} reductase, mitochondrial electron transport complexes, glutathione reductase, and aldehyde oxidase (O'Brien, 2003; Zhitkovich, 2011). A combined activity of Asc, GSH, and Cys in cells

reduced >95% of Cr(VI) into Cr(III) (Zhitkovich, 2011). Which one functions as the primary reducing agent depends on the cellular availability as well as reaction rate. Early studies showed that Asc was the kinetically favored reducing agent and accounted for 80–90% of all *in vivo* Cr(VI) reduction (Suzuki and Fukuda, 1990; Standeven and Wetterhahn, 1991, 1992). At a concentration of 1 mM *in vitro*, Asc-mediated reduction was more rapid ($T_{1/2}$ = 1 min) as compared with 60.7 min for GSH and 13.3 min for Cys (Quievryn et al., 2003). However, the amount of Asc *in vitro* (<50 μM in culture medium) is much less compared to its physiological concentration in white blood cells and epithelial tissues (≈1–2 mM) (Slade et al., 1985; Bergsten et al., 1990; Kojo, 2004). Thus, reduction of Cr(VI) in cultured cells is primarily facilitated by GSH (Zhitkovich, 2011). Mitochondrial electron transport complexes are also potent Cr(VI) reducing agents, but they only reduce Cr(VI) that makes it into mitochondria. Similarly, cytochrome P_{450} reductase reduces Cr(VI) only in the absence of O_2. As such, these enzymes are only minor players in the potential reduction of intracellular Cr(VI) (O'Brien, 2003).

Depending on the nature of the reducing agents in the cell, Cr(VI) undergoes either one- or two-electron reduction (O'Brien, 2003; Zhitkovich, 2005; Yao et al., 2008). Asc reduces Cr(VI) via a two-electron reaction forming the reduction intermediate, Cr(IV). Reduction of Cr(VI) by GSH can be either by one- or two-electron reaction that produces Cr(V) or Cr(IV). Reduction by Cys is almost exclusively a one-electron reaction. Due to its weak membrane permeability, the final metabolite Cr(III) is normally retained in the same cell it was formed. Examples of this are shown in studies that illustrated that intracellular Cr(VI) reduction led to a massive intracellular accumulation of Cr(III). Intracellular level of Cr(III) increased about 10- to 20-fold after 3 h of Cr(VI) exposure and further increased to about 100-fold after 24 h of Cr(VI) exposure (Messer et al., 2006; Reynolds et al., 2007). Though considered relatively nontoxic, nonetheless, high levels of Cr(III) in cells can react with DNA; this is the principal mechanism underlying Cr(VI) genotoxicity (O'Brien, 2003; Zhitkovich, 2005; Nickens et al., 2010). In contrast, Cr(III) generated from extracellular reduction cannot enter the cell and poses little or no toxic/carcinogenic potential.

13.4 TOXICOLOGICAL EFFECTS

13.4.1 Non-cancerous Adverse Health Effects

13.4.1.1 Respiratory Effects The respiratory system is the major target of occupational Cr(VI) exposure. Workers in Cr-related industries like chromate production, chrome plating, pigment production, ferrochromium production, and stainless steel welding are exposed to a variety of Cr(VI) compounds. Inhaled Cr(VI) in PM is predominantly deposited at bronchial bifurcations, with a regional concentration of up to 15.8 mg/g lung tissue having been documented (Ishikawa et al., 1994; Nickens et al., 2010). Acute exposure to Cr(VI) may cause asthma or other respiratory distress in chromate-sensitive workers; intermediate to chronic exposure may lead to increased risks of respiratory pathologies, including chronic bronchitis, fibrosis, nasal ulceration, and bronchial epithelium hyperplasia, as well as nasal and lung cancers [reviewed in IARC (1990) and ATSDR (2012)]. A retrospective study on 2357 chromate production workers from 1950 to 1974 in a Baltimore production plant reported 20–28 μg/m^3 as the median Cr(VI) exposure that cause nasal septum perforation and bleeding as well as nasal irritation and ulceration (Gibb et al., 2000b). Several epidemiologic studies found a correlation between Cr(VI)

concentration and different respiratory complications (Lindberg and Hedenstierna, 1983; Huvinen et al., 1996, 2002a, 2002b). While no nasal perforation was found at chromic acid concentrations $<2\,\mu g/m^3$, nasal ulceration and septal perforations were found at Cr(VI) concentrations $>20\,\mu g/m^3$. Other studies also reported an increased risk of mortality due to non-cancer respiratory diseases in chromate production workers that correlated with duration of employment (Taylor, 1966; Sorahan et al., 1987; Davies et al., 1991). An increased risk of lung and nasal cancers was also found in chromate workers (discussed in more detail in Section 13.4.2).

Similar respiratory effects were also seen in rodents undergoing intermediate or chronic Cr(VI) exposure. Obstructive dyspnea was found in Wistar rats exposed to 200 or $400\,\mu g/m^3$ sodium dichromate aerosols via inhalation for 30 or 90 days (Glaser et al., 1990). Bronchoalveolar hyperplasia and lung fibrosis were also observed in these rats, but only lower levels of hyperplasia persisted to the end of the study. Nasal perforation, tracheal hyperplasia, and emphysema were reported in mice exposed to chromic acid mist for 12 months (Adachi et al., 1986; Adachi 1987). Rats exposed to either five consecutive daily doses of 0.01, 0.05, or 0.25 mg/kg or to one weekly dose of 0.05, 0.25, or 1.25 mg/kg sodium dichromate via intratracheal (IT) instillation for 30 months exhibited no neoplastic pulmonary lesions in the highest dose group (Steinhoff et al., 1986). A marked hyperplasia and atrophy of the pulmonary bronchi as well as emphysema were also reported in mice exposed to $4.33\,mg/m^3$ calcium chromate for life (Nettesheim et al., 1971).

13.4.1.2 Gastrointestinal Effects The GI tract is the main target of oral exposures to Cr (VI). To date, there have been only a few human studies on adverse health effects related to Cr(VI) ingestion. One study reported an increase in stomach cancer mortality in residents of small villages in Liaoning province (China) where the drinking water was heavily Cr(VI) contaminated (>0.5 mg/L) (Zhang and Li, 1987). A study in India evaluated adverse health effects in a population exposed to high levels of Cr(VI) in the water (~20 mg/L) (Shama et al., 2012) and reported a slightly increased incidence of GI and dermatological complaints in the exposed populations. Other types of Cr-induced GI effects have also been reported in animal models following acute or chronic oral exposure. Acute gastritis and enteritis, necrosis of the proximal tubules, and hepatocellular necrosis were observed in rodent models exposed to potassium chromate (Langard and Costa, 2015). Small ulcerations in the stomach and intestinal mucosa were reported in mice undergo long-term inhalation of calcium chromate (Nettesheim et al., 1971).

In 2008, NTP initiated a 2-year rodent study to examine the possible effect of chronic oral exposure to Cr(VI) in their drinking water (NTP, 2008). The groups of 50 males and 50 females of F344/N rats and $B_6C_3F_1$ mice were exposed to various doses of sodium dichromate dihydrate in drinking water for 2 years, and multiple endpoints including neoplastic and non-neoplastic lesions were assessed. In addition to clear evidence of carcinogenic activity of Cr(VI) in both rats and mice, an increased incidence of histiocytic cellular infiltration in several tissues was observed in both species. Diffused epithelial hyperplasia was only observed in the duodenum and jejunum of mice but not rats. A more detailed discussion on the major finding and conclusions from the NTP study were provided in a review article (Witt et al., 2013).

13.4.1.3 Hepatic Effects Liver damage due to cell necrosis was reported in adults exposed to Cr via inhalation (Pascale et al., 1952). Elevated levels of liver enzymes were reported in several cases of acute oral exposure (Fristedt et al., 1965; Kaufman et al., 1970;

Ellis et al., 1982). Increased serum alanine aminotransferase (ALT) activity, a reliable and sensitive marker for liver disease, was found in rats orally exposed to Cr(VI) for acute, intermediate, and chronic duration (Acharya et al., 2001; NTP, 2007; Rafael et al., 2007). Changes in hepatic morphology, including cellular histiocyte infiltration, vacuolization, and necrosis, were observed in rats subchronically-exposed to Cr(VI) compounds for up to 22 weeks (Acharya et al., 2001; NTP, 2007; Rafael et al., 2007). Long-term (2 years; chronic) exposure to Cr(VI) in drinking water led to similar changes in both rats and mice (NTP, 2008). After 2 years of exposure, livers of both species exhibited increased levels of chronic inflammation, histiocytic cellular infiltration, and lipid accumulation (NTP, 2008). Interestingly, female animals exhibited a greater sensitivity to Cr-induced liver changes as compared with males in both species. No obvious liver changes were documented in animals subchronically- or chronically-exposed to Cr via inhalation (Glaser et al., 1988, 1990; Kim et al., 2004).

13.4.1.4 Renal Effects Workers in chromate production industries displayed increased levels of retinol binding protein and other protein antigens in the urine, an early markers of kidney disease (Mutti et al., 1985; Franchini and Mutti, 1988). In addition, urinary *N*-acetyl-β-D-glucosaminidase activity and microalbumin and β_2-microglobulin levels (biomarkers of kidney damage) were significantly increased among chromate workers, an outcome that was positively correlated with Cr levels in measured air, whole blood, and urine samples (Wang et al., 2011). On the other hand, some studies of chromate workers, chrome platers, and stainless steel welders yielded negative results with regard to renal function (Satoh et al., 1981; Littorin et al., 1984; Verschoor et al., 1988). Similarly, no renal effect was observed in small animals exposed to Cr(VI) via inhalation.

Renal failure and tubules necrosis have been reported in multiple cases in which large amount of Cr(VI) compounds was ingested (Fristedt et al., 1965; Kaufman et al., 1970; Ellis et al., 1982; Saryan and Reedy, 1988, Loubieres et al., 1999). The results from animal studies in which rats or mice were orally exposed to Cr(VI) are quite controversial. Some studies revealed either histological or biochemical changes of kidney in rats exposed to Cr(VI) for about 20–28 days (Kumar and Rana, 1984; Diaz-Mayans et al., 1986; Soudani et al., 2011a, 2011b) or 22 weeks (Acharya et al., 2001). However, no histological change of kidney was found in rats or mice following either intermediate or chronic Cr exposure in drinking water (MacKenZie et al., 1958; ATSDR, 2012).

13.4.1.5 Dermal Effects Direct contact with Cr(VI) compound can cause skin irritation, blisters, skin ulcers, and ACD (ATSDR, 2012). An extensive health survey of chromate workers from seven U.S. chromate production plants found skin ulcers or scars in half of the workers (PHS, 1953). A similar result was reported in electroplating workers in Brazil (Gomes, 1972), in which >50% of the workers had skin ulcers on their hands, arms, and feet. A study of 2357 chromate production workers in a Baltimore production plant (over the period from 1950 to 1974) reported that >30% of workers developed ulcerated skin, an outcome that was significantly associated with Cr(VI) exposure (Gibb et al., 2000b). In addition to skin ulcers and burns, direct contact Cr compounds or Cr-containing products can lead to ACD. This Cr-induced ACD contains two essential phases, that is, an induction phase in which initial contact with Cr sensitizes the immune system and activates T-lymphocytes and an elicitation phase in which subsequent Cr exposure triggers a specific immune response. ACD is most commonly observed during occupational dermal contact

with low to moderate levels of chromate; there is a high prevalence of ACD in construction workers (Bregnbak et al., 2015). Both Cr(VI) and Cr(III) are able to induce ACD in Cr-sensitive populations (Hansen et al., 2003; Bregnbak et al., 2017).

13.4.1.6 Immunological Effects The immune system is also a target of Cr exposure. In addition to ACD described above, asthma and pulmonary inflammation are major adverse health effects that have been initiated by Cr targeting lymphocytes. The occupational asthma related to Cr, in combination with other metals such as nickel (Ni), has been reported in two stainless steel welders (Keskinen et al., 1980), a woman in a metalworks company (Cruz et al., 2006), and four Cr-related workers (two electroplating workers, one welder, one cement worker) (Fernandez-Nieto et al., 2006). A study on 62 workers from an aerospace manufacturer reported six cases of occupational asthma and 18 cases of occupational rhinitis, which often precedes asthma, in workers who exhibited significantly increased urinary Cr levels compared with controls (Walters et al., 2012). Early asthma is mediated by antigen binding to IgE-bound mast cells and rapid mast cell degranulation and release of mediators of bronchoconstriction. Late asthma is dependent on proliferating T-lymphocytes secreting lymphokines that promote chemotaxis, bronchoconstriction, and mucous secretion, generally hours after exposure. Both types of asthma have been reported in workers exposed to dichromate, ammonium bichromate, chromic acid, chromite ore, chromate pigments, and welding fumes. In some cases, hypersensitivity to Cr was confirmed by diagnostic patch testing in some, but not in all (suggesting immunologic and non-immunologic origins). Though likely related to dermal hypersensitivity associated with Cr exposure, the underlying mechanisms of these pulmonary reactions remain woefully unexplored.

13.4.1.7 Reproductive and Developmental Effects There is very limited information regarding to the effect of Cr(VI) exposure on human reproductive system and embryo development. Several early studies explored the possible effects of Cr(VI) exposure on the course of pregnancy and the occurrence of spontaneous abortion in chromate workers without any solid conclusion (Hjollund et al., 1995; ATSDR, 2012). A significant decrease on sperm count (47%) and mortality (15%) was found in chrome plating workers ($n = 21$) compared with age-matched unexposed human subjects (Li et al., 2001). A study on 51 chromate workers and 15 unexposed controls in India also reported a significant positive correlation between the percentage of abnormal sperm and blood chromium (Kumar et al., 2005). A recent study investigated reproductive outcomes in women that may have non-occupational exposure to Cr(VI) due to a nearby Cr(VI)-contaminated factory in Willits, CA (Remy et al., 2017). Birth rate of pregnant women from Willits was low compared with those from the rest of county and remained low until 12 years after plant closure, indicating a significantly higher risk of pregnancy loss in Willits women (Remy et al., 2017).

Compared with human data, the animal studies provided much stronger evidence on the adverse effects of Cr(VI) exposure on both reproductive system and embryo development. Reduced sperm count and histopathology changes of testes have been reported in monkeys, rats, mice, and rabbits that were orally-exposed to Cr(VI) (Li et al., 2001; Aruldhas et al., 2004, 2005, 2006; Subramanian et al., 2006). However, no significant morphological change was observed in rats or mice that exposed to various doses of potassium dichromate in the diet or sodium dichromate dihydrate in drinking water for either 3 months or 2 years (NTP, 2007, 2008).

13.4.2 Carcinogenicity

13.4.2.1 Epidemiological Studies

Inhalation Exposure A large number of epidemiological studies have reported a high incidence of lung and nasal/sinus cancers among workers occupationally-exposed to Cr(VI) compounds by inhalation (IARC, 2012; NIOSH, 2013). The increased risk of lung cancer has been found in workers from various industries including chromate production, chrome plating, pigments, ferrochrome production, stainless steel production, and welding. The risk was correlated with the air Cr(VI) concentration, as well as duration of employment. Among these studies, the studies conducted on Baltimore (MD) and Painesville (OH) cohorts provided valuable information on quantitative risk assessments of chromate workers due to large amount of available worker data, the quality of exposure estimate, and years of follow-up (NIOSH, 2013).

The Baltimore cohort was originally composed of 2357 workers employed in the period of 1950–1974 (Gibb et al., 2000a); 3 workers were removed in an extended follow-up study (Gibb et al., 2015). Mean length of employment was 3.1 years; 40% of the cohort was short-term workers that were included as a "low-exposure" group. Annual average exposure was estimated for each job title in the plant from 1950 to 1985 (year plant closed). More than 97% of cohort had well-documented medical records, and vital status was followed until the end of 1992 (Gibb et al., 2000b) and then extended until the end of 2011 (Gibb et al., 2015). With regard to lung cancer deaths, there were 122 cases by the end of 1992 and 217 cases by the end of 2011. The O/E (observed/expected) ratio of lung cancer mortality for the total cohort was 1.63 (95% CI: 1.42–1.68). The mean cumulative Cr(VI) exposures were 0.14 mg/m^3-years for the total cohort and 0.23 mg/m^3-years for 217 lung cancer cases. The O/E ratios of lung cancer mortality were 1.05 (95% CI: 0.77–1.41) for the lowest quartile and 2.19 (95% CI: 1.70–2.77) for the highest quartile, suggesting a positive correlation between lung cancer mortality cumulative Cr(VI) exposure (Gibb et al., 2015). Smoking was the biggest confounding factor of the study as >80% of the cohort were smokers. However, the association between lung cancer O/E ratio and cumulative Cr(VI) exposure was more pronounced in the analysis limited to smokers (Gibb et al., 2015).

A retrospective cohort study was conducted on lung cancer mortality in workers from a chromate production plant in Painesville (Luippold et al., 2003; Proctor et al., 2003). The cohort comprised 493 workers employed at least 1 year between 1940 and 1972. Their mortality was followed till 1997, with an average follow-up length of 31.6 years. A total of >800 air samples were collected from 22 areas of the plant and analyzed for Cr(VI) concentration (Proctor et al., 2003). The cumulated Cr(VI) exposure and highest average monthly exposure levels were determined for each worker. Among 303 death cases, 51 cases were due to lung cancer. The mean cumulative Cr(VI) exposures were 1.55 mg/m^3-years for the total cohort and 3.28 mg/m^3-years for lung cancer cases (Luippold et al., 2003). Among the affected, 59% of workers who died of lung cancer were first hired between 1940 and 1949; airborne Cr(VI) concentrations during that period were very high, with an average value of 0.72 mg/m^3. Average air Cr(VI) levels were reduced to 0.27 mg/m^3 from 1957 to 1964 and to 0.039 mg/m^3 from 1965 to 1972. Lung cancer death cases were reduced to 14% (1955–1964) and 4% (1965–1972), suggesting a correlation between lung cancer mortality and cumulative Cr(VI) exposure.

Oral Exposure Compared to inhalation exposure, there were only a few human studies addressing oral exposure to Cr and its adverse health effects. An initial study from China reported an increase of stomach cancer mortality in the residents of small villages in China

where the drinking water was heavily contaminated with Cr(VI) (>0.5 mg/L) (Zhang and Li, 1987). The increased incidence of stomach cancer in exposed villages was confirmed by a reanalysis of the same data using control population in the whole province (Beaumont et al., 2008). However, the association between Cr(VI) exposure and cancer mortality was not replicated by analyzing the same data using a smaller number of controls from nearby areas with no Cr(VI) in groundwater (Kerger et al., 2009).

An ecological mortality study in the Oinofyta region of Greece, where water was contaminated with Cr(VI) (maximum levels ranging between 41 and 156 μg/L), indicated a significantly increased incidence of liver cancer mortality ($p<0.001$), lung cancer ($p = 0.047$), and cancers of the kidney/genitourinary organs among women ($p = 0.025$) (Linos et al., 2011).

13.4.2.2 Animal Studies A large number of studies have been conducted in animal models, including subchronic and chronic exposures to a variety of Cr(VI) compounds (Cohen and Costa, 2007; IARC, 2012; NIOSH, 2013). No tumor formation was observed as a result of short-term exposures [summarized and discussed in two review articles, i.e., Sedman et al. (2006) and Thompson et al. (2013)]. Tumor formation, particularly lung tumors, was noted following chronic inhalation exposure to a variety of Cr compounds, outcomes that were in line with epidemiological studies.

Inhalation Exposure Nettesheim and Szakal (1972) reported a study in which C57BL/6 mice (both male and female mice) were exposed to calcium chromate dust 5 h/day, 5 days/week, for their lifetime. A fourfold increase in lung tumor incidence was observed in mice exposed to calcium chromate dust compared with air-only group. Consistent with the finding, lung tumors were seen in Sprague Dawley rats exposed to calcium chromate by IT instillations for 30 months (Steinhoff et al., 1986). Levy et al. (1986) conducted a 2-year study to examine the carcinogenic activity of 21 different Cr compounds using an intrabronchial pellet implantation system. The pellets loaded with different Cr agents were surgically implanted into the lower left bronchus of Porton-Wistar rats ($n = 100$ per group). While no tumors were found in the control group (blank pellet loaded with cholesterol), bronchial carcinomas were seen in 25/100, 62/99, and 5/100 in groups exposed to calcium chromate, strontium chromate, and zinc chromate, respectively.

Glaser et al. (1986) investigated lung tumor formation in Wistar rats exposed to submicron diameter aerosols of sodium dichromate via inhalation. The rats were exposed to various doses of sodium dichromate for 18 months and were maintained post-exposure for another year. Of the rats, 3 of 20 rats in the group that received the highest concentration of sodium dichromate (0.1 mg/m^3) developed lung tumors. In another study, sodium dichromate was administered to Sprague Dawley rats by IT instillation. Rats were exposed to either 0.01, 0.05, or 0.25 mg/kg for 5 times/week or 0.05, 0.025, or 1.25 mg/kg once a week for 30 months. In the end, 14/80 rats in the highest dose group (1.25 mg/kg) developed lung tumors (Steinhoff et al., 1986). Adachi (1987) reported a significant number of nasal papilloma developed in mice inhaling 1.8 mg/m^3 chromic acid mist at 2 h/days, 2 days/week, for up to 12 months. Lung tumors were also seen in mice inhaled 3.6 mg/m^3 chromic acid mist at 30 min/days, 2 days/week for up to 12 months (Adachi, 1987).

Oral Exposure The NTP initiated a 2-year rodent study to examine possible effects of chronic oral exposure to Cr(VI) in their drinking water (NTP, 2008). Groups of 50 male and 50 female F344/N rats and $B_6C_3F_1$ mice were exposed to 0, 14, 57, 172, and 516 mg/L

sodium dichromate dihydrate (SDD) [equivalent to 0, 5, 20, 60, and 180 mg/L Cr(VI)] in drinking water. The study was completed after 2 years, and both neoplastic and non-neoplasic lesions were assessed. There was clear evidence of carcinogenic activity of Cr(VI) in both rats and mice. Both female and male F344/N rats that were exposed to the highest concentration of Cr(VI) in drinking water developed cancer in the oral cavity. $B_6C_3F_1$ mice of both sexes, which were exposed to the two highest doses of Cr(VI), developed tumors in the small intestine (duodenum and jejunum) (NTP, 2008; Stout et al., 2009).

Davidson et al. (2004) used hairless (nude) mice to assess toxic and carcinogenic effects from exposure to Cr(VI) in drinking water and in the context of concurrent exposure to ultraviolet radiation (UVR). The mice were treated with low doses of UVR in the absence and presence of potassium dichromate (0.5, 2.5, or 5 ppm) in the drinking water for 6 months. While ingestion of Cr(VI) alone in drinking water did not produce any skin tumors, co-treatment with low-dose UVR resulted in a synergistic effect that gave rise to skin tumors. A dose-related increase in tumor numbers was seen with increased concentration of Cr(VI) in the water. Thus, while Cr(VI) alone was not sufficient to induce tumor formation in the skin in a relative short exposure duration and at low doses that have occurred in human exposure scenarios, Cr(VI) in drinking water can nonetheless promote UV-induced tumorigenesis at a site distal to the entry point (ingestion).

13.5 MECHANISMS OF CHROMIUM TOXICITY AND CARCINOGENICITY

13.5.1 Oxidative Stress

Exposure of a variety of cells types to Cr(VI) agents has been shown to be able to induce oxidative stress via both increased production of reactive oxygen species (ROS) and depletion of cellular antioxidants [reviewed in Yao et al. (2008)]. Several of the reviewed studies showed that metabolic intermediates and products ultimately generated during Cr(VI) reduction could partake in Fenton-type reactions to generate hydroxyl radicals in the presence of hydrogen peroxide. Alternatively, in the presence of endogenous superoxide anion and hydrogen peroxide, Cr(V) and Cr(VI) can produce hydroxyl radicals via Haber–Weiss-like reactions. Depending on the levels of ROS production, Cr(VI)-induced oxidative stress may lead to cell death (cytotoxicity) or tumor formation (carcinogenicity). High levels of ROS production can directly target lipid and DNA to generate lipid peroxidation and DNA damage, as well as many other cellular injuries, leading to cell death by either apoptosis or necrosis. Medium to low levels of ROS in cells may disrupt the cellular redox balance and accelerate cell proliferation, possibly leading to tumor formation and progression. Two major DNA lesions (i.e., 8-oxo-dG or 8-OHdG) that are generated by oxidative DNA damage as well as MDA (a marker for lipid oxidation) have been used as important biomarkers to detect Cr(VI)-induced oxidative stress *in vivo* and *in vitro*. A recent study on 230 non-smoking male workers from "hard Cr" electroplating companies in Taiwan reported increased levels of urinary 8-OHdG and MDA and overall increases in Cr levels in the urine, hair, and fingernails of the workers relative to corresponding levels in control subjects (Pan et al., 2018). Daily cumulated Cr(VI) exposure and urinary Cr levels significantly correlated with urinary 8-OHdG and MDA levels.

Other than directly causing increases in ROS levels, Cr(VI) can also induce oxidative stress by depleting cellular antioxidants, such as Asc and GSH. Dose- and time-dependent deceases in reduced to oxidized glutathione (GSH/GSSG) ratios have been noted in the

duodenum of $B_6C_3F_1$ mice exposed to various doses of Cr(VI) in drinking water for 7 or 90 days (Thompson et al., 2011). This outcome indicated that chronic low-dose exposure of Cr(VI) in drinking water could induce oxidative stress in locally impacted tissues. Moreover, there was also clear evidence of villous cytotoxicity and focal/diffused hyperplasia in cells in the small intestine in animal models in both the NTP study (NTP, 2008) and the 90-day study by Thompson et al. (2011). Such outcomes are a likely consequence of increased levels of localized oxidative stress.

13.5.2 Genotoxicity and Genome Instability

Genotoxicity is a term describing structural changes of genetic materials (DNA, chromosomes, etc.) that affect genome integrity and lead to genome instability. Through either induction of mutagenic or non-mutagenic effects, the genotoxicity of Cr compounds has been documented in cultured cells, experimental animals, and human studies.

In general, Cr(VI) ions readily gain entry into eukaryotic cells via nonspecific anion transporters. Even so, the now internalized Cr(VI) itself does not bind to DNA or other macromolecules in the cells. Instead, its metabolic intermediates, that is, Cr(V), Cr(VI), and Cr(III), are highly reactive and can, upon reaching the nucleus, readily form adducts with DNA. These adducts can be binary (Cr-DNA) or ternary (ligand-Cr-DNA), where the "ligand" can be ascorbate, cysteine/histidine side chains, or glutathione or larger cysteine-containing proteins. Binary Cr-DNA adducts in cells can be repaired rather efficiently, within minutes after formation, by nucleotide excision repair (NER) and ultimately have minimal effects on cell function. As expected, NER-deficient human cells are hypersensitive to Cr(VI) toxicity and tend to display massive accumulation of Cr-DNA adducts (Reynolds et al., 2004). This clearly suggests that DNA repair plays an important role in counteracting potential Cr(VI)-induced DNA damage. In contrast, ternary Cr-DNA adducts are harder to repair and tend to act as strong inhibitors of DNA replication and transcription. Interestingly, replication inhibition by ternary Cr-DNA adducts requires a presence of mismatch repair (MMR) proteins (Reynolds and Zhitkovich, 2007). Accordingly, cells from MMR null mice and some MMR-deficient human cells (e.g., MLH1 null colon cancer HCT116 cells and MSH6 null colon cancer DLD1 cells) were more resistant to cytotoxicity induced by Cr(VI) than corresponding MLH1- or MSH6-bearing cells (Peterson-Roth et al., 2005; Reynolds and Zhitkovich, 2007; Reynolds et al., 2009).

In addition, Cr(VI) reduction can generate several other DNA or chromosome lesions, including abasic sites, single- and double-strand DNA breaks, protein–Cr–DNA cross-links (DPC), DNA inter- or intra-strand cross-links, etc. (O'Brien, 2003; Nickens et al., 2010; Zhitkovich, 2011). Single- or double-strand breaks have been detected in rodent hosts orally-exposed to Cr(VI) by gavage (Sedman et al., 2006; Thompson et al., 2013). DNA–protein cross-links were detected in liver cells—but not lymphocytes—isolated from F344 rats exposed for 3 weeks to potassium chromate via drinking water (Coogan et al., 1991). DNA damage such as DNA intra-strand breaks and cross-links and DPC and Cr-DNA adducts were observed in rats and chick embryo tissues following *in vivo* exposure to Cr(VI), but not to Cr(III) [reviewed in Standeven and Wetterhahn (1989)]. However, DPC were not induced in mice exposed for 9 months to Cr(VI) via drinking water (De Flora et al., 2008). The ability of these DNA lesions to induce DNA mutations has been extensively studied *in vitro* using the shuttle-vector system (O'Brien, 2003; Zhitkovich, 2011) and *in vivo* using the Big Blue mouse model (Cheng et al., 2000). p53 point mutations were reported at higher frequencies in lung tumors from chromate workers than lung samples

from non-chromate workers (Kondo et al., 1997). Very few studies have examined DNA mutation in animals orally-exposed to Cr(VI). In one study, K-Ras codon 12 GAT mutations were observed in both Cr(VI)-treated and untreated mice, without a clear treatment-related trend (O'Brien et al., 2013).

These DNA or chromosomal lesions, which are very prevalent in cells exposed to Cr(VI), are likely to lead to genotoxic/mutagenic consequences. Moreover, two larger-scale genomic changes, that is, microsatellite instability and chromosome aberrations, were shown to be associated with Cr exposure in human and animal studies, as well as *in vitro* in cultured cells. The instability and aberrations were observed in many Cr(VI)-exposed biological systems, both *in vitro* and *in vivo* (Holmes et al., 2008; Wise and Wise, 2012). More than 15 studies analyzed micronuclei formation as a genotoxic endpoint in rodents exposed to Cr(VI) via oral administration at various doses and exposure durations; however, results from these studies were mixed. In most studies, micronuclei formation was analyzed in normochromatic erythrocytes that are not target cells for oral Cr(VI) exposure. Recently, a significant increase of aberrant nuclei was reported at the tips of villi in mice exposed 90 days to Cr(VI) in drinking water; none was detected in the crypt areas of the same mouse (O'Brien et al., 2013).

A number of assays have shown that chromosomal aberrations can be induced by both valence states of Cr. Significant increases in the aberrations were observed in cultured BALB/c mouse and Chinese hamster V79 cells exposed to a number of Cr(III) or Cr(VI) salts (Tsuda and Kato, 1977; Newbold et al., 1979; Leonard and Deknudt, 1981; Loprieno et al., 1985). However, sister chromatid exchange was produced in cultured lymphocytes with Cr(VI) salts exclusively (Ohno et al., 1982; Stella et al., 1982). An increase in the aberration frequency in cells obtained from occupationally-exposed individuals in Cr plating plants paralleled the observations from the *in vitro* studies (Bigaliev et al., 1977; Stella et al., 1982).

13.5.3 Epigenetic Modulation

A growing body of evidence has suggested that epigenetic regulation might mediate Cr(VI)-induced gene expression changes as well as its carcinogenic outcomes (Arita and Costa, 2009; Brocato and Costa, 2013). Epigenetic regulation refers to reversible, but heritable, changes in gene expression that occur *without alterations* in the DNA sequence (Rodríguez-Paredes and Esteller, 2011). DNA methylation, histone modification, and microRNA are major processes/components involved in normal epigenetic regulation.

Exposure to certain forms of Cr(VI) is able to induce a variety of epigenetic changes in cells. One, DNA methylation, is a covalent modification in which a methyl group is added to the 5′-carbon of cytosine. Many studies have reported aberrant DNA methylation in cells/tissues exposed to these Cr(VI) agents. Exposure of a Chinese hamster cell line expressing the bacterial *gpt* reporter gene to potassium chromate induced promoter-specific DNA methylation and silenced the *gpt* transgene (Klein et al., 2002). Coincidently, lung cancer from chromate workers displayed increased DNA methylation in the promoter region of the tumor suppressor gene *P16*, suggesting methylation and silencing of endogenous genes by chronic Cr(VI) exposure (Kondo et al., 2006). Thus, Cr(VI)-induced silencing of tumor suppressor genes has been considered as an important mechanism contributing to its carcinogenicity.

Silencing of the MMR gene *MLH1* in lung cancers of chromate workers was found to be associated with high replication error and microsatellite instability (Takahashi et al.,

2005). Ali et al. (2011) reported hypermethylation of *APC*, *MGMT*, and *hMLH1* gene promoters in chromate worker lung cancers compared with non-chromate-induced lung cancers. Recently, analyzing the promoter methylation of 22 tumor suppressor genes revealed the increased DNA methylation of three gene promoters (e.g., *WIF1*, *APC*, *MLH1*) in peripheral blood mononuclear cells (PBMC) isolated from chromate workers compared with those in cells from referent subjects (Sun et al., 2014). In addition to gene-specific changes, a global hypomethylation was reported in PBMC from chromate workers that had not developed any tumor symptoms (Wang et al., 2012). Such an outcome was in line with the findings of global DNA hypomethylation in two human cell lines exposed to Cr(VI) (Lou et al., 2013). Together, these results could suggest that Cr(VI)-induced DNA methylation changes might alter expression of tumor suppressor genes/oncogenes and promote genome instability via global DNA hypomethylation.

Post-transcriptional modification of histone tails and microRNA expression are also targets of Cr(VI) exposure. It has been reported that Cr(VI) exposure *in vitro* resulted in decreased histone acetylation and increased histone biotinylation (Xia et al., 2011, 2014; Chen et al., 2016), as well as modified histone methylation in both global and gene-specific manners (Sun et al., 2009; Zhou et al., 2009). Moreover, miR-143 was decreased in a Cr-transformed cell line (He et al., 2013), and plasma miR-3940-5p levels were negatively associated with Cr(VI) exposure in chromate workers (Li et al., 2014).

Based on the above-cited evidence, given the capacity of Cr(VI) to modulate epigenetic components it is possible epigenetic mechanisms may mediate, at least in part, some of the carcinogenic effects of Cr(VI) in an exposed host.

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14

DIESEL EXHAUST AND LUNG CANCER RISK

Eric Garshick and Jaime E. Hart

14.1 HISTORICAL OVERVIEW

The compression ignition (diesel) engine was patented by Rudolf Diesel in 1892. The chief difference between diesel engines, fueled by diesel oil, and spark ignition engines, fueled by gasoline, is that the fuel–air mixture in diesel engines is ignited by the heat of compression alone. Diesel engines have several advantages over their gasoline-powered counterparts. They are generally more efficient in converting fuel energy to work because they operate at higher compression ratios and temperatures and burn fuel that is higher in specific energy content. Diesel fuel is heavier and less volatile than gasoline; indeed, compression ignition engines can burn a variety of low-grade fuels. Diesel engines generally have greater durability than gasoline engines, and it is not uncommon to find older diesel engines in regular operation in local commercial fleets. In some circumstances, older diesel engines have been remanufactured in efforts to exempt vehicles from pollution control measures (Miller, 2018). Because diesel fuel has a lower vapor pressure than gasoline, it contributes less to organic air pollution from evaporative emissions and presents a lower explosive hazard.

On the other hand, diesel engines have historically been heavier and noisier than equivalent spark ignition engines and were characterized by more vibration and slower acceleration at low speeds. Diesel engines of the past were also characterized by much more visible and malodorous tailpipe emissions than equivalent gasoline engines. This difference was accentuated when on-road gasoline-powered vehicles were required to be fitted with catalytic converters. These characteristics, coupled with historically low gasoline prices, have limited the use of diesel engines in passenger cars and other light- and medium-duty vehicles in the United States. However, diesel engines have been prevalent in applications in which fuel economy and durability offset the negative factors, such as in heavy-duty trucks, buses, off-road equipment, and railroad locomotives.

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Diesel-powered trucks were introduced into the Western U.S. trucking fleet in the 1940s and into the rest of the country during the 1950s and 1960s. Diesel trucks constituted the majority of heavy-duty truck sales for the first time in 1961 (Motor Vehicles Manufacturers Association, 1962) and are now used in essentially all heavy- and medium-duty trucking, construction, and agricultural applications. Year-end U.S. truck vehicles-in-use data in 2017 demonstrated that about 75% of all commercial vehicles are powered by diesel engines and the largest trucks (class 8) accounted for 99% of the overall number (Diesel Technology Forum, 2019). However, diesel engine use in the U.S. bus fleet has changed dramatically. In 1995, more than 95% of buses were diesel powered. However, by 2015, this had been reduced to almost half (50.8%) of all buses, as natural gas and hybrid buses have been introduced into urban transit fleets (Hughes-Cromwick and Dickens, 2018).

The transition from steam to diesel railroad locomotives occurred primarily after World War II. The approximate midpoint of dieselization was 1952, and, by 1959, 95% of locomotives in the United States were diesel powered (U.S. Department of Labor, 1972). The penetration of diesel engines into aboveground mining applications paralleled their penetration into the trucking and construction industries. The introduction of diesel engines into underground metal and nonmetal mines also began after World War II, with approximately 3000 units in 350 mines in 1970 and 1971, with an increase to 4400 units by 1976 (NIOSH, 1982).

Diesel engines are much more prevalent among light-duty applications in Europe than in the United States, largely because of the higher fuel prices. In addition, starting in the 1990s, European concerns for emissions focused more on global warming than on particulate matter (PM), and diesels emit less carbon dioxide (CO_2) than equivalent gasoline engines. Whereas the Japanese and U.S. markets focused on developing hybrid and electric cars, the European markets focused on the introduction of light-duty diesel vehicles. From a 13% market share Western Europe in 1990, new passenger diesel car sales increased to more than 50% by 2006 and to 55% by 2011 (European Automobile Manufacturers Association, 2019). However, European diesel new car sales decreased to approximately 44% of all sales by 2017. This decrease followed the 2012 announcement by the International Agency for Research on Cancer (IARC) that diesel exhaust is a human carcinogen (IARC, 2014) and the 2015 Volkswagen emissions cheating scandal that highlighted the continuing emissions of diesel exhaust in Europe (Burki, 2015). In India, the sale of diesel car sales (excluding sport utility vehicles) fell to 23% in 2017 from a high of nearly 50% in 2012–2013 as a result of governmental concern about pollution (Doval, 2018). In the United States in 2014, diesel-powered cars only accounted for about 3% of total auto sales and 1.5% of all light-duty vehicles (including passenger cars, sport utility vehicles, minivans, and all but the largest pickup trucks and vans) (Chambers and Schmitt, 2015).

Although still called "diesels," new technology compression ignition engines hardly resemble the diesel engines of the past. There have been remarkable engineering advances in compression ignition engine technology in the United States, driven both by market competition for fuel efficiency, durability, and performance, and in response to progressively more stringent emission standards for PM and nitrogen oxides (NO_x). Through a combination of cleaner fuels, advanced engine technology, and the introduction of exhaust aftertreatment, tailpipe emissions have substantially decreased, while fuel economy and durability have been retained.

There are continued concerns for the adverse health effects of diesel engine exhaust for many reasons. First, human exposures worldwide are common, despite advances in engine pollution control. The introduction of diesel vehicles with modern pollution controls has

been limited by the slow introduction of ultralow sulfur fuel (in the United States 15 ppm and in Europe 10 ppm) in other parts of the world (Miller and Facanha, 2014). Higher sulfur fuel content damages catalysts used in modern PM filters. Second, although overall emissions have been substantially reduced, modern diesel vehicles still emit ultrafine PM (Liati et al., 2018), and new technology diesel emissions still contain trace amounts of toxic compounds (Liu et al., 2018). Third, in 2012, after decades of epidemiologic research, diesel engine exhaust was classified as a carcinogen in humans by IARC based on evidence of its lung carcinogenicity (IARC, 2014). And lastly, toxicologists have found evidence of diesel engine exhaust's toxic effects in animals and cellular toxicity *in vitro* experiments that support the epidemiologic evidence of carcinogenicity (reviewed in the IARC 2014 monograph).

Diesel exhaust is a still a component of traffic-related air pollution (Gu et al., 2018), and the PM fraction has been especially of interest in light of the recent intense focus on ambient fine PM ($PM_{2.5}$). $PM_{2.5}$ was reviewed and classified as a definite human carcinogen by IARC (IARC, 2016). Although the potential for diesel exhaust to present a health hazard has been known since the 1950s, reports in the late 1970s that organic extracts from diesel soot were mutagenic to bacteria launched an international research effort. Research from that time through the 1990s focused almost exclusively on the potential contribution of diesel exhaust to human lung cancer risk. Interestingly, although gasoline emissions contain a similar range of potentially toxic species that most likely present qualitatively similar hazards, there has been very little specific similar research on gasoline engine emissions (IARC, 2014).

The purpose of this chapter is to provide an update on our understanding on the association between lung cancer risk and diesel exhaust. Other good sources of information include the 1995 report by the Health Effects Institute (HEI, 1995), the health assessment documents developed by the California EPA in 1998 (Cal EPA, 1998), U.S. EPA in 2002 (EPA, 2002), the IARC assessment of carcinogenicity (IARC, 2014), and the 2015 Health Effects Institute Diesel Epidemiology Panel report (HEI, 2015a).

14.2 COMPOSITION OF DIESEL ENGINE EXHAUST

Diesel exhaust is a complex mixture of gases, vapors, and PM that contains a very large number of elements and compounds. Exhaust composition can vary markedly with fuel composition, engine type, operating conditions, aftertreatment devices (e.g., PM filters and catalysts), and environmental conditions. Moreover, engines range from small one-cylinder to very large multi-cylinder types, and both the amount and nature of emissions have been changing progressively over the past few decades. Thus, it is not possible consider diesel emissions as a single mixture.

The complete combustion of any petroleum-based fuel produces primarily CO_2, water vapor (H_2O), and nitrogen oxides (NO_x); the other diesel exhaust emissions result largely from incomplete combustion and pyrosynthesis (Scheepers and Bos, 1992). Fuel is injected under pressure into the combustion chamber in variable amounts to achieve different engine speeds and power outputs. Conditions promoting incomplete combustion are exacerbated, before a new steady state is reached, at each power setting, contributing to increases in emissions during load changes. The fuel is aerosolized under pressure by injection nozzles, and the air–fuel mixture is self-ignited by compression. Less than ideal injection timing, fuel aerosolization and distribution, and combustion chamber shape and temperature also contribute to incomplete combustion. The products of incomplete combustion include

carbon monoxide (CO) and unburned fuel and lubricants, together with their additives. NO_x is formed primarily by high-temperature oxidation of nitrogen in the intake air, rather than by incomplete fuel combustion.

The complexity and variability of diesel exhaust makes it difficult with certainty to associate any specific adverse health effect with any single compound or class of compounds. In describing a study of animal exposures, for example, McDonald et al. (2004) listed 6 gases, 14 classes of PM components, and 32 classes of gas- and vapor-phase organic compounds, without listing the numerous individual species within those physicochemical classes. More than 30 years ago, Opresko et al. (1984) reported that over 450 organic compounds had been identified in diesel exhaust.

Adding to the difficulty of associating a specific exhaust composition with health hazards is the fact that few studies of biological effects have included careful characterization of the exposure mixture. Until recently, most laboratory studies of inhaled diesel exhaust only reported the mass concentrations of PM and a few gases such as CO and NO_x. Exposures have frequently been described only by the particle mass concentration, implying an assumption that either PM is solely responsible for observed effects or that PM serves as an adequate surrogate for the causal components of the mixture. Estimates of health risks from epidemiological studies have also usually relied on estimated or measured concentrations of diesel PM (DPM). The reader is referred to previous versions of this chapter that described typical emissions from pre-2007 diesel engines. The U.S. EPA 2007 (PM) and 2010 (NO_x) heavy-duty engine and vehicle emission standards and highway diesel fuel sulfur control rule led to the development of technology to emit 90% less PM constituents compared with traditional diesel engines, which substantially reduced NO_x and hydrocarbon emissions (EPA, 2019). U.S. emission standards for non-road diesel engines, including locomotives, have, until recently, lagged behind on-road diesel vehicles.

14.2.1 Composition of the Diesel PM (DPM)

Concern for the toxicity of diesel exhaust has focused primarily on the PM phase and, until recently, primarily on the potential carcinogenicity of organic hydrocarbons associated with "soot" particles that were emitted from diesel engines lacking modern emission controls. The majority of DPM mass from on-road diesel engines in the United States manufactured before 2007 consisted of "soot," which consists of aggregates of spherical primary particles of 20–30 μm diameter that form in the combustion chamber due to incomplete combustion of fuel (Mathis et al., 2005), grow by agglomeration, and are emitted as clusters having volume median diameters in the 0.05–1.0 μm range (HEI, 1995). Particle size can continue to grow by agglomeration at high concentrations, resulting in mass median aerodynamic diameters of over 0.2 μm at high levels in historic animal studies (Cheng et al., 1984). The small size of diesel soot aggregates makes it readily respirable. The health risks lie in the small, invisible, or poorly visible particles; the larger soot agglomerates comprising most of the visible smoke from older engines during acceleration are too large to be readily respirable, and few would be expected to penetrate to the deep lung during inhalation.

The elemental carbon (EC) core of diesel soot, before the introduction of new diesel technology and emission control, had a high surface area (30–50 m^2/g) (Frey and Corn, 1967) and served as a nucleus for condensation of organic compounds from unburned or incompletely burned fuel or from crankcase oil volatilized from cylinder walls. As emitted, the portion of the mass of diesel soot consisting of adsorbed organic matter can range

from 5 to 90% (Johnson, 1988), with average values of 20–40%, representative of modern engines under most operating conditions. This adsorbed organic matter can be extracted from the EC core by solvents; however, heat and ultrasonic energy are required to thoroughly separate the organic carbon (OC) and inorganic carbon (EC).

Not all DPM is soot. Historically, soot constituted most of the PM mass, but non-soot "nanoparticles" 5–50 nm in size can comprise a large portion of the particle number count (PNC). On-highway measurements have shown PNCs in the range of 200–240 thousand/cc in the immediate vicinity of fresh diesel exhaust (Kittelson et al., 2004). Many of these particles consist largely of organic matter condensed on sulfate nuclei and do not have EC or ash cores, as indicated by their high-temperature volatility and chemical fingerprints (Tobias et al., 2001; Kittelson et al., 2004). Although some may exist in the tailpipe, many form as a "cloud" immediately post-emission as the heavier volatilized compounds hit cooler air. Measuring and characterizing non-EC-based nanoparticles is technically challenging.

14.2.2 Composition of the Gas and Vapor Phases of Diesel Engine Exhaust

The majority of the mass of diesel exhaust consists of gases and vapors. The majority of gases and vapors in diesel are CO_2, H_2O, CO, NO_x [primarily nitric oxide (NO) and nitrogen dioxide (NO_2)], SO_2, and organic (hydrocarbon-based) gases and vapors. Emissions of CO are typically lower than for equivalent gasoline engines, and NO*x* emissions range from similar to higher than those from comparable gasoline engines. Unfortunately, the engine design and operating factors of pre-2010 U.S. diesel engines that substantially reduced emissions of NO_2 tended to increase emissions of PM, and vice versa.

The non-PM fraction of diesel exhaust reflects the chemical composition of the fuel, as is generally true for other combustion emissions. The diesel portion of petroleum distillate consists of a complex mixture of hydrocarbons from ~C_8 to C_{25}. Aromatic compounds such as benzene, methyl-substituted benzenes, and polycyclic aromatic hydrocarbons (PAHs) are also present. The emissions of organic gases and vapors occur as emissions of unburned and partially burned fuel and, to a lesser extent, lubrication oil. Typical emissions from pre-2007 engines included carbonyl compounds, that is, alkenes, alkanes, alkynes, aromatics, and organics acids. The vapor phase contains higher-molecular-weight semivolatile organic compounds that have existed in both the gas and particle phases in fresh emissions. The known vapor-phase organics are primarily straight-chain and branched aliphatics and olefins or their oxidation products (e.g., acids). Smaller amounts of PAHs also exist as vapors and comprise the most thoroughly characterized portion. The PAHs range from two to over seven rings with the majority of the PAH mass in the gas and vapor phases.

14.2.3 Exhaust Aftertreatment

To meet the 2007 U.S. heavy-duty DPM emission standards, engine manufacturers designed DPM filters with or without and diesel oxidation catalysts (HEI, 2011). To meet the 2010 limit for NO_x emissions, selective catalytic reduction devices were introduced. Such devices inject a reductant (such as urea solution) into the engine exhaust after the PM filter that reduces NO_2 to nitrogen. Assessment of the effectiveness of these engines in reducing emissions was demonstrated by the Advanced Collaborative Emissions Study (ACES) (HEI, 2015b) supported by the Health Effects Institute. Particle mass was substantially

(~90%) below the levels specified under the regulation. In PM in exhaust from 1998 engines, EC was the major component (~70%), while the percentage of EC in 2007 and 2010 engine exhaust was substantially lower (approximately 13 and 16%, respectively). There was also a 93% reduction in NO_2 emissions in the 2010 engine compared with the 2007 engine that lacked these controls and a 41% reduction in the PNC in the exhaust.

In contrast to the chronic inhalation studies previously conduced in rats that repeatedly demonstrated a reproducible exposure-related lung tumor response, chronic rat exposure bioassays using exhaust from the new technology engines did not induce tumors or premalignant changes. These findings are in marked contrast to the effects of chronic exposure in rat studies to whole exhaust, in which lung tumors were detected at 30 months (Heinrich et al., 1986) and at 24 months (Mauderly, 1994). Gas-phase diesel engine exhaust did not increase incidence of respiratory tumors in any species tested (Benbrahim-Tallaa et al., 2012). Additional histological findings with exposure to exhaust from older engines included the pulmonary deposition of soot and a chronic inflammatory response. In response to exposure to exhaust from new technology diesel engines, there were some mild histological changes in the lung that that were not precancerous. There was also no evidence of genotoxic effects.

14.3 ENVIRONMENTAL EXPOSURES TO DIESEL EXHAUST

The environmental concentrations of airborne pollutants derived from diesel emissions are estimates, since few, if any, components or atmospheric reaction products of diesel emissions are unique; there are multiple common sources of the same types of PM, gases, and vapors. This is especially true for routinely measured $PM_{2.5}$ mass, NO_x, and CO. The organic pollutants that might provide more specific source profiles are not routinely measured. Compared with the exposure to diesel emissions worldwide, there are relatively few data for diesel soot (EC-based PM) concentrations in specific environmental locations and estimates of average exposures. EC is the surrogate marker most commonly used to estimate diesel particulate mass concentrations. Although gasoline engine (Zielinska et al., 2004) and other combustion-related emissions also contain EC, older diesel engines emitted more EC per distance traveled than other sources. Even in special studies aimed at source apportionment, based on organic compounds used as tracers, concentrations of EC attributable to diesel sources are estimates (Schauer, 2003).

In 2002, EPA reviewed estimates of environmental concentrations of DPM derived from measurements of EC and other markers and generated, using chemical mass balance, positive matrix factorization, dispersion, and other source apportionment and exposure modeling strategies (EPA, 2002). U.S. average urban and rural exposure concentrations from on-road vehicles in the mid-1990s were estimated at approximately 0.8 and 0.5 $\mu g/m^3$, respectively. Contributions from off-road sources and variations in local emissions and meteorology can result in much higher local and regional concentrations. Concentrations in cities commonly ranged from 1 to 5 $\mu g/m^3$, and concentrations of over 40 $\mu g/m^3$ were measured in local "hot spots" such as near busy city bus stops. Between 2012 and 2014, at a Boston central site, mean 24-h mean black carbon (BC) concentrations (which are closely related to EC) were reported as 0.64–0.53 $\mu g/m^3$, which is consistent with the reduction in traffic- and diesel-related emissions over the past 30 years due to improved technology (Hart et al., 2018).

There are few historical data for actual occupational exposures to DPM, although some occupational settings are known to present high concentration exposures. Most estimates are based on filter samples of $PM_{2.5}$ or on analysis of EC concentrations adjusted to estimated soot concentrations on the basis of an assumed carbon–soot ratio. Exposures of railroad workers, truck drivers, and underground miners have been of particular interest because those occupations have provided the majority of epidemiological data from which cancer risks have been estimated. Because neither $PM_{2.5}$ nor EC is unique to diesel exhaust, the accuracy of the estimates of soot exposures depends on the extent to which DPM predominates EC in the workers' environments.

Workers in enclosed spaces, and particularly in underground mines, historically have had the highest exposures. In 1998–2001, in seven mines in the United States, average shift-level respirable EC concentrations ranged from 40 to 384 $\mu g/m^3$ for underground workers and 2 to 6 $\mu g/m^3$ for surface workers (Stewart et al., 2010). Historical estimates in a mine operator job in the 1970s and 1980s ranged from 100 to 600 $\mu g/m^3$ (Vermeulen et al., 2010). Woskie et al. (1988a) used personal and work area air samplers to measure concentrations of total respirable particles in different work environments of four Northern U.S. railroads. They measured the nicotine content of the collected PM and subtracted the estimated contribution of cigarette smoke, but could not adjust for other non-diesel sources of $PM_{2.5}$. The smoke-adjusted geometric mean values for respirable particles were 17 $\mu g/m^3$ for office clerks, 39–73 $\mu g/m^3$ for engineers and firers, 52–191 $\mu g/m^3$ for brakemen and conductors, and 114–134 $\mu g/m^3$ for locomotive shopworkers.

Davis et al. (2007) measured EC in particles 1 μm or less inside the cabs of diesel-powered trucks from 36 different trucking terminals across the United States between 2001 and 2005 during typical driving shifts. Geometric mean levels in diesel heavy-duty pickup and delivery truck cabs were 1.2 $\mu g/m^3$ and in long-haul truck cabs were 1.1 $\mu g/m^3$. These measurements were slightly greater than background exposures measures upwind of trucking terminal yards, which were 0.5 $\mu g/m^3$. These findings suggest that local background exposures to sources of EC contributed to in-cab exposures in addition to exposures from truck-related sources. Estimate historical mean exposures 1971–1980 in drivers ranged from 4.6 to 10.4 $\mu g/m^3$ and 1991–2000 had decreased to 1.8 to 3.1 $\mu g/m^3$ (Davis et al., 2011). Zaebst et al. (1991) used personal samplers to measure exposures of truck drivers to EC. They reported geometric mean values of approximately 4 $\mu g/m^3$ for both city and intercity highway drivers measured in 1989–1990. These data indicated that exposures to traffic-related EC had decreased. In a trucking terminal in St. Louis, USA, where both personal and work area exposure was assessed in 2003 (Sheesley et al., 2008), the percentage of EC attributable to diesel emissions varied from 73 to 96%.

14.4 CANCER

In 2012, a Working Group at IARC reviewed the published epidemiologic studies assessing lung cancer risk and bladder cancer risk attributable to diesel exhaust exposure (IARC, 2014). The epidemiological studies supported a causal association between exposure to diesel exhaust and lung cancer. An increased risk for bladder cancer was also noted in many, but not all, available case–control studies. However, such risks were not observed in cohort studies. The Working Group concluded that there was sufficient evidence in humans for the carcinogenicity of diesel exhaust based on the lung cancer studies (IARC Group I).

The Working Group assessment was based on review of these studies that demonstrated that there was a positive relationship between exposure and lung cancer and that chance, bias, and confounding were ruled out with reasonable confidence. The Working Group also reviewed the animal bioassay data and concluded that there was sufficient evidence in experimental animals for the carcinogenicity of whole diesel engine exhaust, of DPM, and, in additional review, of extracts of DPM (Kunitake et al., 1986). It was also noted that diesel exhaust, DPM, and organic solvent extracts of DPM induced a number of types of DNA damage (IARC, 1989). The Working Group concluded that there is strong evidence for diesel exhaust to induce cancer in humans through genotoxic mechanisms.

Considerable weight was given to the studies of lung cancer risk conducted in human populations. The history of carcinogenicity bioassays (i.e., in animals) of diesel exhaust is interesting because, despite the repeated demonstration of a reproducible exposure-related lung tumor response in rats, current knowledge indicates that this is a species-specific threshold response that is not useful for estimating human lung cancer risk. In the absence of definitive data from humans, estimates of carcinogenic risks typically start with dose–response hazard data from animals and then derive human risk factors by applying adjustments for comparative dosimetry, sensitivity, and safety factors. An increased tumor incidence in animals, especially if it occurs in more than one species, is accepted as an indication of a potential carcinogenic *hazard* for humans. However, extrapolating the animal response to quantitative estimates of environmental cancer *risk* requires confidence that (1) the mechanisms by which cancer occurred in animals are likely to also operate in humans and (2) the exposure–dose–response relationship observed in animals at high levels of exposure can be extrapolated downward to the much lower levels of human exposure. Detailed reviews of the animal bioassay experience have been published in earlier editions of this text (Mauderly, 2000; Mauderly and Garshick, 2009). After much debate, both the California EPA (Cal EPA, 1998) and the U.S. EPA (EPA, 2002) decided against using the rat data as a starting point for estimating human lung cancer risks from diesel exhaust.

14.4.1 Epidemiological Evidence for Lung Cancer Risk

There is a large body of literature supporting the increased risk of lung cancer attributable to diesel exhaust exposure. The IARC Working Group considered at least 47 case–control or cohort studies. The most influential and informative epidemiological studies assessing cancer risks associated with diesel exhaust investigated occupational exposure among nonmetal miners, railroad workers, and workers in the trucking industry. The studies in nonmetal miners (Attfield et al., 2012; Silverman et al., 2012) and those in trucking industry workers (Garshick et al., 2012) were accompanied by estimates of exposure based on EC in the exhaust DPM. The U.S. miners' study included a cohort study and a nested case–control analysis that was adjusted for tobacco smoking. The results of each study were consistent, with an increasing risk of lung cancer with increasing exposure to diesel exhaust based on historical estimates of EC, with little evidence of confounding due to other exposures. In U.S. railroad workers, a 40% increased risk for lung cancer was observed compared with workers with little or no exposures (Garshick et al., 2004). In addition, a pooled case–control study of 11 case–control studies from 41 study centers in 13 countries in Europe and Canada was also strongly supportive (Olsson et al., 2011). These most informative studies are described below and summarized in Table 14.1.

TABLE 14.1 Summary of Most Informative Epidemiologic Studies Assessing Lung Cancer Risk and Diesel Exhaust Exposure

Date	Author	Design	Number of Lung Cancer Cases	Exposure Assessment	Control for Smoking	Number of Cases with Exposure	Relative Risk, Odds Ratio, Hazards Ratio		95% Confidence Interval
1987	Garshick et al.	Case–control study of all active and retired U.S. railroad workers (650,000 workers). Deaths collected 1981–1982	1,256	Yearly job title from retirement board. Next-of-kin smoking history	Next-of-kin smoking history	335	1.41, for 20 years of work in an exposed job		1.06–1.88
1988, 2004	Garshick et al.	Retrospective cohort mortality study of 54,973 railroad workers 1959–1996	4,351	Yearly job title	Indirect adjustment	2,479	1.40, train crews, unadjusted for smoking Estimated smoking-adjusted relative risk 1.17–1.27		1.30–1.51
2006	Garshick et al.	Retrospective cohort mortality study of railroad workers 1959–1996, including 39,388 deceased workers	4,055	Yearly job title	Indirect adjustment; imputation of smoking history using information from 1987 case–control study	2,358	1.35, train crews, unadjusted for smoking 1.22, train crews, smoking adjusted		1.24–1.46 1.12–1.32
2011	Olsson et al.	Pooling of 11 case–control studies from Europe and Canada	13,304	Job exposure matrix developed to assign no, low, or high exposure to diesel motor exhaust × number of years	Smoking history from original studies	5,628	Unit-years <6 6–17.33 17.34–34.5 >34.5 *p* trend <0.01	 0.98 1.04 1.06 1.31	 0.89–1.08 0.95–1.14 0.97–1.16 1.19–1.43

(continued)

TABLE 14.1 *(Continued)*

Date	Author	Design	Number of Lung Cancer Cases	Exposure Assessment	Control for Smoking	Number of Cases with Exposure	Relative Risk, Odds Ratio, Hazards Ratio		95% Confidence Interval
2008	Garshick et al.	31,135 unionized trucking industry workers employed in 1985 for 1+ years mortality follow-up through 2000	779	Years of employment in various trucking industry jobs	Indirect adjustment of smoking based on industry survey		*20 years of work*		
						323	Long-haul drivers	1.65	1.04–2.62
							Smoking, adjusted	1.40	0.88–2.24
						233	Pickup/delivery drivers	2.04	1.28–3.25
							Smoking, adjusted	2.21	1.38–3.52
						205	Dockworkers	1.94	1.18–3.18
							Smoking adjusted	2.02	1.23–3.33
						150	Combination work (local driver/dock)	2.20	1.35–3.61
							Smoking adjusted	2.34	1.42–3.83
2012	Garshick et al.	31,135 unionized trucking industry workers employed in 1985 for 1+ years mortality follow-up through 2000, 29,324 in analysis excluding mechanics	779	Cumulative exposure to elemental carbon, 5-year lag	No adjustment	122	<371 μg/m^3-months	Reference	
						191	371 to <860	1.31	1.01–1.71
						202	860 to <1803	1.38	1.02–1.87
						226	≥1803	1.48	1.05–2.10
						114	<167	Reference	
						183	167 to <596	1.17	0.88–1.57
						205	596 to <1436	1.26	0.90–1.78
						277	≥1436	1.41	0.95–2.11
2012	Attfield et al.	12,315 nonmetal miners in eight mines, 1947–1967	122	Ever-underground	No adjustment	30	<108 μg/m^3-years	Reference	
				Cumulative exposure to elemental carbon, 15-year lag		31	108 to <445	1.50	0.86–2.62
						30	445 to <946	2.17	1.21–3.88
						31	≥946	2.21	1.19–4.09
2012	Silverman et al.	Nonmetal miners in eight mines, nested case–control study, 1947–1967	198	Ever-underground	Next-of-kin history or by interview smoking history	29	<81 μg/m^3-years	Reference	
				Cumulative exposure to elemental carbon, 15-year lag		29	81 to <325	2.26	1.01–6.01
						29	325 to <878	2.41	1.00–5.82
						29	≥878	5.10	1.88–13.87

The other studies considered by IARC included occupationally exposed bus mechanics, other miners, professional drivers, and heavy-equipment operators that were supportive. Although concern has been raised regarding confounding by cigarette smoking in estimates of lung cancer risk, a similarly increased risk was observed in occupational studies whether or not smoking was accounted for. The extent that unmeasured cigarette smoking may confound the assessment of lung cancer risk depends on the association between measures of exposure and smoking. The studies, and issues regarding adjusting for smoking, were reviewed by the Health Effects Institute in 1995 (HEI, 1995) and in two meta-analyses published in the late 1990s (Bhatia et al., 1998; Lipsett and Campleman, 1999).

14.4.2 U.S. Miners' Study

The U.S. nonmetal miners study included a cohort study and a nested case–control analysis that was adjusted for tobacco smoking. Both studies demonstrated an exposure–response relationship with occupational exposure to EC while working in the mine. Attfield et al. (2012) studied mortality in a cohort of 12,315 blue-collar workers who were employed in a one of eight U.S. nonmetal mines for at least 1 year after diesel equipment was introduced. Diesel equipment was introduced 1957–1967, and mortality was assessed through 1997. The mines were selected to minimize potential confounding exposures to silica, radon, and asbestos. There were detailed work histories from company records, and historical estimates of exposure to respirable EC were constructed using an exposure assessment conducted in 1998–2001 and an exposure model based on historical CO measurements. Mine-specific diesel engine horsepower and ventilation rates were used to model CO concentrations, which were, in turn, used to model underground EC concentrations. For ever-underground workers (122 deaths), there was an increase in lung cancer risk with cumulative exposure based on a 15-year exposure lag. Relative to the first quartile of cumulative EC (<108 $\mu g/m^3$-years), the risk in the second quartile (108 to <445 $\mu g/m^3$-years) was 1.50 (0.86–2.62), the third quartile (445 to <946 $\mu g/m^3$-years) was 2.17 (1.21–3.88), and fourth quartile (≥946 $\mu g/m^3$-years) was 2.21 (1.19–4.09). Analysis using finer exposure cut-points confirmed a flattening and a possible attenuation of risk with greater exposure. Based on cumulative exposure, and excluding persons with less than 5 years of work, the risk per 1000 $\mu g/m^3$-years of EC was 1.02 (1.00–1.03, $p = 0.026$). Including estimated cumulative exposures to silica, asbestos, respirable dust, and radon did not meaningfully influence the findings.

In a nested case–control study (Silverman et al., 2012), smoking histories were obtained by direct interview or from next of kin in 198 lung cancer cases and 562 control subjects. Controls were assigned based on random sampling from all members of the cohort who were alive before the day the case subject died. Subjects were matched on birth year within 5 years, gender, ethnicity, and mine. With cumulative exposure unlagged and lagged 15 years, there was an increased risk of lung cancer with greater exposure in the ever-underground workers. For a 15-year lag, relative to the first quartile of cumulative EC (<81 $\mu g/m^3$-years), the risk in the second quartile (81 to <325 $\mu g/m^3$-years) was 2.46 (1.01–6.01), the third quartile (325 to <878 $\mu g/m^3$-years) was 2.41 (1.00–5.82), and fourth quartile (≥878 $\mu g/m^3$-years) was 5.10 (1.88–13.87), adjusting for smoking, history of respiratory disease 5 or more years before death, and an occupational exposure in another high risk job for lung cancer for at least 10 years.

14.4.3 U.S. Trucking Industry Study

A cohort of trucking industry workers was assembled from work records for 31,135 male workers employed in the unionized U.S. trucking industry for at least 1 day in 1985 (Garshick et al., 2008) of workers age 40 and over with at least 1 year of employment. Lung cancer mortality was assessed through 2000 using the National Death Index. An industrial hygiene review of previous exposures and current exposures to EC was conducted in a national exposure survey to identify eight job categories that included long-haul driver (35%), pickup and delivery driver (19%), dockworker (18%), combined driver and dockworker (17%), and mechanic (6%). Cigarette smoking histories were available from a survey of approximately 5000 current workers and recent retirees in 2003. Indirect methods based on job title-associated smoking rates were used to adjust for smoking (Schlesselman, 1978; Axelson and Steenland, 1988). A healthy worker survivor effect was considered by adjusting time on- and off-work. Lung cancer mortality risk was elevated in workers with jobs associated with regular exposure to vehicle exhaust and increased linearly with years of employment in long-haul drivers, pickup and delivery, dockworkers, and combination dockworker/drivers. Estimates of risk for 20 years of employment in a job, adjusted for smoking, were 1.40 (0.88–2.24) for long-haul drivers, 2.21 (1.38–3.52) for pickup and delivery drivers, 2.20 (1.23–3.33) for dockworkers, and 2.34 (1.42–3.83) for combination driver/dockworkers.

In the same cohort in Garshick et al. (2012), a quantitative assessment of lung cancer risk in the trucking industry cohort was done based on a reconstruction of EC exposure. An exposure assessment was conducted 2001–2006 by collecting >4000 cross-shift samples of EC measured in PM less than or equal to 1.0 μm in diameter (PM_1) in representative trucking terminals and jobs. Job-specific EC was modeled based on trucking terminal characteristics, background EC, weather, distance from a highway, land use, and U.S. Census region (Davis et al., 2006, 2007, 2011). Historical trends were modeled based on historical trends in coefficient of haze (COH), a measurement of PM based on optical density that is highly predictive of EC. However, since lung cancer risk was inversely associated with total duration of work, associations with cumulative EC exposure were stronger after adjusting for duration of employment (to control for the healthy worker survivor effect). Mechanics were excluded due to uncertainty of the accuracy of reconstruction their historical exposures. Adjusting for race, calendar year, census region, and duration of employment, risks for 5- and 10-year lagged exposures increased with each cumulative exposure quartile, with estimated HR of 1.48 (95% CI: 1.05, 2.10) and 1.41 (95% CI: 0.95, 2.11) for the highest versus lowest quartiles of 5- and 10-year lagged exposures, respectively. There was suggestion of a linear exposure–response relationship using cumulative EC as a continuous covariate and incorporating splines into the models. For each 1000 $\mu g/m^3$-months of cumulative EC, based on a 5-year exposure lag, HR = 1.07 (95% CI: 0.99, 1.15) with a similar association for a 10-year exposure lag, HR = 1.09 (95% CI: 0.99, 1.20).

14.4.4 U.S. Railroad Worker Case–Control and Cohort Studies

As noted earlier, the U.S. railroad industry converted from steam to diesel-powered locomotives mainly starting in the late 1940s, and by 1959, 95% of the locomotives in service were diesel powered. Garshick et al. (1987) collected death statistics over one year (1981–1982) from a population base of 650,000 active and retired male U.S. railroad

workers with 10 years or more of service using records from the Railroad Retirement Board. Their study included a total of 1256 exposed cases and 2385 controls, assigned on the basis of job records and contemporary measurements (early 1980s) of diesel exhaust concentrations in similar job environments (Woskie et al., 1988a, 1988b). Lung cancer cases and controls were matched by birth and death date. The cases and controls were classified by age, length of service, smoking (via next-of-kin history), and likely exposure to asbestos. Exposed workers included train crews and locomotive shopworkers; unexposed workers included workers not in these job groups. The cases were divided at age 64 into younger and older groups, with the younger group presumed to have more years of diesel exposure because of the dates of railroad dieselization, and years of work starting in 1959 in a diesel-exposed job was used as a continuous exposure variable. After adjustment for smoking (pack-years) and asbestos exposure (yes/no), the odds ratio (OR) for lung cancer among 335 cases and 637 controls for working 20 years in an exposed job was 1.41 (95% CI = 1.06–1.88). After similar adjustments, the OR for workers with 20 or more years of diesel exposure was 1.64 (95% CI = 1.18–2.29), and in analyses examining mortality in the train crews with 20 or more years of exposure was 1.55 (95% CI = 1.09–2.21). To exclude the effects of recent diesel exhaust exposure, the data were analyzed excluding exposures during the 5 years preceding death, and the relative risk (RR) for lung cancer remained similarly elevated.

Garshick et al. (1988, 2004) also conducted a retrospective cohort study of lung cancer mortality among 54,973 white male U.S. railroad workers, 40–64 years old in 1959, who had begun work 10–20 years earlier. In the original publication, mortality was assessed through 1980 and then later updated through 1996. The cohort was selected on the basis of job title in 1959 using records from the U.S. Railroad Retirement Board. As in the case–control study, exposed workers included train crews and locomotive shopworkers, and the unexposed group included clerks, ticket and station agents, and signalmen. Jobs with the most likely exposure to asbestos were excluded. There were 45,593 deaths over the 38 years of follow-up, including 4351 lung cancer deaths. Workers on operating trains (train crews) had an RR of lung cancer mortality of 1.40 (95% CI = 1.30–1.51). There was no increase in lung cancer mortality with greater years of work that was attributed to a healthy worker survivor effect and insufficient information regarding historical changes in railroad exposures. Locomotive shopworkers did not have an increased lung cancer risk, but it was later noted that the job titles of the workers included were not specific for diesel locomotive shops, thereby reducing the ability to detect an effect of exposure.

Although there was no specific cigarette smoking history information available from the workers in the cohort, smoking information was available from surveys of railroad workers, including the 1987 case–control study conducted by Garshick et al. (1987; Larkin et al., 2000). This information was used to estimate an effect of smoking that reduced the RR to 1.17–1.27 (Garshick et al., 2004). The authors also conducted an additional analysis to assess whether differences in smoking behavior between diesel-exposed and unexposed workers influenced the risk of lung cancer (Garshick et al. 2006). A simulation of smoking behavior using cause of death, birth cohort, age, and job-specific smoking prevalence from the 1987 lung cancer case–control study was conducted for 39,388 deceased railroad workers. The risk of lung cancer among exposed workers unadjusted for smoking was 1.35 (95% CI = 1.24–1.46), and after adjustment an excess risk remained (1.22; 95% CI = 1.12–1.32).

In order to improve the estimation of historical exposures during the transition from steam to diesel locomotives, historical information on diesel locomotives used by each

railroad was obtained (Laden et al., 2006). Starting in 1945, annual railroad-specific weighting factors for the probability of diesel exposure were calculated. Among workers hired after 1945, as diesel locomotives were introduced, the RR of lung cancer for any exposure was 1.77 (95% CI = 1.50–2.09), and there was evidence of an exposure–response relationship with exposure duration.

14.4.5 Pooled Case–Control Study

Olsson et al. (2011) reported a pooling and reanalysis of 11 case–control studies from 41 study centers in 13 countries in Europe and Canada. The pooled study included 13,304 cases (10,812 men and 2492 women) and 16,282 controls (13,031 men and 3251 women). The study used the lifetime occupational and smoking histories from the original studies, but these were recoded to allow a common classification. A job exposure matrix was developed and assigned no, low, or high exposure to diesel exhaust (with assigned relative intensities 0, 1, and 4) for every work period. ORs were adjusted for age, sex, study, exposure to known carcinogens based on job, pack-years, and time-since-quitting smoking. The lung cancer OR was significantly increased in the highest quartile of cumulative exposure to diesel exhaust (OR = 1.31; 95% CI = 1.19–1.43) with evidence of a dose–response trend ($p < 0.01$). Analysis of among never-smokers showed an increased OR in the highest quartile of cumulative exposure to diesel exhaust, OR, 1.26 (95% CI: 0.90–1.78).

14.4.6 Pooled Quantitative Assessment of Lung Cancer Risk

Using studies with exposure–response data based on EC, including an earlier trucking industry in the 1990s (Steenland et al., 1998; Garshick et al., 2012; Silverman et al., 2012), the coauthors conducted a meta-regression to estimate the excess lifetime risks (ELRs) of lung cancer mortality based on assumed occupational and environmental exposures to EC from diesel exhaust (Vermeulen et al., 2014) (Fig. 14.1). Estimated numbers of excess lung cancer deaths through 80 years of age for lifetime occupational exposures of 1, 10, and 25 $\mu g/m^3$ EC were 17, 200, and 689 per 10,000, respectively. For an assumed lifetime environmental exposure to 0.8 $\mu g/m^3$ EC, there were 21 excess lung cancer deaths per 10,000. Using the regression equation provided (Vermeulen et al., 2014), estimates of risk may be estimated for different exposure scenarios, including at ambient exposures. Although these results are based on occupational studies, the exposures experienced by the trucking worker cohort assessed by Garshick et al. (2012) overlap considerably with general population environmental exposures in the United States and Europe.

14.5 CONCLUSIONS

Due to the study of the health effects of diesel exhaust and the positive assessment of carcinogenic risk, new diesel technology has emerged that has the ability to substantially reduce exposure to traditional diesel soot and associated NO_x. There are many parts of the world where this technology has not been introduced since it requires (a) transition to ultralow sulfur fuel, (b) costly emissions controls, and (c) the maintenance of these controls. In these parts of world, continuing exposure to diesel exhaust from engines using traditional technology would be expected to contribute to lung cancer rates. In addition,

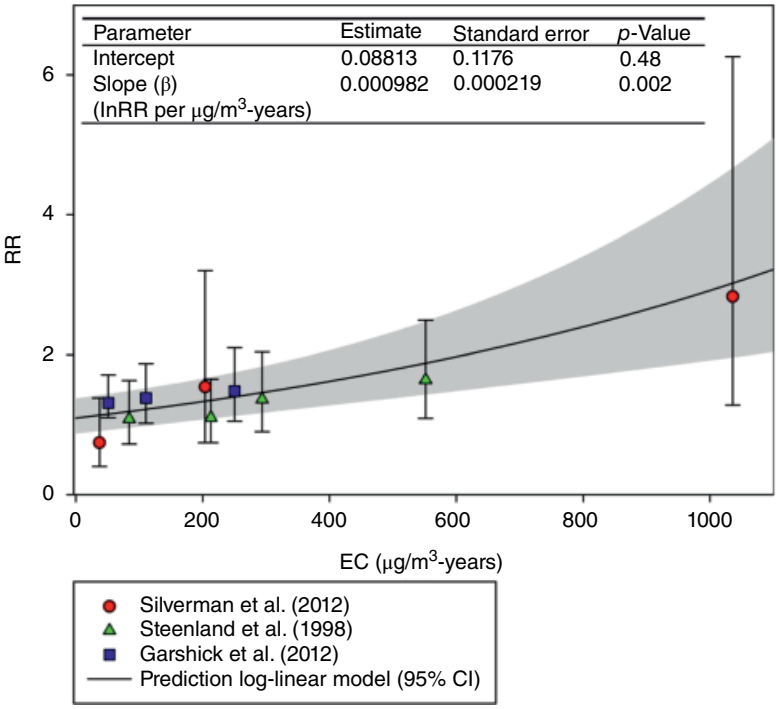

FIGURE 14.1 Predicted exposure–response curve based on a log-linear regression model using RR estimates from three cohort studies of EC exposure attributable to diesel exhaust and lung cancer mortality. Individual RR estimates [based on HRs reported by Garshick et al. (2012) or ORs reported by Silverman et al. (2012) and Steenland et al. (1998)] are plotted with their 95% CI. The shaded area indicates the 95% CI estimated based on the log-linear model. The insert presents the estimates of the intercept and beta slope factor, the SE of the estimates (Vermeulen et al. 2014).

due to their recent introduction, there are no long-term studies of potential health effects attributable to the continuing emissions of exhaust-related PM and gases from new technology diesel engines.

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15

ENDOCRINE-DISRUPTING CHEMICALS

THADDEUS T. SCHUG AND LINDA S. BIRNBAUM

15.1 INTRODUCTION

Endocrine-disrupting chemicals (EDCs) are a special group of compounds that can bind to the body's endocrine receptors to activate, block, or alter natural hormone synthesis and degradation by a number of mechanisms resulting in abnormal hormonal signals that can increase or inhibit normal endocrine functioning. EDCs are ubiquitous in our environment, food, and consumer products, making exposure to trace levels of these toxicants common and widespread in non-occupational settings (Waring and Harris, 2005). Exposure to many chemicals, including EDCs, during early stages of development, can permanently disrupt normal patterns of development and produce adverse neurological, reproductive, cardiovascular, metabolic, and immune effects in humans. Some studies have shown that these alterations can be transmitted across generations (Wolstenholme et al., 2012). Recent studies show that EDCs are capable of acting through nuclear and membrane receptors and transcriptional coactivators, as well as in enzymatic pathways involved in steroid metabolism and numerous other mechanisms that converge upon endocrine systems, including neural, reproductive, metabolic, and others. Other less studied mechanisms of action of EDCs include effects on cell signaling and trafficking (Moral et al., 2008) and alterations in epigenetic programming (Anway and Skinner, 2008). Thus, the wide-ranging actions of EDCs may result in subtle or severe disruptions in development or perturbations of normal physiology and homeostasis.

There are several characteristics of the endocrine system that must be understood in order to develop a full understanding of the mechanisms of actions and the consequences of exposure to EDCs (Table 15.1). For instance, similar to hormones, EDCs can function at very low doses in temporal and tissue-specific manners. EDCs may also exert nontraditional dose responses due to the complicated dynamics of hormone receptor occupancy and saturation. Therefore, low-dose exposures may have significant effects on hormonally

Environmental Toxicants: Human Exposures and Their Health Effects, Fourth Edition.
Edited by Morton Lippmann and George D. Leikauf.

TABLE 15.1 Common Properties of Endocrine-Disrupting Chemicals

- Bind to hormone receptors to cause downstream effects
- Are not hormones or pharmacological agents
- May alter endogenous hormone availability or action, independent of receptors
- Often have latent effects that vary with age at exposure
- Need to be considered in the context of the window of exposure, age at analysis, the sex, and the endpoint
- May have inter- and transgenerational effects
- May produce nonlinear dose responses
- Often found in mixtures

sensitive tissues. The age at which an individual is exposed to an EDC also has important consequences for health. Exposure to EDCs during development can result in different effects than exposures during adulthood (WHO, 2012), and these effects can perpetuate or even amplify over the lifetime. For this reason, the fields of endocrine disruption and developmental origins of health and disease (DOHaD) are closely associated (Diamanti-Kandarakis et al., 2009). EDCs can also affect hormone synthesis, breakdown and clearance, and transport of hormones across membranes and through tissues and influence proliferation and differentiation in hormone-producing cells and tissues.

Evidence from animal models suggests that EDCs may affect not only the exposed individual but also its offspring and subsequent generations. The likely mechanism of transmission involves non-genomic modifications of the germ line, such as changes in DNA methylation, histone modifications, and changes in microRNA populations. Altogether, EDCs pose a significant challenge to our industrialized society and to the health of humans and other receptors in the environment. In 2009 the Endocrine Society issued a statement identifying EDCs as a top area of concern in the field of endocrinology. This statement proved to be a key milestone in lending legitimacy to the EDC field in the eyes of physicians and other scientists (Diamanti-Kandarakis et al., 2009). Recent reviews and consensus documents, including a 2012 World Health Organization report and a 2013 statement by the European Food Safety Authority, have reinforced these messages (WHO, 2012). In this chapter, we review the modes of action of EDCs and selected disease endpoints.

15.2 MODES OF ACTION

15.2.1 Nuclear Receptor Signaling

EDCs are structurally similar to many hormones and function at extremely low concentrations, and many have lipophilic properties. EDCs are capable of mimicking natural hormones and exerting similar modes of action, transport, and storage within tissues (Fig. 15.1). The properties of these chemicals, while unintended, make them particularly well suited for activating or antagonizing nuclear hormone receptors. Thus, there is virtually no endocrine system immune to these substances because of the shared properties and similarities of receptors and enzymes involved in the synthesis, release, and degradation of hormones (Diamanti-Kandarakis et al., 2009).

The nuclear hormone receptors are a superfamily of transcription factors that play important roles in both normal physiology and disease. In humans, there are some 48 nuclear receptors, and many are termed “orphans,” as their endogenous ligands are yet to

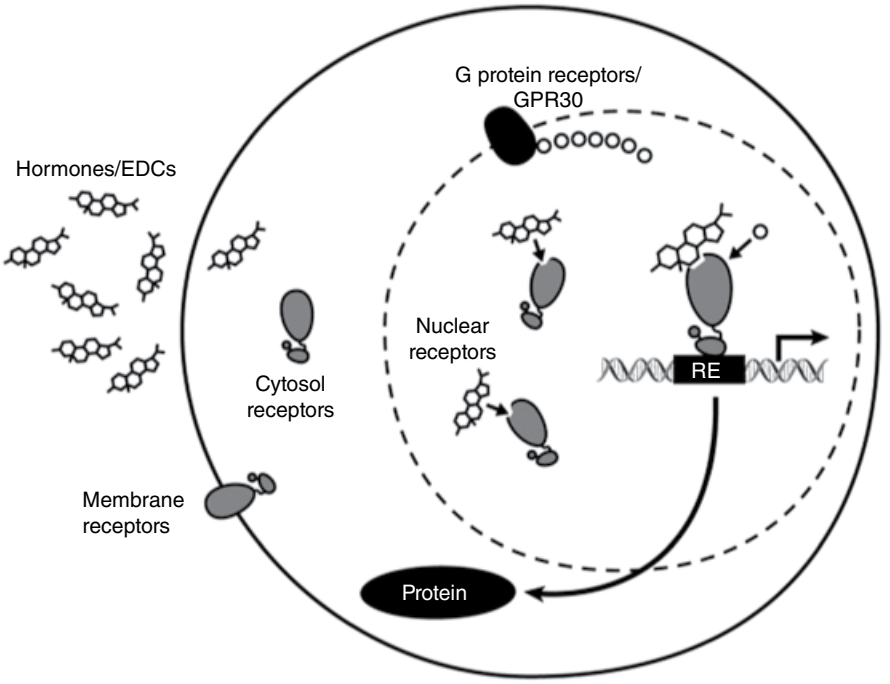

FIGURE 15.1 Illustration of steroid hormone receptor signaling pathway. Hormones, or hormone mimics, bind to membrane or cytosol receptors, which in turn shuttle to the nucleus and attach themselves to response elements (REs), where they work to regulate gene transcription and ultimately protein production. Some receptors reside solely in the nucleus atop REs in inactive forms and become activated upon hormone binding. EDCs can alter this signaling process by binding to steroid receptors and either activating or inhibiting transcriptional response.

be determined. Research on the roles of orphan nuclear receptors has been limited largely to the use of synthetic agonists to test receptor binding efficiency and target gene expression levels. This contrasts with the estrogen, androgen, and thyroid receptors (ER, AR, TR), which bind to their endogenous hormone ligands with high affinity (Heldring et al., 2007). These receptors remain at the center of endocrine disruption research, as outlined below, and results from these studies may provide a model for how other nuclear receptors interact with hormone mimics.

The estrogens are a group of steroid hormones produced by enzymatic modification of cholesterol. The primary estrogen of the reproductive years in females is 17β-estradiol (estradiol), which is derived from testosterone by aromatase activity. There are multiple estrogen receptors, some in the cell membrane as well as the nucleus. Natural estrogens include those produced by plants (phytoestrogens) and fungi (mycoestrogens). Synthetic ER activators include those intentionally produced for use in humans [e.g., diethylstilbestrol (DES), BPA] as well as chemicals targeted for other uses that have unintended ER-modulating activities [e.g., 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), methoxychlor]. Identifying chemicals that display estrogenic activity has been a major focus of the research done on endocrine disruption, in large part because of their obvious impact on reproduction. Estrogenic compounds disrupt normal development via interaction with one of the estrogen receptors. There are three types of receptors for estrogens: the

nuclear estrogen receptors (nERs), the membrane-bound estrogen receptors (mERs), which are variants of the nuclear ERs, and the estrogen G protein-coupled receptor (GPR30), which is a membrane-bound protein with a high affinity toward estrogen. The main function of the nER is as a DNA-binding transcription factor that regulates gene expression and subsequent downstream responses (Fig. 15.1).

While some EDCs act as estrogen mimics, others have variable estrogenic activity, and some act as selective estrogen receptor modulators (SERMs). For example, BPA, arguably the most studied EDC, was designed as a synthetic estrogen and has been shown to bind to the estrogen receptors ERα, ERβ, membrane ER, the estrogen-related receptor (ERRα), and even the G protein-coupled receptor, resulting in cellular signal transduction cascades that are indicative of an estrogenic response (Thomas and Dong, 2006; Watson et al., 2007; Szafran et al., 2017). However, detailed examination of its effects in a variety of tissues indicates that while there is significant overlap, it does not function at the same doses or necessarily stimulate the same suite of genes as estradiol. EDCs have also been shown to mimic or block normal androgen signaling in males, which is crucial for reproductive development, maturity, and various male secondary characteristics such as bone mass, musculature, fat distribution, and hair patterning. For example, several DDT metabolites inhibit androgen binding to the androgen receptor (AR) and androgen-induced transcriptional activity leading to delayed pubertal development in rats (Kelce et al., 1995).

In addition, there is mounting evidence that EDCs can be promiscuous and thus interact with other nuclear receptors, albeit at higher concentrations. For example, one study found that BPA binds to the thyroid hormone receptor (TR) with a lower affinity than the estrogen receptor (Moriyama et al., 2002). However, others believe that BPA acts as an indirect antagonist of thyroid hormone (TH) and that its effects on TH action *in vivo* are likely dependent on the composition and relative abundance of cofactors available in the cell (Gauger et al., 2004). Thus, EDCs are not hormones, but they do display hormone-like properties that can have wide-ranging effects on cellular systems.

The majority of EDC research has been on estrogens, androgens, and thyroid agonists and antagonists, but it is now clear that there are EDCs that affect other receptors and cellular signaling systems. Another nuclear hormone receptor targeted by EDCs is the peroxisome proliferator-activated receptor gamma (PPARγ) [reviewed by Janesick and Blumberg (2011)]. PPARγ has been shown to be a key regulator of adipogenesis, both *in vitro* and in *vivo*, and is important clinically as a target for drugs that ameliorate insulin resistance in type 2 diabetes. PPARγ functions as a heterodimer with the retinoid "X" receptor (RXR); the RXR-PPARγ heterodimer is a ligand-modulated transcription factor that directly regulates the expression of its target genes (Tontonoz et al., 1994). PPARγ is thought to be the master regulator of adipogenesis because it plays an important role in nearly all aspects of adipocyte biology [reviewed by Evans et al. (2004) and Tontonoz and Spiegelman (2008)]. Activation of PPARγ in preadipocytes induces them to differentiate into adipocytes; PPARγ is required for this process *in vitro* and *in vivo* (Rosen et al., 1999; Rosen and MacDougald, 2006). Moreover, expression of PPARγ is sufficient to transform susceptible stem cells into preadipocytes (Tang et al., 2008). Activating the PPARγ pathway drives multipotent stromal stem cells to enter the adipogenic pathway, whereas inhibition of PPARγ expression promotes an osteogenic fate [reviewed by Takada et al. (2009, 2010)]. It is known that humans whose diabetes is being treated with rosiglitazone (a drug that activates PPARγ) develop more adipocytes and gain weight (Shim et al., 2006). Therefore it is reasonable to hypothesize that chemicals capable of activating PPARγ might have the same effect [reviewed by Janesick and Blumberg (2011)]. More research is needed to determine the extent to which EDCs interact with all nuclear receptors.

15.2.2 Low-Dose and Non-monotonic Dose Effects

For many years, toxicologists have relied on the presumption that "the dose makes the poison," first proposed by the Swiss physician and alchemist Paracelsus in the 1500s. This view predicts that higher doses of a chemical will cause greater harm than low doses. This model is traditionally used to establish risk assessment profiles of chemicals. It is based on a monotonic dose–response curve generated from high and moderate dose measurements that are linearly extrapolated downward to predict toxicity at lower doses. However, multiple studies on EDCs challenge this concept and question whether higher-dose effects can be used to adequately predict low-dose effects of hormone active chemicals. These reports suggest that, similar to hormones, EDCs are capable of eliciting biphasic dose responses for many endpoints at many levels of organization [reviewed by Vandenberg (2014)]. These U-shaped and inverted U-shaped non-monotonic dose–response (NMDR) curves, which are common with many vitamins and minerals, are used as evidence that very low concentrations of EDCs can affect endpoints such as cell proliferation and organ development.

NMDR curves have been described for numerous EDCs (Conolly and Lutz, 2004). However, much controversy surrounds determining internal concentrations, the active metabolites, and the actual daily exposure levels of EDCs. The duration and route of exposure may also have a big influence on how the chemical is metabolized and whether or not the chemical remains biologically active. Additionally, the "low-dose" levels at which these chemicals function are often lower than those typically used in standard toxicology testing because animal exposures often require higher delivered doses to reach the same tissue levels observed in human. This makes it difficult to use traditional rodent models to predict relevant endpoints for human exposures, which are often several magnitudes lower than the lowest administered doses used in animal studies.

Despite the controversy surrounding the application of the "low-dose" concept to risk assessment, there are several reasons why dose response curves to toxicants may be non-monotonic. For example, the induction of metabolizing enzymes or conjugation of substrates my result in a U-shaped dose response for some endpoints. Gualtieri et al. (2010), using Sertoli cells exposed to various doses of BPA (0.5 nM–100 μM), demonstrated that only intermediate doses (10–50 μM), not high or low doses, induced an incremental increase in cell-protective glutathione levels. Their findings show that detoxification through direct conjugation was enhanced at intermediate levels and cell viability (cellular toxicity) was negatively affected at high and low doses where the cells were incapable of eliciting a protective response mechanism.

Several studies have suggested that non-monotonic responses can be explained by the downregulation, or feedback inhibition, of receptors at higher hormone levels (Medlock et al., 1991; Tibbetts et al., 1998). There is also evidence that NMDR curves are generated by the integration of two or more monotonic dose–response curves that occur through different pathways affecting a common endpoint with opposing effects (Soto et al., 1995; Vandenberg et al., 2009). Furthermore, adaptive responses through complex cell signaling pathways could cause biphasic effects that are inconsistent with traditional dose–response curves. For example, Bouskine et al. (2009) reported that BPA stimulates JKT-1 cell proliferation *in vitro* in an inverse U-shaped dose–response curve. The authors propose that BPA activates two different signaling pathways that are distinct in both signaling mechanism and time frame of response. Low-dose and non-monotonic dose responses have important implications for EDCs, since it well known that most hormonal signaling pathways are regulated by negative feedback and it has been demonstrated that EDCs differentially affect the stability of nuclear receptor proteins and ligands [reviewed by Tabb and Blumberg (2006)].

15.2.3 Developmental Windows of Susceptibility

Adult exposure to EDCs is certainly an important factor in adverse health outcomes; however *in utero* and early-life exposures are of primary concern since developing organisms are extremely sensitive to perturbation by chemicals with hormone-like activity. Adverse effects may be most pronounced in the developing organism and occur at concentrations of the chemical that are far below levels that would be considered harmful in the adult (Newbold et al., 2006, 2007a). Some of the reasons for this increased sensitivity include the fact that the protective mechanisms that are available to the adult such as fully functional metabolic systems, DNA repair mechanisms, a competent immune system, detoxifying enzymes, and establishment of the blood–brain barrier are not fully functional in the fetus or newborn. In addition, the developing organism has an increased demand for nutrients as compared with an adult, which, in some cases, may result in increased toxicity (Newbold et al., 2007b). Finally, prenatal exposure to environmental factors can modify normal cellular and tissue growth trajectories through developmental programming, such that individual may have a higher risk of health disorders later in life.

While fetal development is commonly known to be a period of increased sensitivity to chemical insult, childhood and adolescence are also marked by continued maturation of key endocrine systems and are therefore also susceptible to chemical exposure. The DOHaD hypothesis, first proposed by David Barker in 1997, showed that poor *in utero* nutrition resulted in high rates of disease later in life (Barker and Clark, 1997). This concept has been expanded to include non-nutritional early-life exposures that have been shown to alter the body's physiology. Thus the DOHaD paradigm provides a framework to assess the effect of not only early nutrition but also EDCs on long-term health (Silveira et al., 2007).

Of special concern are man-made hormone-mimicking chemicals capable of misdirecting developmental processes. Recent studies document detectable amounts of a variety of EDCs such as phthalates, polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons, and BPA in pregnant women, fetuses, newborns, young children, and adolescents (Murphy and Jirtle, 2003; Klose and Bird, 2006; Tang and Ho, 2007; Bhan et al., 2014; Ho et al., 2015; Tauheed et al., 2017). Since each organ system has a different developmental trajectory, and the sensitive window for exposures to cause toxicity is during tissue development, the effects of exposures are dependent on both timing and dose of exposure (Gore et al., 2015). Studies demonstrate that developmental exposures can lead to subtle physiological changes that may not emerge until later in life (Liu et al., 2013). Studies have linked early-life chemical exposures to subsequent increases in the rates of many common human diseases, including asthma, learning and behavioral problems, early puberty, infertility, breast and prostate cancer, Parkinson's disease, obesity, and other adverse health conditions (Cowin et al., 2010; Liu et al., 2013; Derghal et al., 2016).

15.2.4 Epigenetic Programming

One of the most commonly studied mechanisms of DOHaD is the effect of EDCs on the levels of enzymes that regulate epigenetic patterns, particularly of the DNA methyltransferases (DNMTs). Many studies implicate alteration of epigenetic marks, which have a central role in mediating transfer of information that is stored in the genome (Jirtle and Skinner, 2007). The term epigenetics refers to the factors "above" DNA that regulate its activity but are independent of the DNA sequence. Epigenetic changes can occur through

chemical modifications to the DNA (e.g., DNA methylation), histone modifications, and noncoding RNAs, rather than mutations of the DNA sequence itself. Epigenetic markers carried over from parents are typically wiped clean during events that happen early in embryonic development; however, emerging evidence shows that EDCs may interfere with this process (Walker and Gore, 2011). DNA methylation takes place at the carbon-5 position of cytosine in CpG dinucleotides due to DNMTs (Klose and Bird, 2006). Methyl-binding proteins then attach to these sites and subsequently attract other chromatin-modifying proteins, with the end result being a silencing of the methylated gene. On the other hand, hypomethylated genes tend to be more accessible to transcriptional machinery and can generate increased and inappropriate gene expression (Tang and Ho, 2007).

There are also several other epigenetic factors that can modify DNA to alter gene expression, including histone remodeling and regulation by small noncoding RNAs. For example, a recent study conducted in Bangladesh, a country with high environmental arsenic exposure through contaminated drinking water, reported significant correlations between arsenic exposure and posttranslational histone modifications in plasma of expectant mothers (Tauheed et al., 2017). The group also reported that folate-deficient women exposed to arsenic had altered plasma histone modification levels and elevated risks of having children born with spinal cord defects. Other studies have reported EDC-induced modifications of noncoding RNAs (Bhan et al., 2014; Ho et al., 2015). For example, Ho et al. found that BPA exposure to prostospheres resulted in repressed expression of small nucleolar RNAs with C/D motif (SNORDs), ncRNAs that regulate ribosomal RNA assembly and function. BPA induced changes in H3K9me3, H3K4me3, and H3K27me3 histone methyl marks at several SNORDs while having little effect on DNA methylation. Altogether, these studies of exposure-induced changes of the epigenome by EDCs are providing new insights into epigenomic plasticity that is vulnerable to disruption by environmental exposures.

During development, the epigenome cycles through a series of precisely timed methylation changes designed to ensure proper development. The appropriate timing and extraordinary accuracy of methylation in the gametes and following fertilization make this highly concerted system particularly vulnerable to interference from environmental exposures (Murphy and Jirtle, 2003). The highly orchestrated organizational processes that occur during these critical developmental periods give rise to concerns about vulnerability during early stages of life. For instance, epigenetic marks such as methylation patterns are laid down during development and are responsible for the programming necessary to transform stem cells into differentiated cells and tissues. The loss and subsequent reestablishment of the epigenetic profile in the developing embryo comprise a critically sensitive period during which the system is particularly vulnerable to environmental influences. Exposures to environmental chemicals, many with endocrine activity, can alter the epigenetic programming of both somatic and germ cells inducing subtle functional changes leading to disease later in life and in future generations.

Although epigenetic effects of EDCs have been well documented, the exact mechanisms by which they interfere with epigenetic marks are not well understood. The most commonly studied mechanism is the effect of EDCs on the levels of enzymes that regulate epigenetic patterns, particularly of the DNMTs. Several studies describe effects of EDCs on DNMT expression, on both gene and protein levels (Gore et al., 2015), and others have reported regulation of methyltransferases through modulation of mRNA expression. For example, aryl hydrocarbon receptor-deficient mice have decreased levels of *Dnmt1* and *3b* mRNA (Liu et al., 2013). The endocrine disruptor vinclozolin, an AR antagonist, was

shown to induce a marked increase in *Dnmt* mRNA expression *in vivo* through an AR-mediated pathway (Cowin et al., 2010). Another mechanism by which EDCs affect DNMTs is via small interfering microRNA (miRNA) expression. For instance, it has been shown that neonatal exposure to BPA increases the expression of miRNA-29, which in turn decreases *Dnmt1*, *Dnmt3a,* and *Dnmt3b* mRNA levels, concomitant with other gene expression changes, for example, decrease in the anti-apoptotic myeloid cell leukemia sequence 1 (Mcl-1) protein (Derghal et al., 2016). Importantly, epigenetic endpoints have the potential to provide early indications of later-life adversities as epigenetic changes often precede cellular and organismal alterations. Therefore, there is potential for using epigenetic endpoints as biomarkers for EDC exposures.

15.3 SELECTED DISEASE ENDPOINTS

15.3.1 Male Reproduction and Development

Given the fact that both hormone production and action are regulated in large part by the reproductive tissues, it is not surprising that EDCs contribute to many adverse reproductive health outcomes in developing and adult humans (Table 15.2). Epidemiological data has revealed an increase in male reproductive function disorders over the past 50 years, suggesting a correlative relationship with the increasing amounts of EDCs in the environment (Delbes et al., 2006). In the context of male reproductive health, EDCs have been linked to (1) disrupted reproductive function, displayed as reduced semen quality and quantity and infertility; (2) altered fetal development, displayed as urogenital tract abnormalities, including hypospadias and cryptorchidism; and (3) testicular germ cell cancer (TGCC) (Vos et al., 2000; Diamanti-Kandarakis et al., 2009; Levine et al., 2017). As previously mentioned, the potential lag between exposure to EDCs and the manifestation of a clinical reproductive disorder is of critical concern. In humans, this period may be years or decades

TABLE 15.2 Common Endocrine-Disrupting Chemicals

Compound	Use/Source	Disease Links
Bisphenol A	Plastics, thermal receipts	Breast and other cancers, metabolism, puberty, neurobehavioral
Phthalates	Plastics, fragrances	Low sperm count, metabolism, birth defects, asthma, neurobehavioral
Polychlorinated biphenyls (PCBs)	Electrical coolant and other uses	Cancer, developmental issues
PBDEs	Flame retardants	Thyroid disruption, neurological issues
Lead	Drinking water, paint, gasoline	Neurological issues, premature birth, kidney disorders
Mercury	Burning coal, seafood	Neurological issues, diabetes
Dioxin	Formed in industrial processing	Cancers, sperm quality, fertility, neurobehavioral
DDT/DDE/DDD	Pesticides	Cancers, developmental toxicity
Arsenic	Drinking water, animal feed, herbicides, fertilizers	Cancers, diabetes, immune suppression, neurodevelopment, cardiovascular disease

post-exposure because sexual maturity and fertility cannot be assessed until the exposed individual has attained a certain age (Diamanti-Kandarakis et al., 2009).

Skakkebaek et al. (2001) have suggested that the incidences of cryptorchidism, hypospadias, and poor semen quality are risk factors for one another and that they are all predictive of developing testicular germ cell cancers. This quartet is defined as the testicular dysgenesis syndrome (TDS). They propose that the etiology of TDS lies in the diminished androgen action in fetal developmental periods and has a negative impact on the proper functioning of Sertoli cells (the cells supporting germ cells) and Leydig cells (where androgen synthesis occurs). This hypothesis proposes a strong association between environmental exposures and development of TDS.

Identifying environmental causes of TDS in humans is difficult because developing fetal tissues are inaccessible for examination. Thus, the majority of mechanistic evidence linking EDCs to TDS comes from animal experiments. It is possible to experimentally induce all the elements of TDS, except for germ cell cancer, by exposing pregnant rats to phthalates and other chemicals that block androgen action (Foster, 2005). This model is referred to as the "phthalate syndrome" model, and it comprises non-descent of testis, malformations of the external genitalia, poor semen quality, and malformations of other sex organs (Foster, 2006). The causes of phthalate syndrome center on suppression of fetal androgen action, which is the key driver of male reproductive organ development. Phthalates lower levels of testosterone and its derivatives by interfering with the uptake of steroid hormone precursors into fetal Leydig cells where steroid synthesis takes place. The net results are malformations of internal reproductive organs, hypospadias, retained nipples, and feminized anogenital distance (AGD) (Foster, 2006). Swan (2006) reported similar effects in humans prenatally exposed to phthalates, as well as declines in semen quality in adult males exposed to phthalates. Certain pesticides, such as vinclozolin, are able to block the AR or interfere with the conversion of testosterone into dihydrotestosterone, thus producing effects similar to phthalate syndrome. Androgen action also is essential for proliferation and development of Sertoli cells, which are necessary for sperm production. Altogether, EDC-mediated disruption of androgen action during fetal development results in reduced fertility later in life (Sharpe, 2010).

Epidemiological studies have identified an association between chemical exposure [e.g., to phthalates, polychlorinated biphenyls (PCBs), dioxins, and certain nonpersistent pesticides] and reduced semen quality. In a U.S.-based study, Duty et al. (2003) found links between monobutyl phthalate exposure and poor sperm motility and concentrations. A study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure conducted by Mocarelli et al. (2008) suggests that timing of exposure has a significant impact on semen quality. This study was based on men exposed to high levels of TCCD as a result of a chemical plant explosion in 1976 in Seveso, Italy. Men exposed prepubertally (1–9 years of age) demonstrated poor semen quality as adults. Interestingly, men exposed between 10–17 and 18–27 years of age showed slightly positive or no differences in semen quality, respectively. Several occupational studies have found associations between pesticide exposure and reduced semen quality (Whorton et al., 1979; Larsen et al., 1998; Juhler et al., 1999; Abell et al., 2000; Padungtod et al., 2000; Oliva et al., 2001; Lifeng et al., 2006). In a study on male partners of pregnant women, Swan et al. (2003) found elevated odds ratios for poorer semen quality in relation to urinary concentrations of several common pesticides. Meeker et al. (2004) also found an inverse relationship between urinary pesticide levels and sperm concentration and motility in men. While there are clear associations between EDCs and diminished male reproductive health, there is a need for further epidemiological studies

to identify the classes of chemicals, exposure levels, and the most critical windows of susceptibility important to male reproductive health.

15.3.2 Female Reproduction and Development

The ability of EDCs to alter reproductive function and health in females has been clearly demonstrated by the consequences of DES use in pregnant women. The daughters of women given DES while pregnant were shown to have rare vaginal adenocarcinomas (Herbst et al., 1971; Barclay, 1979), decreased fertility, increases in rates of ectopic pregnancy (Goldberg and Falcone, 1999), and early menopause (Hatch et al., 2006). Many of these disorders have been replicated in laboratory animals treated with DES during gestation (Newbold et al., 1999, 2007b; Newbold, 2004, 2008; Kim et al., 2009; Miyagawa et al., 2011).

Proper development of ovarian follicles in the fetus is dependent on estrogen exposure during critical periods of development. For instance, mice treated with DES on postnatal day 1–5 develop multi-oocytic follicles as adults (Kipp et al., 2007). Therefore, maintaining a homeostatic balance of local and systemic hormones during follicle development is necessary for normal follicle development and germ cell quality (Crain et al., 2008). Perturbations in hormone signaling resulting from chemical exposures during developmental periods could contribute to ovarian disorders and declining conception rates in human populations (Braw-Tal, 2010). While the mechanisms by which EDCs alter follicle development are not fully understood, there is evidence that these chemicals are contributing to increased rates of aneuploidy (Hunt et al., 2003), polycystic ovary syndrome (PCOS) (Knochenhauer et al., 1998; Yildiz et al., 2008), premature ovarian failure (POF), and altered cyclicity and fecundity (Markey et al., 2003; Bonassi et al., 2004; Jefferson et al., 2005; Munoz-de-Toro et al., 2005; Rubin et al., 2006). For example, studies have shown that prenatal exposure to BPA causes irregular cycles in mice, which is likely due to hypothalamic alterations in the circuitry that controls luteinizing hormone (LH) secretion and ovulation (Rubin et al., 2001; Markey et al., 2003). In humans, altered cyclicity has been reported in individuals exposed to organochlorine pesticides. Indeed, cycle irregularities have been noted in women whose mothers were exposed *in utero* to DES (Titus-Ernstoff et al., 2008).

Some EDCs are known to target the ovary and cause reproductive health problems such as infertility and POF. POF leads to early infertility and is associated with an increased risk of osteoporosis, depression, cardiovascular disease, and early death (Patel et al., 2015). Phthalates have been shown to alter follicle development and growth and impair follicle functionality. Studies in mice have shown that exposure to phthalates may lead to acceleration of primordial follicle recruitment (Patel et al., 2015). For example, exposure to di(2-ethylhexyl) phthalate (DEHP) was reported to accelerate primordial follicle recruitment through a mechanism that involves overactivation of the phosphatidylinositol 3-kinase (PI3K) pathway (Hannon et al., 2014). Exposure to mono(2-ethylhexyl) phthalate (MEHP), the bioactive metabolite of DEHP, accelerated primordial follicle recruitment, thus accelerating folliculogenesis (Hannon et al., 2015). These studies provide evidence that exposure to certain phthalates affects ovarian folliculogenesis, but more data are needed to determine if other phthalates inhibit follicle growth and to determine the mechanisms by which they do so.

Uterine fibroids (leiomyomas) are the most common tumor of the female reproductive system (Walker and Stewart, 2005), occurring in 25–50% of all women. The risk of the

development of uterine fibroids increases with age during premenopausal years, but tumors typically regress with the onset of menopause (Cramer and Patel, 1990). Obesity, age at menarche, and unopposed estrogen signaling have been shown to increase the risks for fibroids (Kjerulff et al., 1996). The best characterized animal model for the study of uterine fibroids is the Eker rat. A mutation of the tuberous sclerosis complex 2 (Tsc2) tumor suppressor gene causes females to develop spontaneous uterine fibroids at a high frequency (Everitt et al., 1995). Studies using this model have shown that exposure to EDCs increases the incidence of fibroids in these animals (Newbold et al., 2002). Population studies have shown that exposures to natural soy-derived phytoestrogen alter morphology and cell proliferation of uterine leiomyoma and normal myometrial cells (Moore et al., 2007). In the Sister Study involving 19,972 non-Hispanic white women who were 35–59 years of age, researchers found that greater risk of early fibroid diagnosis was associated with soy formula during infancy (D'Aloisio et al., 2010). Also, in a cohort of African-American women ages 23–34 years, Upson et al. (2016) reported that women fed soy formula as infants have larger fibroids than unexposed women. Developmental exposure to DES causes rats that are genetically predisposed to uterine tumors to develop even more tumors of a larger size, but fails to induce tumors in wild-type rats. Importantly, DES exposure imparts a hormonal imprint on the developing uterus that causes an increase in estrogen-responsive gene expression (Crain et al., 2008). The potential for DES to cause uterine fibroids in humans is less clear. Two studies on DES daughters came to different conclusions. In a study of 2570 women born during the period DES was prescribed, no association was found between prenatal exposure and uterine fibroids (Wise et al., 2005). Another study of 1,188 women found a significant relationship between DES exposure and uterine fibroids (Baird and Newbold, 2005). On analysis of these studies, Baird and Newbold concluded that there was a definitive increase in uterine fibroids in DES daughters and the discrepancies between the studies were due to the differences and sensitivities of the methods used to detect the tumors.

In summary, both animal and human studies suggest a role of EDCs in altering female reproductive development. Data from animal experiments show that EDC exposure during critical periods of development, both prenatal and neonatal, can induce functional changes that appear later in life. There are data gaps in understanding the mechanisms by which EDCs carry out their action, but it is clear that to reduce the risk of reproductive disorders, we must take action to reduce exposure to these chemicals.

15.3.3 Metabolic Disorders

There is now compelling evidence linking prenatal exposures to a variety of chemicals with altered developmental programming that may lead to weight gain (Janesick and Blumberg, 2011) and metabolic disturbances, such as diabetes, later in life (Alonso-Magdalena et al., 2010, 2011). One well-studied example concerns the effects of maternal tobacco smoking. Babies born to mothers who smoke are typically born with a low birth weight but experience increased risk of obesity, cardiovascular disease, and metabolic syndrome later in life; thus some components of tobacco smoke that are transported to the fetus are "obesogens" (Power and Jefferis, 2002).

Obesogens are functionally defined as chemicals that promote weight gain by acting directly on adipocytes (to increase their number or the storage of fat) or indirectly by altering mechanism through which the body regulates appetite and satiety, by altering basal metabolic rate, or by altering energy balance to favor the storage of calories (Grun and

Blumberg, 2009a; Janesick and Blumberg, 2011). Many known obesogens are EDCs that can act as direct ligands for nuclear hormone receptors or affect components in metabolic signaling pathways under hormonal control (Janesick and Blumberg, 2011). Indeed, environmental chemicals such as tributyltin (TBT) and triphenyltin (TPT) are known to stimulate adipogenesis *in vitro* and *in vivo*. TBT and TPT are nanomolar affinity ligands for the RXR–PPARγ heterodimer (Kanayama et al., 2005; Grun et al., 2006) and stimulate 3T3-L1 preadipocytes to differentiate into adipocytes (Inadera and Shimomura, 2005; Kanayama et al., 2005; Grun et al., 2006) in a PPARγ-dependent manner (Kirchner et al., 2010; Li et al., 2011). The ligand-binding pocket of PPARγ is large and considered to be promiscuous (Nolte et al., 1998); therefore, it is not surprising that an increasing number of other chemicals with dissimilar structures have been shown to be PPARγ ligands (Janesick and Blumberg, 2011). It is currently unknown how many environmental chemicals activate PPARγ and whether some or all of these will ultimately turn out to be obesogens. However, there is little doubt that activating PPARγ is an important pathway for adipogenesis and obesity (Janesick and Blumberg, 2011).

There is growing concern in the scientific community that EDCs may be contributing to the rapid increased rates of diabetes and metabolic syndrome. It is of particular concern that the incidence of both obesity and type 2 diabetes is rising rapidly in the young. While there can be no argument that eating calorie-dense, nutrient-poor food in large portions combined with lack of exercise plays an important role, the rapid rise in obesity and diabetes in the young raises the question of whether early-life exposures to certain chemicals may also be playing an important role. A substantial body of evidence suggests that a subclass of EDCs, which interfere with endocrine signaling, can disrupt hormonally regulated metabolic processes, especially if exposure occurs during early development. These chemicals, so-called obesogens, might predispose some individuals to gain weight despite their efforts to limit caloric intake and increase levels of physical activity (Heindel et al., 2007). Studies have linked exposure to EDCs such as BPA, dioxins, and organochlorine and organophosphate pesticides with the incidence of metabolic syndrome and diabetes (Alonso-Magdalena et al., 2010, 2011; Hectors et al., 2011). It is well known that obesity and type 2 diabetes are associated pathologies and several publications have associated weight gain/obesity (Grun and Blumberg, 2009a, 2009b; Janesick and Blumberg, 2011) and diabetes. Some studies suggest that EDC exposures can alter epigenetic patterns in adipose generating stem cells, leading to increased fat cell size and numbers (Grun et al., 2006). Others suggest EDC-induced alterations in germ cells leading to permanent shifts in metabolic set points (Chamorro-Garcia et al., 2017). While the precise metabolic pathways targeted by most of these chemicals are uncertain at present, the number of studies linking EDCs with obesity, metabolic syndrome, and diabetes is growing. Understanding the molecular mechanisms involved in the role of epigenetics and early-life exposures will provide important insights into the etiology of these chronic disorders and should play an important role in designing effective prevention strategies.

15.3.4 Preconception and Transgenerational Effects

Some chemicals, including some EDCs, have the potential to cause health effects in the offspring of exposed individuals through environmentally induced epigenetic modifications. Experiments by Michael Skinner and colleagues demonstrate that male rats whose female ancestors were exposed to the fungicide (and EDC) vinclozolin are less attractive to females and show accelerated onset of cancer, prostate disease, kidney disease, and immune

defects (Anway et al., 2005; Crews et al., 2007). Other studies have shown that high doses of a variety of EDCs could also elicit transgenerational effects in third-generation offspring (Crews et al., 2012; Manikkam et al., 2012; Wolstenholme et al., 2013).

The mechanisms by which environmental exposures cause transgenerational effects are unclear. One hypothesis leans toward epigenetic inheritance patterns, which involve chemical modifications to the DNA (DNA methylation), histone modifications, and noncoding RNAs, rather than mutations of the DNA sequence itself. Most epigenetic marks carried over from parents are typically wiped clean during events that happen early in embryonic development. Researchers have found that transgenerational effects can result from chemical dosing at precise windows in fetal development—specifically, at the time of sex determination, which occurs around embryonic days 41–44 in humans (Anway et al., 2005). Together, this emerging body of research suggests that exposure to EDCs could have consequences not only for our own health and for that of our children but also for the health of generations to come.

Germ cell development during the preconceptional time period involves rapid cell growth, meiotic division, and hormonal and epigenetic changes. It is likely that maternal and paternal exposures occurring during this window of susceptibility can have lifelong effects on the offspring. Evidence shows that EDC exposures can have subtle effects including interference with meiosis, alteration of redox states, and changes in the epigenome (Gely-Pernot et al., 2015). These effects can influence developing organs, tissues, and cells in a process known as reprogramming and can lead to differences in adult phenotypes (Walker, 2016).

There is a significant body of evidence showing that the paternal and maternal preconceptional exposure to drugs, social instability, nutritional status, and smoking can have adverse effects on the offspring. For example, maternal smoking is associated with increased risk of congenital heart defects (Correa et al., 2015), and paternal smoking is associated with type 2 diabetes in the offspring (La Merrill et al., 2015). However, to date, only a few studies have reported associations between preconceptional environmental chemical exposures and their effects on lifelong health.

15.4 CONCLUSION

Humans are exposed to thousands of chemicals during their lifetime through the air, food, and water. A significant number of these chemicals can be toxic, since they can disrupt the endocrine system. Over the past decade, the list of chemicals with endocrine-disrupting activity has dramatically increased. Evidence has shown that EDCs compromise the reproductive system, thyroid signaling mechanisms, tissues and organs associated with energy metabolism, glucose control, fat cell development, and hormone systems in the central nervous system that regulate satiety and eating behaviors (Desai et al., 2018). Since EDCs activate the same receptors and signaling pathways as hormones and act at low concentrations, they are subject to the same biological regulatory systems as hormones. And since hormones control all aspects of physiology across the lifespan, the same can be expected from EDCs.

Hormones play a critical role in tissue development and the programming of stem cells and tissues during the developmental process. The same can be said for EDCs. The DOHaD paradigm illustrates that many, if not all, diseases have their origin during development. EDCs pose the most risk during the development period as they alter programming, which

leads to increased susceptibility to disease later in life. Testing for chemicals with endocrine-disrupting activity can be challenging, as the effects are often subtle (functional changes such as alterations in epigenetic marks and changes in gene expression), and they can manifest effects later in life, long after the EDC is eliminated from the body. Over the past 40 years, there has been a significant increase in a variety of endocrine-associated diseases including, infertility, premature puberty, ADHD, obesity, and diabetes and endocrine cancers such as prostate, ovarian, and breast. It is biologically plausible that EDCs are playing a significant role in these and other diseases.

More research is needed to better understand the health effects of EDCs, and additional effort is needed to understand critical roles of dosing and timing of exposures. Nevertheless, there should be concerted efforts to reduce exposures to EDCs across the lifespan, with particular emphasis on men and women of reproductive age, pregnant women, infants, and children.

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16

FORMALDEHYDE AND OTHER SATURATED ALDEHYDES

GEORGE D. LEIKAUF

16.1 BACKGROUND

Defined by a reactive, polarized carbonyl group (–CHO), low-molecular-weight aldehydes are a family of organic compounds useful in a large number of industrial processes. The simplest aldehyde, formaldehyde (HCHO), is one of the top 10 organic chemical feedstocks and one of the top 20 chemicals produced in the United States. Other widely used saturated aldehydes include acetaldehyde (CH_3CHO), propanal aka propionaldehyde [CH_3CH_2CHO], butanal aka butyraldehyde [$CH_3(CH_2)_2CHO$], pentanal aka valeraldehyde [$CH_3(CH_2)_3CHO$], and hexanal [$CH_3(CH_2)_4CHO$], which differ from formaldehyde in carbon chain length. Additional low-molecular-weight saturated aldehydes include the dialdehydes: malondialdehyde [$OHCCH_2CHO$] and glutaraldehyde [$OHC(CH_2)_3CHO$]. The toxicity of these compounds cannot be predicted by their structure alone. For example, formaldehyde, the metabolite of methanol, is more toxic than acetaldehyde, the metabolite of ethanol, in most assays.

16.1.1 Human Environmental Exposure

Human aldehyde exposures result from exogenous sources and endogenous formation (i.e., biogenesis through metabolism or oxidative stress) (Benedetti et al., 1980, 1984; NRC, 1981, 2011, 2014; Nilsson and Tottmar, 1987; Marnett, 1988; Uchida et al., 1998; Langevin et al., 2011; Rosado et al., 2011; Garaycoechea et al., 2012; Schroeter et al., 2014; Yu et al., 2015b). Exogenously formed or environmental aldehydes can be generated naturally through tropospheric reactions of terpenes and isoprene released from foliage with hydroxyl radicals. In addition, major sources are the incomplete combustion of alcohols or the release from polymeric substances and solutions. Concern over the health

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effects of environmental aldehydes continues because of formation from automotive fuels containing alcohols (ethanol and methanol) (Othmer, 1987). Sources other than motor vehicle exhaust include power plants, incinerators, paper mills, and refineries. Other valuable reviews on aldehyde toxicity include reports by the National Research Council (NRC, 1981, 2014), Feinman (1988), Marnett (1988), Council on Scientific Affairs (1989), World Health Organization (WHO, 1989, 1995, 2010), Heck et al. (1990), McLaughlin (1994), U.S. Environmental Protection Agency (USEPA, 2010), and International Agency for Research on Cancer (IARC, 1982, 1985, 1995a, 1995b, 1999, 2006, 2012).

16.1.1.1 Indoor Air Estimated contributions from three indoor sources of formaldehyde are listed in Table 16.1. Considering the average time–activity pattern, poor indoor air quality in the home is the most common source of aldehyde exposure. Aldehyde-generating activities in the home include tobacco smoking, wood burning, and cooking. Other common sources include release from paint, structural materials, furnishings, clothing, cosmetics, and insulation (Pickrell et al., 1983; Feinman, 1988). We typically spend less than 1 h per day (18–42 min) outside (Fig. 16.1), so that outdoor exposures constitute only 3% of our average daily exposure (Chapin, 1974; Samet et al., 1987; Samet and Spengler, 1991). Excluding the other activities, such as time in transit (1.0–1.6 h per day) and occupational exposure for those working outside the home 5.2–6.7 h per day, the remaining and primary exposure for most individuals occurs at home. Indoor exposure therefore equals 55–65% for those working outside the home and 85% for those working inside the home (Songco and Fahey, 1987).

Of the volatile organic compounds measured indoors, formaldehyde (Salthammer, 2011; Sarigiannis et al., 2011) and acetaldehyde (Bradman et al., 2017; Langer et al., 2017) are typically the most common. In the past, urea-formaldehyde foam insulation was a source leading to the highest home exposures, including exposures averaging 120 ppb (Gupta et al., 1982). Concentrations in older homes without foam insulation typically range ≥20 ppb, whereas high-level exposures of up to 4200 ppb have been recorded in mobile homes (Georghiou et al., 1983; IARC, 1985, 1995a, 1995b, 2006, 2012). Newer homes and structures have levels about 30% higher than older homes (Guo et al., 2009; Coombs et al., 2016; Dodson et al., 2017). Most study of homes reported concentrations averaged

TABLE 16.1 Indoor Sources of Formaldehyde Exposure

Sources	Concentration
Cigarette smoke (40 ppm in 40 mL puff)	
Dose per pack for smoke	0.38 mg per pack
Environmental tobacco smoke	0.25 ppm
Clothing made with synthetic fibers	
Men's polyester cotton blend	2.7 μg/g per day
Women's dress	3.7 μg/g per day
Furnishings	
Particle board[a]	0.4–8.1 μg/g per day
Plywood	1.5–5.3 μg/g per day
Paneling	0.9–21.0 μg/g per day
Draperies	0.8–3.0 μg/g per day
Carpet/upholstery fabric	≤0.1 ppm

Sources: Pickrell et al. (1983) and Feinman (1988).
[a]Made with urea-formaldehyde resin.

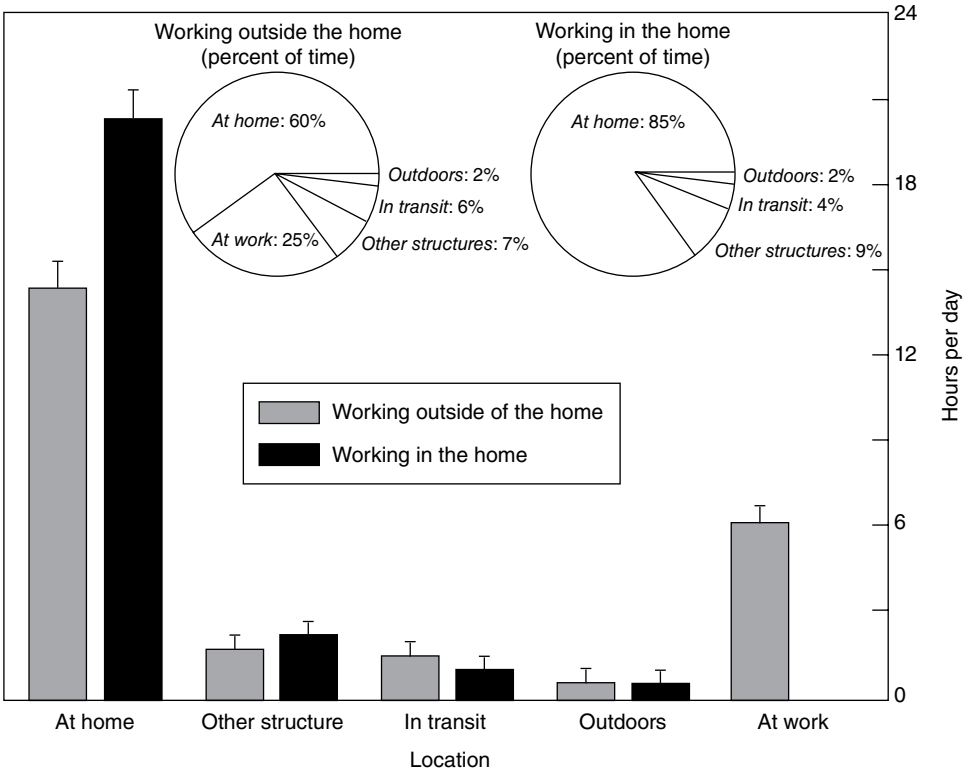

FIGURE 16.1 Time spent per day at various locations for adults. Values for working outside home are for men and women and for working in the home are for women. *Sources*: Adapted from Chapin (1974) and Samet et al. (1987).

about 20–25 ppb (Clarisse et al., 2003; Geiss et al., 2011; Langer et al., 2015). Higher median concentration (30 ppb) have been found in new U.S. single-family detached homes (Offermann, 2009), and much higher concentrations of ~70 ppb were found in both old and new residences in Japan (Park and Ikeda, 2006) and Hong Kong (Guo et al., 2009).

Formaldehyde is also released from polymeric resins such as urea-formaldehyde (used in particle board, plywood, paper and textile treatments, and surface coatings) or melamine formaldehyde (used in laminates, surface coatings, wood adhesives, and molding compounds) (WHO, 1989; Gerberich and Seaman, 1994). Emissions from synthetic binders like phenol-formaldehyde adhesive (used in plywood adhesives and insulation) or natural binders like tannin-formaldehyde are lower (Chaudhary and Hellweg, 2014). Elevation of temperature or humidity can increase release rates of formaldehyde and therefore lead to higher exposure concentrations. Penetration of outdoor ozone and reaction with indoor materials can be an additional source of aldehydes (Morrison and Nazaroff, 2002; Destaillats et al., 2006).

By far, the leading indoor source of airborne formaldehyde and several other aldehydes today is mainstream cigarette smoke (the portion inhaled by the smoker), sidestream (the portion emitted from a burning cigarette), and environmental tobacco smoke (the aged combination of sidestream and exhaled mainstream smoke) (Table 16.2). Aldehydes are principally associated with the vapor phase of mainstream smoke but have also been

TABLE 16.2 Low-Molecular-Weight Aldehydes in Cigarette Smoke

	Amount Released (mg/pack)		
		Environmental	
Aldehyde[a]	Mainstream	Sidestream	Tobacco Smoke[b]
Formaldehyde	3.4–3.7	14.5	1.3
Acetaldehyde	12.5–55.6	84.7	3.2
Propionaldehyde	1.3–6.1	18.8	0.9
Butanal	2.2–23.2	6.1	ND
Pentanal	0.6	ND	ND
Hexanal	2.6–9.5	ND	ND

Sources: R. J. Reynolds Tobacco Company (1988), Miyake and Shibamoto (1995), Haussmann (2012), and Stabbert et al. (2017).

[a]Other aldehydes in cigarette smoke include acrolein, crotonaldehyde, isobutylaldehyde, isovaleraldehyde, methacrolein, 2-methyl-2-pentanal, and trans 2-hexenal.

[b]Environmental tobacco smoke = 2-h integrated amount per pack. ND = not determined.

measured in the particulate phase (Ayer and Yeager, 1976; Godish, 1989; Nazaroff and Singer, 2004; Stabbert et al., 2017).

Of the various saturated aldehydes present in mainstream smoke, acetaldehyde is the most abundant compound by weight, followed by formaldehyde and propionaldehyde (Fujioka and Shibamoto, 2006). Importantly, the aldehyde concentrations released in smoke from cigarettes between puffs at a lower temperature (600°C) are greater than those produced during smoking (900°C). Thus, sidestream aldehyde emissions are 5–35 times greater than mainstream levels. Because smokers inhale only about 45% of the smoke from each cigarette, sidestream smoke can add significantly to the indoor aldehyde burden. For example, the contribution of cigarette smoke alone to indoor aldehyde concentrations can be ≥100 ppb formaldehyde (Gammage and Gupta, 1984; Schaller et al., 1989) in rooms where several individuals are smoking. The estimated formaldehyde and acetaldehyde exposure to nonsmokers living with a smoker would be 2.5–5.6 and 6.3–14 μg/m^3, respectively. This would produce a dose of 30–67 μg formaldehyde/day and 75–170 μg acetaldehyde/day (Nazaroff and Singer, 2004).

16.1.1.2 Occupational Exposures Workplace exposure to low-molecular-weight aldehydes is extensive, primarily because of the usefulness of their reactive carbonyl in chemical synthesis. Of the several aliphatic aldehydes available, formaldehyde has the greatest production and usage. Annually production exceeds 10 billion pounds in the United States and over 45 billion pounds worldwide. Global formaldehyde consumption continues to grow with about 90% being used as a chemical feedstock or an intermediate in the synthesis of a wide number of chemicals including urea- and phenol-formaldehyde resins. Formaldehyde end-use applications include resins (adhesive and coating) for wood products, textile and paper treatment, vulcanization accelerators (rubber), polyurethane foam (insulation), explosives, elastic fibers, paint resins, household and electronic appliances, plasticizers, synthetic lubricants, fertilizers, dyes, disinfectants, germicides, hardening agents, and a preservative in water-based paints, cosmetics, and hair shampoos. Among the greatest usage of urea- and phenol-formaldehyde resins is as adhesives in the manufacture of particleboard, fiberboard, and plywood.

TABLE 16.3 Occupational Exposure Limits

Compound	Recommended Exposure Limit (REL), ppm (mg/m^3)	Ceiling (C), ppm (mg/m^3)	Immediately Dangerous to Life or Health (IDLH), ppm
Saturated Aldehydes			
Formaldehyde	0.016 (0.02)	0.1 (0.12)	20
Acetaldehyde	—	25 (45)	2000
Chloroacetaldehyde	—	1.0 (3.0)	45
Propionaldehdyde	20 (50)	—	—
Butanal	—	—	—
Pentanal	50 (175)	—	—
Hexanal	—	—	—
Glutaraldehyde	—	0.2 (0.08)	—

Sources: REL and C, National Institute of Occupational Safety and Health; IDLH, Centers for Disease Control.

Over one million people in the United States are estimated to be occupationally exposed to formaldehyde alone. This includes persons working in medical and health services (approximately one third), funeral homes, textiles, furniture, paper, and agriculture industries (Consensus Workshop on Formaldehyde, 1984; IARC, 1995a). Over 20,000 individuals have been exposure to concentrations greater than 1000 ppb, with over 500,000 exposed to concentrations of 500–1000 ppb (Occupational Safety and Health Administration, 1985; Noisel et al., 2007). Along with inhalation, occupational exposures to low-molecular-weight aldehydes can also be topical, as these compounds are used as aqueous solutions (e.g., formalin, which is typically 37% formaldehyde in water and methanol) or as a polymerized solid (e.g., paraformaldehyde). Absorption through the skin is limited (typically <10%) because of the high reactivity and volatility of aldehydes.

Additional saturated aldehydes with the greatest industrial production include acetaldehyde, chloroacetaldehyde, and glutaraldehyde. The current recommended occupational threshold limit values (TLV) for formaldehyde and these compounds are presented in Table 16.3. In 2016, the European Union (EU) changes the classification of formaldehyde from carcinogen category 2 "suspected to be a human carcinogen" to carcinogen 1B "presumed human carcinogen." The EU also classifies formaldehyde as germ cell mutagen category 2.

Acetaldehyde is used primarily as a chemical intermediate, principally for the production of acetic acid, crotonaldehyde, pyridine and pyridine bases, glycols, and esters (IARC, 1985), and as a food additive (vinegar is 5–20% acetic acid) or in the synthesis of flavors and fragrances. Acetaldehyde has been used commercially in the synthesis of aniline dyes, synthetic rubber, resins, disinfectants, drugs, explosives, lacquers, and photographic chemicals, and annual U.S. production exceeds 700 million pounds. Chloroacetaldehyde is used to facilitate bark removal from tree trunks and as an intermediate in the synthesis, for example, of 2-aminothiazole, or other pharmaceutical compounds. Glutaraldehyde is used as a disinfectant to sterilize medical instruments and a histological fixative. It is also used as biocide in the oil and gas industry, in the paper industry, in x-ray processing, in embalming fluid, and for leather tanning.

Other aldehydes of lessor use are proprioaldehyde, butanal, pentanal, and hexanal. Proprioaldehyde is used in the manufacture of propionic acid, polyvinyl, and other plastics and synthesis of rubber chemicals, disinfectant, and preservative with 100–500 million pounds being produced in or imported to the United States in 2002. Butanal is used

commercially in rubber accelerators, synthetic resins, solvents, and plasticizers with over 1 billion pounds being produced in or imported to the United States in 2002. Pentanal is used in flavoring compounds, resin chemistry, and rubber accelerators with 10–50 million pounds being produced in or imported to the United States in 2002. Hexanal is also used as a flavoring agent and in the synthesis of plasticizers, dyes, synthetics resins, and insecticides. It is present in low concentrations to impart fruity odor to perfumes with 10–500 thousand pounds being produced in or imported to the United States in 2002.

16.1.1.3 Ambient Air and Alternative Fuels Low-molecular-weight aldehydes are low-level contaminants of the urban environment. Concentrations in ambient air are typically much lower than those encountered in occupational or indoor settings (McCarthy et al., 2006). Along with the direct release of aldehyde from stationary sources (coal-fired power plants, home wood fires, and incinerators), these compounds are either released directly from mobile sources (car, truck, or jet engines) or formed by secondary photochemical reactions from emitted hydrocarbons. Nonetheless, aldehydes are important components of atmospheric chemistry because, as products of oxidation of almost all hydrocarbons, they are precursors of free radicals, ozone, and peroxyacyl nitrates (Grosjean, 1991; Grosjean et al., 1996; Tuazon et al., 1997). As such, aldehydes are an excellent species to evaluate predictions of ozone formation in kinetic models of urban air quality (Carter, 1990; Grosjean et al., 1992; Harley and Cass, 1995; Liu et al., 2006).

In urban areas, aldehyde levels, like those of other hydrocarbons, exhibit diurnal variations that precede ozone peaks. In the past, total aldehyde levels have occasionally reached maximums of 300 ppb but more typically are less than 40 ppb. Approximately 20–50% of the total aldehyde is in the form of formaldehyde, with typical values ranging from 5 to 30 ppb (with maximal values as high 50–86 ppb in urban air masses contained by atmospheric inversions) (Dasgupta et al., 2005; Bruinen de Bruin et al., 2008). Historical episodic values recorded in Los Angeles for formaldehyde have reached 90–150 ppb; in contrast, ambient values in rustic environments can be 1.0 ppb.

Because other aldehydes are not routinely measured in urban air, few data exist on the ambient concentrations of most aldehydes. Acetaldehyde levels are often 2–39 ppb, which is about 75% of formaldehyde levels (range 32–155%) (Grosjean et al., 1996). These levels are sufficient to make acetaldehyde the major aldehyde in the removal of hydroxyl radicals from the atmosphere. Other aldehydes that are important in this process include formaldehyde and nonanal and those for other aliphatic aldehydes between 35 and 40% of total aldehyde concentrations. Of these, higher-molecular-weight carbonyls (nine species: C_8–C_{14}) typically account for 10–15% of the total aldehydes (Grosjean et al., 1996).

Between 55 and 75% of ambient aldehydes are from mobile sources, and proximity to such sources may produce greater localized exposures. For example, exposures near garages, tunnels, or city street canyons may be much greater than average, since exhaust from passenger cars equipped with either spark-ignition or diesel engines can produce aldehydes (Zweidinger et al., 1988; Grosjean and Grosjean, 2002). Estimated concentrations in emissions from warm gasoline-fueled automobiles (running at 40-mph cruising speeds) are about 1000 and 100 ppb for formaldehyde and acetaldehyde, respectively (Swarin and Lipari, 1983). In contrast, starts of cold engines yield levels in which formaldehyde can exceed 5000 ppb. Diesel engines also produce significant amounts of formaldehyde and acetaldehyde, with levels produced by warm engines equaling about 4000 and 1000 ppb, respectively.

Ambient propinoaldehyde levels have been measured to be 0.1–14 ppb in Los Angeles (Grosjean, 1982; Kawamura et al., 2000) and 0.08–6.7 ppb in Mexico City (Báez et al., 2003). Ambient butanal levels have been measured to be 0–7 ppb with a median of 1.5 ppb in Los Angeles (Grosjean, 1982) and 2.9–2.7 ppb along a roadside in Raleigh, NC (Zweidinger et al., 1988). Ambient pentanal levels have been measured to be 0.03–0.4 ppb in Los Angeles (Grosjean, 1982; Kawamura et al., 2000) and detected in 7 of 8 winter and 3 of 18 summer samples at 0.0–3.0 and 0.0–0.27 ppb around and in Boston, MA (Reiss et al., 1995). Ambient hexanal levels have been measured to be 0.06–0.3 ppb in Los Angeles (Kawamura et al., 2000) and 0.2–0.93 ppb in Europe (Guicherit and Schulting, 1985; Ciccioli et al., 1993).

Aldehydes are enriched in motor vehicle emissions when fuels containing oxygenated additives (especially alcohols) are combusted (Carter, 1990; Grosjean, 1990; Shah and Singh, 1998; Kawamura et al., 2000; Magnusson et al., 2002; Dasgupta et al., 2005; Ban-Weiss et al., 2008). In some incidences these sources contribute to ambient levels of formaldehyde that can exceed carcinogenic benchmark concentrations (Tam and Neumann, 2004). The formulation of these alternative fuels may vary and contain either methanol (from methane) or ethanol (from agricultural sources); these are likely to be mixtures of alcohol and gasoline (e.g., M-85, 85% methanol–15% gasoline mixture). The addition of methanol to gasoline can increase formaldehyde emissions, whereas ethanol is likely to increase acetaldehyde concentrations. Acetaldehyde concentrations, for example, for internal combustion engines using ethanol rather than gasoline can increase from <100 to over 190,000 ppb following cold starts and from <100 to 8400 ppb acetaldehyde from warm engines (Marnett, 1988). Formaldehyde concentration can also increase from 480 to 3600 ppb.

In a study by Lioy et al. (2011), personal exposures and ambient concentrations of formaldehyde and acetaldehyde were characterized in a pollution "hot spot" and an urban reference site in Camden, NJ. The hot spot was the city's Waterfront South neighborhood; the reference site was about 1 km away near Copewood and Davis streets. Using personal exposure measurements, residential ambient air measurements, statistical analyses, and exposure modeling, the impact of local industrial and mobile pollution sources, particularly diesel trucks, on personal exposures and ambient concentrations in the two neighborhoods was assessed. Formaldehyde and acetaldehyde concentrations were high in both neighborhoods. Mean concentration of formaldehyde was 16.9 ppb in Waterfront South and 19.8 ppb in Copewood–Davis, which indicated that mobile sources (i.e., diesel trucks) had a large effect on formaldehyde and acetaldehyde concentrations in both neighborhoods (i.e., both were aldehyde "hot spots").

16.1.2 Cellular Exposure and Dosimetry

16.1.2.1 Nasal Deposition and Penetration Regional deposition of an inhaled gas is controlled by water solubility, concentration, and physiological and pathological features of the respiratory tract. Measurements of formaldehyde deposition in rats (Dallas et al., 1985; Patterson, 1986) and in dogs (Egle, 1972b) found essentially 100% total respiratory tract deposition. One interesting observation made in these studies was the secretion of low levels from control animals/individuals, presumably from endogenous (biogenic) formation. Aldehydes are highly soluble in water compared with other common air pollutants (Table 16.4), and the principal site of deposition of these compounds is the upper

TABLE 16.4 Physical and Chemical Properties of Saturated Aldehydes Compared to Other Environmental Agents

Compound	Structure	Mol. Wt.	Aqueous Sol. (g/L)	Organic Sol. (Most–Least)
I. Saturated aldehydes				
Formaldehyde	HCHO	30.03	560.0	Eth, ace, bz, al, chl
Acetaldehyde	CH_3CHO	44.05	200.0	Eth, al, bz
Chloroacetaldehyde	$ClCH_2CHO$	78.50	161.0	Meth, ace
Propionaldehyde	CH_3CH_2CHO	58.08	160.0	Eth, al
Butanal	$CH_3CH_2CH_2CHO$	72.10	50.9	Ace, bz, chl
Pentanal	$CH_3(CH_2)_3CHO$	86.13	14.8	Eth
Hexanal	$CH_3(CH_2)_4CHO$	100.16	4.5	Eth, ace, bz
Glutaraldehyde	$OHC(CH_2)_3CHO$	100.12	64.0	Eth, bz
II. Other pollutants				
Sulfur dioxide	SO_2	64.07	106.4	Chl, ether meth, al
Carbon dioxide	CO_2	44.01	1.7	—
Carbon monoxide	CO	28.01	0.03	Eth, meth, eth ace
Oxygen	O_2	32.00	0.04	—
Ozone	O_3	48.00	0.02	Meth cl

Sources: Comey and Hahn (1921), Seidell (1940, 1941), and Windholz (1983).
Abbreviations: Eth, ethanol; ace, acetone; bz, benzene; al, alcohol; chl, chloroform; meth, methanol; eth ace, ethyl acetate; meth cl, methylene chloride.

respiratory tract (Aharonson et al., 1974). When inhaled through the nose in low concentrations, almost all (>98%) of formaldehyde is deposited in the moist layers covering the nasal mucosa. This deposition occurs during the inspiration, with little (<2%) or no formaldehyde being exhaled (Egle, 1972b; Heck et al., 1983; Dallas et al., 1985). In rats, Heck et al. (1983) reported that radiolabeled formaldehyde deposition was proportional to the concentration and length of exposure. Deposition rate decreased over a 6-h exposure period resulting, in part, from a decrease in ventilatory rate during exposure (Kimbell et al., 2001).

Aqueous solubility of aliphatic low-molecular-weight aldehydes decreases with increasing carbon chain length. For this reason, greater nasal penetration has been noted for propionaldehyde (CH_3CH_2CHO) and for acetaldehyde (CH_3CHO) than for formaldehyde (HCHO) (Asgharian et al., 2012). Egle (1972a, 1972b), for example, reported approximately 60% retention (40% penetration) for propionaldehyde and acetaldehyde as compared with >90% retention for formaldehyde in the canine upper respiratory tract during inspiration. Aliphatic aldehydes with carbon chain lengths greater than four carbon atoms are less miscible in water, and penetration is therefore more likely to be even greater than that for propionaldehyde. Values vary for other gases of low solubility (e.g., only about 20% of ozone is retained in the upper respiratory tract of dogs), and few measures are available for aldehydes other than formaldehyde (Overton et al., 2001).

Additional physiological factors, such as altered clearance mechanisms (epithelial metabolism, mucociliary clearance, and possibly regional air and blood flow), also affect aldehyde dosimetry. This has been suggested for formaldehyde, as the magnitude of the decrease in the rate of deposition was not solely related to the decrease in ventilation.

Studies with other inhalation hazards (sulfur dioxide and ozone) have suggested that other factors influencing nasal penetration include vascular congestion, respiratory flow rate, concentration, duration of exposure, and airway caliber.

Morris (1997) proposed that aldehyde penetration, at least for the rat nasal passages, is strongly influenced by the metabolic capacity of the epithelium. Aldehydes are handled by a number of specific and nonspecific enzymes, including aldehyde dehydrogenases (ALDHs), aldehyde oxidases, or xanthine oxidase (Bohren et al., 1989). The predominant elimination pathway for aldehydes by dehydrogenation yields a carboxylic acid (e.g., formic acid from formaldehyde) through an irreversible step that requires glutathione (Lam et al., 1985; Bhatt et al., 1988) and a hydrogen carrier, for example, nicotinamide adenine dinucleotide or its phosphate, NAD(P). Formaldehyde deposits mainly (93%) within the respiratory epithelium with little in the olfactory epithelium of the rat nose (Kimbell et al., 1993, 1997; Cohen Hubal et al., 1997). Therefore, the dose rate at low concentrations (<5000 ppb at a breathing rate of 200 mL/min) produces a tissue dose rate approximately equaling the maximal metabolic rate (V_{max}) of formaldehyde dehydrogenase [aka alcohol dehydrogenase 5 (class III), chi polypeptide] estimated capacity 40 nmol/min reported by Casanova-Schmitz et al. (1984) for the rat nasal epithelium. Likewise, Morris (1997) proposed that 800,000 ppb acetaldehyde could produce a dose rate at the V_{max} of ALDH of the rat respiratory epithelium. Accordingly, inhaled concentrations greater than 800,000 ppb would have greater penetration. This proposition is in general agreement with the nasal depositions of 76, 48, 41, and 26% for inspired acetaldehyde concentrations of 1000, 10,000, 100,000, and 1,000,000 ppb (1, 10, 100, and 1000 ppm), respectively.

In addition, differences in airflow patterns within the nasal passages produce different doses to various types of epithelium within the nose. For example, due to difference of the metabolic capacity and airflow over the olfactory epithelium, the acetaldehyde concentration necessary to overwhelm metabolism would be lower, 300,000 ppb, than of the respiratory epithelium, 800,000 ppb (Morris, 1997). Because formaldehyde deposits more in the anterior respiratory epithelium, this difference has less of an effect for this compound (Monticello et al., 1996).

Morris (1998) also demonstrated that aldehydes interact in co-exposures. For example, simultaneous exposure to acrolein resulted in non-steady-state acetaldehyde uptake behavior, with uptake efficiencies steadily decreasing with extension of exposure. Acrolein also produced a concentration-dependent reduction in net uptake during the exposure. For example, at a flow rate of 200 mL/min, net upper respiratory tract acetaldehyde uptake efficiency averaged 43, 39, 24, and 24% in animals simultaneously exposed to 0, 2000, 10,000, or 20,000 ppb acrolein, respectively. The mechanisms of these responses are unknown. However, these results demonstrate that caution is necessary in utilizing dosimetric data obtained during exposure to individual vapors to predict relationships that may exist under complex exposure scenarios to multiple vapors.

Relative reactivity of the inhaled aldehyde also will influence retention in the upper respiratory tract (Aharonson et al., 1974). In the dog, nasal retention of acrolein (80%) was intermediate between those of formaldehyde (>95%) and propionaldehyde (60%). The difference between acrolein and propionaldehyde, both three-carbon molecules, can be explained only partially by acrolein's higher solubility (acrolein 210 g/L and propionaldehyde 160 g/L). The higher chemical reactivity of acrolein may also contribute significantly. Presumably, covalent binding with surface macromolecules, like mucin, is greater for acrolein than propionaldehyde and would prevent reentry of acrolein more than reentry of propionaldehyde (Bogdanffy et al., 1987).

Aldehydes that penetrate the nasal cavity or enter through oral breathing are deposited almost completely in the lower respiratory tract. Heck et al. (1983) examined deposition of [^{14}C]HCHO in nasal breathing rats and mice. In 6-h tests with formaldehyde concentrations up to 24,000 ppb, approximately 250 nmol [^{14}C]HCHO/g tissue was deposited in the trachea as compared with 2200 nmol/g tissue in the nasal mucosa (the amount of deposition was nonlinear in relation to dose, with saturation at doses exceeding 6000 ppb). Autoradiography of mouse and rat airways by Swenberg and associates (Chang et al., 1983; Swenberg et al., 1983) demonstrated the presence of radioactivity in the trachea and main bronchi following [^{14}C]HCHO exposure. Deposition of formaldehyde in the trachea and bronchi of monkeys was greater than that of rats, presumably because rodents breathe principally through their nose. Humans typically breathe between 30 and 60% through their mouth at rest and more during exercise.

Using a model developed to estimate the deposition of inhaled gases in the human lung, Asgharian et al. (2012) predicted that formaldehyde would be absorbed (~97%) by the mucus membranes. Most formaldehyde is estimated to deposit in the trachea (airway generation 0), and the remaining formaldehyde is deposited within first eight airway generations. Aldehydes with moderate solubility such as acetaldehyde and acrolein were estimated to penetrate deeper into the lung, reaching the alveolar region, and had a higher probability of passing through the thin alveolar-capillary membrane to reach the blood. For all aldehydes, tissue concentrations reached a maximum at the end of inhalation at the air–tissue interface. The depth of peak concentration moved within the tissue layer due to gases desorption during exhalation.

16.1.2.2 DNA–Protein Cross-Links and Molecular Dosimetry The toxicity of formaldehyde in cells arises primarily from its reactivity as an electrophile that targets amine (–NH_2) (Feldman, 1973; Conaway et al., 1996) and free thiol (–SH) (Klatt and Lamas, 2000; Moran et al., 2001; Paget and Buttner, 2003) groups on proteins and nucleic acids. The nucleophilic addition of an amine to formaldehyde generates an *N*-methylol adduct that may subsequently condense to an imine. The imine carbon can then have a second nucleophilic addition by another amine, forming an irreversible cross-link composed of a methylene bridge. In formaldehyde reactions with thiols, the nucleophilic addition of the sulfur atom to the aldehyde forms a hemithioacetal (*S*-hydroxymethyl adduct), which may cyclize rapidly and irreversibly with a neighboring amine to generate a thiazolidine adduct (Kallen, 1971; Higgins and Giedroc, 2014).

Initial covalent interactions at the sites of exposure could serve as a sensitive indicator of the dose to the critical biological target site. If such interactions are irreversible and stable, these methods could enable a measure of regional molecular dosimetry. Aldehydes can form cross-links, bridging macromolecules through attack of the electrophilic carbonyl carbon with various reactive groups on proteins, RNA, or DNA (French and Edsall, 1945; Naylor et al., 1988). One reactive group contained in proteins is the amino group, which is also contained in each of these types of macromolecules. Thus, this reaction site can lead to mixed cross-links (e.g., DNA–protein cross-links) via a two-step process initiated by the formation of an unstable hydroxymethyl intermediate (through loss of water from a methylene Schiff base), followed by a nucleophilic attack on the carbon donated by formaldehyde by a second amino group contained on either DNA or a protein. Bifunctional aldehydes, for example, glutaraldehyde, are more effective at forming cross-links. The resulting methylene bridge can be repaired rapidly or can become stable temporally. Thus, an integrated measurement would reflect these competitive processes of formation and degradation (Graftstrom et al., 1984).

Although DNA–DNA cross-links are possible, attempts at detection in intact cells have met with only limited success (Feldman, 1973; Chaw et al., 1980; Hemminki, 1981). On the contrary, DNA–protein cross-links have been more readily measured (Wilkins and Macleod, 1976; Magana-Schwenke et al., 1978; Magana-Schwenke and Ekert, 1978; Graftstrom et al., 1984; Casanova et al., 1994; Heck and Casanova, 1999). In contrast, the relationship between formaldehyde dose and DNA–protein cross-links has, however, been found to be nonlinear over the range of 300–1000 ppb (Swenberg et al., 1983). This nonlinearity makes evaluation of cellular dose by measurements of DNA–protein alone difficult. Lastly, protein–protein cross-links have been detected and are linear with dose; however, evidence suggests that this covalent binding is primarily between extracellular proteins and therefore does not reflect intracellular dose. This limitation may be important in the evaluation of dose in certain toxicological processes, leading to cancer.

In addition to the instability of cross-links formed by formaldehyde exposure (Graftstrom et al., 1984), a principal reason for the nonlinearity in the formation of DNA–protein cross-links is likely to be simply the rapid enzymatic removal of aldehyde before cross-links are formed. Depletion of glutathione alone increases the amount of detectable DNA–protein cross-links and nearly restores the linearity of the dose–response relationship (Lam et al., 1985; Casanova and Heck, 1987), suggesting that this is an important defense mechanism at lower doses. Ultimately the carboxylic acid formed from each aldehyde is converted to CO_2 and H_2O, either through a multiple-step process involving tetrahydrofolate (initiated by formyl-THF synthetase) or a reaction catalyzed by catalase.

Conceivably, other methods also will prove useful in assessing regional aldehyde dosimetry. Such an indicator could be DNA adduct levels, and at least two adducts, N^6-hydroxymethyl-deoxyadenosine (HOMedA) and N^2-hydroxymethyldeoxy guanosine (HOMedG), are possible. However, these hydroxymethyl adducts, like DNA–protein cross- links, are not stable. Fennel (1990) has presented a method that stabilize these adducts so that they would be suitable for ^{32}P or electrophore postlabeling. Such a technique could reduce the need for the use of radiolabeled formaldehyde. Subsequently, the problems of DNA–protein cross-linking estimation of dosimetry have been somewhat circumvented by a combined approach, which includes cytolethality–regenerative cellular proliferation, which is also used to model formaldehyde dosimetry in rats (Conolly et al., 2003, 2004).

Edrissi (2017) measured N^6-formyllysine adducts in the nasal epithelium of Fisher 344 rats exposed to 2 ppm [$^{13}C^2H_2$]-formaldehyde for 7, 14, 21, and 28 days (6 h/day) and investigated adduct loss over a 7-day post-exposure period using liquid chromatography-coupled tandem mass spectrometry. Exogenous adducts could be distinguished from endogenous adducts, and exogenous adducts were detected in the nasal epithelium and the trachea but not in the distant lung, circulating white blood cells, or bone marrow. Exogenous adducts increased twofold over endogenous N^6-formyllysine over the 3-week exposure period. This was followed by analysis of decay of N^6-formyllysine in proteins extracted from different cellular compartments. Decay was biexponential with half-lives of ~25 and ~182 h for the fast and slow phases, respectively, in cytoplasmic proteins. These results were similar to decay of N^2-hydroxymethyl-dG DNA adducts and DNA–protein cross-links (Lu et al., 2010, 2011; Moeller et al., 2011; Edrissi et al., 2013), with protein adducts cleared faster than DNA−protein cross-links, and supported the utility of N^6-formyllysine protein adducts as biomarkers of formaldehyde exposure.

Although many studies have focus on formaldehyde, acetaldehyde has gained attention recently. Acetaldehyde reacts with 2′-deoxyguanosine in DNA to primarily form *N*(2)-ethylidene-2′-deoxyguanosine (*N*(2)-ethylidene-dGuo). The subsequent reaction of

N(2)-ethylidene-dGuo with another molecule of acetaldehyde gives rise to 1,*N*(2)-propano-2′-deoxyguanosine (1,*N*(2)-propanodGuo). Garcia et al. (2014) exposed rats to 12, 33, and 96 ppb acetaldehyde in atmospheric air for 50 days. A significant increase in the levels of 1,*N*(2)-propanodGuo was observed in lung tissues of rats exposed to 12 ppb (7.8×10^8 dGuo), 33 ppb (8.9×10^8 dGuo), and 96 ppb (11.6×10^8 dGuo) compared to controls (4.2×10^8 dGuo). For comparative purposes, the levels of 1,*N*(2)-etheno-2′-deoxyguanosine (1,*N*(2)-edGuo), which is produced from α,β-unsaturated aldehydes formed during the lipid peroxidation process, were also measured. Elevated levels of 1,*N*(2)-edGuo were observed only in lung tissues of animals exposed to 96 ppb acetaldehyde. 1,*N*(2)-propanodGuo also differed quantitatively in the liver but not in the brain.

Similarly, Sanchez et al. (2018) found that [$^{13}C_2$]-acetaldehyde induces DNA modification with the formation of isotopically labeled 1,N^2-propano-2′-deoxyguanosine adducts in the brain and lungs of rats exposed to 10 ppb labeled acetaldehyde for 50 days. The adduct, with the addition of two molecules of isotopically labeled acetaldehyde [$^{13}C_4$]-1,N^2-propano-dGuo, was detected in the lung and brain tissues of exposed rats by micro-HPLC/MS/MS. Structural confirmation of the products was unequivocally performed by nano-LC/ESI$^+$-HRMS^3 analyses.

16.1.3 Populations of Concern

At present, no specific human subpopulation has been clearly identified to have an increased risk for adverse effects from environmental aldehyde exposures. But persons with genetic variants that altered aldehyde and precursor alcohol metabolism are clearly susceptible to adverse health effects. Previously, persons with asthma were identified as being of special concern when the National Ambient Air Quality Standards was established for sulfur dioxide. By analogy, formaldehyde or other aldehydes with bronchoconstrictive properties may also represent an added risk to this population (see later for more details). An aspect of this concern includes persons who have developed an immunologic hypersensitivity, but this is a complex issue (see later for details).

Aldehydes other than formaldehyde that can penetrate the upper respiratory tract, much like ozone, may produce similar adverse responses. Currently, children have been suggested as a population of concern for ozone and environmental tobacco smoke exposure, and thus aldehyde effects among this population deserve attention.

Genetic differences in metabolism are predisposing risk factors for adverse responses to aldehyde exposure. Aldehydes can be oxidized to carboxylic acids or reduced to alcohols by aldose (aldo-keto) reductases. Elimination of aldehydes is typically rapid and dependent on these enzymes and pathways that regulate levels of glutathione, tetrahydrofolate, and nicotinamide adenine dinucleotide (NAD^+). As an example, consider acetaldehyde metabolism, which involves alcohol dehydrogenase (ADH) and ALDH that contribute to the oxidative metabolism of alcohols in the liver. Various ADHs convert ethanol to acetaldehyde that is metabolized rapidly to acetic acid (acetate) by ALDHs. Acetic acid is either excreted in the urine or reincorporated into intermediary metabolism as acetyl-CoA. These reactions involve an intermediate carrier of electrons, NAD^+, which is reduced to form NADH. The accumulation of acetaldehyde in human tissues is dependent on the balance of the rate of its formation from alcohol by ADHs and the rate of its removal by ALDHs.

Alteration of the metabolism responsible for normal function of these defense mechanisms could therefore place individuals at risk. Currently, 19 ALDH genes have been identified in humans, and aldehydes are substrates for ADHs as well (Yoshida et al., 1998;

Vasiliou et al., 2004). In humans, there are 11 ALDH protein families (ALDH1–ALDH9, ALDH16, and ALDH18). The two major enzymes are cystolic aldehyde dehydrogenase 1 family member A1 (ALDH1A1) and mitochondrial ALDH2. ALDH2 has a low Km for acetaldehydes.

The accumulation of circulating acetaldehyde is responsible for a dysphoric response among individuals with reduced acetaldehyde elimination following ethanol ingestion, which leads to sympathomimetic responses of facial flushing, tachycardia, and muscle weakness and a rise in circulating catecholamines. ALDH can also be inhibited by disulfiram (tetraethylthiuram disulfide) (DeMaster and Stevens, 1988; Helander et al., 1988) used clinically for the treatment of alcohol abuse. The potential pathophysiological significance of depressed ALDH activity, and its relationship to adverse reaction to aldehydes in the lung, may be important in understanding individual susceptibility.

Previously, studies of adverse effects due to poor ethanol metabolism have identified specific polymorphisms in ALDH enzymes among East Asians and Native Americans (Hsu et al., 1987; Card et al., 1989; Farres et al., 1989; Goedde and Agarwal, 1989; Kurys et al., 1989; Shibuya et al., 1989). Ethanol also is converted to acetaldehyde by microsomal ethanol-oxidizing system (mainly by cytochrome P450, family 2, subfamily E, polypeptide 1: CYP2E1) or catalase (Crabb, 1990; Vasiliou et al., 2004). Normally, acetaldehyde is converted rapidly to acetic acid. However, because of an additional alteration ALDH2, concentrations of this intermediate are elevated. This alteration is often due to a polymorphism in ALDH2 (guanine to adenine transition in exon 12) that produces a glutamine to lysine substitution. This allelic variant is in high frequency (40–60%) among, and may be confined to, Asians (Yoshida et al., 1998). Based on blood acetaldehyde levels following alcohol consumption, individuals with this polymorphism have about a 20-fold increase in acetaldehyde levels (Mizoi et al., 1994).

Besides having a role in systemic aldehyde metabolism, ALDH2 may have a tissue-specific role dependent on the route of exposure. For example, gene-targeted mice lacking functional ALDH2 protein are more susceptible to adverse pulmonary responses during inhalation of 5 ppm acetaldehyde (Isse et al., 2005). Approximately half of Asians with asthma experience an exacerbation of the symptoms of asthma after drinking alcohol, that is, alcohol-induced asthma (Shimoda et al., 1996). Importantly, although individuals carrying ALDH2 variants are resistant to alcoholism, they are at increased risk for several cancers including esophageal, head and neck, oropharyngolaryngeal, and gastrointestinal cancers (Muto et al., 2002; Yokoyama and Omori, 2005). Persons with ALDH2 deficiency who drink alcohol excessively have increased acetaldehyde-derived DNA adducts (Matsuda et al., 2006). Brooks and Theruvathu (2005) also noted that the repair of such adducts is complex, involving multiple pathway, and inherited variation in the genes encoding the proteins involved in the repair acetaldehyde secondary adducts may contribute to susceptibility to alcoholic beverage-related carcinogenesis.

Genetic variants in Fanconi anemia DNA repair pathway may also increase individual susceptibility to aldehydes (Langevin et al., 2011). Mice with combined inactivation of aldehyde catabolism (gene-targeted Adh2−/−) and the Fanconi anemia DNA repair pathway (Fancd2−/−) display developmental defects, a predisposition to leukemia, and are susceptible to the toxic effects of ethanol, an exogenous source of acetaldehyde. Garaycoechea et al. (2012) determined that aged Aldh2(−/−) Fancd2(−/−) mutant mice develop aplastic anemia, with the concomitant accumulation of damaged DNA within the hematopoietic stem and progenitor cell (HSPC) pool. Only HSPCs, and not more mature blood precursors, required Aldh2 for protection against acetaldehyde toxicity. A 600-fold reduction was

noted in the hematopoietic stem cells pool of mice deficient in both Fanconi anemia pathway-mediated DNA repair and acetaldehyde detoxification. These findings suggest that bone marrow failure noted in Fanconi anemia is probably due to aldehyde-mediated genotoxicity restricted to the HSPC pool.

Also of note is that acetaldehyde/alcohol metabolism requires and alcohol induces CYP2E1 (Seitz and Mueller, 2015). CYP2E1 has a role in the metabolism of retinoids to their polar metabolites and of procarcinogens to their ultimate carcinogens. The loss of retinol and retinoic acids leads to dedifferentiation and hyperproliferation. In addition, ethanol metabolism via CYP2E1 results also in the generation of reactive oxygen species (ROS) with lipid peroxidation and the occurrence of lipid peroxidation products such 4-hydroxynonenal (4HNE) and malondialdehyde (MDA), which can form carcinogenic exocyclic etheno DNA adducts.

Malondialdehyde (MDA) and acetaldehyde (AA) can be created by following ethanol metabolism and tobacco pyrolysis to form a stable protein adduct, malondialdehyde–acetaldehyde (MAA) adduct. Sapkota et al. (2017) hypothesized that lungs of individuals with alcohol use disorders (AUDs) are a target for the effects of combined alcohol and cigarette smoke metabolites. MAA adducts were increased in lung cells of AUD subjects who smoked cigarettes (+AUD/+smoke) as compared with subjects who smoked without AUD (−AUD/+smoke). No significant increase in MAA adducts was observed in −AUD/+smoke or in +AUD/−smoke compared with −AUD/−smoke. Serum from +AUD/+smoke had significantly increased levels of circulating anti-MAA IgA antibodies. After 1 week of alcohol, that MAA-adducted protein is formed in the lungs of those who smoke cigarettes and abuse alcohol, leading to a subsequent increase in serum IgA antibodies. The authors concluded that MAA-adducted proteins could play a role in pneumonia and other diseases of the lung in the setting of AUD and smoking.

In addition to altered ethanol-related aldehyde formation and elimination, other dehydrogenases include alcohol dehydrogenase 5 (ADH5, as known as chi-ADH, formaldehyde dehydrogenase, and *S*-nitrosoglutathione reductase) may have a role in individual susceptibility to the adverse health effects induced by aldehyde exposure. Despite being named an alcohol dehydrogenase, ADH5 has a high affinity for formaldehyde but only a low affinity for ethanol and other alcohols. ADH5 catalyzes the oxidation of *S*-hydroxymethylglutathione, a spontaneous adduct between formaldehyde and glutathione, into *S*-formylglutathione in the presence of NAD^+ (Koivusalo et al., 1989). ADH5 activity is increased by fatty acids (Engeland et al., 1993). Interestingly, ADH5 is also responsible for regulating the levels of *S*-nitrosoglutathione (GSNO), which is formed from and provides a source nitric oxide (Liu et al., 2004). Gene-targeted mice lacking ADH5 protein have increased nitric oxide available and therefore are protected from airway hyperreactivity following inhaled antigen challenge (Que et al., 2005).

Other aldehydes have tissue-specific metabolism, which can differ from that of formaldehyde. The exact enzyme subtypes have yet to be determined but probably include multiple ALDH members including ALDH2, ALDH3A1, and ALDH5A1 (Vasiliou et al., 2004) and possibly cytochrome P450 isozymes including CYP3A. Glycidaldehyde is a substrate for lung and liver cytosolic glutathione S-transferases (GSTs) (EC 2.5.1.18) and can also be hydrated to glyceraldehyde (Patel et al., 1980). Glyceraldehyde can be metabolized via the glycolytic pathways.

Adverse effects from genetic susceptibility to ethanol and therefore acetaldehye may not be limited to Asians. Clarke et al. (2017) performed a genome-wide association study (GWAS)

of self-reported alcohol consumption in 112,117 individuals in the United Kingdom and found significant genome-wide associations at 14 loci. These include single-nucleotide polymorphisms (SNPs) in alcohol metabolizing genes, including *ADH1B*, *ADH1C1*, and *ADH5*. The latter suggest a possible genetic risk for formaldehyde but this was not tested.

In the lung, the primary elimination pathway of many aldehydes involves glutathione conjugation by GSTs. The cytosolic GSTs exist as monomers and are catalytically active as homo- or heterodimers and are divided into seven classes: alpha, mu, omega, pi, sigma, theta, and zeta (Nebert and Vasiliou, 2004; Hayes et al., 2005; McIlwain et al., 2006). Of these, members of the mu (GSTM1), theta (GSTT1), and pi (GSTP1) families have garnered the most attention. Both GSTM1 and GSTT1 have null genotypes that yield no protein and these genotypes are common in Europeans, being present in about 50 and 25% individuals, respectively. In addition, the GSTT1 null variant is present in about 40% of Asian populations (Gilliland et al., 2002; Hayes et al., 2005). The GSTP1 gene has two common polymorphisms (with a frequency of about 5–10%) that have been found to alter catalytic efficiency with isoleucine 105 valine (ile105val) and alanine 114 valine (ala114val) transitions (Ali-Osman et al., 1997; Watson et al., 1998).

In terms of aldehyde conjugation, GSTM1 is more efficient using 4-hydroxyalkenals as substrates, whereas GSTP1 is more efficient using acrolein or crotonaldehyde as substrates (Berhane et al., 1994) and the Val105 Val114 isoform of GSTP1 is less efficient in catalyzing acrolein (but not crotonaldehyde) than other variants (Pal et al., 2000). GSTP1 is a major form found in the lung (Watson et al., 1998), and the Val105 genotype is associated with an increase susceptibility to lung cancer from environmental tobacco smoke (Miller et al., 2003) especially when combined with GSTM1 null genotype (Wenzlaff et al., 2005). Polymorphisms in GSTP1 are associated with younger age of onset of lung cancer (Miller et al., 2006). In addition to being associated with lung cancer, the Val105 GSTP1 genotype is associated with decreased lung function in smokers that have a serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1-deficiency, suggesting GSTP1 has a modulatory role in chronic obstructive pulmonary diseases (Rodriguez et al., 2005). It should be noted that aldehydes, when conjugated to glutathione, have toxic potential, because the aldehyde group remains intact (Horvath et al., 1992).

The catalytic activity and protein levels of these enzymes can be altered by exposures to aldehydes. For example, nasal metabolic activity was studied in male Wistar rats (5–6 per group) exposed 6 h/day for 3 consecutive days to formaldehyde (1.0, 3.2, and 6.4 ppm), acetaldehyde (750 and 1500 ppm), acrolein (0.25, 0.67, and 1.40 ppm), or mixtures of these compounds (Cassee et al., 1996b). Mixture (rather than single chemical exposure) effects were noted on regional nasal histopathological and biotransformation enzymes measured in homogenates of nasal tissue. Only in the 1.40 ppm acrolein exposure group was GST activity depressed, while ADH5 and ALDH activities increased. Similar, *in vitro* acrolein can covalently modify and inactivate GSTs (Berhane and Mannervik, 1990) and carbonic anhydrase II (Tu et al., 1989). In contrast, acrolein, a substrate for aldo-keto reductase family members (including AKR1A1, AKR1B10, AKR1C3, and AKR7A1) (yielding allyl alcohol), can activate this enzyme *in vitro*, but this is not likely to occur *in vivo* because it is prevented by $NADP^+$ (Lan et al., 2004; Fukumoto et al., 2005). Other aldehyde substrates for aldo-keto reductase included crotonaldehyde and cinnamaldehyde (Ellis and Hayes, 1995; Gardner et al., 2004).

16.2 SINGLE-EXPOSURE HEALTH EFFECTS

16.2.1 Formaldehyde

16.2.1.1 Symptomatic Responses in Controlled Human Exposures Moderate concentrations of formaldehyde produce rapid responses including eye (50–2000 ppb) and upper respiratory tract (100–25,000 ppb) irritation that can become intolerable (Table 16.5). Eye irritation progresses to a greater extent with continuous exposure than with discontinuous exposure, whereas nose and throat irritation were significantly greater in discontinuous exposures (Weber-Tschopp et al., 1977). Tolerance to nose and throat irritation can develop in the same individual who continues to become increasingly sensitive to eye irritation during exposure (Anderson and Molhave, 1983).

The characteristic odor of formaldehyde is detectable at concentrations below those that produce irritation (Bender, 2002; Arts et al., 2006). However, as with many compounds, the odor threshold (20–500 ppb) can vary widely between individuals, and acclimatization can occur during exposure (Berglund and Nordin, 1992). For these reasons, the detection of odor (or more importantly, the lack thereof) must not be used as an indicator of safety.

Sequelae to massive acute exposure, an aversion response elicited merely by the odor, can develop (Shusterman et al., 1988). This can be viewed as a psycho-protective response

TABLE 16.5 Symptomatic Responses in Humans to Formaldehyde

Formaldehyde Concentration (ppm)	Response	References
Eye irritation (blinking rate, lacrimation, conjunctivitis)		
0.01	Detectable by some	Schuck et al. (1966)
0.30	Slight but tolerable response	Rader (1977)
0.50	Intermediate response	Bourne and Seferian (1959), Lang et al. (2008)
0.80	Severe response	Wayne et al. (1977)
1.0–3.0	Moderate symptoms	Kulle et al. (1987)
1.7–2.0	Marked eye blinking	Bender et al. (1983)
Upper respiratory tract irritation (nasal secretion or dryness, throat irritation)		
0.03	Minimal or no effect	Weber-Tschopp et al. (1977)
0.25–1.39	Moderate irritation	Kerfoot and Mooney (1975), Schoenberg and Mitchell (1975), Anderson and Molhave (1983), Kulle et al. (1987), Lang et al. (2008)
1.7–2.1	Significant throat irritation	Weber-Tschopp et al. (1977)
3.1	Severe to intolerable irritation	Kane and Alarie (1977)
Odor threshold		
0.05	Odor threshold	Pettersson and Rehn (1977)
0.17	Detected by 50% exposed	Pettersson and Rehn (1977)
1.5	Detected by all subjects	Pettersson and Rehn (1977)

resulting from behavioral conditioning in which the initial single acute exposure accompanied by observable pathophysiological effects serves as the unconditional stimulus. The odor (at subirritant concentrations) during a secondary reexposure after the initial toxic acute exposure then serves as the conditioned stimulus. Following such a regimen, conditioned subjects can develop subjective (including those outlined above as well as a perception of poor air quality, discomfort, or a "desire to leave the room") or objective (changes in heart rate and breathing pattern) responses. Although the enhanced response is psychogenic in origin, response generation is often involuntary and may involve minimal personality components.

In general, formaldehyde exposures of 50–150 ppb produce mild, transient, concentration-dependent responses in 10–50% of the persons exposed (Bernstein et al., 1984). These types of responses are common and often are the primary or solitary finding associated with low-level environmental exposure (see below). In one study, 410 ppb formaldehyde exposure of 2 h increased mucosal inflammation (leukocytes and albumin recovered in nasal lavage recovered 4 and 18 h after exposure) in healthy and asthmatic subjects (Pazdrak et al., 1993). Eye irritation develops frequently in the range of 1000–3000 ppb in most subjects, and upper respiratory irritation is frequent at concentrations of 1000–11,000 ppb (NRC, 1981; IARC, 1995a; Yang et al., 2001; Arts et al., 2006). At concentrations of 4000–5000 ppb, many subjects cannot tolerate prolonged exposure (IARC, 1982, 1995a; WHO, 1989).

16.2.1.2 Respiratory Mechanics in Laboratory Animals Inhalation of formaldehyde decreases minute volume in laboratory animals, primarily as a result of a decrease in respiratory breathing frequency (more than through a decrease in tidal volume, which may even increase) (Table 16.6). Concomitantly, pulmonary resistance increases with or without a slight decrease in pulmonary compliance. These responses are rapid in onset, can remain constant during exposure (response plateau), and are readily reversible with exposure cessation.

The magnitude of the "response plateau" developed during exposure is dose dependent. The persistence of the response, however, varies somewhat with species. In studies with rats (F-344) and mice (Swiss Webster but not B6C3F_1), decreases in minute volume are not maintained during exposure, suggesting tolerance. In B6C3F_1 mice, the induced decrease in minute volume remains more consistent during exposure. These differences in minute

TABLE 16.6 Acute Pulmonary Responses in Animals to Formaldehyde

	Concentration (ppm)			
Species	Threshold	$ED_{25\%}$	$ED_{50\%}$	References
Decreased minute volume/respiratory rate				
Mice (Swiss-Webster)	0.50	0.8	3.1	Kane and Alarie (1977)
(B6C3F_1)*a*	0.50	1.8	4.4	Barrow et al. (1983)
Rats (F-344)[a]	0.95	3.1	13.1	Chang et al. (1981)
Guinea pig	0.30	11.0	>49.0	Amdur (1960)
Increased airway resistance				
Guinea pig	0.30	2.0	11–49	Amdur (1960)
Cynomolgus monkey	2.5	ND	ND	Biagini et al. (1989)

[a]Tidal volume was noted to increase with exposure in animals during exposures to ≥6.4 ppm.

volume, as noted by Barrow et al. (1983), could alter the dose from exposure to 15 ppm (a dose found sufficient to induce nasal carcinoma in rats but not mice). In $B6C3F_1$ mice, minute volume is decreased 75% as compared with 45% in F-344 rats. This would reduce the estimated dose to the mouse nasal epithelium from 0.156 to 0.076 μg/min/cm^2. Changes in breathing pattern, therefore, may be viewed as protective. In a study with male cynomolgus monkeys, Biagini et al. (1989) recorded a 42% increase in airway resistance within 2 min of exposure to 2.5 ppm formaldehyde. At 10 min airway resistance increased by 77%.

The formaldehyde dose producing decreases in respiratory rate in mice is comparable with the dose producing increases in symptomatic responses in humans (see above). This led Alarie and associates to propose that evaluation of sensory irritation in mice might serve as a useful test system to evaluate relative irritation potential of various compounds (Alarie, 1973; Kane and Alarie, 1977; Arts et al., 2006). From these finding, the authors suggested that the TLV should be set in the range of 30–300 ppb to prevent irritant responses.

16.2.1.3 Respiratory Mechanics in Humans After Single Exposure When inhaled alone in controlled exposure chambers, formaldehyde produces few changes in respiratory mechanics among healthy subjects either during or shortly after a single exposure at environmentally relevant levels (Table 16.7). Following exposures ranging from 0.3 to 7.5 ppm, essentially no change has been noted in this group. For example, Day et al. (1984) exposed 18 volunteers to 1.0 ppm formaldehyde, with nine subjects having complained of various nonrespiratory adverse effects from the urea-formaldehyde foam insulation in their homes. No effects were noted in subjects who exposed to 1.0 ppm formaldehyde for 90 min or 1.1 ppm formaldehyde (produced from urea-formaldehyde foam insulation) for 30 min. Exposure to 3.0 ppm for 60 min did produce a small change in forced expiratory volumes and flows (Green et al., 1987), but this response was transient and reversed when exposures were extended to 120–180 min (Sauder et al., 1986).

Formaldehyde's ability to alter pulmonary function has also been tested additional, possibly more susceptible populations. The first population, persons with asthma, was selected based on the observation that another soluble irritant, like SO_2, had lower-dose thresholds to initiate bronchoconstriction in this group compared to control subjects (Sheppard et al., 1980). Studies have involved 0.4–3.0 ppm for 40–120 min in chamber exposures with and without exercise (Harving et al., 1986; Green et al., 1987; Witek et al., 1987; Krakowiak et al., 1998; Ezratty et al., 2007) or oral breathing (mouthpiece) for 10 min to 1.0–3.0 ppm (Sheppard et al., 1984). In each study, subjects failed to develop an increase in pulmonary resistance or a decrease in flow rate when tested at rest or with exercise. In one study (Witek et al., 1987), however, airway reactivity may have been altered (see below).

The second clinical population tested were persons with previous occupational exposures (Table 16.7). The major objective of these studies was to reproduce, in the laboratory, observed across-shift changes in pulmonary function. This effect is often evident and is discussed later under Section 16.3. Another objective of these studies was to uncover an immunologically based response in subjects referred to the clinic for a direct bronchial provocation. Such investigations have documented immediate and delayed responses, much like those found in antigen-induced immediate hypersensitivity reactions, but these responses appear to be rare (e.g., occurring in 12 of 230 persons tested, or 5%, in one study). Given the widespread exposure to formaldehyde and documented formaldehyde skin sensitization (see below), these results suggest that there are remarkably few cases of pulmonary hypersensitivity. This implies that sensitization by aldehyde inhalation is not a

TABLE 16.7 Respiratory Function of Humans Exposed to Formaldehyde

Formaldehyde Concentration (ppm)	Length of Exposure (min)	Route of Exposure	Findings (No. of Subjects)	References
Healthy individual (nonsmoking) in clinical studies				
0.3–1.6	300	Oronasal (chamber)	No change in FVC, $FEV_{1.0}$, $FEF_{25–75\%}$ or R_{aw} ($n = 16$)	Anderson and Molhave (1983)
0.5–3.0	180	Oronasal (chamber, with or without exercise)	No change in FVC, $FEV_{1.0}$, $FEF_{25–75\%}$ sG_{aw} ($n = 9–10$)	Kulle et al. (1987)
2.0	40	Oronasal (chamber)	No change in FVC, $FEV_{1.0}$, MMEF ($n = 15$)	Schachter et al. (1986)
3.0	60	Oronasal (chamber, with exercise)	2.5–3.8% change in FVC and $FEV_{1.0}$ ($n = 22$)	Green et al. (1987)
3.0	180	Oronasal (chamber, with exercise)	2–7% change in FVC, and $FEV_{1.0}$ in 60 min; no change in FVC and $FEV_{1.0}$ at 180 min	Sauder et al. (1986)
7.5	2	Oral (mouthpiece)	No change in $FEV_{1.0}$ or R_{aw}	Rader (1977)
Individual with asthma in clinical studies				
0.4	60	Nasal (chamber, without exercise)	No change in FVC, $FEV_{1.0}$	Ezratty et al. (2007)
0.4	120	Nasal (chamber, without exercise)	No change in FVC, $FEV_{1.0}$	Krakowiak et al. (1998)
0.1–0.7	90	Nasal (chamber, without exercise)	No change in R_{aw}, $FEV_{1.0}$ ($n = 15$)	Harving et al. (1986)
2.0	40	Oronasal (chamber, with exercise)	No change in FVC, $FEV_{1.0}$, MMEF ($n = 12$)	Witek et al. (1987)
3.0	10	Oral (mouthpiece, with exercise)	No change in sR_{aw}	Sheppard et al. (1984)
3.0	60	Oronasal (chamber)	No change in FVC, $FEV_{1.0}$, $FEF_{25–75\%}$ sGa_w	Green et al. (1987)
Individual with occupational exposure				
0.3–0.6	8 h work	Oronasal (at work)	Small (≥200 mL) change in FVC and $FEV_{1.0}$ ($n = 38$)	Alexandersson and Hedenstierna (1988)
2.0	40	Oronasal (chamber, with exercise)	No change in FVC, FEV1.0, MMEF ($n = 15$)	Schachter et al. (1987)
0.1–3.0	20	Oronasal (face mask)	12 ± 2% decrease in $FEV_{1.0}$ as compared to 8 ± 3% in control; little or no change in MMEF and other measures	Frigas et al. (1984)
1.8	30	Oronasal (chamber)	13% decrease in $FEV_{1.0}$ ($n = 4$)	Burge et al. (1985)
2.0	20	Oronasal (chamber)	Decreases in R_{aw} in 12 of 230 (5%) persons	Nordman (1985)
3.2	30	Oronasal (chamber)	12% decrease in $FEV_{1.0}$ ($n = 8$); three with decrease >25% in $FEV_{1.0}$	Burge et al. (1985)

Abbreviations: FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory flow at 1.0 s; $FEF_{25–75\%}$, mean of forced expiratory flow at 25 and 75% vital capacity; (s)R_{aw}, (specific) airway resistance; MMEF, midmaximal expiratory flow; (s)G_{aw}, (specific) airway conductance.

primary mechanism for a respiratory effect, but rather observed effects are associated with a direct (nonimmuno-specific) irritant effect. This limited role for immunologic sensitization is particularly evident if hypersensitivity is narrowly defined as a response to extremely low doses of a compound, well below the dose necessary to induce irritation.

Few data exist comparing possible formaldehyde effects on minute volumes in humans with this well-documented effect noted in laboratory animals. This is unfortunate, because this information would be useful if directly compared to the symptomatic responses noted within the same species.

16.2.1.4 Airway Reactivity Aldehydes can influence the underlying bronchial reactivity of the airways following an initial, transient bronchoconstriction [typically produced only at high (≥10 ppm) concentrations]. Several other irritants (sulfur dioxide, ozone, and toluene diisocyanate) that induce an immediate bronchoconstriction also can induce bronchial hyperreactivity. Hyperreactivity is experimentally defined as heightened responsiveness to inhaled methacholine (a stable form of acetylcholine) or histamine and is a diagnostic feature of asthma (Boushey et al., 1980; Barnes et al., 1989). Following a single initial exposure of healthy individuals, this condition is typically reversible and lasts for 12–48 h. In persons with asthma, however, this condition can persist for several years. Hyperreactivity frequently lacks an immunologic component and is thus termed nonspecific. In these cases no identifiable causative antigen can be found, and a general heightened response is noted after a wide range of irritant stimuli. The relationship between each phase of the dual responses (e.g., an immediate and/or a delayed decrease in $FEV_{1.0}$) after antigen presentation/challenge and hyperreactivity is currently unclear.

Animal models have been informative in the past. Formaldehyde exposure of guinea pigs for 2 h produced a small change in pulmonary resistance, with an estimated half-maximal change in bronchial reactivity at 8.0 ppm (Swiecichowski et al., 1993). When the duration of exposure was extended to 8 h, 1.1 ppm formaldehyde produced a significant increase in reactivity, greater in magnitude as that produce when exposure was short but to an eightfold higher concentration. This study suggests that low-level exposures of several hours may have effects not detectable with shorter (≤2 h) exposures.

In another animal model, ICR strain mice were sensitized intraperitoneally with dust mite allergen (Der f) prior to exposure to 0.5% formaldehyde mist once a week for 4 weeks (Sadakane et al., 2002). Mice were then challenged by intratracheal instillation with Der f and airway inflammation examined. When combined with following Der f challenge, formaldehyde exposure enhanced the histopathology (including eosinophil infiltration and goblet cell formation) and lung levels of two cytokines associated with asthma [interleukin-5 (IL-5) and chemokine (C–C motif) ligand 5 (CCL5) aka regulated on activation, normal T cell expressed, and presumably secreted (RANTES)].

Bronchial reactivity has been examined in humans, and no changes have been recorded in healthy persons after exposures up to 3000 ppb formaldehyde for 3 h (Sauder et al., 1986; Green et al., 1987; Kulle et al., 1987). In studies with persons with asthma, however, Witek et al. (1987) reported a decrease in the dose of methacholine necessary to produce a 20% decrease in forced expiratory volume in 1.0 s ($FEV_{1.0}$) in 8 of 12 individuals following a 40-min exposure to 2.0 ppm formaldehyde. The mean response for all 12 subjects was not statistically different from control; thus, further study is required before any conclusions on the effect of formaldehyde on airway reactivity can be drawn. Burge et al. (1985) also has suggested that a relationship exists between bronchial responses of subjects with previous occupational exposure to formaldehyde and their underlying bronchial reactivity.

Lemière et al. (1995) reported that three individuals developed decreased $FEV_{1.0}$ after being exposed to formaldehyde resin dust, with one of these subjects also being responsive to formaldehyde gas. Kim et al. (2001) presented a case report of an individual with occupational asthma. Working in an environment with a mean formaldehyde level of 60 ppb (with occasional peaks of 120–130 ppb), this worker had across-shift decreases in $FEV_{1.0}$, which was reversed by inhalation of a bronchodilatory (beta2-adrenergic agent). In addition, short-term exposure (20 min) at concentrations of 500 ppb in the laboratory led to an immediate and delay (lasting over 20 h) decreased $FEV_{1.0}$. No circulating antibody or cutaneous reactivity specific to formaldehyde–human serum albumin conjugate was detected. In another case report, by Vandenplas et al. (2004), a single accidental high-level formaldehyde exposure led to changes in $FEV_{1.0}$ and a persistent (lasting over 1 year) increase in bronchial hyperactivity to histamine, and again this seemed not to involve a specific immunological mechanism.

To explore the possible mechanism, Kim et al. (2002) examined human mucosal microvascular endothelial cells following *in vitro* exposure to formaldehyde and found an increase in the surface expression of intercellular adhesion molecule 1 (ICAM1) and vascular cellular adehesion molecule 1 (VCAM1). In addition, the adhesiveness between endothelial cells and eosinophils was also increased by formaldehyde exposure. This implies that formaldehyde is acting as an irritant of the nasal mucosa that may lead to an increasing the expressions of adhesion molecules and interaction that increase eosinophil trafficking (an even critical to allergic rhinitis and possible asthma).

In sensitized persons with asthma, Casset et al. (2006a) found that a short, low-dose pre-exposure (30 min × 30 ppb formaldehyde) lowered the threshold dose of dust mite need to induce allergen-mediated bronchoconstriction. In addition, the late-phase reaction (defined as a ≥15% decrease in $FEV_{1.0}$) was also more common in persons exposed to both allergen and formaldehyde as compared with persons exposed to allergen and filtered air. Consistent with an increased asthmatic response, the level of eosinophil cationic protein in serum or induced sputum was greater following exposure to formaldehyde with antigen. In a review of the literature, these authors also noted that the risk for development of asthma is increased (approximately 1.4-fold) in homes that exceed 50 ppb formaldehyde (Casset et al., 2006b). Similarly, in several other studies, children exposed to formaldehyde levels of >50 ppb were at increased risk of having asthma (see Section 16.3.1.5).

Larsen et al. (2013) investigated the role of air humidity and allergic sensitization on the acute airway response to inhaled formaldehyde. Mice were sensitized to the immunogen ovalbumin (OVA) by intraperitoneal injections followed by aerosol challenges, giving rise to allergic airway inflammation. Control mice were sham sensitized by saline injections and challenged by saline aerosols. Once sensitized, the mice were housed at high (85–89%) or low (<10%) relative humidity, respectively, for 48 h prior to a 60-min exposure to either 0.4, 1.8, or about 5 ppm formaldehyde. Before, during, and after exposure, nose and lung irritation and the expiratory flow rate were measured. The sensory irritation response in the upper airways was not affected by allergic inflammation or changes in humidity. At high relative humidity, the 5 ppm formaldehyde-exposed OVA-sensitized mice had a decreased expiratory airflow rate compared with the saline control mice. At low humidity, the opposite trend was observed with the control mice having a significantly decreased expiratory airflow rate compared with OVA-sensitized mice exposed to 4 ppm formaldehyde. The latter may be due to increase nasal capacity of sensitized to humidify the dry, but this remains to be determined.

16.2.1.5 Mucociliary Clearance Formaldehyde markedly inhibits mucociliary clearance in animals and humans (Table 16.8). This effect was noted as early as 1942 by Cralley (1942), who observed an inhibition and complete stasis of ciliary beating after formaldehyde exposure.

As concerns arose about the health effects of cigarette smoke, several investigators (Guillerm et al., 1961; Falk, 1963; Kensler and Battista, 1963; Wynder and Hoffman, 1963; Carson et al., 1966; Dalhamn and Rosengren, 1971; Sisson and Tuma, 1994) confirmed that aldehydes (including formaldehyde and acetaldehyde) in smoke can alter ciliary function and extended this observation, finding that formaldehyde exposure as short as 12 s could reversibly inhibit ciliary activity.

The onset and duration of the effects of formaldehyde on mucociliary clearances are dose dependent. Formaldehyde exposures initially diminish the movement of the surface mucus layer before decreasing ciliary beat frequency (Morgan et al., 1984); a result could

TABLE 16.8 Formaldehyde Inhibition of Mucociliary Clearance in Laboratory Animals

Concentration	Duration of Exposure	Response	Species	References
20 ppm	10 min	Depression of ciliary activity without recovery	Rabbit	Cralley (1942)
10 ppm	—	Cilia stasis in 20%	Rabbit	Dalhamn and Rosengren (1971)
52 ppm	—	Cilia stasis in 90%	Rabbit	Dalhamn and Rosengren (1971)
32 ppm	11.5 min	Cilia stasis	Guinea pig	Oomichi and Kita (1974)
20 ppm	4 h	Decrease early clearance of particles; no effect on delayed clearance (alveolar)	Rat (Sprague–Dawley)	Mannix et al. (1983)
9.6 ppm	30 min	Increase mucus flow rate at 2 min; muco- and cilia stasis at 2 and 3 min	Frog palate	Morgan et al. (1984)
5.0 ppm	60 min	Decreased mucociliary clearance and cilia beat frequency	Frog palate	Fló-Neyret et al. (2001)
4.4 ppm	30 min	Mucostasis in 8 min	Frog palate	Morgan et al. (1984)
2.5 ppm	60 min	Decreased mucociliary clearance and cilia beat frequency	Frog palate	Fló-Neyret et al. (2001)
1.4 ppm	30 min	Increase in clearance	Frog palate	Morgan et al. (1984)
1.25 ppm	60 min	Increased cilia beat frequency	Frog palate	Fló-Neyret et al. (2001)
0.2 ppm	30 min	No effect	Frog palate	Morgan et al. (1984)
66 mg/cm^2	60 min	Reduced ciliary activity within 10 min	Rabbit and pig	Hastie et al. (1990)
33 mg/cm^2	60 min	Reduced ciliary activity within 10–20 min	Rabbit and pig	Hastie et al. (1990)
16 mg/cm^2	60 min	Reduced ciliary activity within 30–40 min	Rabbit and pig	Hastie et al. (1990)

be due to covalent reaction of formaldehyde with mucus macromolecules that alters the physical (rheologic) properties essential for effective energy coupling with the underlying cilia. Doses of formaldehyde sufficient to inhibit ciliary activity can also reduce the number of cilia extractable from exposed tissue preparations (Hastie et al., 1990). In cilia recovered from exposed epithelia, the specific activity of ATPase and dyneins and tubulin protein content are decreased. These responses were reversible in less than 2 h after exposure, suggesting that recovery is not dependent on *de novo* protein synthesis and that ciliary loss and recovery are a dynamic process. In studies with a related aldehyde of concern due to its formation from alcohol and possible interaction with cigarette smoke, Wyatt et al. (1999) found that acetaldehyde diminished ciliary beat frequency through a protein kinase C-dependent pathway.

In isolated tissue preparations, responses to formaldehyde are dose dependent. In the past, a single exposure to a concentration of 1.0–1.25 ppm was thought to have no effect or increases mucus transport rates and cilia beat frequency, whereas concentrations between 2.5 and 10 ppm depress transport (Table 16.8). However, Schafer et al. (1999) reported a decrease in ciliary beat frequency in nasal epithelial cells isolated from persons exposed to 4.1 ppm formaldehyde for 2 h. Similarly, Fló-Neyret et al. (2001) performed a dose–response analysis and found that 2500 ppb formaldehyde inhibited mucociliary transport rate within 30 min. In animals, depression of overall particle clearance has been noted at a concentration of 20 ppm (4-h exposure) in rats (Mannix et al., 1983; Adams et al., 1987). In humans, however, nasal particle clearance was depressed at concentrations as low as 240 ppb (4–5 h), with maximal effects observed at concentrations of 400 ppb (Anderson and Molhave, 1983). These effects on clearance are most prominent in the anterior portion of the nose.

Clearance is dependent on mucus composition, mucus quantity, and ciliary function. Clearance can be compromised in humans at concentrations below those necessary to produce single effects in *in vitro* preparations (i.e., decreased ciliary beat frequency). This suggests that this compound acts through interference with more than a single cellular or extracellular process. For example, it is probable that ciliary proteins are sensitive to formaldehyde (Hastie et al., 1990), protein–protein methylene cross-links in mucus can alter the tertiary structure of this glycoprotein (Morgan et al., 1984), or protein kinase C activation diminishes ciliary function (Salathe et al., 1993; Wong et al., 1998; Wyatt et al., 1999). Acting together, these effects could diminish particle clearance at lower doses.

16.2.1.6 Effects of Aerosol–Formaldehyde Co-exposures Amdur (1960), a founder of inhalation toxicology, reported that formaldehyde-induced increases in the respiratory resistance of guinea pigs were potentiated by the simultaneous administration of submicrometer sodium chloride aerosol (sodium chloride alone produced no effect). A response, induced by a formaldehyde concentration delivered as a formaldehyde–aerosol mixture when breathed through the nose, was greater than the response to the same formaldehyde dose (gas alone) when administered directly to the lung through a tracheal cannula. This interesting observation suggests that the increment added by the aerosol is not solely a result of the transfer of more formaldehyde to the lungs but may reflect additional factors. Similarly, Kilburn and McKenzie (1978) reported that co-exposure of formaldehyde with carbon particles produces greater recruitment of leukocytes into the airway epithelium and epithelial cytotoxicity (cytoplasmic vacuolization and nuclear aberrations) in trachea and bronchi of Syrian golden hamsters. This effect peaked 24–48 h after exposure. Because many environmental exposures involve co-exposure to ambient

and occupational aerosols, for example, resin particulate matter (Lemière et al., 1995), future investigations clarifying these issues would add greatly to our understanding of how complex mixtures act in concert to exert aldehyde toxicity.

16.2.2 Other Aldehydes

16.2.2.1 Symptomatic Responses and Respiratory Mechanics The decrease in respiratory rate, induced by a wide array of aldehydes, has been studied in mice by Steinhagen and Barrow (1984). In this experimental system, half-maximal concentration (ED50) for respiratory rated was 1.0, 3.1, and 3.5 ppm for acrolein, crotonaldehyde, and formaldehyde, respectively. In contrast, the half-maximal dose for acetaldehyde was greater (>100 ppm). Acrolein is consistently more potent than formaldehyde in multiple assays. Saturated aliphatic aldehydes with two or more carbons (e.g., butyraldehyde or propionaldehyde) had half-maximal concentrations of 0.75–4.2 ppm, whereas cyclic aldehydes (e.g., 3-cylcohexane-1-carboxyldehyde or benzaldehyde) exert its effects in intermediate doses ranging from 60 to 400 ppm. Thus, the relative potency was acrolein > crotonaldehyde ≥ formaldehyde > benzaldehyde ≫ acetaldehyde. This apparent relationship holds for most other toxic endpoints including measures of pulmonary function in humans (Pattle and Cullumbine, 1956); half-maximal lethal dose in mice, guinea pigs, and rabbits (Salem and Cullumbine, 1960); and nasal pathology in rats (Lam et al., 1985; Roemer et al., 1993; Cassee et al., 1996b).

Microelectrode recordings of trigeminal nerve fibers during inhalation of aldehydes (Kulle and Cooper, 1975) are consistent with involvement of this neural pathway in the decrease in respiratory rate (Kane and Alarie, 1977). The role of specific sensory afferent pathways was further examined by Lee et al. (1992), who reported that acrolein inhalation evoked an inhibitory effect on breathing with a prolongation of expiration and bradycardia. As determined by a number of interventions, acrolein activated both vagal C-fiber active afferents and rapidly adapting irritant receptors and suggested that the elongation of expiration was due to stimulation of the former afferent pathway. Similar to acrolein, formaldehyde stimulates C-fiber nerves and can stimulate the release of substance P, which may induce a neurogenic inflammatory response. In rat airways, this is marked by an increase in vascular permeability that is mediated predominantly by stimulation of the tachykinin NK1 receptor (Ito et al., 1996). In addition, Fujimaki et al. (2004) found that mice exposures to 2.0 ppm formaldehyde for 12 weeks increased substance P levels in plasma.

16.2.2.2 Airway Reactivity Human studies have been performed with acetaldehyde and its metabolic precursor, ethanol. Watanabe (1991) found that 55% of the Japanese asthmatics he tested had a significant fall in $FEV_{1.0}$ after ingestion of 10% ethanol (responder). The induced blood ethanol concentration was equal between the responder group and nonresponder group. Acetaldehyde and histamine concentrations in the responders were significantly higher than that of the nonresponders. Using a leukocyte histamine release assay, 2–100 μM acetaldehyde caused dose-dependent increase in histamine release, whereas 2–100 mM ethanol had no effect on histamine release.

Myou et al. (1993) found that nine subjects with asthma had a dose-dependent decrease in $FEV_{1.0}$ following 2 min inhalations of acetaldehyde (0, 5, 10, 20, and 40 mg/mL) aerosol. In control subjects without asthma, acetaldehyde had no effect on lung function. Five of the subjects with asthma also had alcohol-induced bronchoconstriction, common among the Japanese population. This group also found that 8.0 mg/m acetaldehyde inhalation for

4 min potentiating effect of inhaled acetaldehyde on bronchial responsiveness to methacholine in asthmatic subjects (Myou et al., 1994).

As stated above, alcohol sensitivity is due to a dual inheritance of a rapid isoform of ADH with a slow isoform of aldehyde dehydrogenase-2. This allelic combination increases the formation of acetaldehyde and decreases subsequent clearance and is carried by ≥20% of persons of Oriental lineage. It is further enriched among Japanese asthma patients, with over 50% of these individuals having alcohol intolerance (Gong et al., 1981; Watanabe, 1991). However, this factor did not explain the effect of inhaled acetaldehyde observed by Myou et al. (1993), because four of the nine asthma patients were not alcohol intolerant, yet they responded like those patients that were. Although the sample size of these populations is small, persons with asthma appeared to be at greater risk to the bronchoconstrictive effects of acetaldehyde. These results are like the immediate bronchoconstriction that has been noted after sulfur dioxide exposure (Sheppard et al., 1984).

Similarly, an oral ethanol provocation test was performed by Matsuse et al. (2007) in Japanese asthmatics, and pulmonary function, blood ethanol, acetaldehyde, and histamine were measured. Acetaldehyde dehydrogenase 2 (ALDH2) genotype was determined by polymerase chain reaction (PCR) and ethanol patch test. In addition, human bronchi and mast cells were stimulated with acetaldehyde *in vitro*, and mite allergen-sensitized mice were inoculated with intranasal acetaldehyde. Approximately half the asthmatic subjects developed bronchoconstriction with concomitant increases in blood acetaldehyde and histamine, which was associated with genetically reduced ALDH2 activities. *In vitro* acetaldehyde stimulation induces bronchial constriction and degranulation of human mast cells. It also induced granulocyte macrophage colony stimulating factor (GM-CSF) production and nuclear factor-kappa B (NF-κB) activation in human bronchi and increased mite allergen-sensitized allergic inflammation in mice. Thus, acetaldehyde has the potential to induce airway mast cells to release histamine, which results in exacerbation of asthma in susceptible populations, and to induce cytokine formation, which results in airway inflammation.

In a study of response to methacholine and acetaldehyde challenges measured in 81 nonsmoking European adults (61 asthmatics and 20 normal controls), Prieto et al. (2000) found that that airway hyperresponsiveness to acetaldehyde is a sensitive and specific indicator for separating asthmatic and normal subjects. The results of this larger group support the initial finding of Myou et al. (1993) and demonstrate that persons with asthma are clearly at risk of acetaldehyde airway hyperresponsiveness. In a follow-up study of 62 nonsmokers with asthma and 59 smokers with chronic bronchitis, 92% of the persons with asthma were hyperresponsive to acetaldehyde, whereas only 5% of the persons with chronic bronchitis were sensitive to acetaldehyde bronchoprovocation (Sánchez-Toril et al., 2000). In addition, this group found that subjects with allergic rhinitis are less responsive to inhaled acetaldehyde than asthmatic subjects, but more than healthy controls (Prieto et al., 2002).

16.2.2.3 Mucociliary Clearance and Defense Mechanisms Along with studies with formaldehyde, mucociliary function has been studied with other aldehydes. Unlike reported bronchoconstriction, Dalhamn and Rosengren (1971) found that the effects and dosimetry of formaldehyde were comparable with acrolein and crotonaldehyde in potency in the inhibition of cilia beating. Acetaldehyde, although much less potent, can alter ciliary functions. Changes in ciliary beat can also be accompanied by alteration in mucus load. Sisson et al. (1991) reported that acetaldehyde induced concentration- and time-dependent

slowing of cilia beating and cilia-derived dynein ATPase activity in primary cultures and isolated axonemes of bovine airway epithelial cells. Cilia slowing (potency greatest to least: benzaldehyde = isobutyraldehyde > butyraldehyde = propionaldehyde = acetaldehyde) and ATPase inhibitory effects (potency greatest to least: benzaldehyde = butyraldehyde > propionaldehyde = acetaldehyde = butyraldehyde) were observed with aldehydes but not with ethanol. Acetaldehyde binding, assessed by gel electrophoresis using [^{14}C] acetaldehyde, occurred with the dynein heavy chains and with tubulin and closely paralleled ATPase inhibition. Thus, acetaldehyde directly impairs bronchial cilia function causing slowing of cilia beating, inhibits cilia dynein ATPase activity, and binds to cilia proteins critical for motion including dynein and tubulin. These data suggest that acetaldehyde-induced cilia dysfunction may be related to direct cilia ATPase inactivation and adduct formation with cilia dynein and tubulin.

16.3 EFFECTS OF MULTIPLE EXPOSURES

16.3.1 Formaldehyde

16.3.1.1 Carcinogenesis According to IARC (2006, 2012), formaldehyde is carcinogenic to humans (group 1) on the basis of sufficient evidence in experimental animals and in human. This is a higher classification than the initial IARC evaluations of group 2A—probably carcinogenic to humans, which were based on sufficient evidence in experimental animals but insufficient evidence in humans (IARC, 1995a). In addition, *in vitro* and *in vivo* genotoxic studies support this classification. The major reason for the stricter classification was due to updated epidemiological studies that found that occupational formaldehyde exposure was associated with induction of nasopharyngeal cancer (Hildesheim et al., 2001; Hauptmann et al., 2004) and possibly leukemia (Zhang et al., 2010; IARC, 2012).

In 2006, IARC found that a strong but not sufficient evidence to associate formaldehyde exposure to leukemia. However, in 2012, IARC (2012) included the association with leukemia, concluding: "There is sufficient evidence in humans for the carcinogenicity of formaldehyde. Formaldehyde causes cancer of the nasopharynx and leukaemia." The inclusion of leukemia was based on evidence that formaldehyde induces genetic changes in blood cells and pancytopenia of exposed workers that are characteristic of myeloid leukemia and myelodysplastic syndromes.

Formaldehyde has genotoxic effects in a wide number of *in vitro* systems (Auerbach et al., 1977; Kreiger and Garry, 1983; Ma and Harris, 1988; WHO, 1989; Feron et al., 1991; IARC, 2012; She et al., 2013). The level of activity in many experimental systems is often either weak or moderate compared with other known mutagens and can depend on what are sometimes unique experimental conditions. Regardless of these caveats, formaldehyde is clearly capable of reacting with cellular macromolecules (see above), and DNA damage induced by formaldehyde includes DNA–protein cross-links and single-strand breaks (Fornace, 1982; Fornace et al., 1982; Graftstrom et al., 1984; IARC, 1995a). DNA–protein cross-links have been found *in vivo* in the nasal passages of Fisher 344 rats (Heck et al., 1989; Casanova et al., 1994) and rhesus monkeys (Casanova et al., 1991). Graftstrom et al. (1984) also found that DNA single-strand breaks could accumulate in the presence of DNA excision repair inhibitors and that formaldehyde can inhibit repair.

Studies examining genotoxic effects in bacteria, yeast, fungi, and drosophila produce varied responses. For example, mutations in drosophila are only noted in male larvae under culture conditions requiring adenosine, adenylic acid, or RNA medium supplementations. In mammalian systems the results are also subtle, complex, and varied. As noted by Boreiko and Ragan (1983), formaldehyde-induced sister chromatid exchange in Chinese hamster ovary cells has been found by one investigator (Obe and Beck, 1979), but not by another (Brusick, 1983). In addition, formaldehyde is not mutagenic in either of these cells (Hsie et al., 1979). Another illustration of the complexity associated with the evaluation of formaldehyde's genotoxic effects is that it can induce unscheduled DNA synthesis, which has been recorded in HeLa cells (Martin et al., 1978), but not in monkey kidney cells (Nocentini et al., 1980). *In vivo* dominant lethal assays in mice are negative or transiently positive (Epstein, 1972; Fontignie-Houbrechts, 1981). In any case, formaldehyde can facilitate malignant transformation of mouse embryo C3H10T1/2 C18 fibroblasts, acting through either an "initiating" or "promoting" mechanism (Ragan and Boreiko, 1981; Boreiko and Ragan, 1983; Frazelle et al., 1983), and produce DNA single-strand breaks and DNA–protein cross-links (Graftstrom et al., 1984).

Mutational spectra induced by formaldehyde have been studied in human lymphoblasts *in vitro*, in *Escherichia coli*, and in naked pSV2gpt plasmid DNA (Crosby et al., 1988). In lymphoblasts large visible deletions of some or all the X-linked hprt bands were detect by Southern blot analysis (Liber et al., 1989). In *E. coli*, mutations in the xanthine guanine phosphoribosyl transferase (*gpr*) gene included large insertions (41%), large deletions (18%), and point mutations (41%). Many of the point mutations were GC transversions. Formaldehyde-induced genetic alterations in *E. coli* are concentration dependent, with higher doses yielding 92% point mutations, 62% of which were single AT transitions. In a plate assay with *Salmonella typhimurium* strain TA100 in the absence and presence of mammalian metabolic S9 mix, a weak mutagenic response was observed (Schmid et al., 1986). Exposure of a SV2gpt plasmid DNA (with transformation into *E. coli*) resulted in frameshift mutations. Through a comparison of DNA repair proficient and deficient heterokaryons of *Neurospora crassa*, de Serres and Brockman (1999) examined formaldehyde-induced specific-locus mutations at two closely linked loci in the adenine-3 (ad-3) region and inactivation of heterokaryotic conidia. As in many other systems, formaldehyde was a weak mutagen in the DNA repair proficient strain. However, in the DNA repair-deficient strain, formaldehyde caused about a 35-fold higher frequency of ad-3 mutations and pronounced inactivation of heterokaryotic conidia. In addition, formaldehyde induced a 5.4-fold higher frequency of ad-3 mutations resulting from multilocus deletion mutation. As with x-ray-induced multilocus deletion mutations, the formaldehyde-induced ad-3 mutations could lead to deleterious heterozygous effects. Overall, the *in vitro* assays demonstrate the feasibility that formaldehyde could be mutagenic, but the results are complex and not always consistent.

16.3.1.2 Nasal Cancer in Laboratory Animals In contrast to the studies above, studies with laboratory animals are clearer. Chronic inhalation of formaldehyde induces squamous cell carcinoma in the nasal cavity of rats (Swenberg et al., 1980; Albert et al., 1982; Kerns et al., 1983; Sellakumar et al., 1985; Feron et al., 1988; Woutersen et al., 1989). Exposure to 14.3 ppm formaldehyde (6 h/day × 5 days/week × 24 weeks) produced carcinomas in 103 of 240 (43%) Fischer 344 rats and in 2 of 240 (rv1%) C57BL/6 × C3H/He F1 mice

(Kerns et al., 1983; Morgan et al., 1986). (Note the C57BL/6 mouse strain is a resistant to many lung carcinogens and the F1 offspring of this strain is likely to be resistant.) Similarly in a 28-month study (15 ppm formaldehyde × 6 h/day × 5 days/week), Kamata et al. (1997) reported that male Fisher 344 rats developed nasal tumors that were macroscopically evident within 14 months, with 8 of 32 rats developing tumors (squamous cell papillomas and carcinomas) at 28 months. No nasal tumors were observed with lower concentrations (0.3 and 2.0 ppm groups), but nasal epithelial cell hyperplasia, hyperkeratosis, squamous metaplasia, inflammatory cell infiltration, erosion, and edema was evident in all groups.

To understand how formaldehyde exposure might lead to cancer, Recio et al. (1992) examined 11 rat nasal squamous cell carcinomas and found point C or G mutations in regions II–V of the tumor suppressor gene, p53, in five tumors. Proliferating cell nuclear antigen (PCNA) staining was similar in pattern and distribution to p53 immunoreactivity (Wolf et al., 1995). These mutations [particularly a CpG dinucleotide at rat codon 271 (codon 273 in humans)] were mutational hot spots that occur in many human cancers.

Studies with different strains of rats suggest that a gene–environment interaction may be controlling the cancer response. For example, Sprague–Dawley rats also respond to 14.2 ppm formaldehyde, but the time to onset was delayed, and the total carcinoma incidence was less [38 squamous cell carcinoma (38%) and 10 polyps or papillomas in 100 rats] (Albert et al., 1982). Holmström et al. (1989a) noted nasal squamous cell metaplasia and one squamous cell carcinoma in Sprague–Dawley rats exposed to 12.4 ppm formaldehyde for 104 weeks. Wistar rats yielded six tumors in 132 animals exposed to 20 ppm formaldehyde for 4–13 weeks and followed by observation to 126 weeks (Feron et al., 1988). Although the exposure period and concentration varied, these results suggest that strain differences exist in rats (Fisher 344 = Sprague–Dawley > Wistar strain) and that rats are more sensitive than one mouse strain (possibly derived from a resistant cross). Future studies to identify the genetic loci controlling these responses would be very informative.

The formaldehyde concentration–carcinoma response relationship is nonlinear in rats and also varies among strains. In Fisher 344 rats, exposure to 5.6 ppm formaldehyde resulted in two rats (of 240 exposed) with squamous carcinomas, and 2 ppm produced no carcinomas (Kerns et al., 1983). Similarly, Woutersen et al. (1989) found squamous cell carcinoma in 1 of 26 Wistar rats exposed to 10 ppm for 28 months. (However, 15 of 58 rats develop carcinomas when the nasal epithelium was injured before exposure.)

Swenberg et al. (1983) ascribed this nonlinearity in response to be due to nonlinearity in the formaldehyde dosimetry to its macromolecular target site. This nonlinear dosimetry results in part from variability of the breathing pattern during exposure, which influences the inhaled dose. (As noted earlier, formaldehyde deposition in mice exposed to 14 ppm was equivalent to that in rats exposed to 5.6 ppm. Consistent with this dosimetry estimate was the incidence of carcinomas: 2/240 in mice after 14 ppm and in 2/240 rats after 5.6 ppm.) In addition to altered breathing pattern, these investigators have ascribed the nonlinearity in the dose–response relationship to the nonlinearity of cytotoxicity and overloading of protective mechanisms (including metabolic biotransformation, mucociliary clearance, and DNA repair, all of which might be altered by multiple genetic variants) induced at higher formaldehyde concentrations.

In Syrian golden hamsters, 10 ppm formaldehyde alone (5 h/day × 5 days/week for life) did not produce nasal carcinomas (Dalbey, 1982). In the exposed group, however, the mortality was marked, with only 20 of 88 animals surviving 10 weeks of treatment. In these animals, nasal epithelial cell hyperplasia or metaplasia was observed. When hamsters were exposed to formaldehyde before exposure to another carcinogen, diethylnitrosamine, the

incidence of respiratory carcinomas increased. Formaldehyde (30 ppm) exposures were for 48 h before subcutaneous injections with 0.5 mg diethylnitrosamine (each once a week for 10 weeks). About twice as many tracheal adenomas (per tumor-bearing hamster) were observed when compared to hamsters exposed to diethylnitrosamine alone. When formaldehyde was given after diethylnitrosamine, no increase in adenomas was observed. Formaldehyde clearly produces nasal carcinomas in rats, and although mice and hamsters may be less sensitive than rats, nasal tumors have been observed in mice, and nasal hyper- and metaplasia has been observed in hamsters.

The nasal epithelial disruption has been investigated in several species including mice, rats, hamsters, and monkeys (Chang et al., 1983; Rusch et al., 1983; Al-Abbas et al., 1986; Maronpot et al., 1986; Morgan et al., 1986; Zwart et al., 1988; Monticello et al., 1989, 1991; St Clair et al., 1990; Bhalla et al., 1991; Cassee et al., 1996b; Cohen Hubal et al., 1997). In each species tested, the anterior nasal epithelium portion is most affected, although some species differences exist in the regional distribution of epithelial lesions. Repeated exposures to nearly comparable concentrations (about 5–6 ppm) produced loss of cilia and various stages of hyperplasia and squamous metaplasia of pseudostratified columnar respiratory epithelium and leukocyte infiltration. In Fischer 344 rats, these changes were most severe in the maxilloturbinate, the lateral aspect of the nasoturbinate, and the wall of the lateral meatus (Morgan et al., 1986), whereas in the rhesus monkey changes were most severe in the middle turbinate. In both species, effects noted after a single exposure become more persistent, and the percentage of nasal surface area affected progressed, when exposures are extended. The Fisher 344 appears to be more sensitive to formaldehyde-induced nasal pathology than the Brown Norway strain of rat following a single exposure (Ohtsuka et al., 1997).

Formaldehyde also increases nasal epithelial proliferation rate. In monkeys, effects were more persistent than those observed in rats. In rats, the proliferation rate returned to control values after 3–9 days at 1000–6000 ppb (Morgan et al., 1986; Cassee et al., 1996a, 1996b). In contrast, in monkeys the proliferation rates remained elevated longer, and areas of effected epithelium were more widespread. In an *in vitro* correlated study by Tyihak et al. (2001), 10 mM formaldehyde caused extensive cell damage to human colon carcinoma (HT-29) and human endothelial (HUV-EC-C) cell cultures, 1.0 mM enhanced apoptosis and reduced mitosis, and 0.1 mM enhanced cell proliferation and decreased apoptotic in both cell types but more so in the tumor cells. Changes in rat epithelium were mimicked by changes in cell cycle transcript expression (Hester et al., 2003). Cell proliferation increased in the nasopharynx, trachea, and carina of the lungs of monkeys, as compared to only in the anterior nasal cavity of rats (Monticello et al., 1989). Since monkeys, like humans, breathe through both the nose and the mouth, the involvement of the lower respiratory tract also may be possible in humans.

16.3.1.3 Respiratory Tract Cancer: Epidemiological Studies Epidemiological studies have examined the cancer mortality among persons with occupational and residential exposure to formaldehyde. This topic remains controversial and has been reviewed extensively elsewhere (Blair et al., 1990; Feron et al., 1991; Partanen, 1993; IARC, 1995a, 2012; Collins et al., 1997; Coggon et al., 2003; Heck and Casanova, 2004; Cole and Axten, 2004; Tarone and McLaughlin, 2005; Binetti et al., 2006; Marsh et al., 2007). Based on animal toxicological data, most concern has been directed at carcinomas of the nasal cavity because formaldehyde is thought to be so reactive that the target tissue must directly be exposed to inhaled formaldehyde. Evidence supporting an association between

formaldehyde and nasal cancer in humans was lacking for several years. While increased relative risk for nasal cancer had been associated with formaldehyde exposure in many case–control studies (Hayes et al., 1986; Olsen and Asneas, 1986; Roush et al., 1987; Vaughan et al., 1986a, 1986b, 2000; Luce et al., 1993; West et al., 1993; Armstrong et al., 2000; Hildesheim, 2001), there were concerns about consistency in this association because it was clearly not found in two other studies (Hernberg et al., 1983; Brinton et al., 1985).

In addition, this association was not always been supported by comparable increases in the standard mortality ratio in several cohort studies for nasal cancer (Marsh, 1982; Acheson et al., 1984a, 1984b; Levine et al., 1984; Blair et al., 1986; Stroup et al., 1986; Hayes et al., 1990; Gardner et al., 1993; Marsh et al., 1994). However, a reanalysis of formaldehyde-exposed workers that combined the Marsh et al. (2002) cohort with that of Hauptmann (2004) supported associations with cancers of the upper respiratory tract and nasal cavity with formaldehyde. Importantly, this association remained even after adjusting for 11 potential confounding substances (Hauptmann et al., 2003, 2004). In addition, these associations are supported by several case–control studies (Hauptmann et al., 2005). Nonetheless, the controversy has yet to be resolved, and reanalyses of these data suggest that uncertainties still remain (Tarone and McLaughlin, 2005; Marsh et al., 2007).

In contrast to nasal cancer, positive associations of formaldehyde exposure have been noted more frequently with nasopharyngeal cancer. This association has been noted in five of seven case–control (Olsen et al., 1984; Olsen and Asneas, 1986; Vaughan et al., 1986a, 1986b, 2000; Roush et al., 1987; West et al., 1993; Armstrong et al., 2000; Hildesheim et al., 2001) and two of five cohort (Blair et al., 1986; Hayes et al., 1990; Gardner et al., 1993; Marsh et al., 1994; Andjelkovich et al., 1995) epidemiological studies. Three meta-analyses (Blair et al., 1990; Partanen, 1993; Collins et al., 1997) of the later data suggest a small to moderate increase in relative risk (1.2–2.1).

Other epidemiologic studies have not found an association of formaldehyde exposure with nasopharyngeal cancer (Walrath and Fraumeri, 1983; Coggon et al., 2003, 2014; Pinkerton et al., 2004; Meyers et al., 2013). Included in the latter group are two large industrial cohort studies of 14,014 chemical workers in the United Kingdom and 11,039 workers in the garment industry in the United States. However, the expected number of deaths in these studies was small (2.0 and 0.96, respectively). Other negative studies have assessed the Finnish Cancer Registry (Siew et al., 2012) and workers in an Italian plastic factory (Pira et al., 2014).

Of the positive studies, one showed an increase in squamous and unspecified epithelial cell carcinomas with increasing formaldehyde exposure, but not with undifferentiated or non-keratinizing carcinomas of the nasopharynx (Vaughan et al., 2000). This relationship was further reconsidered by Collins et al. (1997), who proposed that underreporting (when no nasopharyngeal cancer is observed in a number of studies of small sample size) influenced the outcome. They found that when studies of small sample size were added to the meta-analysis, the metarelative risk for cohort studies decreased to 1.0 (with 95% confidence interval of 0.5–1.8). It is also important to consider the small number of observed cases (less than 10 nasopharyngeal cancer in total) used in these analyses. Nonetheless, the reanalysis by Hauptmann et al. (2004) also supported associations between nasopharyngeal cancer and formaldehyde exposure (including adjustment for potential confounding substances), whereas Marsh et al. (2007) challenged these conclusions.

The NCI cohort was followed up by Beane Freeman et al. (2013). A total of 25,619 individuals contributed 998,239 person-years to this follow-up of the cohort. Of these, 22,493 (88%) were men and 23,758 (93%) of European descend. The median duration of

follow-up was 42 years and median employment was 2.6 years. Through December 31, 2004, there were 13,951 deaths. Although the study included plants where formaldehyde was used or produced, not all workers were exposed. Overall, 10.5% of the workers in the cohort were never exposed. The cohort had 3703 cancer deaths with 3146 among people exposed to formaldehyde and 557 among unexposed people. Compared with the U.S. population, a small but statistically significant increased risk of death from all causes existed among those exposed to formaldehyde (SMR = 1.03; 95% CI: 1.01, 1.05) and a significant deficit among the unexposed (SMR = 0.90; 95% CI: 0.87, 0.94). The previous noted excesses for nasopharyngeal cancer persisted for peak, average intensity and cumulative exposure with relative risks in the highest exposure categories equaling 7.66 (95% CI: 0.94, 62.34), 11.54 (95% CI: 1.38, 96.81), and 2.94 (95% CI: 0.65, 13.28), respectively. For all cancer, solid tumors and lung cancer, SMRs among exposed workers were elevated, but internal analyses revealed no associations with formaldehyde exposure. Again, the NCI study had been challenged by Tarone and McLaughlin (2005), but this challenge subsequently rebutted by Beane Freeman et al. (2014).

Cytology of the nasal mucosa has also been examined in formaldehyde-exposed workers (Berke, 1987; Edling et al., 1988; Holmström et al., 1989b; Boysen et al., 1990). Abnormalities observed include loss of cilia, goblet cell hyperplasia, squamous metaplasia, and mild dysplasia, but, as with cancer mortality, these changes do not exhibit dose–response relationships and are confounded by co-exposure to particulate matter including wood dust. Increases in squamous metaplasia in individuals living in homes with urea-formaldehyde insulation have also been inconsistent with level of exposure (Broder et al., 1988, 1991). In contrast, Ballarin et al. (1992) found significant levels of epithelial abnormalities in 15 subjects exposed to urea-formaldehyde glue in a plywood factory. Exposed workers had higher frequencies of micronuclei and dysplasia of nasal epithelial cells and nasal inflammation (leukocyte infiltrates) when compared with age- and sex-matched control subjects.

Formaldehyde exposure may lead to these effects through disruption of several cellular processes. DNA–protein cross-links can arrest DNA replication and lead to the induction of other genotoxic effects such as sister chromatid exchanges in proliferating cells (Merk and Speit, 1998). Incomplete repair of DNA–protein cross-links can lead to the formation of mutations (Barker et al., 2005), which can be detected as chromosomal aberrations and micronuclei, rather than gene mutations at specific loci. These errors, in turn, can lead to larger deletions and recombinations and therefore increase micronuclei frequency (Speit and Merk, 2002). As noted above, several studies suggest that increased micronuclei frequency occurs in the nasal or buccal mucosa cells of formaldehyde-exposed workers (Ballarin et al., 1992; Burgaz et al., 2001; Ye et al., 2005). However, the effects are not consistent across studies (e.g., the threshold dose may vary greatly). Speit and Schmid (2006) have suggested that this may be due to the lack of standardization of micronucleus tests, the high assay variability, and the fact that effects can be focal and that sampling may be incomplete.

None to slight increases in risks for other respiratory tract cancers (e.g., buccal cavity, oropharynx, pharynx, and lung) (Walrath and Fraumeri, 1983; Acheson et al., 1984b; Liebling et al., 1984; Sterling and Arundel, 1985; Bertazzi et al., 1986; Stayner et al., 1986; Vaughan et al., 1986b; Blair et al., 1987) and nonrespiratory sites (brain, prostate, and skin) (Harrington and Shannon, 1975; Walrath and Fraumeri, 1984; Blair et al., 1986; Hagmar et al., 1986; Stayner et al., 1988; Hall et al., 1991; Hauptmann et al., 2004; Heck and Casanova, 2004; Pinkerton et al., 2004). However, most of the increases in cancer risk at

these sites lack correlation with duration or intensity of exposure. This has been explained, in part, by a strong self-selection effect because of irritant responses (healthy worker effect in occupational populations), difficulties in retrospective assessments of exposure, and potential confounding factors (e.g., wood dust exposure) (Acheson et al., 1967; Olsen et al., 1984; Holmström and Wilhelmsson, 1988; Blair and Stewart, 1990; IARC, 1995a; Collins et al., 1997; Hauptmann et al., 2003; Pinkerton et al., 2004). For example, an increased risk of nonrespiratory cancer mortality has often been associated with formaldehyde exposure in embalmers, and these individuals have exposures to complex mixtures of materials.

Formaldehyde produces a complex array of genotoxic effects in *in vitro* systems and consistently has been found to induce squamous carcinoma in the nasal cavity of laboratory animals (at concentrations exceeding 5 ppm) with correlated histopathological changes (at concentrations exceeding 0.5 ppm). Available epidemiological evidence is conflicting but has been deemed to be sufficient to support the hypothesis that formaldehyde leads to an increased risk of cancer in exposed humans. Controversy exists as to whether formaldehyde is carcinogenic to humans (group 1), but there is little controversy that formaldehyde is probably carcinogenic to humans (group 2A). This is because the evidence of an increased risk of nasopharyngeal cancer in humans from epidemiological studies is supportive but controversial and evidence for nasal cancer is limited.

16.3.1.4 Nonrespiratory Tract Cancer: Epidemiological Studies The possible association occupational formaldehyde exposure with leukemia has been noted in some (Stern et al., 1987; Pinkerton et al., 2004; Beane Freeman et al., 2009; Hauptmann et al., 2009) but not all epidemiological studies. This association was subsequently supported by a meta-analysis of existing studies by Schwilk et al. (2010), but not by Bachand et al. (2010). These studies have been controversial and lead to several extensive critical reviews (Cole and Axten, 2004; Collins and Lineker, 2004; Heck and Casanova, 2004; Marsh and Youk, 2004; Golden et al., 2006; Bosetti et al., 2008; Duhayon et al., 2008; Pyatt et al., 2008; Bachand et al., 2010; Lu et al., 2010; Speit et al., 2010; Goldstein, 2011, 2018; Rhomberg et al., 2011; Checkoway et al., 2012; Gentry et al., 2013; Swenberg et al., 2013; Mundt et al., 2017, 2018a, 2018b; Rothman et al., 2018).

The possible link between formaldehyde and hematotoxicity is supported by multiple reports of increased frequency of sister chromatid exchange in peripheral lymphocytes and other genetic alterations (e.g., increased micronuclei) in leukocytes noted in formaldehyde-exposed workers/students (Bauchinger and Schmid, 1985; Yager et al., 1986; Suruda et al., 1993; Kitaeva et al., 1996; Shaham et al., 1997; He et al., 1998; Ying et al., 1999; Shaham et al., 2002; Ye et al., 2005; Iarmarcovai et al., 2007; Costa et al., 2008, 2013, 2015; Jakab et al., 2010; Ladeira et al., 2011) and the general genotoxicity noted with exposure (Titenko-Holland et al., 1996; Burgaz et al., 2001; Shaham et al., 2009). Some studies have failed to find these effects (Fleig et al., 1982; Thomson et al., 1984; Ying et al., 1997; Pala et al., 2008). Others have suggested that alterations in circulatory leukocytes may arise from endogenous formaldehyde formation (Lu et al., 2010, 2011; Moeller et al., 2011).

Zhang et al. (2010) have reported a pancytopenia in Chinese formaldehyde factory workers. Note that the decreased cell count was still within normal limits, but if repeated, the finding of pancytopenia is a hallmark of leukemia (Goldstein, 2018). Proliferative capacity of myeloid progenitor cells isolated from peripheral blood of formaldehyde workers was also diminished (Zhang et al., 2010; Bassig et al., 2016), which suggests

toxicity to these cells in the bone marrow. Leukocyte count decreased in nurses occupationally exposed to ≤0.3 ppm formaldehyde compared with non-expose nurses (Kuo et al., 1997). In addition, levels of aneuploidies increased (Zhang et al., 2010; Lan et al., 2015; Bassig et al., 2016). In another group of formaldehyde-exposed workers, Costa et al. (2015) also noted increased aneuploidies. Conversely, Jakab et al. (2010) noted a decreased aneuploidies among workers exposed to formaldehyde in a pathology department. In this case, the observed lower frequency of aneuploid cells may be attributed to an increase in apoptotic cells found in these workers. Workers exposed to formaldehyde had decreased circulating levels of CXCL11 and CCL7 (aka thymus and activation regulated chemokine) consistent with immunosuppression (Seow et al., 2015).

In general, it is uncertain and perhaps very unlikely that inhaled formaldehyde could reach distal cells in the bone marrow (Swenberg et al., 2013). A lack of evidence that inhaled formaldehyde reaches the bone marrow has been one of the major arguments proposed by some against the classification of formaldehyde as a leukemogen. Sensitive methods for detecting specific DNA adducts and distinguishing those arising from endogenous formaldehyde sources did not detect exogenous adducts at distant sites, including the bone marrow and blood in rats and monkeys (Lu et al., 2010, 2011; Moeller et al., 2011; Swenberg et al., 2013).

However, hemolymphoreticular neoplasias can be induced by 50–1500 mg/L formaldehyde in drinking water in male rats or ≥10 mg/L in females rats (Soffritti et al., 2002). Moreover, this increase shows a dose–response relationship. Therefore, formaldehyde has the capacity to induce neoplasia. In addition, other studies reported that formaldehyde induces DNA–protein cross-links and oxidative stress in the bone marrow of mice (Cheng et al., 2010; Ye et al., 2013). In ICR mice exposed very high levels of formaldehyde (16–65 ppm, for 2 h/day × 15 days), leukocyte and platelet counts were decreased at ≥33 ppm (Yu et al., 2014a). In the bone marrow, oxidative stress increased (i.e., superoxide dismutase and MDA increased) at ≥16 ppm. Following ≥33 ppm exposures, bone marrow pro-apoptotic biomarkers [i.e., BCL2 associated X, apoptosis regulator (Bax), and cytochrome c] increased and anti-apoptotic [i.e., B-cell leukemia/lymphoma 2 (Bcl2)] decreased (Yu et al., 2015a) and glutathione peroxidase decreased (Yu et al., 2014b). Following 65 ppm, a decrease occurred in nucleated cells, in mitochondrial membrane potential, and in colony formation at *in vitro* cultivation of the bone marrow. Note that the formaldehyde concentrations in this study were extremely high compared to human exposures.

In another study, lower concentration of formaldehyde (0.4–2.4 ppm 5 days/week × 2 week) produced reduced blood cell counts in several lineages in BALB/c mice (Zhang et al., 2013) and was similar to hematotoxicity findings in formaldehyde-exposed workers (Zhang et al., 2010). Biomarkers of oxidative stress (ROS, glutathione depletion, and cytochrome P4501A1 and GSTT1 expression), inflammation [NF-κB, tumor necrosis factor-alpha (TNF), IL-1beta], and apoptosis [activity of cysteine-aspartic acid protease 3 (caspase 3)] in the bone marrow were increased following formaldehyde exposure. Subsequently, Wei et al. (2017) reported that formaldehyde exposure decreased nucleated bone marrow cells and bone marrow-derived colony-forming unit-granulocyte-macrophage (CFU-GM) cells and burst-forming unit-erythroid (BFU-E) cell and increased bone marrow ROS, apoptosis in nucleated spleen, and CFU-GM cells. It also decreased hematopoietic growth factor and receptors. The latter was proposed as a possible mechanism of formaldehyde-induced bone marrow cytotoxicity.

Because exogenous formaldehyde DNA adduct cannot be detected in the bone marrow, it remains uncertain as to how formaldehyde targets the bone marrow. As noted above, formaldehyde exposure was associated with aneuploidy including chromosome 7 monosomy in cultured circulating myeloid progenitor cells *in vivo* (Zhang et al., 2010). Notably, monosomy 7 is a frequent cytogenetic change in myeloid leukemia and myelodysplastic syndromes. However, the importance of a specific chromosomal abnormality has been controversial (Goldstein, 2018; Mundt et al., 2018a; Rothman et al., 2018). Aneuploidy was not consistently induced in myeloid progenitor cells exposed to formaldehyde *in vitro* (Kuehner et al., 2012; Ji et al., 2014). In addition, monosomy and trisomy of chromosome 7 in expanded human erythroid progenitors was negligible following formaldehyde treatment *in vitro* (Ji et al., 2014).

The negative *in vitro* data with myeloid and erythroid progenitor cells and the positive *in vivo* data in lymphocytes and myeloid progenitor cells of exposed workers again suggest that formaldehyde may produce mediators at the site of injury that are transmitted to the bone marrow. Recent finding suggest that deregulated canonical NF-κB signaling in hematopoietic stem cells causes a complete depletion of the hematopoietic stem cell pool, pancytopenia, bone marrow failure, and premature death (Nakagawa and Rathinam, 2018). Thus, chronic respiratory injury that induced circulating mediators would have to alter NF-κB signaling in bone marrow hematopoietic stem cells. Currently, this is only a hypothetical possibility; however, it does support biological feasibility. A possible model of action is presented in Fig. 16.2.

In mice, formaldehyde depresses respiratory ventilation and therefore could induce hypoxia. Nielsen et al. (2017) have proposed that the hypoxia, in turn, induces systemic oxidative stress. He noted that the bone marrow is sensitive to oxygen tension and could exhibit adverse effects that are somewhat consistent with the studies in mice mentioned above. The respiratory depression, which occurs in mice more than in rats, could also explain the lower frequency of nasal cancer in mice than in rats.

Another hypothesized mechanism of bone marrow toxicity is that precursor myelopoietic cells in the nasal passages could be alter and travel to bone marrow. This assumption supposes chloroma formation, which occurs as acute myloid leukemia develops. Chloromas can occur in several tissues but rarely in the nose. As noted by Goldstein (2011), chloromas are very rare or absent, even in the case of a known leukemogen, benzene. Alternatively, Lan et al. (2015) hypothesized that formaldehyde or the released mediators could damage cells traveling in the blood as they pass through the nose, lung, and other portions of the respiratory tract. This would explain the generation of aneuploidy in human blood cells *in vivo* but its relative absence *in vitro*. It would also explain why endogenously generated formaldehyde in the liver and elsewhere does not appear to be genotoxic as it is rapidly metabolized to formate and does not produce inflammation.

Noteworthy to this discussion is the recent discovery of a hematopoietic progenitor cells residing in the lung interstitium (i.e., the extravascular spaces of the lung) (Lefrançais et al., 2017). Furthermore, during relative stem cell deficiency in the bone marrow and thrombocytopenia, these progenitors can migrate out of the lung, repopulate the bone marrow, completely reconstitute platelet counts, and contribute to multiple hematopoietic lineages (Lefrançais et al., 2017). Thus, damage to this pool could lead to diminish hematopoietic capacity (Borges et al., 2017). The lungs' role in formaldehyde-induced hematotoxicity requires additional attention inasmuch as human, especially in occupational settings, is likely to have increased formaldehyde deposition in the lung through oral breathing (as compared to rodents that are obligatory nasal breathers).

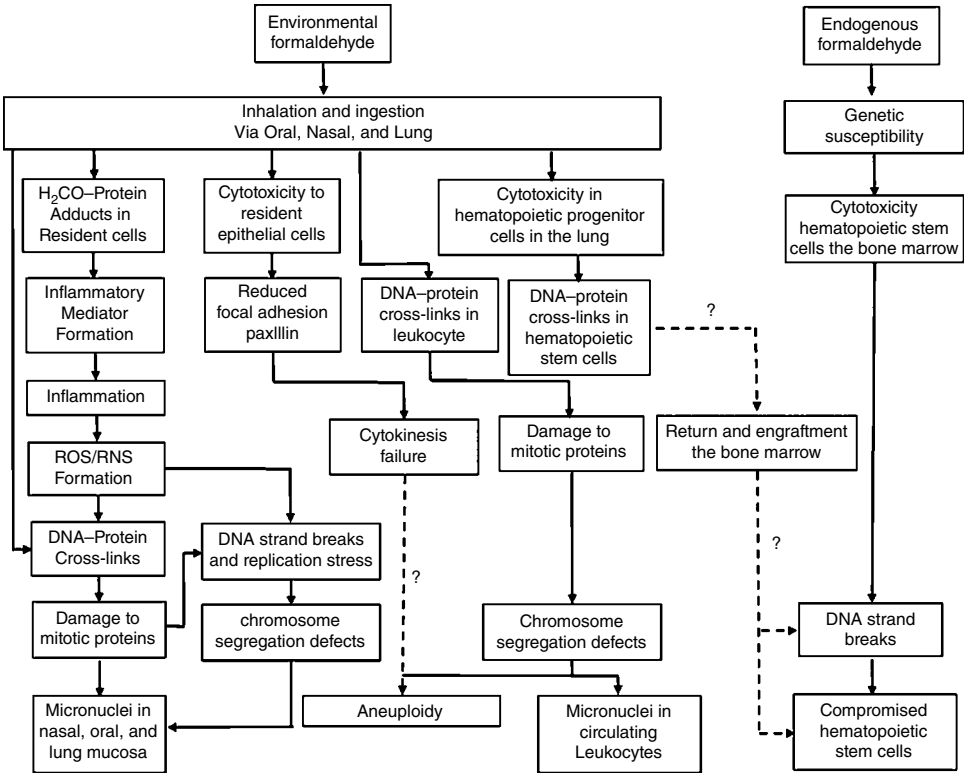

FIGURE 16.2 Potential mechanism for formaldehyde-induced damage to target tissues and circulating leukocytes. Environmental (exogenous) formaldehyde is inhaled and forms covalent bonds within resident cells at the site of contact, which in turn leads to a cascade of effects induced by inflammation and reactive oxygen species (ROS) and reactive nitrogen species (RNS). These intermediates can damage DNA and mitotic proteins, leading to aberrant chromosome segregation and micronuclei formation in the nasal, oral and lung mucosa and in circulating leukocytes. Formaldehyde cytotoxicity to resident cells decreases focal adhesion paxillin that is consistent with cytokinesis failure. This a potential mechanism for aneuploidy. Endogenous formaldehyde formation can lead to compromised hematopoietic stem cell function, especially in individuals with genetic variants that diminish aldehyde metabolism and DNA cross-link repair. Interestingly the lung has recently been identified as a major source of hematopoietic progenitor cells, and these cells are capable of returning to and engrafting into the bone marrow. Thus, a potential mechanism of compromised hematopoietic stem cells could be a combination of endogenous and exogenous formaldehyde exposure. Solid lines are relationship support by extensive investigations; dashed lines are potential mechanism requiring further investigation.

16.3.1.5 Other Responses to Multiple Exposures: Occupational Studies Although much attention and controversy has been placed on the carcinogenic potential of formaldehyde, the irritant capacity of this compound is irrefutable. Repeated formaldehyde exposure can result in eye and upper respiratory tract irritation, declines in pulmonary function, and can initiate skin sensitization. Except for skin sensitization, these effects are often readily reversible with cessation of exposure and depend more on the exposure concentration (threshold dose) than on the exposure duration (cumulative dose). Such effects may be viewed as repeated immediate responses to acute exposure rather than

persistent, irreversible dysfunction produced by cumulative degradation of defense mechanisms and initiation of compensatory processes.

Eye, nose, and throat irritation are the most common complaint of individuals with occupational or residential formaldehyde exposures. In occupational studies, Alexandersson and Hedenstierna (1988) found that exposures to 300–500 ppb during a work shift led to symptoms. Likewise, Horvath et al. (1988) found that across-shift responses of sore throat and burning nose increased at concentrations ≤400 ppb. Control responses were 3–4%, and after exposure 8–15% responded. These responses followed a dose–response relationship with 22–36% and 33–55%, responding at <1000 and <3000 ppb, respectively. Although these exposures occurred in a population with repetitive exposure, the threshold dose or frequency of reported symptoms does not differ remarkably from that observed after a single exposure (Anderson and Molhave, 1983; Bender et al., 1983).

To evaluate the possible mechanisms for formaldehyde-induced discomfort of the upper airways, Wilhelmsson and Holmström (1992) determine whether chronic exposure to formaldehyde causes annoying symptoms by direct irritation and whether it affects all exposed people (through hyperreactivity in atopic persons, through formaldehyde-induced hyperreactivity also in nonatopic persons, or through an immune-mediated, immediate type 1 reaction to formaldehyde itself). Of 66 workers occupationally exposed to formaldehyde (mean = 0.21 ppm; range = 0.04–0.48 ppm), 53% experienced nasal discomfort through hyperreactivity. This was compared with 3% noted in a control group of workers not exposed to formaldehyde. Atopics were not significantly overrepresented among the persons with occupational nasal symptoms in the formaldehyde group. Two workers with isolated occupational nasal discomfort, and sensitized by long-term inhalation, had a positive radioallergosorbent test (RAST) for formaldehyde. This is consistent with an immune-mediated reaction and was reported previously in other formaldehyde workers (Imhof and Wüthrich, 1988). Eye and skin discomfort was also more common in the formaldehyde group studied by Wilhelmsson and Holmström (1992). In the formaldehyde group, 44% reported general lower airway discomfort indicated by wheezing, intermediate cough, or symptoms of chronic bronchitis. This was significantly more than the 14% noted in the control group.

Residential exposures also demonstrate similar results, with one difference: effects, particularly eye irritation, are noted with greater frequency at low concentrations. For example, whereas Horvath et al. (1988) found about 8–15% positive respondents after occupational exposure, Hanrahan et al. (1984) and Ritchie and Lehnen (1987) found 22–32% positive respondents for eye irritation after residential exposure to nearly equivalent concentrations (300 ppb). Ritchie and Lehnen (1987) also found nearly 90% of persons exposed to ≥300 ppb reporting eye irritation. One reason for this difference may be that residential exposures are of longer duration (Fig. 16.1). As mentioned previously, Anderson and Molhave (1983) also found that reports of eye irritation increase, whereas nose and throat irritation complaints decrease during extended exposure. Because these responses are subjective, this difference may also be explained by a greater willingness to tolerate symptoms at work than at home. Another reason for this difference is the "healthy worker effect" in that irritant sensitive workers might seek employment elsewhere.

Pulmonary function after repeated formaldehyde exposure in occupational settings has also been examined. One type of study involves comparison of forced expiratory volumes before and after an 8-h work shift. Exposures in these studies ranged from 100 to 3000 ppb (typically averaging 500 ppb) and yielded slight declines that are associated with single

exposures (Schoenberg and Mitchell, 1975; Gamble et al., 1976; Alexandersson et al., 1982; Kilburn et al., 1985; Horvath et al., 1988; Uba et al., 1989; Khamgaonkar and Fulare, 1991).

Baseline values obtained Monday morning (after no exposures for 2 days) in each of these studies, however, typically were not different from control values, indicating that the cross-shift declines are reversible and are unlikely to lead to a persistent pulmonary dysfunction. In one study by Alexandersson and Hedenstierna (1988), however, Monday morning decrements in $FEV_{1.0}$ and forced vital capacity (FVC) were noted, suggestive of a persistent change. However, these changes correlate neither with peak exposure nor with duration of employment and thus only offer limited support for the contention that persistent changes are a consequence of repetitive exposure. In a follow-up study Alexandersson and Hedenstierna reported that formaldehyde exposure causes transient lung function impairment over a work shift, with a cumulative effect over the years. The impairment, however, can be reversed with 4 weeks of no exposure.

In each of the above cross-shift studies, small but measurable decreases in $FEV_{1.0}$ were observed. In most cases, these changes resulted from exposure to threshold concentrations of about 500 ppb. This threshold is in good agreement with the 300 ppb threshold for acute respiratory effects reported in guinea pigs by Amdur (1960). Individuals may vary in dose threshold, however. For example, Kim et al. (2001) presented a case report of an individual with occupational asthma. Working in an environment with a mean formaldehyde level of 60 ppb (with occasional peaks of 120–130 ppb), this worker had across-shift decreases in $FEV_{1.0}$ that were reversed by inhalation of a bronchodilatory (beta-2 adrenergic) agent. Thus, individual susceptibility may vary greatly, and occupational environments with irritant atmospheres are likely to be selective for healthy workers who tolerate exposures upon acclimiation.

This type of complex effect may not always readily apparent in exposed populations. In numerous studies of healthy subjects, or persons with asthma (exposed in inhalation chambers), effects observed on respiratory function are difficult to demonstrate, even at concentrations of 3000 ppb. Possible explanations include the following: (a) occupational exposures involve a complex atmosphere that may or may not include particulate matter or other contaminants (Amdur, 1960; Kilburn and McKenzie, 1978); (b) occupational exposure can be to transient peak levels that produce irritation but are undetected by time-weighted averaging (Ryan et al., 2003); (c) the extended occupational exposures (8 h) produce greater effects than 10–120-min tests even at equivalent doses (concentration time) (Leikauf and Doupnik, 1989; Swiecichowski et al., 1993); or (d) repeated exposure lowers the threshold of responsiveness without producing chronic effects.

16.3.1.6 Other Responses to Multiple Exposures: Childhood Asthma In a study of schoolchildren, 1.6 ppm formaldehyde was associated with ocular throat symptoms and fatigue (Norbäck et al., 2017). The exact mechanism of eye irritation is not fully understood, but studies in rabbits suggest that topical formaldehyde can lead to release of secretoneurin, a 33-amino-acid chromogranin neuropeptide (Kralinger et al., 2003). Together these studies imply that irritant-related responses will occur in humans at concentrations of ≥300 ppb.

Because symptomatic responses (e.g., eye irritation) occur at a greater frequency following residential exposures to 50–300 ppb formaldehyde, when compared with occupational exposures to equivalent concentrations, residential exposure to 50–300 ppb could also produce pulmonary effects. Inasmuch as the current occupational TLV

(time-weighted average) has been set at 100 ppb (ACGIH, 2017), a prudent indoor air level might be between ≤100 ppb, a value also recommended by Ritchie and Lehnen (1987) and Arts et al. (2008). Indeed the World Health Organization established an indoor air quality guideline from short- and long-term exposure to formaldehyde of 80 ppb (WHO, 2010). This level was supported by a review of the literature presented more recently by Nielsen et al. (2017). This value may be difficult to achieve, however, unless indoor cigarette smoking is curtailed (see Section 16.1.1.1).

Epidemiological evidence supportive of a conservative environmental standard has been obtained by Krzyzanowski et al. (1990), who found an increased pulmonary morbidity (prevalence of asthma and bronchitis) among children living in homes with 60–120 ppb formaldehyde when compared with children living in homes with ≤40 ppb. Decrements in peak expiratory flow rates were also correlated with formaldehyde exposure. Similarly, a study of health outcomes for children that that seasonal differences in formaldehyde levels found that children exposed to formaldehyde levels of >50 ppb are at increased risk of having asthma (Rumchev et al., 2002). Previously, this effect had been reported in an abstract by Czap et al. (1993). These investigators found that the prevalence of physician-diagnosed asthma and asthma-related symptoms were about double for individuals living in home with formaldehyde levels averaging about 50 ppb as compared to controls that averaged about 5 ppb.

Similarly in a cohort of 2940 infants in Paris, Roda et al. (2011) reported that for every interquartile formaldehyde increase of 10 ppb, there is an estimated 32% [95% confidence interval (95% CI): 11–55] and 41% (95% CI: 14–74) increase in lower respiratory tract infections and wheezy lower respiratory tract infections. In an additional study of this cohort, these investigators found an association of residential formaldehyde exposure to nocturnal dry cough among infants without parental history of allergy, but not among infants with a parental history of allergy (Roda et al., 2013).

In a study of schoolroom freshly paneled with particleboard, formaldehyde levels measured are 75, 69, and 48 ppb (Wantke et al., 1996). Three children attending the classroom with 75 ppb formaldehyde had elevated RAST >2.0. Elevated RAST classes of ≥1.3 were found in another 21 pupils. Thirty-eight children as well as 19 control children showed RAST classes in the normal range of ≤1.2. The children with formaldehyde exposure reported headache, nose bleeding, rhinitis, fatigue, cough, dry nasal mucosa, and burning eyes that correlated with the formaldehyde concentrations in the classrooms. However, elevated IgE levels to formaldehyde did not correlate with symptoms. In another study of children (9–11 years) exposed to formaldehyde, Erdei et al. (2003) reported a significant increase in bacteria-specific IgGs associated with formaldehyde exposure. However, Doi et al. (2003) found only 2 of 122 asthmatic children have formaldehyde-specific IgE and concluded that formaldehyde is not a risk factor for childhood asthma. In addition, Symington et al. (1991) compared the prevalence of respiratory symptoms exhibited by children living within 1 mile of a formaldehyde-emitting foundry with that of children living in other areas and reported no differences.

Nonetheless, in a meta-analysis of several studies (Krzyzanowski et al., 1990; Smedje et al., 1997; Garrett et al., 1999; Smedje and Norbäck, 2001; Rumchev et al., 2002; Doi et al., 2003; Pati and Parida, 2005; Mi et al., 2006; Zhao et al., 2008) including 6347 participants of which 635 had self-reported of diagnosed asthma, McGwin et al. (2010) reported a positive association between formaldehyde exposure and childhood asthma. For an increase of 10 μg/m^3 (8.1 ppb) formaldehyde, fixed and random effects models revealed odds ratios of 1.03 (95% CI: 1.02–1.04) and 1.17 (95% CI: 1.01–1.36),

respectively. Similarly, Yao et al. (2015) performed a meta-analysis of six studies (Norbäck et al., 1995; Yan and Yibulayin, 2008; Hulin et al., 2010; Lou et al., 2010; Wu et al., 2010; Jia et al., 2012) including 356 cases and 199 controls of childhood asthma. Formaldehyde exposure was associated with increased risk of childhood asthma, with a random effects model yielding a pooled weighted mean difference of 0.021 (95% CI: 0.009–0.033). In addition, Delfino et al. (2003) evaluated formaldehyde exposure of Hispanic children and reported an adjusted odds ratios for bothersome or more severe asthma symptoms from interquartile range increases in 3.16 ppb formaldehyde, 1.37 (95% CI: 1.04–1.80).

Airway inflammation/oxidative stress can be measured by exhaled nitrogen oxide (eNO). To investigate possible inflammatory effects of formaldehyde at levels typically found in the home, Franklin et al. (2000) measured eNO in 224 healthy children and monitored residential formaldehyde concentrations. Formaldehyde levels measured in homes did not effect changes in lung function. However, eNO levels were significantly elevated in children living in homes with average formaldehyde levels ≥50 ppb. Exhaled NO levels (geometric mean) were 15.5 ppb (95% CI: 10.5–22.9 ppb) for children from homes with formaldehyde concentrations ≥50 ppb compared with 8.7 ppb (7.9–9.6) for children from homes with formaldehyde concentrations <50 ppb ($p < 0.05$). These results suggest that exposure to formaldehyde in homes may invoke a subclinical inflammatory response in the airways of healthy children.

Other investigators, including Norbäck et al. (1995) and Wieslander et al. (1997), also have reported formaldehyde exposure (mixed with other volatile organic compounds) is associated with an increase in prevalence of asthma, asthma-related symptoms, and blood eosinophil counts. In addition, sensitized persons with asthma develop bronchoconstriction to lower threshold dose of dust mite following short, low-dose 30 ppb formaldehyde exposure (Casset et al., 2006a). In a review of the literature, Casset et al. (2006b) also noted that the risk for development of asthma is increased (approximately 1.4-fold) in homes that exceed levels of 50 ppb formaldehyde. Thus, these studies suggest that persistent respiratory effects can result from low-level indoor formaldehyde exposure and environmental exposures produce effects at concentration below those that produce observable effects in short-term clinical studies.

16.3.1.7 Other Responses to Multiple Exposures: Immunological Reactions Along with direct irritation, topical application of formalin (37% formaldehyde in methanol/water) can initiate allergic reactions including contact dermatitis or systemic responses (anaphylactic shock). In the past the frequency of contact dermatitis may have been underreported because the previous test for skin sensitivity may have produce false negatives in as many as half of the formaldehyde-allergic patients (Pontén and Bruze, 2015). The prevalence of contact dermatitis is low (1–2%) but still in common in the general population (Fasth et al., 2018; Veverka et al., 2018). Such reactions may result in workers from formaldehyde usage in industrial processes including histological laboratories, dental procedures, or kidney dialysis (Feinman, 1988; Bardana and Montanaro, 1991; Braun et al., 2003). Skin allergic reactions have also occurred in the general population from formaldehyde release from clothing (Bourne and Seferian, 1959; de Groot et al., 2010a, 2010b) and personal care products (Hauksson et al., 2016a, 2016b).

The mechanisms of these immunologic reactions are still somewhat unclear, but it appears that formaldehyde reactions involve both immediate (antibody–antigen-mediated)

and delayed (cell-mediated) hypersensitivity. In immediate hypersensitivity, formaldehyde acts as an incomplete antigen (hapten) through its covalent binding to constituent proteins in the skin or blood, forming new antigenic determinants (Horsfall, 1934). Antibodies to formaldehyde–hemolytic red blood cell membrane protein or formaldehyde–human serum albumin conjugates have been identified and include immunoglobin (Ig)E, IgG, and IgM subtypes (Sandler et al., 1979; Lynen et al., 1983; Maurice et al., 1986; Wilhelmsson and Holmström, 1987; Thrasher et al., 1987, 1988; Broughton and Thrasher, 1988; Patterson et al., 1989; Hilton et al., 1996; Braun et al., 2003).

Concentrations of formaldehyde causing immediate or delayed skin hypersensitivity (30–55 ppm) in human volunteers are typically lower than those causing irritation (20 ppm) (Feinman, 1988). These changes in acquired immunity may also modulate innate immunity. For example, Górski et al. (1992) reported that neutrophils isolated from persons with formaldehyde contact sensitivity that had been exposed to 0.44 ppm formaldehyde for 2 h had higher responses (activation measured by chemiluminescence) than neutrophils isolated from control subjects. Similarly, Lyapina et al. (2004) found that the neutrophil respiratory burst activity was diminished in workers exposed to formaldehyde, especially among those with chronic mucosal inflammation.

Modulation of cell-mediated immunity, on the other hand, is not as well documented (Pross et al., 1987; Thrasher et al., 1988; Patterson et al., 1989). It also remains unclear whether allergic reaction involving the lung can be initiated solely by inhalation exposure (Hendrick and Lane, 1975, 1977; Hendrick et al., 1982; Lee et al., 1984; Patterson et al., 1989; Bardana and Montanaro, 1991; Liteplo and Meek, 2003), but it does appear that, although rare, documented dual (immediate and delayed) pulmonary reactions can be evoked in persons with previous occupational exposure (Burge et al., 1985; Nordman, 1985; Kim et al., 2001).

Although formaldehyde is a weak sensitizing agent in allergic reactions, it may modulate immunity to other more common allergens. For example, combined exposure to 0.2 or 1.0 ppm formaldehyde (6 h/day × 5 days/week × 4 weeks) inhalation and topical house dust mite (HDM) stimulation increased HDM-induced total plasma IgE and IgG2a production, Th1, Th2, and Th17 cytokine as well as prostaglandin-endoperoxide synthase 2 mRNA expressions in the skin of NC/Nga mice (Kim et al., 2013). Enhancement of sensitization has been demonstrated in mice by Tarkowski and Górski (1995), who found higher IgE OVA antibody titers following exposure to 1.6 ppm formaldehyde for 10 days after presentation of antigen. Similarly, guinea pigs exposed to 130–250 ppb formaldehyde for 5 days before presentation of antigen developed higher IgG OVA antibody levels (ELISA units) when compared with guinea pigs exposed to filtered air (Riedel et al., 1996). Likewise, repeated transnasal administration of formaldehyde also enhanced allergic bronchoconstriction and potentiated IgG production in guinea pigs (Kita et al., 2003). These changes in the Riedel et al. (1996) study were associated with greater epithelial pathology and eosinophilic infiltrates. Repeated dermal formaldehyde exposure of mice that had an IL4 promoter-reporter transgene indicated that challenge can activate this system (Kwak et al., 2014). Ear thickness and serum IgE concentration increased following exposure.

Together, these investigations suggest that immunologically based bronchial hypersensitivity can develop following formaldehyde exposure alone but that this response is rare (based on human studies of clear responses among a few individuals). More frequently, formaldehyde may enhance sensitization to other more common respiratory antigens (based mainly on animal evidence).

16.3.2 Repeated Exposure to Other Aldehydes

16.3.2.1 Mutagenicity and Carcinogenicity of Other Aldehydes Like formaldehyde, several other aldehydes have been found to be genotoxic in microbial, insect, and mammalian systems (Graftstrom, 1990; Feron et al., 1991; Graftstrom et al., 1994; WHO, 1995; Feng et al., 2006). Of the more common environmental aldehydes, acetaldehyde, furfural, and glutaraldehyde can be mutagenic in *in vitro* assay systems (Vegheli and Osztovics, 1978; Obe and Beck, 1979; Hemminki et al., 1980; Marnett and Tuttle, 1980; Neudecker et al., 1981; Bird et al., 1982; Marnett et al., 1985; Cooper et al., 1987; Galloway et al., 1987; Reynolds et al., 1987; Hadi et al., 1989; He and Lambert, 1990; Graftstrom et al., 1994; Vaca et al., 1998; Feng et al., 2006; Lorenti Garcia et al., 2009).

IARC classifies acetaldehyde as a Group I human carcinogen mainly because of the cancer risk associated with ethanol consumption (IARC, 1999; Secretan et al., 2009). In drinking water, acetaldehyde was administered to 50 male and 50 female Sprague–Dawley rats beginning at 6 weeks of age at concentrations of 2500, 1500, 500, 250, 50, or 0 mg/L. Formaldehyde was administered for 104 weeks in drinking water supplied ad libitum at concentrations of 1500, 1000, 500, 100, 50, 10, or 0 mg/L to groups of 50 male and 50 female Sprague–Dawley rats beginning at 7 weeks of age. Control animals (100 males and 100 females) received tap water only. Animals were kept under observation until spontaneous death. Formaldehyde and acetaldehyde were found to produce an increase in total malignant tumors in the treated groups and showed specific carcinogenic effects on various organs and tissues. Inhaled acetaldehyde is a suspected human carcinogen because acetaldehyde can induce nasal adenocarcinoma and squamous cell carcinoma in rats (Woutersen et al., 1986a; Woutersen and Feron, 1987) and laryngeal carcinoma in hamsters (Feron et al., 1982). The sites of tumors are somewhat different with acetaldehyde than formaldehyde inasmuch as acetaldehyde (750 ppm) produced adenocarcinomas of the olfactory epithelium in rats (Woutersen et al., 1986a, 1986b). Histopathological correlates at lower acetaldehyde concentrations (400–1000 ppm) include hyper- and metaplasia, principally in the nasal olfactory epithelium rather than the anterior respiratory epithelium (Appelman et al., 1982; Woutersen et al., 1984, 1986a, 1986b; Cassee et al., 1996b; Morris, 1997). In addition acetaldehyde shares an ability, with formaldehyde, to enhance the tumorigenicity of benzo(a)pyrene in hamsters (Feron, 1979).

Reaction of crotonaldehyde or two molecules of acetaldehyde with DNA generates 3-(2′-deoxyribos-1′-yl)-5,6,7,8-tetrahydro-8-hydroxy-6-methylpyrimido[1,2-a]purine-0(3H). When transfected into cells this oglionucleotide produces miscoding events including G to T transversions, G to A transitions, and G to C transversions (Stein et al., 2006). Interestingly, [$^{13}C_4$]-1,N^2-propano-2′-deoxyguanosine, a DNA adduct with the addition of two molecules of isotopically labeled acetaldehyde, was detected in the lung and brain of rats breathing 10 ppb of [$^{13}C_2$]-acetaldehyde (24 h/day × 50 days) suggesting that acetaldehyde inhalation can lead to DNA damage in at least one distant organ (Sanchez et al., 2018).

Studies comparing formaldehyde with glutaraldehyde also may provide insights in to the comparative carcinogenic potential of aldehydes. In a direct comparison with formaldehyde, glutaraldehyde-induced genotoxicity occurred at concentrations of 2–10 μM depending on the endpoint measured, while formaldehyde-induced genotoxicity occurred at 25–100 μM (Speit et al., 2008). Formaldehyde is cytotoxic and produces rat nasal epithelial cell tumors after 12 months, whereas glutaraldehyde, while also cytotoxic, is not carcinogenic to nasal epithelium, even after 24 months. Both aldehydes induce similar

acute and histopathology that is characterized by inflammation, hyperplasia, and squamous metaplasia. Hester et al. (2005) found differences among gene expression patterns in rat nasal tissue after instillation of glutaraldehyde and formaldehyde and suggested that glutaraldehyde has a greater toxicity through diminishing DNA repair and increasing mitochondrial damage and apoptosis.

Inhalation of glutaraldehyde induces cell proliferation in nasal tissue in rats and mice, but DNA damage is not detected at this site in rats (Zeiger et al., 2005). Male rats had increased cell proliferation (^{3}H-thymidine-labeled cells) in the squamous epithelium at ≥250 ppb. Lesions induced by glutaraldehyde were more anterior in the nose than those reported for formaldehyde, they differed in character, and no evidence of "pre-neoplastic" lesions or karyomegaly, reported for formaldehyde, was observed with glutaraldehyde. Chromosome aberrations in bone marrow cells of rats or mice have been reported in one of eight studies, micronuclei were not induced in bone marrow cells of mice, and dominant lethal mutations were not induced in mice (Zeiger et al., 2005). Glutaraldehyde does not induce cell transformation *in vitro*. Bone marrow hyperplasia and low, but statistically significant, levels of leukemia were seen in one chronic drinking water study in rats, but not in a chronic inhalation study in rats or two chronic inhalation studies in mice (Zeiger et al., 2005).

Lastly, another aldehyde, malondialdehyde, has also been found to be mutagenic in *in vitro* assays (Mukai and Goldstein, 1976; Shamberger et al., 1979; Yau, 1979; Bird et al., 1982; Basu and Marnett, 1983; Marnett et al., 1985) and carcinogenic to mice (Shamberger et al., 1974). Like acrolein, this naturally occurring compound, a 3-carbon dialdehyde (O═CH–CH_2CH═O), is produced from autoxidation (peroxidation) of unsaturated fatty acids (Bernheim et al., 1948; Esterbauer et al., 1982), but unlike acrolein, malondialdehyde is also generated during synthesis of prostaglandins (Hamberg and Samuelsson, 1967; Marnett and Tuttle, 1980). Malondialdehyde levels in plasma (Gonenc et al., 2001) and malondialdehyde–DNA adducts in bronchial tissue (Munnia et al., 2006) increase in lung cancer patients. Other related compounds, β-ethoxyacrolein and β-methoxyacrolein, are 25–40 times more mutagenic than malondialdehyde. Inasmuch as these compounds are the result of common biogenic pathways, acrolein, crotonaldehyde, and malondialdehye and other possible aldehydes carry potential importance as mediators of spontaneous carcinogenesis, particularly with respect to background level age-related tumor incidence.

16.3.2.2 Other Reponses to Repeated Exposure The noncarcinogenic effects of repeated exposure to low-molecular-weight aldehydes other than formaldehyde are less studied. Extended exposure to certain aldehydes may lead to similar symptomatic (upper respiratory tract irritation) effects as formaldehyde (NRC, 1981; Feinman, 1988). These compounds may also produce respiratory effects (WHO, 1995).

The chronic effects of acetaldehyde are well studied. Chronic acetaldehyde exposure may produce persistent changes in lung function in that in Wistar rats exposed to 243 ppm, acetaldehyde (8 h/day × 5 days/week × 5 weeks) had altered functional residual capacity, residual volume, total lung capacity, and respiratory frequency (Saldiva et al., 1985).

High (≥250 ppm) acetaldehyde concentrations diminished lung function about as much as >2 ppm formaldehyde. Acetaldehyde produced nasal lesions in the rat at concentrations of 0.40 ppm (6 h/day × 5 days/week × 4 weeks) (Appelman et al., 1982) and in the hamster at concentration of 40 ppm (6 h/day × 5 days/week × 13 weeks) (Kruysse et al., 1975). The nasal area most damaged with acetaldehyde was the olfactory epithelium.

In hamsters, tracheal epithelial metaplasia was noted following the 4.0 ppm and a lower 1.34 ppm exposure, suggesting that the nasal passages of the hamster are less sensitive than those of the rat.

Less is currently known about the potential pulmonary effects of repeated exposures to other aldehydes. Longer-chain saturated aliphatic aldehydes (e.g., propionaldehyde) are less toxic, but cyclic aldehydes (e.g., benzaldehyde) have intermediate toxic potency when compared with formaldehyde. Inasmuch as a number of environmental exposures involve the cogeneration of these aldehydes with formaldehyde, need exists for more details on the extent of environmental exposure (through routine environmental sampling) and toxicological structure–activity relationships of these compounds. One aldehyde that may need further study is glutaraldehyde because occupational asthma has been reported following exposure (Chan-Yeung et al., 1993; Gannon et al., 1995; Ong et al., 2004). Patients with occupational asthma had increased bronchoalveolar lavage eosinophils and bronchoconstriction following inhalation challenge with 0.1 ppm glutaraldehyde (Palczynski et al., 2005).

Lastly, a number of aldehydes, including malondialdehyde or hexanal, are naturally occurring or produced metabolically during oxidative metabolism, which is likely to be augmented by chronic inflammation when combined with exogenous exposure to another aldehyde. A better understanding of the molecular and cellular toxicology of these and other aldehydes is warranted and may be useful to establish a relationship between human exposure and spontaneous carcinogenesis and perhaps even chronic obstructive pulmonary disease.

To investigate the effects of route of exposure to aldehyde sensitizers, BALB/c mice were exposed by inhalation to 6 or 18 ppm glutaraldehyde generated as a vapor or as an aerosol (van Triel et al., 2011). Other groups received 0.25 or 2.5% glutaraldehyde on the skin of the ears. Lymphocyte proliferation and cytokine production were measured in the draining lymph nodes. Glutaraldehyde was positive in the skin and its cytokine profile (IL4/interferon-gamma) skewed toward a Th2-type immune response with increasing dose. Inhalation exposure did not result in increased lymphocyte proliferation or increased cytokine levels, despite comparable tissue damage (irritation) in the skin and respiratory tract. The investigators suggested that the highly reactive and hydrophilic glutaraldehyde oligomerizes in the protein-rich mucous layer of the respiratory tract, which impedes sensitization but still facilitates local irritation. Within the context of risk assessment in respiratory allergy, our results stress the importance of prevention of skin exposure.

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17

LEAD AND COMPOUNDS

Lester D. Grant†

17.1 INTRODUCTION

Lead (Pb), a heavy metal with numerous useful properties (low melting point, highly malleable, etc.), has been put to many diverse uses by both ancient civilizations (e.g., the Roman Empire) and modern societies. The extensive expanded use of the metal in modern times (in water distribution systems, in additives to paints and gasoline, for electronics applications, etc.) caused widespread increases in lead exposures for human populations around the world, especially during the twentieth century. Despite its broad usefulness, however, the metal has also been long recognized to be acutely toxic at high-dose exposures (e.g., see Aub et al., 1925; Beechx, 1986).

Diagnosis of classically defined acute Pb poisoning (often life-threatening) historically typically involved clinical observation in individual medical cases of signs and symptoms of (1) marked impairment of red blood cell (RBC) formation/function, (2) severe central nervous system (CNS) and/or peripheral nervous system (PNS) damage/functional impairment, and/or (3) notable kidney damage/renal dysfunction. Extensive research findings emerging since the 1970s (comprising major advances in our understanding of Pb toxicity) indicate that Pb can exert toxic effects of concern on many organ systems and at exposure levels far lower than those producing clinically evident signs and symptoms of overt Pb intoxication. Such "subclinical" Pb effects are not identifiable through routine clinical examination, but rather their characterization generally requires more sophisticated methods, for example, identification through longitudinal observation. Also, although

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many such subclinical effects may be subtle, their ultimate impact on a population basis can be substantial.

For more than 60 years, there have been demonstrations of "subclinical" toxic effects at lower and lower exposure levels, as well as increasing recognition of their potential public health implications, leading to more conservative revisions of Pb exposure levels posing unacceptable risks for adverse human health impacts. Extensive remedial actions have produced notable reductions in Pb exposure among many population groups. Clinically evident acute Pb intoxications attributable to high-dose exposures continue in some geographic areas, but subclinical Pb toxicity is now of broader public health interest. This chapter is focused on (1) lower-level Pb exposure effects, (2) factors affecting susceptibility/vulnerability to such effects, and (3) progress in developing biokinetic models for predicting likely increased risk of Pb-related toxicity among human population groups.

Older reviews of Pb exposures and health effects were provided by Davis and Svendzgaard (1987) and Lippmann (1990), as well as by and exhaustive reviews in the EPA Air Quality Criteria documents for Pb (EPA, 1977, 1986a, 1986b, 1990, 2006) that supported the setting and periodic review of U.S. National Ambient Air Quality Standards (NAAQS) for Pb.

17.2 PHYSICAL/CHEMICAL PROPERTIES AND BEHAVIOR OF PB AND ITS COMPOUNDS

Pb is a member of subgroup IVA of the periodic table, is a typical heavy metal (Greninger et al., 1978), has a relatively high atomic weight, and has a stable oxidation state (Pb^{2+}) that furnishes a divalent ion. Its metabolism, in a redox sense, does not appear to be particularly important in determining its biological properties. Bivalent Pb forms well-delineated, often highly crystalline basic salts of both anhydrous and hydrated types, for example, white lead (a pigment of past widespread commercial use). The inorganic chemistry of bivalent Pb resembles that of alkaline earth elements. Several Pb salts (e.g., carbonate, nitrate, and sulfate) are isomorphous with corresponding strontium (Sr) and barium (Ba) compounds. Pb forms highly insoluble salts of phosphate, carbonate, and sulfide. Pb can also form salts with organic acids, which is the basis for the use of certain chelating agents for treating Pb intoxication. Elemental Pb, as a metal, is readily oxidized in biological systems and is not considered as a separate form here.

Pb's position in the periodic chart favors formation of covalent rather than ionic bonds in Pb^{4+} compounds, as in Pb tetrachloride and tetraacetate. Predominately covalent bonding is also seen with organo-Pb compounds (with up to four Pb–C bonds). An organometallic compound differs in both chemical and biological properties from an ionic compound of the same metal. Thus, determining only the total amount of the metal in a biological sample can be misleading with regard to estimating the potential for toxic effects. In general, inorganic Pb has been more extensively studied than organometallic Pb. The biological effects of organo-Pb compounds were reviewed by Grandjean and Grandjean (1984).

An appreciation of some general features of metal chemistry is very helpful in understanding specific aspects of Pb chemistry (Hanzlik, 1981). Among the most important criteria differentiating metal ions from each other, and from electrophilic organic species, is their bonding to biological ligands. Metal–ligand bonds can be as strong, in a thermodynamic sense, as bonds formed when a reactive epoxide alkylates a nucleophilic group in DNA or protein. Regardless of the mechanism by which a metal ion enters a biological

system, complexation plays a role in both its distribution within and elimination from the organism. Metals' ions are Lewis acids, and one very important determinant of their affinity for ligands is their charge–radius ratio. Increasing the metal's oxidation state increases its Lewis acidity and its affinity for a given ligand (assuming that it does not ionize the ligand).

Most metal complexes undergo ligand exchange by processes that involve a dissociative rate-limiting step analogous to the Sn1 solvolysis of alkyl halides. For a given metal, the rates are rather sensitive to the nature of the departing ligand and are essentially independent of the entering group. The dependence of biological activity on ligand exchange rates reflects the fact that the complex must be sufficiently inert to survive long enough *in vivo* to reach critical reactive target molecules and yet, once having reached those sites, be sufficiently labile to react. A given metal–macromolecular interaction may persist enough to have biological consequences if the equilibrium constant of the rate for dissociation of the complex is very small.

The hard–soft, acid–base dichotomy provides a rationale underlying many features of the behavior of metal systems in chemistry and biology. This parameter is correlated qualitatively with the charge–size ratio of the ion in that large ions of low ionic charge have easily polarizable or deformable (i.e., soft) electrostatic fields about them, whereas small highly charged ions, with relatively intense electrostatic fields, are hard. Flexibility in hard–soft ligand preferences is a key property underlying the biological activity/toxicity of metals in living systems. Several toxic heavy metal ions (i.e., Pb^{2+}, Hg^{2+}, Th^{1+}, Ni^{2+}, and Sb^{3+}) are classified as soft or have borderline properties in this classification scheme. The ligands, analogously, can also be classified by these hard–soft criteria.

In this regard, the rate of transport of a given metal ion across model liquid membranes can vary by several orders of magnitude simply by altering the anion (ligand) present in the original salt solution (Christensen et al., 1978). Like the other group IVA metals, tin (Sn) and germanium, Pb forms complexes in which the donor atom is chiefly oxygen (Greninger et al., 1978). It also forms stable complexes with sulfur and halogens as the donor. Carbon and nitrogen donors are less common. Pb generally forms complexes with the coordination number 6 (having octahedral geometrical structure), whereas other geometries are less common. On the basis that hard metal ions prefer to bind ligands and vice versa, one might expect Pb to bind halides in the order I > Br > Cl > F. This is consistent with the early use of potassium iodide to enhance Pb removal from the body (Aub et al., 1925).

Another important aspect of metal chemistry is the potential for metal compounds to act as initiators or catalysts *in vivo* (Hanzlik, 1981). Inhibition of enzyme molecules by stoichiometric quantities of tightly bound metal ions can reduce the flow of vital metabolites through a pathway and, thus, cause toxicity. In addition to stimulating or inhibiting the synthesis of enzymes, as well as the enzymes' activities, many simple metal ions and compounds have catalytic activity in their own right. Electrochemical gradients across biological membranes have the potential for a foreign metal ion to act as an "antimetabolite," which may be significant in view of existence of a mechanism for coupling biological oxidation/reduction pathways to ion transport and the control of membrane potential. In many cases, apparently nonessential metals are absorbed into an organism and not excreted at all; rather, they are simply concentrated and deposited in granular, insoluble complexes with or without accompanying proteinaceous material.

There are several ways to express the relationship of Pb to other metals, both foreign and endogenous. There is a resemblance of bivalent Pb chemistry to that of the alkaline earth metals in general. Of particular note is the similarity to calcium (Ca). Pb and Ca both form insoluble carbonates and insoluble phosphates. However, Pb phosphate is

much more insoluble than Pb carbonate, whereas Ca phosphate is more soluble than Ca carbonate. Pb phosphate is one of the few insoluble phosphates that do not react with most chemical reagents. The extreme insolubility of Pb phosphate may serve as a driving force for Pb to function as a phosphate scavenger in biological systems, which may include inorganic forms of phosphate as well as the various important phosphate esters. The ultimate deposition of Pb in the skeleton is consistent with Pb's chemical relationship to Ca and the formation of highly insoluble salts (Aub et al., 1925). Sr can also compete with Ca in bone tissue (Smith et al., 1985), and this chemical similarity of Pb and Sr may be related to their ionic radii and the stable 2^+ oxidation state relative to that of Ca^{2+}. Intestinal calcium-binding proteins have been shown to bind Pb with high affinities and in preference to Ca (Fullmer et al., 1985). These proteins bind several other cations (notably Sr^{2+}, Ba^{2+}, and Cd^{2+}) in a fashion apparently related to metal ionic radii relative to Ca.

Another important factor that can determine metal chemistry and biology is the hard–soft, acid–base property (amphoteric nature). In this regard, Pb is similar to iron (Fe), copper (Cu), zinc (Zn), mercury (Hg), and thallium (Th), among others. The amphoteric property, along with redox cycling, appears to account, in large part, for the importance of Fe in biological systems and its adsorption, storage, and transfer in these systems (Hanzlik, 1981). Such effects are attributable to the greater polarizabilities (function of number of electrons) of these cations and subsequently greater covalent character of the bonds they form with donor ligands relative to that expected for alkaline earth cations of the same size. The situation with Pb^{2+} is very analogous to that of Th^{1+} (Izatt et al., 1976).

Similarities in size and coordination chemistry may be important factors that determine the ability of metals to act antagonistically (Hill and Matrone, 1970). The chemical similarity of Pb to certain alkaline earth metals (particularly Ca) and Pb's ability to form highly insoluble salts (particularly of carbonate and phosphate) along with increased affinities to biological donors (enriched in oxygen and possibly nitrogen) due to favorable polarizabilities may well account for much of the relevant biological/toxicological chemistry of Pb compounds. In an overall sense, the importance of the ligand exchange chemistry of divalent Pb is emphasized in the expression of toxicity. Ligands can include simple anions or more complex donors that can form chelates or organic complexes.

The biological activity of a given metal is a consequence of the way in which the metal's compounds (salts, complexes, etc.) and cells interact. It is this interaction that is governed by intrinsic chemical properties (modulated by certain physical properties) of both the particular metal compound and the cell. In addition, the extent of cellular interaction can be affected by the same or different chemical properties that determine the *in vivo* absorption, distribution, and elimination of the compounds. Importantly, Pb forms highly stable bonds with S and S-containing compounds, but somewhat less stable ones with carboxylic acids (O-based ligands) and imidazoles (N-based ligands) (Claudio et al., 2003). Also, Pb competes very effectively in biological systems with native or homeostatic metal ions for binding with sulfhydryl, carboxyl, and imidazole side chains that comprise enzyme active sites, and this competition leads to inhibition of enzyme activity, the replacement of Ca in bone, and many other deleterious health impacts. Other key features of lead coordination chemistry, their roles in biological systems, and relationships to Pb-induced adverse health effects are delineated in the review by Claudio et al. (2003).

17.3 LEAD IN THE ENVIRONMENT AND HUMAN EXPOSURE

Trace metals, such as Pb, can be present in the environment in various forms (Boline, 1981), such as free hydrated ions, ion pair salts/complexes, organic complexes/chelates, surface-adsorbed material, and undissolved compound. Although differences may occur in valence states and associated ligands (including mixed ligands) across these various forms, the metal identity is still retained, and the chemical reactivity of the metal is a function of the combined physicochemical properties of the metal and its associated ligands. This chemical reactivity can be modulated by physical properties, such as the surface properties of the metal compound itself. Thus, under certain physical, chemical, and biological conditions, it is possible for a given metal to assume more than one form, which can follow new pathways of chemical reactivity. This same reactive potential contributes to the posing by certain metals of possible toxic threats to the environment and living systems that concentrate them. The range of chemical properties and reactivities associated with various types of metal compounds is thus much greater than that of simple organic compounds. Since the metal is never destroyed, the potential to exert this complex chemistry always exists. Inorganic Pb compounds to which humans are likely to be exposed include halides and oxides, sulfides and sulfate, carbonate, and chromate.

With the exception of a few sporadic measurements in air, marine fish, sediments, birds, and human brains, there is relatively little information available on organic Pb compounds (Jawrerski et al., 1987). It appears that most organic Pb compounds in the environment come from the release of organo-Pb compounds (such as those used as gasoline additives) prior to or during their use rather than being derived from inorganic Pb compounds. With the restriction of the use of Pb-based gasoline additives, an increasingly greater proportion of inorganic Pb compounds has come to dominate. Thus, the global movement of inorganic Pb and its compounds, as well as human exposure to them, is of much greater concern, has been far more extensively studied, and is the main focus here.

The biogeochemical cycling of Pb and routes of human exposure were described by Schlag (1987) and EPA (2006). Key types of environmental reservoirs of Pb can be identified, and quantitative estimations exist of inputs to them from various natural or human sources, but rates of transfer within and between the reservoirs are generally known only qualitatively or semiquantitatively. The temporal pattern of changing worldwide Pb emissions has been documented by examination of natural historical records of Pb accumulated over time in ice packs or other layered natural materials, and estimates of natural and anthropogenic Pb emissions to air and to the oceans were made on a global basis by Nriagu and Pacyna (1988), showing that anthropogenic sources were responsible for at least one to two orders of magnitude more than natural ones. Detectable long-term elevations in global Pb emissions began at the time of the Roman Empire, followed by a relatively slow increase over many centuries, with a steep rise starting in the eighteenth century. They peaked at around 400,000 tons/year during 1970–1980 and then declined to about 100,000 tons/year (Nriagu, 1998), with analogous peaking in the 1970–1980 period being followed by declining Pb levels. For example, the pattern of atmospheric Pb deposition in a peat bog in the Swiss Jura Mountains paralleled historical increases in Pb deposition attributable to the introduction in 1947 of leaded gasoline into Switzerland, with later bog layer samples corresponding to 1991 showing a decline in atmospheric Pb deposition and a shift in isotopic ratios (Shotyk et al., 1998) reflective of subsequent phasing out of leaded gasoline in Europe. Analogous patterns of variations in sediment Pb levels (i.e., reaching peak concentrations in layers reflecting deposition during the 1970s, followed by ensuing declines)

were observed for a variety of other locations in North America and Europe. For more detailed reviews of natural historical records reflecting temporal variations in Pb emissions and deposition, see Boutron et al. (1994), Weiss et al. (1999), and Garty (2001).

Human Pb exposure generally occurs via one or more of four main components of the human environment: inhaled air, ingested soil and dust of various types, drinking water, and food. The primary medium for widespread dispersal of Pb in the ambient (outdoor) environment tends to be air, because Pb-containing fine particles (emitted mainly by high-temperature anthropogenic sources) can travel long distances before settling out via wet, dry, or cloud deposition. Pb deposition from air is most intense near a given source, but the zone of readily detectable elevated deposition can extend many kilometers. Substantial decreases in ambient airborne Pb levels have occurred in parallel with the phase down in usage of organic Pb compounds as additives in gasoline, >90% decrease in U.S. ambient air lead levels from the mid-1970s to 0.10–0.25 $\mu g/m^3$ range by 2000–2002 (EPA, 2006). However, accumulation of Pb in roadside soil and other soils due to past deposition of airborne Pb from gasoline, smelters, and other sources constitutes a persisting reservoir of anthropogenically generated Pb. Most Pb particles deposited on soil are retained and, eventually, are mixed into the surface layer, with the Pb accumulated at the soil surface becoming available to be taken up by plants, grazing animals, or soil microorganisms and thereby entering terrestrial food chains. Also, direct exposure of children to Pb in soil can occur by oral intake of Pb-contaminated dust and dirt during normal hand-to-mouth activity. In addition, Pb-contaminated soil particles can be resuspended in air and as such become a long-term source of airborne Pb exposure for humans via inhalation.

Pb in rivers comes from runoff, erosion, and direct deposition from air. Freshwater generally contains more inorganic and organic suspended PM than marine water, and this suspended material has a strong tendency to adsorb any dissolved Pb, with adsorption potential being much greater for smaller than larger particles (Rhoads and Cahill, 1999). Pb concentrations in sediment typically increase with humic (organic matter) content (Kiratli and Ergin, 1996; Rhoads and Cahill, 1999) and can be sequestered on Fe or Mn oxides (Schintu et al., 1991; Peltier et al., 2003; Gallon et al., 2004) or its adsorption increased by sulfides, especially under anoxic conditions (Kiratli and Ergin, 1996; Perkins et al., 2000). Most of the Pb entering the open oceans comes from atmospheric deposition rather than from rivers. The lower concentrations in marine waters, as well as high salt (chloride, bromide, etc.) levels, tend to favor a larger proportion of dissolved Pb in the marine water column. Deep ocean sediments may thereby represent a sink for Pb, since there is little evidence to suggest notable remobilization from such sediments. On the other hand, it appears that Pb in sediments in freshwater lakes and rivers can be remobilized (e.g., see Steding et al., 2000; Hlavay et al., 2001; Peltier et al., 2003; Gallon et al., 2004; Kurkjian et al., 2004), especially under acidic conditions. Thus, in some areas, Pb in freshwater sediments may continue to be a potential source of water-related exposure to Pb deposited many years earlier from the air, via runoff, and so on.

Pb in drinking water supplied by municipal water distribution systems typically derives mainly from corrosion of Pb pipes, Pb-based solder, or bronze and brass fixtures (e.g., faucets) within residences or workplaces, with little Pb generally coming from properly buffered utility supplies (Lee et al., 1989; Gulson et al., 1994; Singley, 1994; Isaac et al., 1997). Low pH (acidic) water enhances the leaching of Pb from indoor plumbing components, which can be greatly reduced by buffering utility water supplies toward neutral pH conditions. Of much importance, the addition for disinfection purposes of chlorine (Cl) to drinking water (which can lower the pH) does not generally increase leaching of Pb into the water

because of a Cl reaction with Pb^{2+} that results in precipitating out of a highly insoluble, red-brown-colored Pb solid (Edwards and Dudi, 2004). On the other hand, the insoluble Pb solid is not formed in the presence of chloramines, and the introduction of chloramine disinfectants in place of Cl (especially in the absence of other adequate water chemistry adjustments) appears to have contributed to increases in drinking water Pb levels seen in some U.S. communities since 2000, as noted in EPA (2006), and more recently in Flint, MI.

Potential human exposure via drinking of Pb-contaminated water can still be a nontrivial contributor to Pb exposures. As an example, tap water Pb was the main correlate of elevations in maternal blood Pb levels in a study of mothers and infants in Glasgow, Scotland (Watt et al., 1996). In a U.S. prospective study, Lanphear et al. (2002) found that children exposed to water with Pb levels over 5 ppb had blood Pb levels 1.0 μg/dL higher than children exposed to water with Pb concentrations less than 5 ppb. Although exposure to Pb via drinking water still occurs, it is often difficult to readily determine the importance of this exposure route in contributing to any specific overall toxic insult, due in part to the considerable potential for wide variations in the concentration and bioavailability of Pb in water. Furthermore, the bioavailability of Pb and other metals in water depends not only on trace metal solubility but also on numerous complex chemical equilibria affected by the presence of other trace inorganic and organic compounds (Jackson and Sheiham, 1986). The bioavailability of Pb in soils, food, and inhaled air may depend on similar factors that determine the ligand exchange chemistry once in contact with the biota and aqueous phase. Accordingly, metal speciation analyses and solubility modeling are likely to yield further insights and improved understanding of the potential for toxic insult (Hunt and Creasey, 1980) and therefore contribute to improved scientific bases for Pb abatement strategies [e.g., reducing the bioavailability of trace metals in water via manipulation of their solubility and aqueous chemistry, as suggested by French and Hunt (1988)].

Pb in food derives from plant and animal exposure to contaminated air, soil, and water and from products used in the processing and storage of foods. Pb in foods in many countries declined dramatically during recent decades, from typically 100 to 200 μg/day (Mahaffey, 1977) to typically less than 5 μg/day by the mid-1990s (Bolger et al., 1996). This decline resulted from virtual bans on the use of Pb solders in food and beverage containers and to more widely practiced limits on the use of Pb glazes in pottery and food storage containers. Pb contamination of raw food resulting from air and water contamination has also notably declined.

Marked decreases noted above for Pb concentrations in several environmental media reflect, in large part, the strong steps taken to prevent Pb exposure. This notable progress in reducing Pb in environment media during the last several decades represents a major success story for a number of countries. For example, dramatic reductions in blood Pb levels among U.S. population groups were demonstrated by the National Health and Nutrition Examination Survey (NHANES) II (conducted 1976–1980) and phase I of NHANES III (conducted 1988–1991), the geometric mean blood Pb level for ages 1–74 years declined from 12.8 to 2.9 μg/dL, and the prevalence of elevated blood Pb levels (i.e., >10 μg/dL) decreased from 77.8 to 4.4% (Mahaffey et al., 1982; Pirkle et al., 1994). Also, between NHANES III phase II and phase III (1991–1994), the comparable geometric mean decreased by 22% (CDC, 1997). It was further noted that the blood Pb concentrations of children were reduced by more than 80% over the prior two decades, and EPA (2006) noted that marked decreases in environmental Pb during the 1980s–1990s were paralleled by decreases in concurrent blood Pb levels of U.S. children (from a geometric mean of 15 μg/dL in 1980 to ~1–2 μg/dL in 2004).

Such declines in blood Pb levels among the U.S. general population and children not only reflect changing economic circumstances (e.g., declining numbers of operating U.S. primary or secondary Pb smelters) but also the success of highly effective primary interventions (virtual elimination of Pb additives from gasoline, shifting to low Pb plumbing solder and fixtures, removal of Pb solder from food and beverage cans, etc.) and secondary prevention strategies, such as public health screening/education programs and improved nutrition. Despite this overall success, however, some U.S. children still experience blood Pb concentrations above10 μg/dL, and disproportionate numbers of black and low-income children continue to exhibit elevated blood Pb levels. The most extensive remaining source of Pb exposure for U.S. children is Pb-based paint in older residences, which are more likely to contain Pb-based paints (Jacobs, 1995). The CDC (1997) estimated that in the 1990s, 890,000 U.S. children had sufficiently high (>10 μg/dL) levels to impair learning.

Elevated blood Pb concentrations also occur among adults attributable to the use of "folk" remedies, cosmetics, and Pb-glazed pottery. The other common cause of elevated blood Pb among adults is occupational exposures. The National Institute for Occupational Safety and Health (NIOSH) maintains an Adult Blood Lead Epidemiology and Surveillance (ABLES) program, and based on data reported from 27 states, the cumulative number of reports in 1996 was 16,551 adults with blood Pb concentrations of 25 μg/dL or higher, with some concentrations >60 μg/dL (NIOSH, 1998). The ABLES website indicated that geometric mean of all reported concentrations is <3 μg/dL, much lower than the 25 μg/dL level that the U.S. DHHS recommends not to be exceeded by adults.

The transfer of Pb from the environment to cells and subsequent interactions with cell components occur as functions of the physical/chemical properties of Pb and of physiological factors. The delineation of physiological pathways involved in determining Pb uptake and internal distribution to various tissues, accumulation in particular types of tissue and internal redistribution, and factors affecting uptake, internal distribution, metabolism, or excretion are all important, especially (1) to help identify tissues and mechanisms underlying different toxic effects and (2) to enhance estimation or prediction of adverse responses resulting from varying exposure patterns and intensities. The absorption of Pb via portals of entry and the important factors that affect absorption from different exposure routes as well as other aspects of the Pb biokinetics are summarized next as a prelude to discussion of Pb-related health effects. More detailed reviews of Pb toxicokinetics can be found in Mushak (1991, 1993, 1998) and EPA (1986a, 1986b, 2006).

17.4 LEAD ABSORPTION

17.4.1 Gastrointestinal (GI) Pb Absorption

Most of the pertinent research on gastrointestinal (GI) mechanisms has been carried out in rodents, especially the rat. Aungst and Fung (1981), Henning and Cooper (1988), and Fullmer (1997) emphasized the complexity of the process and the extent to which the specifics depend on factors such as dose, physiological state, nutritional condition, and age.

There are at least two mechanisms for GI absorption. One exhibits characteristics of energy-dependent, carrier-mediated, active transport (Aungst and Fung, 1981); whether this process is saturable or not depends on the Pb dose used (Keller and Doherty, 1980a, 1980b). This absorption has active transport mechanism characteristics, since intestinal uptake and flux depend on metabolic energy. At a buffer concentration on the mucosal side

of 0.5 μM, capacity-limited processes contributed nearly 200 times more to the mucosal-to-serosal flux than diffusion (Aungst and Fung, 1981). At a Pb concentration three orders of magnitude higher (48.3 μM), diffusion accounted for less than 20% of the flux (Aungst and Fung, 1981).

Others concluded that the major control level for GI absorption likely resides in the intestinal mucosal cell and that the interrelationships between the elements may affect bioavailability at both luminal and mucosal levels. Ragan (1983) suggested three components to the absorptive phase: uptake by the mucosal cell, transfer through the cell, and movement into the plasma. Pb absorption also has a dose-dependent component. In addition to the work of Aungst and Fung (1981), other work includes *in situ* studies by Barton et al. (1978a, 1978b) in which the percentage of Pb absorbed depends on the magnitude of the dose and is increased in Fe-deficient animals (Hamilton, 1978) in relation to fed or Fe-replete animals.

17.4.2 Effects of Age on Lead Absorption

Age substantially influences Pb absorption in human and nonhuman primates. Willes et al. (1977) reported that infant monkeys at 10 and 150 days of age retained 64.5 and 69.8% of an oral dose of $^{210}Pb(NO_3)_2$ adult monkeys only retained 3.2%. Similar age-related differences have been observed for humans. Using classic balance study techniques, Kehoe (1961) found the GI absorption by adult males to be 5–10% of ingested Pb. This range for usual food intake patterns (i.e., not fasting) was confirmed to be in the range of 5–15% based on studies with short-lived radioisotopes (Hursch and Suomela, 1968) or stable Pb isotopes (Rabinowitz et al., 1975, 1976). James et al. (1985) subjects (aged 26–77 years) included both females and males (12 women and 11 men) and reported Pb absorption from foods and beverages, but did not discuss any sex-related differences.

Oral exposure uptake rates for children have been less clearly documented than those for adults and were derived mainly from two mass balance studies with small numbers of children. Alexander et al. (1974) conducted balance studies in eight subjects (aged 3 months to 8 years) with Pb intakes averaging 10.6 μg Pb/kg/bw/d (bodyweight/day). Absorption averaged 53% of intake, and retention averaged 18% of intake. Also, Ziegler et al. (1978) studied Pb absorption by 12 infants (aged 14–746 days) whose Pb intakes exceeded 5 μg/kg/bw/d. These fractional absorption estimates, having been derived from studies in the 1970s, may not be directly applicable to current estimates of kinetics.

It is unclear over what age period in childhood do the Pb absorption characteristics become more like those of adults than infants. Although studies specifically evaluating age-related changes in fractional absorption by children older than infants are not yet available, some insight might be drawn from other studies. Based on analyses of stable Pb isotope profiles of nine immigrant children from Eastern Europe living in Australia, Gulson et al. (1997) found the fractional absorption by the children (aged 6–11 years) to be comparable to absorption patterns of adult females in the 29–37 age range. Whether the 40–50% absorption values for ingested Pb obtained for subjects under 2 years old apply to 2- to 6-year-old children remains unclear. Lower absorption values for 2- to 6-year-old children are supported by data from Angle et al. (1995), who suggested that absorption of ingested Pb among 2- to 3-year-old children was 10–15%. See Mushak (1991) for more detailed discussion of factors (including both physiological and dietary) potentially underlying age-related differences in GI absorption of inorganic Pb.

17.4.3 Influence of Nutritional Status and Dietary Factors on Lead Absorption

The influence of nutritional status and dietary factors on blood and on tissue Pb distributions was most clearly observed at low levels of exposure, for example, those more likely to exist in the post-2000 than in the pre-1980 period. Since the mid-1980s, Pb exposure declined markedly in countries that discontinued the use of Pb solder in food and beverage cans and phased out the use of Pb-based gasoline additives. The beneficial effects of optimal nutrition are enhanced under these lower exposure circumstances (Mahaffey, 1995; Bogden et al., 1997).

17.4.3.1 Total Food Intake Adults in the fasting state absorb a substantially greater fraction of Pb compared with the fraction absorbed in the nonfasting state (Blake and Mann, 1983). However, there is a lack of data regarding comparable information on the effects of fasting state on Pb absorption by children or young nonhuman primates.

17.4.3.2 Influence of Calcium Dietary Ca can influence GI absorption of Pb through both acute and long-term effects of low dietary Ca intake. Studies of experimental animals fed low Ca diets established that Ca deficiency increased both tissue retention and toxicity of Pb (Mahaffey-Six and Goyer, 1970). Long-term Ca deficiency produces physiological adaptations, including increased concentrations of various binding proteins and the stimulation of endocrine and regulatory systems, for example, 1,25-dihydroxycholecalciferol and parathyroid hormone. These secondary changes produced by Ca deficiency also affect the Pb biokinetics.

Overall, Ca deficiency generally increases Pb uptake. Experimental animal studies showed that simultaneous ingestion of Pb with reduced Ca in the incubation medium (i.e., comparable to a low Ca meal) enhanced Pb absorption. For example, Barton et al. (1978a), using ligated intestinal loop techniques to measure Pb absorption, found that when the Ca concentration in the incubation medium varied within physiological ranges, Pb absorption decreased with increasing Ca concentration. Prior conditioning by low or high Ca diets did not significantly alter the rat's Pb absorption *in vivo*. Lower Pb absorption was observed in rats and chicks (Smith et al., 1980) during studies on the role of vitamin D in Pb absorption. Reduced Pb uptake from ligated gut loops upon addition of Ca to incubation media was reported by Barltrop and Khoo (1975), and Meredith et al. (1977) found that oral Ca, given immediately before Pb, very effectively decreased Pb absorption in rats. Analogously, higher dietary Ca intake decreased Pb absorption in humans (Ziegler et al., 1978; Heard and Chamberlain, 1982; Blake and Mann, 1983).

Mechanisms that produce changes in Pb absorption due to Ca status have become increasingly better understood (Fullmer, 1997). Aungst and Fung (1985) found that the apparent systemic availability of 1, 10, and 100 mg/kg oral Pb doses were three- to fourfold greater in Ca-deficient than in control animals. However, the intestinal absorption of 10 kg/mg doses of oral Pb was unaffected by Ca supplements. Such differences reflect the roles in Pb and Ca absorption of dietary vitamin D (cholecalciferol) and metabolically active vitamin D (1,25-dihydroxycholecalciferol). Mykkanen et al. (1982) reported that, in chicks, both cholecalciferol and 1,25-dihydroxyvitamin D_3 affected both the ^{203}Pb and ^{47}Ca absorptive processes, but the nature of these responses differed, suggesting differences in the transport path or the macromolecular interactions of these ions during the course of absorption. Studies of Pb conclusively verified specific, high-affinity binding of Pb to several Ca-binding proteins and suggested that it may be a general property of certain intestinal Ca-binding proteins (Fullmer et al., 1985).

Fullmer (1997) further investigated time courses and dose–response relationships for these interactions. By feeding five different levels of Ca and five levels of Pb to chicks, he found that Pb ingestion and Ca deficiency, either alone or in combination, generally increased serum 1,25-dihydroxyvitamin D levels over most of the range of dietary Pb and Ca intake. However, with severe Ca deficiency, consumption of Pb produced marked decreases in 1,25-dihydroxyvitamin D levels. Overall similarities in the responses of 1,25-dihydroxyvitamin D, intestinal Ca absorption, and calbindin-D indicate that the predominant interaction between Pb and Ca is mediated via changes in circulating 1,25-dihydroxyvitamin D concentrations, rather than directly through the intestine. Kidney and bone Pb levels also changed in response to these dietary manipulations, suggesting that added effects occur that do not fully depend on the concentrations of 1,25-dihydroxyvitamin D, although this appeared to be the predominant control mechanism for intestinal absorption.

17.4.3.3 Influence of Iron Fe deficiency increases Pb tissue deposition and toxicity (Mahaffey-Six and Goyer, 1972). Ragan (1983) demonstrated sixfold increases in tissue Pb in rats when body Fe stores were reduced but before frank Fe deficiency developed. Also, Hamilton (1978) and Flannagan et al. (1979) reported significantly increased Pb absorption from the GI tract of Fe-deficient animals. Lastly, based on results obtained by *in situ* ligated gut loop techniques, Barton et al. (1978b) reported that Fe deficiency (secondary to bleeding and to Fe-deficient diets) increased Pb absorption and that Fe loading decreased Pb absorption.

Ferritin was shown to bind Pb both *in vivo* and *in vitro*. In rats fed an Fe-deficient diet, the ferritin concentration was low, permitting increased transfer of Pb to blood rather than retention in the small intestine bound to ferritin. Transferrin is increased in Fe deficiency anemia as a result of increased synthesis. Although transferrin binds Fe preferentially, transferrin also transports a number of trivalent and divalent cations such as Pu, Am, Cr, Co, Mn, and Cu, among others. A protein that specifically binds Pb, as well as Fe, was isolated from both the rat and the human duodenal mucosa (Conrad et al., 1992).

The influence of Fe status on Pb absorption has also been studied in human subjects, with mixed results (Flannagan et al., 1979; Watson et al., 1980). To date, it is not clear whether these mixed results reflected differences in the severity of Fe deficiency, differences in analytical approaches, or other undefined factors. Despite the lack of clarity regarding mechanisms, Fe therapy is a valuable adjunct in the treatment of low-level Pb toxicity (Granado et al., 1994).

17.4.3.4 Influence of Chemical Forms of Pb on Gastrointestinal (GI) Absorption The effect of the chemical forms of Pb on GI absorption of the metal can described in general terms. Pb bound to alkyl compounds is readily absorbed and concentrates in tissues high in lipid, such as the brain. The percentage of absorption and tissue distribution of alkyl Pb differs markedly from those of inorganic Pb compounds. Among inorganic Pb compounds, the particle size of ingested Pb plays a major role in determining the fractional GI absorption. Barltrop and Meek (1979) showed a fivefold enhancement in Pb absorption by rats when the particle size was reduced from 196 to 6 μm. Healy et al. (1982) reported that Pb sulfide (one of the least soluble Pb compounds) had increased solubility in gastric fluid (as a result of chemical conversion to the more soluble chloride) when the particle size was reduced from 100 to 30 μm (Healy et al., 1982).

Information about the influence of the chemical form of Pb on its GI absorption is frequently complicated by limited information on the experimental conditions, including other factors in the diet, particle size of the inorganic Pb source, physiological condition of the animal, and so on. *In vitro* solubility does not predict well the degree of *in vivo* absorption (Sartorelli et al., 1985). Despite this limitation, the use of multiple *in vitro* methods to estimate solubility continues. Interpretation of results from these *in vitro* methods is further complicated by organ-to-organ differences in bioaccumulation. For example, when immature swine were fed two fully characterized soil samples from a Western U.S. Superfund site (Casteel et al., 1997), the bioavailability ranged from about 50 to 90%, depending on the organ system used to express dose (e.g., blood, liver, or renal Pb concentrations).

In mechanistic terms, Pb absorption depends on such factors as chelation, membrane permeability, solubility, and particle size (Huisingh and Huisingh, 1974; Brezinski, 1976). The coordination chemistry of Pb^{2+} is likely to play an important role. Coordination with proteins may be a determining factor for the Pb availability for absorption and transfer across the mucosal cell. It is important to know in which form(s) Pb is (are) available and what ligands exist in mucosal cells that may be vehicles for absorption or inhibition. Metals can precipitate or coagulate proteins in solution. Also, metal salts often show increased solubility in body fluids (Fairhall, 1924), Pb carbonate being, for example, about 300 times more soluble in serum than in water. Such metal–protein interactions depend on factors such as the radius, charge, and coordination number of the metal, as well as factors intrinsic to the proteins, such as size and basicity. In a protein-rich environment, the local metal ion power and complexing ability of the food should be considered.

17.4.4 Absorption Following Inhalation

Absorption of Pb from the pulmonary system depends, in a major way, on particle size. It is difficult to determine what fraction of Pb dust in inhaled air actually gets deposited in the gas exchange airways to be taken up by alveolar cells or what fraction is deposited on the conducting airways and is eventually passed out through the trachea and swallowed with mucus from the trachea or nasal passages. It is clear, nevertheless, that the pattern of Pb deposition in the respiratory tract is affected by the particle size and ventilation rate (Chamberlain, 1983). The rate of Pb absorption from particles also depends on solubility of the chemical species. In humans, Pb absorption from the lung is usually rapid and complete within 24 h. Even relatively insoluble Pb compounds can be taken up directly into the general circulation. Ligand exchange ability is also a key factor, since it relates to physiochemical properties of the available Pb species and the surface properties of the particles.

All species of Pb compounds deposited in the alveolar region are thought to be more or less completely absorbed into the bloodstream (Morrow et al., 1980), with distinctly greater alveolar deposition typically occurring for particles $<2.5\,\mu m$ in diameter than for larger-sized particles. However, as is often seen with various environmental or occupational exposures, it is not unusual for Pb dusts in inhaled air to contain particles $>2.5\,\mu m$ diameter, so many are cleared from the conductive airways by mucociliary action. Swallowed particles can affect the GI absorption of Pb, significantly influencing their bioavailability. Thus, depending on the particle size and Pb concentration associated with a particular air exposure source, the digestive tract can also be an important avenue of Pb absorption following inhalation.

Chemical speciation of Pb dust in occupational settings has shown marked variability in the size of particles generated in primary smelters (Spear et al., 1998). Depending on the process performed (e.g., samples from ore storage, sintering, or blast and dross furnaces), the particle size, mineralogy, and extractability of the constituent Pb can differ substantially, and, consequently, changes to the ore during processing can influence the biological availability of the Pb.

17.4.5 Dermal Absorption of Lead

Skin absorption is not usually considered to be a significant mode of Pb uptake (Minot, 1929), unless it is present in its more lipid-soluble organic forms (such as tetraethyl Pb). Florence et al. (1988) found that inorganic Pb can be absorbed through skin and rapidly distributed throughout the body, with the distribution varying from that of ingested Pb. Skin absorption gave rise to increased Pb excretion in sweat, although similar increases in blood and urine were not observed.

17.5 DISTRIBUTION

Pb is absorbed into blood plasma, where it rapidly equilibrates with extracellular fluid. More slowly, but within minutes, Pb is transferred from plasma into blood cells (Chamberlain, 1985; Simons, 1986). The typical Pb concentration in whole blood is about 10^{-6} M. Because 95–98% is bound in RBC, about 10^{-8} M is present in plasma. If the distribution of Pb between plasma and cytosol is similar to that of Ca (10,000:1), the cytosolic concentration of Pb in exposed individuals should be in the picomolar range. In animal experiments, no constant relationship has been found between Pb concentrations in blood and in soft tissue. Thus, as earlier noted by Kazantzis (1988), controversy exists with regard to whether Pb in blood represents biologically active Pb and, indeed, the extent to which the two may be linearly related.

Improved detection limits for analytical methods have increased our ability to determine Pb concentrations present in plasma. Concerns remain, however, that even very slight hemolysis of erythrocytes, during the separation process, can transfer Pb into the plasma fraction. Consequently, data on plasma Pb concentrations needs to be treated with caution unless the technique can establish that what is thought to be plasma Pb does not simply represent the *in vitro* transfer of Pb from erythrocytes.

From the blood plasma, absorbed Pb is distributed to different organs, with liver and kidney attaining the highest concentrations. The PNS may accumulate much more Pb than the CNS. Also, marked variation occurs in Pb distribution within other tissues and organs (Drasch, 1974, 1997; Barry, 1975, 1981; Drasch et al., 1987; Drasch and Ott, 1988). In this regard, there are several important corollary observations. First, Pb tends to accumulate wherever high Ca levels are found. Highest Pb concentrations are, therefore, found in bone, especially in dense cortical bone. Second, within and among soft tissues, highest Pb concentrations accumulate in those tissues and organs having the highest mitochondrial activity. Likewise, within a given organ, the highest concentrations occur in regions with the highest mitochondrial activity (e.g., in the renal tubule and in the choroid plexus and cerebellum of the CNS).

The skeleton contains more than 90% of the Pb body burden at steady state. However, this pool is neither homogeneous (Kehoe, 1961; Rabinowitz et al., 1976; Chamberlain,

1985) nor static (Gulson et al., 1995). This association with bone is related to Pb's similarity to Ca and formation of insoluble Pb phosphate. There are many Pb bone pools, and bone is a very complex organ, having varying density and structure, depending on skeletal site and function. The turnover of tissue Pb is high throughout life. Among young adult women, between 50 and 75% of blood Pb comes from tissue stores (i.e., skeletal) rather than the current environment (Gulson et al., 1995, 1996). Under conditions of physiological stress for Ca (including pregnancy and lactation), the release of bone Pb becomes even higher (Gulson et al., 1998a, 1998c); that is, the initial source of the Pb is environmental Pb that has accumulated in tissues over previous years. Estimates of the fraction of current blood Pb derived from bone were provided by Smith et al. (1996), reporting that the skeleton contributed 40–70% of Pb in blood among five subjects who had trabecular bone samples obtained at surgery.

There are now thought to be three basic types of bone Pb pools: (1) rapidly exchanged Pb in very metabolically active portions of bone, (2) Pb in trabecular or spongy bone, and (3) Pb in dense cortical bone. Pb turnover in these three pools was to roughly parallel the relative rates of Ca turnover. Marked variation in the distribution and turnover rates within these pools depends on the particular region of the skeleton studied. Although Pb concentration is lower in trabecular than in cortical bone, the mass of trabecular bone is ~4 times that of cortical bone and that it is approximately four times as labile. Therefore, trabecular bone may represent a much more metabolically important pool of Pb.

The distribution of Pb in tissues reflects a state of constant, dynamic equilibrium. Many methods of enhancing Pb excretion are also influenced by Pb's redistribution within the body (Cory-Slechta et al., 1987). Clearly, any situation that mobilizes the very large, relatively stable Pb pools within the body, particularly those in the bone, will lead to the redistribution of Pb to a variety of tissues. This redistribution is thought to explain the increased symptomatology that is frequently noted in Pb-poisoned children during acute illnesses. Redistribution is known to occur during pregnancy and, even under usual circumstances, can result in increased risk to the fetus, particularly in women with prior Pb poisoning. There is also some evidence that the osteoporosis of aging may be accompanied by the significant mobilization of Pb from bone pools. It is clear that much additional information is needed to clarify more fully the various physiological and pathological conditions of enhanced mobilization and redistribution of Pb.

17.5.1 Excretion

Pb is excreted from the body mainly by urinary and fecal routes, with fecal excretion representing the sum of unabsorbed endogenous Pb from saliva, bile, and (to a lesser extent) other GI secretions, plus the unabsorbed portion of inhaled and ingested Pb. Less excretion occurs through sweat and integumentary losses (including skin, hair, and nails), and these routes account for only a small portion of total excretion. Under conditions of fairly constant exposure to low Pb concentrations, a steady-state condition evolves, wherein excretion approximates intake (Rabinowitz et al., 1976) and ~70% of intake is excreted via urine. Under short-term conditions of low-level increased exposure (Chamberlain, 1985), ~60% is retained by the body and 40% excreted. In a 14-day study of human volunteers receiving a single dose of ^{203}Pb, ~18% of the dose was excreted, whereas ~35% was retained in blood, with a residual of ~47% in soft tissues and bone storage. Extrapolating this result would predict an eventual ~30% excretion and 70% retention of this single dose.

Urinary excretion of Pb is quite complex, depending on the situation, but is most likely a function of plasma Pb levels. Under most conditions of exposure to relatively low Pb concentrations (e.g., at blood Pb levels below ~25 μg/dL), the concentration of Pb in plasma is very low (about 0.01 μg/dL) and not related to whole blood Pb. Above this concentration, plasma Pb increases significantly, as does urinary Pb excretion. At blood Pb concentrations <25 μg/dL, urinary clearance of Pb was estimated at ~1.1% per day. As blood Pb rises above this, urinary clearance appears to rise at a rate reasonably related to the increase in plasma Pb (Chamberlain, 1985). At very high blood Pb concentrations, renal dysfunction may decrease Pb urinary excretion.

In considering fecal Pb excretion, one must differentiate between fecal Pb that is unabsorbed from ingestion or inhalation and fecal Pb that truly represents endogenous fecal excretion. Endogenous excretion was measured by Rabinowitz et al. (1976) using stable isotope studies and by Chamberlain (1985) after inhalation and/or parenteral administration of ^{203}Pb. Endogenous excretion is often estimated by comparing renal clearance with apparent total body clearance. All such estimates suggest a clearance of ~0.5% per day at blood lead levels >25 μg/dL. It is likely that this rate of clearance is basically independent of blood Pb and does not significantly increase with increasing blood or plasma Pb. Excretion by all other routes is at a rate of ~0.2% per day is essentially independent of blood Pb concentration. The total for all these excretion routes is ~1.8% per day at blood Pb concentrations <25 μg/dL and somewhat greater at higher blood levels because of increased urine Pb excretion.

A special form of excretion is that which occurs via breast milk. Maternal breast milk Pb concentrations correlate well with those of maternal blood. Plasma Pb concentrations in mice (Keller and Doherty, 1980a, 1980b) suggested that breast milk Pb concentrations were closely related to plasma Pb concentrations and can be as much as 25 times that of plasma Pb. This suggests that at lower blood Pb concentration in the mother, breast milk would likely represent a minor route of excretion and is usually a minor exposure route for the infant. However, at higher blood Pb concentrations with increasing plasma Pb concentrations, it is plausible that a significant amount of Pb could be mobilized from the maternal skeleton in lactating women and that breast milk could represent an important exposure pathway for breast-fed infants. In fact, using stable isotope methods, Gulson et al. (1998c) demonstrated this mobilization among women with blood Pb concentrations less than 10 μg/dL. Breast milk lead appears to be linearly related to blood Pb and has concentrations similar to plasma. For blood Pb levels in the range of 2–34 μg/dL, breast milk contained <3% of the Pb in blood. The amounts of Pb released from the skeleton show person-to-person variation, suggesting that among women who have had substantial prior Pb exposure, it is important to assess this as a possible exposure source for the infant.

A variety of specific methods have been used to alter the Pb clearance. Aub et al. (1925) showed that urinary excretion could be enhanced by acidification, presumably by Pb mobilization from relatively stable pools. After some initial enthusiasm for the therapeutic potential of this intervention, it was abandoned because such therapy often also enhanced symptomatology, presumably by redistributing Pb to soft tissues. A variety of chelating agents have been used to enhance Pb clearance. The majority of chelating agents, including ethylenediaminetetraacetic acid (EDTA), dimercaptosuccinic acid, and D-penicillamine, enhance clearance by binding Pb and promoting urinary clearance. Dimercaprol is another reasonably effective Pb chelator, but it predominantly enhances biliary Pb excretion.

17.6 KINETICS

Basic to an understanding of the effects of Pb exposure on animals and humans is an appreciation of its kinetics in living animals. This requires recognition and knowledge of the various phases of Pb kinetics, including absorption, distribution, and clearance. Although Pb may exist in the body in various ionic forms and compounds, it is not properly considered, as a material, to be "metabolized" by the body. Rather, it is transported by various more or less metabolically active complexes and compounds. Useful terms to describe this distribution are Pb kinetics and biokinetics.

Several diverse approaches have been used to assess Pb kinetics in mammals, but all of the approaches have produced similar conclusions. The most important of these is that delineation of Pb kinetics requires a multi-compartment model, with some compartments being rather large and relatively stable, whereas other compartments are smaller and comparatively labile. Also, it appears most likely that the Pb kinetics at lower concentrations of exposure may be considered linear, whereas at high exposure concentrations, they appear to be nonlinear. This has potentially important implications in considering the biological effects of Pb at varying exposure levels.

Several types of approaches have been used to investigate the Pb biokinetics. Such approaches mainly include the following:

1. Exposure of both experimental animals and human subjects to Pb orally or via inhalation, wherein increasing amounts of Pb are introduced and the resulting accumulation and excretion of Pb are measured by a variety of methods.
2. Introduction of radioactive or stable Pb isotopes to determine kinetics without increasing exposure and disturbance of the steady-state situation.
3. Study of the spontaneous Pb clearance in situations where exposure to a high concentration has been terminated.
4. Study of the Pb distribution in various tissues by postmortem examination.

The last approach has been very useful in animal studies, but little used in studying Pb distribution in humans. It is important to remember that there is notable interspecies variation with regard to the Pb kinetics due to many factors, for example, diet, physiology, and relative tissue mass. For these reasons, it is clear that studies of animals must be viewed with great caution in attempting to understand human kinetics. Since the greatest concern is with human exposure, human studies are emphasized below.

The earliest studies of Pb balance in humans and animals were by Aub et al. (1925), who used several of the above-noted approaches in a series of studies of Pb exposures of animals by both inhalation and ingestion. Aub concluded that Pb was most readily absorbed from the respiratory tract, particularly by the inhalation of "finely divided particles," and he noted that Pb was somewhat less well absorbed by ingestion. In his animal studies, Aub also measured Pb concentrations in a variety of tissues after exposure to determine distribution within the body. In addition, he performed autopsies on human bodies to evaluate the internal Pb distribution. Aub concluded that, especially after termination of exposure, all the Pb is "permanently" stored in bone and, accordingly, that Pb was harmless to a person unless there was "recent absorption from an external source or mobilization of a skeletal store." He conducted extensive further studies of Pb excretion in animal and human subjects and found spontaneous excretion to be very low and variable, but noted that

Ca-deficient diets and administration of acids markedly increased Pb excretion (especially if both factors are applicable at the same time).

Kehoe (1961) subsequently conducted a landmark series of experiments that involved long-term Pb exposure of human volunteers by inhalation and ingestion. The subjects were observed prior to, and subsequent to, exposure to determine both baseline Pb balance and balance during a "recovery" stage from exposure. Kehoe's studies were summarized by Gross et al. (1975), who made several important observations consistent with Aub's earlier findings. For example, there was considerable variability of observations within the subjects during the control period, which appeared to relate, at least in part, to variability in dietary Pb exposure. Also, these variations in ingested Pb (about a mean of ~191 μg/day) were paralleled by variations in fecal and urine Pb, with an overall net negative Pb balance (in most subjects), as calculated by intake versus urine and stool output. It is not clear whether this reflected an actual net negative balance or was simply a result of not being able to measure airborne Pb exposures during the control period.

A later study by Rabinowitz et al. (1976) clarified our understanding of absorption and distribution of Pb in the body. The results of this study, among the most carefully obtained data available, have been extensively used by the subsequent kineticists. Rabinowitz et al. (1976) studied "normal" volunteers under standard conditions of diet and activity. These volunteers were fed a low Pb diet supplemented to approximate their usual level of intake by addition of ^{204}Pb as a tracer. Since this isotope is rare in most usual sources of exposure, it provided a stable tracer for purposes of defining the kinetics of Pb in the body. Pb isotope distributions in samples were determined by mass spectroscopy. These adult volunteers had pre-study blood Pb concentrations of 16–25 μg/dL and had intakes of Pb between 156 and 215 μg/day during the study, which approximated their pre-study intake. One of the five subjects was studied for 10 days. The other four were studied for longer periods, ranging from 108 to 210 days. The range of absorption observed was 6.5–13.7% of the ingested dose. Of this absorbed Pb, 54–78% was excreted in the urine, with most of the rest being excreted via bile and integumentary losses.

The translocation of the tracer Pb was best described by a three-compartment kinetic model. The first compartment, which included 1.5–2.2 times the amount of Pb in blood, contained an average of 1900 μg of Pb and was turned over in about 36 days. A second one, which comprised most of the soft tissue Pb, contained about 600 μg and was turned over every 40 days. These authors noted that the total of less than 3 μg of Pb in these two labile pools was much less than the 10–30 μg found in autopsy studies, suggesting that most of the soft tissue Pb must have been in a more stable compartment. The third, large and comparatively stable, compartment was primarily composed of bone Pb. In these subjects, it contained about 200 μg of Pb and was turned over approximately every 10^4 days.

Of interest with regard to this pool was the comparison of total Pb and tracer Pb to total Pb ratios in cortical and trabecular bone from the iliac crest. The total Pb concentration in the cortical bone by weight was approximately twice that in trabecular bone. However, trabecular bone had a two- to threefold greater ratio of tracer to total Pb, suggesting that it was turned over much more rapidly than the cortical bone. Of further interest was their calculation, based on these measurements, that the iliac bone received Pb three to seven times more rapidly than did the very stable pool as a whole. These observations make it clear that bone cannot be regarded physiologically as a single pool. Furthermore, Rabinowitz et al. (1976) emphasized that all of the pools are in dynamic equilibrium with each other and, therefore, any changes in the movement of Pb from one to another can cause significant

changes in the measured amount. Factors that might cause movement of Pb from the large stable pool into blood, and hence to target soft tissues, are of particular interest.

Steenhout (1982) developed a kinetic model of Pb distribution based on data regarding tooth Pb in children and adults in three Belgian regions. Her model, basically consistent with the Rabinowitz model, suggested that the rate of transfer of Pb to teeth is 1.85 ppm/year/μg/100 mL blood. According to the model, Pb accumulation in teeth and dense (cortical) bone is linear and continuous over age, suggesting very slow loss from dense bone (approximately 0–0.005 ppm/year/μg/100 mL). In contrast, estimates of Pb loss from "porotic" bones, such as ribs and vertebrae, are on the order of 0.06 ppm/year/μg/100 mL. Steenhout concluded that the apparent nonlinearity of Pb transfer in some other studies reflected this relatively rapid Pb loss from such porotic bone. She also suggested that her data support the concept that for dense bone, there is no Pb loss with increasing age and, therefore, the osteoporosis of age should not represent a risk for Pb mobilization.

Chamberlain (1985) used data from a variety of data sets, including some from other investigators, as well as some he had developed to assess several aspects of Pb kinetics in humans. He focused primarily on volunteer feeding experiments to observe the response of blood Pb to either airborne exposure or to dietary intake. His discussion concerned largely inorganic Pb and relatively short-term studies. He found that Pb is absorbed rapidly into plasma and then into extracellular fluid in minutes, based on experiments with the injection of radioactive tracers. He noted that in a time frame measured in tens of minutes, Pb in plasma, and Pb from extracellular fluid via plasma, becomes largely bound to RBC. Approximately 58% of a Pb dose was bound to red cells after 20 h.

Chamberlain (1985) also found that Pb excretion after a single dose occurs over a month and that Pb storage in tissues and bone persists for months to years. He further noted that Pb accumulation and distribution differed in several ways from those of Sr. Most importantly, the attachment of Pb to red cells appeared to retard, rather than to promote, the distribution of Pb to storage sites. Chamberlain's autopsy studies showed that relative to "dose," there is more Pb than Sr or Ca in soft tissues. All the studies Chamberlain reviewed agreed that Pb transfer to excreta from blood occurs over a period of about one month. He also noted that at low-level exposures, urinary excretion is two or more times greater than excretion via stool. In his discussion of Pb transfer to bone, he analyzed both the discrepancies and consistencies among available data sets. Of most importance for kinetic modeling was that the Pb concentration in trabecular bone was similar to that in cortical bone, while, in other data sets, the Pb in trabecular bone was much higher than in the cortical bone. Some of this discrepancy may have been related to the study duration, since short-term (vs. longer) studies may show relatively more Pb in the presumably more labile trabecular pool. Chamberlain's resorption rates from storage in bone were inferred indirectly, that is, based on studies of Sr turnover, given the assumption that the rates do not significantly differ for various trace minerals. However, considering his review of differences between blood, plasma, and tissue distribution of Sr and Pb, this assumption may not be valid.

Based on these studies, Chamberlain (1985) suggested a mean life of Pb to be 12.5 years in trabecular bone and 50 years in cortical bone. His estimate for the mean life of Pb in soft tissues, derived from autopsy data, was 500 days. The relationship between urinary clearance and blood Pb appeared to be constant in the range of "normal" blood Pb concentrations, but to increase proportionately at blood Pb levels above 20–25 μg/dL. This implied a decreasing apparent relative uptake with increasing blood Pb. Chamberlain reviewed some data on intestinal absorption, noting that uptake of soluble Pb tracers was markedly

affected by a period of fasting, with an average (in several studies) of 8% uptake when Pb was taken with a meal and 60% when taken after an overnight fast, when the fast continued for several hours after the Pb ingestion. He noted that insoluble Pb sulfide absorption was less affected by fasting (12% absorbed in fasting vs. 6% with meals). He also reviewed data showing that the addition of Ca and phosphorus (P) salts markedly decreases the absorption of soluble Pb. Chemical incorporation of Pb with foodstuffs did not alter Pb absorption below the levels observed when Pb was administered with another metal.

The discussion of airborne Pb exposure by Chamberlain (1985) focused on three major factors of exposure to inorganic Pb only: (1) airborne Pb concentration, (2) ventilation rate/volume, and (3) fractional distribution of the aerosols. He did not consider airborne exposure to organic Pb. The particle size in the inhaled aerosol and the "residence time" in the pulmonary region, determined largely by respiratory rate, appeared to be relatively unimportant. Once retained in the lungs, the deposited Pb was essentially completely absorbed into the bloodstream within 24 h. Many larger particles, deposited higher in the conductive airways, were returned by mucociliary clearance to the pharynx and thereafter ingested, reducing this mainly to the case of ingested inorganic Pb.

Bernard (1977) suggested a somewhat more complex model. His "reference man" had a total Pb body burden of ~120 mg. Of this total burden, ~110 mg was in bone and the rest in soft tissues. His model proposed at least two bone pools, a slow pool in cortical bone and a relatively labile pool in trabecular bone. He further proposed two soft tissue pools, one of which was relatively large and slow and another was quite large and very rapidly turned over. Although this model was logical, and based on experimental observations, its actual validity is subject to question because it was based exclusively on studies in rats and non-human primates, which may markedly differ physiologically from humans.

Subsequently, Schutz et al. (1987a) observed the decline of blood Pb after termination of occupational exposure by studying two separate and somewhat different groups. The first group included workers who no longer worked in the Pb industry, whereas the second included those who were removed from work due to a blood Pb increase to above 3 μmol/L (~60 μg/dL). The first group was older, had longer periods of exposure, and generally had lower mean blood Pb levels than the second. The subjects also had bone Pb concentrations estimated by the use of X-ray fluorescence (XRF) of the middle phalanx of the left index finger. Schutz et al. (1987a) found a two-compartment model to provide a satisfactory fit, with the "fast" compartment having a half-life of 30 days and the "slow" compartment a half-time of ~5.6 years. The notable intersubject variation was suggested to represent "considerable variation in risk at a given exposure level." The "slow" pool was turned over somewhat more rapidly than other reported rates. The bone Pb observations by XRF correlated positively with estimates of the slow pool, but the coefficient of correlation was rather low ($r = 0.36$). Schutz hypothesized that the slow pool may have actually consisted of a combination of two bone pools, one of trabecular and the other of cortical bone Pb. This hypothesis was used to explain why the measurement of bone Pb somewhat differed between the two groups. He suggested that this difference may have related to differing proportions of Pb in the cortical versus trabecular bone. If he was correct, it has implications to the widespread application of noninvasive methods of bone Pb measurement to research and clinical assessments.

The biological redistribution of Pb pools within the body has been described in a series of reports based on the evaluation of differences between stable isotope profiles of body Pb compartments in separate geographic locations and during different life periods. Earlier investigation by Manton (1977, 1985) described the process. Further research was

conducted by Gulson et al. (1995, 1997), who investigated a cohort of adult women who immigrated to Australia from Eastern Europe and Russia during the early 1990s. They had accumulated tissue Pb in Europe with a stable isotope ratio distinctly different from that in Australia. These differences enabled them, through meticulous measurement of stable isotope ratios via thermal ionization mass spectroscopy, to identify the proportion of blood Pb from the contemporaneous environment and that from tissue Pb stores accumulated earlier in Europe. Among these young adult women, 45–70% of blood Pb came from the long-term tissue stores, presumably in bone. These proportions occurred at blood Pb concentrations that averaged ~5 μg/dL as the result of environmental Pb exposures typical of developed countries where steps had been taken to restrict Pb exposures.

The above study also aimed to determine the influence of pregnancy and lactation on this mobilization. During pregnancy, blood Pb concentrations in these subjects increased by about 20%, on average, with individual changes ranging from ~14 to 83% (Gulson et al., 1997). Among those subjects whose blood Pb levels increased during pregnancy, the mean increase in mobilization of long-term tissue Pb stores varied from 26 to 99%, averaging ~30% (Gulson et al., 1997). Skeletal Pb mobilization continued to be elevated after the pregnancy. Observations for infants born to these mothers showed that the long-term tissue Pb in the mothers had been transferred to the fetus and that among those infants who were breast-fed, additional Pb transfer continued to occur during breast-feeding (Gulson et al., 1998a, 1998c). The transfer of maternal skeletal Pb to the fetus as shown by stable isotope analyses was also confirmed among nonhuman primates (Franklin et al., 1997).

Barry (1985) measured Pb concentrations in tissues of 129 subjects at autopsy and presented extensive data on Pb content in various tissues. Consistent with other studies, the content of bone Pb was much higher than in soft tissues, and levels of Pb in dense bone were much higher than in more cancellous bone. For example, petrous bone had the highest levels, and ribs the lowest (by an approximate ratio of 4:1), a finding important in understanding the generally nonhomogeneous distribution of Pb in bones. Barry noted that in those soft tissues with the higher amounts, the concentrations in males exceeded those in females by ~30%. He stated that soft tissue Pb concentrations increased with age only through the second decade of life and were thereafter stable. Children were reported to have soft tissue concentrations similar to adult females, but had much lower bone concentrations. He also stated that in adults, over 90% of the Pb was in bone, with >70% being in dense bone. Among occupationally exposed adult males, 97% was in bone.

Barry noted that the increase of bone Pb with age, with respect to stable soft tissue levels, was consistent with the hypothesis that bone Pb is not available to soft tissues. He also indicated that the lack of decline in the bone Pb, in the face of demineralization with increasing age, suggested that Pb was not mobilizable even under conditions of massive Ca turnover. However, Barry expressed bone Pb changes with age only as concentration and not as total Pb, and he ignored the fact that demineralized bone would have decreased total mass. Thus, the apparent constant concentration of Pb in bone may actually reflect a markedly decreased total amount of Pb in bone.

Predicting quantities of Pb mobilized from bone requires bone Pb data for both cortical and trabecular bone. Data sets providing bone Pb among adults and children described above were obtained during time periods in which environmental Pb exposures were much higher. Drasch (1974, 1997), Drasch et al. (1987), and Drasch and Ott (1988) obtained data on bone Pb in adults for cases coming to autopsy in Munich between the early 1970s and 1994 for subjects living in the same geographic vicinity in southern Germany. Between 1974 and 1994, trabecular bone Pb decreased from 2.5 μg/kg (1974) to 1.7 μg/kg (1984) to

0.7 µg/kg (1994). Compact bone Pb decreased from 5.5 µg/kg (1984) to 2.8 µg/kg (1994). Changes in bone Pb can be expected to be even more dramatic among young children, who (unlike adults) do not have long-term stores of Pb accumulated during decades of much higher Pb exposures.

Drawing upon the above types of advances in regard to Pb kinetics and associated modeling thereof, substantial further progress was made in developing and refining biokinetic model systems to project likely increased risk of Pb-related toxicity in human population groups due to various exposure scenarios. Such biokinetic models involve stipulation of mathematical relationships among biological processes (e.g., absorption, distribution, redistribution, clearance, elimination) that determine variations in internal concentrations of the metal and associated potential for causing toxic effects. In recent decades, progress has been made in developing various biokinetic models for predicting "blood Pb" as the most widely accepted internal biomarker (discussed subsequently) traditionally used to index Pb exposure/dose and to gauge consequent potential for Pb-related pathophysiological responses. Examples of progress made in such modeling efforts include those of Marcus (1985a, 1985b, 1985c), in which available data sets were used to derive multi-compartment kinetic models for Pb, and that of Bert et al. (1989) who developed a compartmental model for adult males. Leggett (1993) published an age-specific biokinetic model for Pb that was developed originally for the International Commission on Radiological Protection (ICRP), but was later expanded to include additional features of use for Pb as a chemical toxicant. In it, the Pb transport between compartments was assumed to follow linear first-order kinetics, provided that the Pb concentrations in RBC remained below a nonlinear threshold level. A nonlinear relationship between plasma Pb and RBC Pb was modeled for concentrations above that level. Several other physiologically based models for bone-seeking elements published by O'Flaherty (1993, 1995, 1998) utilized information about age dependence of bone formation rate and take into account increasing localization of bone remodeling activity with age.

In addition to the above models, EPA developed a widely used "Integrated Exposure Uptake Biokinetic" (IEUBK) Model for Lead in Children (EPA, 1994a, 1994b; Hogan et al., 1998; White et al., 1998). As noted in chapter 4 of EPA (2006), the IEUBK Model simulates Pb exposure and biokinetics from birth to age 7 years and predicts quasi-steady-state average blood Pb concentrations corresponding to daily average Pb exposures averaged over periods of one or more years. Composed of four subcomponent models (an exposure model, an uptake model, a biokinetic model, and a blood Pb probability model), the IEUBK Model (1) calculates average daily Pb intakes (µg/day) for each exposure concentration (or rates) for different multimedia exposure routes (via air, water, diet, dust, or soil); (2) converts the media-specific intake rates (calculated from the exposure model) into media-specific time-averaged rates of uptake (µg/day) into blood plasma as the central compartment, followed by (3) biokinetic modeling that simulates transfer of absorbed Pb between blood and other body tissues (bone, brain, kidney, etc.) and excretion from the body (via urine, feces, skin, hair, or nails), and predicts an average blood Pb concentration for the exposure time period of interest; and (4) utilizes a blood Pb probability submodel that applies a lognormal distribution (with specific geometric mean/geometric standard deviation parameters) to predict probabilities for the resulting occurrence of a specified blood Pb concentration in a population of similarly exposed children.

Such models have had varied success in predicting blood Pb concentrations. Typically, such models have been more successful in predicting mean/median blood Pb than the overall distribution of blood levels. Dose-dependent differences in fractional absorption

and distribution of Pb complicate application. Person-to-person variabilities (intensity of hand-to-mouth activity, nutritional status, etc.) can also modify the relationships between external (environmental) Pb doses and internal (blood, bone, soft tissue) concentrations. Among those models that recognize the importance of bone Pb contribution to blood Pb, the very limited available data on contemporary bone Pb needs to be more fully addressed. The availability of pooled analyses of epidemiological data from childhood Pb studies in the United States identified Pb-contaminated dust loadings within the residence as a very strong predictor of blood Pb among children (Lanphear et al., 1998). Further, the child's age, race, mouthing behaviors, and study- or site-specific factors are influential in predicting blood Pb at a given level of exposure (Lanphear et al., 1998).

Analysis of available information on Pb kinetics revealed some problems in using the available models to study Pb in the mammals. Many of the models fit observed data for Pb absorption, distribution, and excretion reasonably well and often described observed changes in blood Pb quite well. However, available models are generally not fully adequate for purposes of anatomic/physiologic description and prediction, thus limiting their usefulness in devising experimental models or developing useful approaches for clinical diagnosis/management of Pb intoxication. For example, the assertion that bone Pb is a single homogeneous pool, or two relatively homogeneous pools composed of cortical and trabecular bone, does not comport well with available data. The Rabinowitz models, and others derived from his data, suggest that the most stable pool, thought to be largely bone, had a mean life of 30–50 years (Kehoe, 1961; Rabinowitz et al., 1976; Chamberlain, 1985; Cory-Slechta et al., 1987; Kazantzis, 1988; Simons, 1989). However, when studied directly by bone biopsy (Rabinowitz et al., 1976), the same subjects appeared to have much more rapid trabecular and cortical bone turnover than predicted. The clear conclusion is that either the iliac bone is not part of the stable pool or that there must be exceptionally stable portions of the bone pool that outweigh the relative lability of iliac bone. In either case, bone is not a single homogeneous pool nor is either major type of bone (cortical or trabecular) likely to represent a homogeneous Pb pool.

17.7 BIOMARKERS

An evaluation of potentially useful biomarkers of Pb exposure requires consideration of a variety of specific issues. In common with all toxic exposures is the basic dose–response issue. In any such system, if dose–response characteristics are typical, well defined, and predictable, it makes little difference whether one focuses on measuring the dose or prefers to focus on a response variable. In the case of Pb, which has many diverse effects, it is usually necessary to define the response variable(s) of most importance to the investigator.

In the case of mammalian (particularly human) studies, it is often useful to try to define the "critical" organ, tissue, or system. This definition presupposes that it is indeed possible to define the most sensitive, or most important, effect of the toxic agent. In the case of Pb toxicity, especially childhood Pb toxicity, the nervous system has been most commonly identified as being the "critical" organ or organ system. To the extent that the nervous system is accepted as the critical organ system, then any indicator of Pb exposure/dose, whether measurement of Pb *per se* in one or another tissue component or of some closely varying biological response, should closely define or reflect the extent of nervous system exposure to Pb. Ideally, any response variable(s) used as the Pb exposure/dose marker(s) should be based on nervous system toxicity or, at least, strongly correlate with

nervous system toxicity. Also, it is often more difficult to detect a response in an individual than it is for groups of individuals. Hence, some particular measures of dose and/or response that may be useful in epidemiological studies may not necessarily aid much in the diagnostic categorization of the individual subject. An in-depth analysis of analytical methods, important considerations in selection of Pb biomarkers, and interpretation of data for sensitive populations (e.g., maternal/fetal pairs, women of childbearing age, infants, and young children) was published by the NRC Committee on Measuring Lead in Critical Populations (1993).

Pb exposure occurs via multiple routes, at variable external dose rates, and results in variable absorption depending on route(s) of absorption and other factors (described elsewhere in this chapter). It is, therefore, generally very difficult to directly specify the Pb "dose" to which a subject is (or has been) exposed. The exception to this has been studies using stable radioactive Pb tracers, wherein the absorbed dose can be quite carefully calculated by isotope dilution methods (Rabinowitz et al., 1976) or through careful measurement of diverse external sources (e.g., see Gulson et al., 1996). With the exception of such tracer methods, approaches to characterizing Pb dosage in living subjects have generally been limited to measurements in relatively easily obtained biological samples. All such methods encounter difficulties in sample contamination and analytical technique because of the very small amounts of Pb typically found in the samples.

Measurement of whole blood Pb has been most widely used, but has been criticized on practical and theoretical grounds. Difficulties potentially arising from contamination, or due to technical aspects of measurement, have largely been averted when analyses are done by a competent laboratory, and by the late 1990s, many laboratories could accurately detect Pb concentrations <1 μg/dL. On the other hand, blood Pb represents only a small fraction of the total Pb body burden, and there is extensive turnover in the body. Thus, the blood Pb at any given point of time can be considered to reflect both current and longer-term past Pb exposure (Mushak, 1989; Gulson et al., 1995, 1997, 1998a, 1998b, 1998c; Smith et al., 1996). It has been posited that blood plasma Pb is a better measure of Pb available for internal transport to target tissues (e.g., neural tissues) and that such Pb may, therefore, be a preferable measure of Pb dose. However, it has been very hard to measure Pb in plasma free of contamination, mainly due to the destruction of red cells in preparation of plasma samples. Because more than 95% of Pb in blood is in red cells (DeSilva, 1981), even a small degree of contamination by red cell material could markedly alter plasma Pb results.

Urine Pb reflects Pb in blood in the sense that their stable Pb isotope profiles are highly correlated, but Pb concentrations in these two biological fluids are typically only weakly correlated (Gulson et al., 1998b). Thus, urine Pb concentrations cannot serve to predict blood Pb concentrations, particularly at exposures associated with values <10 μg/dL. Measurements of Pb in urine may pose significant problems for a variety of reasons. First, urine Pb has a complex relationship with Pb dose. Also, the concentration of urine itself is affected by fluid intake. Thus, the variability of urinary Pb excretion during the day has been found to be rather problematic (Gulson et al., 1998b). Variations in the volume of urine itself (related to fluid intake) and the marked variability of urine Pb excretion throughout the day mandate careful quantitative urine collections, which can be quite difficult to accomplish for children and experimental animals. Yet another problem is the contamination of urine samples with feces or other bodily discharges.

A variant of urine Pb measurement, often used diagnostically, but infrequently in research studies, is the measurement of the amount of Pb in urine after administration of a chelating agent. Such measurements presumably sample a larger pool of Pb than

unstimulated urinary excretion and have been held to define the "chelatable" pool of lead that should be available for further therapeutic chelation therapy. However, the "chelatable" pool varies with the agent used, and it is not particularly clear, in any real sense, as to what the "chelatable" pool represents biologically, or how it can be directly related to response measurements.

Because, with few exceptions, it is not routinely possible to directly sample body tissues other than blood or urine in living subjects, the measurement of Pb in samples of other tissues has generally seen only very limited use. Pb in the dentine of teeth represents a stable pool of reflecting cumulative past Pb exposure, and the measurement of Pb in dentine of shed deciduous teeth has been used for some research on the effects of early Pb exposure in children (Needleman et al., 1979). Also, bone biopsies have occasionally been used to assess Pb in the more stable and larger pools in the body (Rabinowitz et al., 1976; Aufderheide and Wittmers, 1992). Lastly, although sporadic efforts have also been made to use samples such as hair, nails, or saliva to assess Pb exposure or dose in living subjects, all have been found to be unsatisfactory for general use for one reason or another and, at best, have only very limited applicability.

Most direct Pb measurements in internal organs and tissues have been done on autopsy subjects (e.g., Drasch, 1974, 1997; Barry, 1975, 1981; Drasch et al., 1987; Drasch and Ott, 1988). However, the development of methods for noninvasive Pb measurement in tissues of living subjects has emerged as a potentially viable alternative means for assessing Pb levels in internal organs. In particular, the development of approaches to measurement in bone Pb in living subjects has progressed (Nordberg et al., 1991). The human skeleton contains the great majority of Pb body burden. The inactivity of the skeletal Pb deposits was thought to reflect a very long half-time in bone, and it was generally assumed that bone was homogeneous as a Pb compartment and that the very long half-time would greatly delay Pb transfer from bone back to other tissues. However, based on data from stable isotope studies, this is not a defensible concept. Bone is a set of compartments for Pb deposition, but also a target of Pb toxicity, with at least two kinetic compartments. Trabecular (spongy) bone Pb is more mobile than Pb stored in long, dense, or cortical bone (Skerfving, 1998). Also chelatable Pb is well correlated with trabecular, but less so with cortical bone (Schutz et al., 1987a, 1987b). In adults, long-term tissue (largely bone) stores of Pb contributed between 50 and 75% of the Pb present in blood (Gulson et al., 1995, 1997; Smith et al., 1996). Young children, due to constant skeletal turnover during physiological remodeling processes that accompany somatic growth, recycle Pb between bone and other tissue compartments. Rosen et al. (1989) reported that cortical bone (e.g., tibia) Pb is correlated with, and predictive of, chelatable Pb.

XRF methods were developed to measure Pb in bone noninvasively (NRC, 1993). Two general groups of XRF techniques can be distinguished based on their sampling of the fluorescence emitted either by K-shell or by L-shell electrons following radiation from an X-ray machine or other radiation sources. Such XRF methods have gained some use in epidemiological studies (e.g., see Hu, 1998), having been applied most successfully to groups with high Pb exposures, for example, for persons living in high Pb exposure environments, those occupationally exposed, or overtly Pb-poisoned children. One concern is that the quantitation limits and precision of the XRF instruments may be too high for use in a general population (Rosen and Pounds, 1998). The rate of improvement in quantitation limits and precision of these instruments appears slower than the rate of declining levels of Pb bone burden in many countries.

A wide range of methods have been employed, with varying success, to define Pb biological effects. Such methods range from *in vitro* biochemical testing to observation and measurement of behavioral attributes of exposed subjects. Among the widely diverse array of biological effects caused by Pb, the impairment of heme synthesis has long been recognized as being a key class of pathophysiological effects highly responsive to variations in Pb exposure/dose. With the reliable detection of Pb-induced impacts at several points along the heme synthesis pathway in RBC being facilitated by sampling of readily available blood in living subjects, certain indicators of Pb-induced impairment at salient points in the heme synthesis pathway were used for many years as biochemical measures (i.e., biomarkers) of Pb exposure and/or toxicity. Particular emphasis has been placed on the inhibition of two enzymes in the heme biosynthetic pathway, porphobilinogen synthetase and heme synthetase (Piomelli, 1973; Sassa et al., 1973). Porphobilinogen synthetase was measured by activity levels and characterized by electrophoresis as to its phenotypic variability (Doss et al., 1982). Inhibition of heme synthetase, as a mitochondrial-bound and dependent enzyme, was gauged primarily by the accumulation of its porphyrin precursors, especially protoporphyrin IX. Accumulation of zinc protoporphyrin is strongly and logarithmically correlated with blood Pb concentrations in both children (e.g., Piomelli et al., 1973, 1982; Roels et al., 1976) and adults (e.g., Grandjean and Lintrup, 1978; Lilis et al., 1978). Among children, the threshold for response is thought to fall within a blood Pb concentration range of 15–20 μg/dL whole blood (Piomelli et al., 1982; Hammond et al., 1985). Analysis of free erythrocyte protoporphyrin (FEP) had previously been used in screening children to identify Pb poisoning. However, because accumulation of protoporphyrin is also seen with iron deficiency, increased FEP is not a change specific only to Pb, and because Fe deficiency is sufficiently common among Pb-exposed populations, the measurement of erythrocyte protoporphyrin (EP) poorly distinguishes between Fe deficiency and Pb excess (Mahaffey and Annest, 1986). This, as well as the threshold for EP change being higher than for blood Pb levels now recognized to be of concern based on neurobehavioral changes, has led to curtailing of use of EP as a Pb exposure screening method.

Another area of biochemical investigation, that is, the evaluation of levels of neurochemical mediators in blood and urine, has also been of some interest, but such markers have not produced, to date, reliable and valid measures of response to Pb exposure, at least in regard to neurological effects of Pb. It remains to be seen if any other types of Pb-induced changes in signature indicators of Pb-related neurotoxicity, especially any of those resulting from decreasingly lower Pb exposure levels, may emerge as useful biomarkers. One possibility might be the use of certain electrophysiological changes, for example, altered brainstem auditory evoked potentials (BAEPs) (as discussed in the next section). Or, perhaps, further advances in the pioneering use of magnetic resonance imaging (MRI) or of magnetic resonance spectroscopy (MRS) methods to detect Pb-induced neurotoxic effects (as discussed below) may ultimately generate new results that may prove to be useful in deriving new Pb biomarkers.

17.8 HEALTH EFFECTS

As noted earlier, it has long been known that high-level Pb exposures can cause quite serious health effects in human children and adults. Such effects include severe neurological, renal, and hematological impairments that typify classically defined Pb “poisoning”

or "intoxication." However, extensive research has led to the recognition that Pb exerts notable impacts on many different tissues and organ systems, including some impacts seen at very low exposure levels, extending down to only slightly above current U.S. population means. This has been accompanied by parallel shifts in public health protection/medical attention to focus on reducing human Pb exposures as the primary approach to dealing with Pb as a continuing important public health issue. Reflecting this widespread shift of public health/medical interest in many countries, key emphasis is accordingly placed here on the discussion of health effects induced by low-level Pb exposures. Rather than attempting to summarize the entire broad scope of documented Pb effects, the discussion focuses most strongly on neurotoxic impacts that, as an overall class, have come to be seen as key "signature" or "critical" effects of low-level Pb exposures. Examples of demonstrated low-level Pb exposure impacts on other organ systems (e.g., renal, cardiovascular, immune) are also highlighted.

17.8.1 Neurotoxic Effects of Lead

Among the best-known and most widely recognized "subclinical" impacts of low-level Pb exposures are decrements in IQ and impacts on other global measures of neurocognitive abilities. Following pioneering work in the 1970s, for example, by Needleman et al. (1979), numerous epidemiological studies during the 1980s and 1990s evaluated Pb effects on the higher-order integrated neurological functions (as indexed by impacts on intelligence measures, perceptual-motor coordination, and various other neurobehavioral endpoints). Such studies (e.g., see Bellinger et al., 1986; Dietrich et al., 1990, 1993a, 1993b; Baghurst et al., 1992; Wasserman et al., 1997) substantiated that Pb has adverse neurocognitive effects at very low levels of exposure, especially of the fetus and infant in multiple cultures. Several major prospective, longitudinal epidemiological studies have shown impaired intellectual functioning in childhood following increases in blood Pb across a range of approximately 10–30 μg/dL whole blood, even after control for social and demographic conditions associated both with Pb exposures and with lowered (slowed) development scores (Bellinger et al., 1986; Baghurst et al., 1992; Dietrich et al., 1993a, 1993b; Wasserman et al., 1997). These studies found an approximate 4–6-point decrease in subsequent IQ (when measured at about age of 6 or 7 years, but not earlier). Of interest, analogous to some earlier reports by Baghurst et al. (1995) and Dietrich et al. (1993) of impairment of visual-motor integration at this range of exposures, Pb impacts on perceptual-motor skills were also found by Wasserman et al. (1997) and were suggested as possibly being more sensitive to Pb exposure than language-related aspects of intelligence. Despite the demonstration of neurocognitive effects across geographic areas, social class, and cultures by these studies, it should be noted that such findings were not obtained by all other studies.

Studies that found evidence of impaired cognitive abilities associated with low-level Pb exposures of children identified neurotoxic effects of Pb in children and adults were reviewed in EPA (2006) and in the overview of neurotoxic effects of Pb in humans by Bellinger (2008). As discussed in EPA (2006), in the largest available cross-sectional study, Lanphear et al. (2000) found relationships between the blood Pb concentrations and the cognitive deficits in a nationally representative sample of 4853 U.S. children (all NHANES III participants), aged 6–16 years (having a geometric mean blood Pb of 1.9 μg/dL, with 97.9% being <10 μg/dL). In multivariate analyses, significant covariate-adjusted associations were found between the blood Pb levels and the two subtests for visual-motor skills

and for short-term and working memory for all children and for those with blood Pb <10 μg/dL, as well as with the visual-motor subtest for children with blood Pb <7.5 μg/dL. EPA (2006) also noted that numerous other longitudinal studies were consistently observed effects on IQ in children at blood lead levels <10 μg/dL. Perhaps of most importance, a large international pooled analysis of 1333 children from seven different cohorts by Lanphear et al. (2005) was highlighted as estimating a 6.2-point decline in full-scale IQ per increase in blood Pb from 1 to 10 μg/dL. Across several of these studies, there were nonlinear relationships between blood Pb and IQ or other neurobehavioral outcomes, with larger impacts being seen per unit increase in blood Pb for levels below 10 μg/dL than those above that level.

This otherwise nonintuitive dose–response relationship may be plausible, as noted by EPA (2006), if different underlying biological mechanisms (e.g., early CNS neurodevelopmental processes) are initially affected at relatively lower Pb exposures than other processes that may be disrupted in producing classic indicators of frank Pb poisoning, with the dominant mechanisms at low exposure levels perhaps being very rapidly saturated versus less rapidly saturated mechanisms becoming predominant at higher exposure levels. However, it should be noted that although the reported nonlinear relationship between Pb effects and neurocognitive functions may have gained wide acceptance, some associated controversy remains, as reflected by Bowers and Beck (2006) and the ensuing series of comments published in *Neurotoxicology*, Vol. 27, 2006, and Vol. 28, 2007.

Other neurocognitive changes and long-term educational, behavioral, and social consequences of low-level Pb exposure have also been identified. Bellinger et al. (1992) reported a higher rate of retention in grade and other results reflecting learning difficulties among higher blood Pb children. Other studies, also summarized in EPA (2006), confirmed analogous low-level Pb exposure impacts on academic achievement. Lanphear et al. (2000) used multiple linear regression analyses of standardized academic achievement measures for the 4853 NHANES III children aged 6–16 years (mean blood lead, 1.9 μg/dL) and found blood Pb to be significantly related to decrements in both reading and arithmetic achievement scores. Such decrements were also found in analyses stratified by blood Pb to be inversely related to blood Pb for those children with concurrent blood Pb values <5 μg/dL. In yet another study noted by EPA (2006), from among 533 girls aged 6–12 years in Riyadh, Saudi Arabia (having a mean blood lead of 8.1 μg/dL), percentile of class rank was significantly associated with blood Pb level in a subset of those with blood lead levels <10 μg/dL (Al-Saleh et al., 2001). Also, significant associations were noted for blood Pb (mean of 11.4 μg/dL) and poorer math and vocabulary scores achieved by 594 second graders in Mexico, with segmented regression analyses showing the slope for the Pb effect to be significantly steeper for blood Pb <10 μg/dL (Téllez-Rojo et al., 2006). The effects on academic achievement seen in the above studies were statistically significant even after adjustment for IQ, thus raising the possibility (as posed by EPA, 2006) that the impairment of neurocognitive functions besides those indexed by global intelligence measures may contribute to Pb-induced impacts on learning and academic achievement. It should also be noted that academic achievement decrements seen in the above studies may be attributable to earlier (but unmeasured) higher pediatric blood Pb levels (that usually peak before 3 years of age, then decline).

As for Pb impacts on neurobehavioral endpoints besides the above global measures of intelligence or academic achievement, EPA (2006) noted that epidemiological studies have evaluated Pb effects on more specific cognitive abilities, for example, attention, memory, visual-spatial processing, and executive functions (impulse control, planning, other

integration of higher-order cognitive processes, etc.). Such studies were further noted as having shown relationships between blood Pb and impacts on attentional behaviors and executive function among cohorts of children (varying in age range from 4–5 years to 19–20 years), even in those cohorts with more than 80% of subjects having concurrent blood Pb values <10 μg/dL. Epidemiological studies have demonstrated childhood Pb exposure to be associated with disruptive/antisocial behavior, with such effects apparently persisting into adolescence and early adulthood. Needleman et al. (1996) found that Pb exposure increased risk for antisocial and delinquent behavior at 11 years of age. Dietrich et al. (2001) reported behavioral disturbance and/or delinquency among young adults to be significantly related to blood Pb measures obtained at various earlier time points (prenatally, at intervals during infancy and childhood, etc.) during participation in the Cincinnati prospective cohort Pb study. Analogous long-term effects were reported by Burns et al. (1999), who observed that increasing blood Pb across the range of 10–30 μg/dL adversely affected the behavioral and emotional development of children in the Port Pirie, Australia, cohort when evaluated at ages 11–13 years. In the same children, increasing blood Pb across the range of 10–20 μg/dL was associated at such ages with a three-point decline in mean IQ (Tong et al., 1996). Needleman et al. (2002) found bone Pb to be one of the strongest predictors of adjudicated delinquency among high-school-aged white and African-American subjects living in the Pittsburgh, PA, area. However, EPA (2006) indicated that Lidsky and Schneider (2003) noted that Pb affects numerous brain sites and processes involved in impulse control, and Needleman et al. (2002) proposed that Pb impacts on cognitive function and academic performance may indirectly contribute to antisocial behavior/delinquency.

In addition to the above types of effects on intelligence and other higher-order integrative functions, Pb also exerts effects on more basic levels of sensory, motor, and sensorimotor integrative functions in children. As noted by EPA (2006), epidemiological studies showed Pb effects on hearing thresholds and other features of auditory processing in children that appeared to persist into young adulthood, including (1) observation by Schwartz and Otto (1987) of significant elevations in pure tone hearing thresholds (at frequencies within the range of human speech) among more than 4500 NHANES II participants (aged 14–19 years); (2) replication by Schwartz and Otto (1991) of such findings in a sample of approximately 3000 subjects (6–19 years old) in the Hispanic Health and Nutrition Examination Survey (HHANES), even at blood Pb levels below 10 μg/dL; (3) observation by Dietrich et al. (1992) of associations between higher prenatal, neonatal, and later postnatal blood Pb concentrations in 215 children from the Cincinnati Lead Study and poorer scores on a test of central auditory processing (SCAN) at age of 5 years; and (4) demonstration by Osman et al. (1999) of significant associations between concurrent blood Pb levels and increased hearing thresholds among 155 children (aged 4–14 years) in Poland, which remained significant in analyses restricted to data for blood Pb levels <10 μg/dL. Bellinger (1995) suggested that such Pb-induced impacts on hearing and auditory processing may be a mechanism contributing to learning impairment by Pb. These studies indicated that such sensory effects occur at rather low exposure levels (e.g., blood Pb concentrations <10 μg/dL), although it should be noted again that some of the observed auditory effects could, potentially, derive from somewhat higher earlier peak blood Pb concentrations.

Several epidemiological studies have also characterized Pb-related neuromotor deficits at relatively low levels of exposure. Dietrich et al. (1993a, 1993b) reported that both

pre-and postnatal blood Pb concentrations were significantly related to poorer scores on tests of bilateral coordination, visual-motor control, upper limb speed and dexterity, fine motor control, and postural stability among Cincinnati Lead Study children at 6 years of age, with strongest associations being seen with concurrent Pb levels (mean of 10.1 μg/dL). Later, 78-month postnatal blood Pb levels were associated with poorer fine motor skills at the age of 16 years. Other studies were also noted as finding (1) associations between lifetime average blood Pb levels through 54 months of age and poorer fine motor and visual-motor function among Yugoslav children (Wasserman et al., 2000) and (2) significant associations between blood Pb values (mean 5.0 μg/dL) and increased reaction time, postural sway oscillations, and action tremor among 110 preschool Inuit children in Canada, even when data from the 10% of the children having blood Pb levels <10 μg/dL were excluded from analyses.

In addition to the above types of impacts, Pb has also been shown to affect various electrophysiological measures of sensory and motor neurological responses. Peripheral nerve conduction (Seppalainen et al., 1979) and visual (Araki et al., 1987) and auditory (Schwartz and Otto, 1987) brainstem responses were altered at relatively low Pb exposure levels. Other epidemiological findings of significant Pb-related impacts on visual-evoked potential interpeak latencies were seen (Altmann et al., 1998) in an environmentally exposed population of 6-year-old children, with mean blood Pb of 4.2 μg/dL and 95th percentile value of 8.9 μg/dL (Walkowiak et al., 1998).

Innovative approaches are being used to evaluate neural substrates and neurochemical processes potentially perturbed by Pb and possibly contributing to neurobehavioral impacts. EPA (2006) noted that MRI and MRS have come to be used to evaluate Pb-exposed children, with several studies comparing subjects with elevated blood Pb levels (<20 μg/dL) with control subjects (blood Pb <10 μg/dL). It was highlighted that although all subjects had normal MRI, the elevated Pb subjects showed that significant reductions in the ratios of N-acetylaspartate to creatine and phosphocreatine in frontal gray matter relative to the lower Pb control subjects (Trope et al., 2001) and reduced peak values of choline, creatine, and N-acetylaspartate were seen in all the four brain regions of the Pb-exposed children (Meng et al., 2005). One or the other such Pb effects could be related to lower neuronal density/neuronal loss, decreased cell membrane turnover, myelin alterations (possibly leading to CNS hypertrophy), or less neuronal cell viability. Also, in another study using functional MRI methods to follow a subsample of 48 young adults (aged 20–23 years) from the Cincinnati Lead Study, higher childhood average blood Pb levels were associated with reduced activation in Broca's area (a brain area involved in speech production) while performing an integrated verb-generating/finger tapping task (Cecil et al., 2005; Yuan et al., 2006).

The acute neurological damage in adults due to high levels of inorganic Pb exposure and neurological consequences of exposure to organic Pb compounds have been recognized for decades. Among the additional findings on adverse neurologic effects of Pb, including at exposure levels not previously recognized as harmful to the adult, is that of Hanninen et al. (1998), who reported that, in studies of workers whose blood Pb levels had never exceeded 2.4 μmol/L (50 μg/dL), Pb was associated with decrements in visual-spatial and visual-motor functions, verbal comprehension, and attention, as well as increased symptoms of impaired well-being as rated by psychological assessments of mood, whereas blood Pb increases of ~2.4 μmol/L (50 μg/dL) to 4.9 μmol/L (100 μg/dL) caused persisting, possibly permanent impairment of CNS function.

17.8.2 Other Effects

Adverse renal system effects have been described by Loghman-Adham (1997) in a review of high Pb exposure effects. Acute effects of exposure to high concentrations resulted in proximal tubule damage manifested by glycosuria and amino acid urea and overt nephropathy developed when blood Pb levels exceeded a threshold of 60 μg/dL [~2.9 μmol/L. However, early renal tubule dysfunction secondary to far lower Pb levels exposure was also indicated, by Loghman-Adham (1997), as having been detected by measuring urinary excretion of low-molecular-weight proteins, microglobulins, retinol-binding protein], or the lysosomal enzyme NAG, as well as other brush border proteins. A cross-sectional study by Fels et al. (1998) compared changes in urinary or serum markers of function or integrity of specific nephron segments in children with those having a mean blood Pb concentration of ~13 μg/dL showing increased excretion rates for prostaglandins and thromboxane B_2, epidermal growth factor, microglobulin, and Clara cell protein compared with children with mean blood Pb levels averaging <4 μg/dL. This overall pattern of glomerular, proximal, and distal tubular and interstitial markers was noted as being similar to that earlier found among adults, but occurring at lower blood Pb concentrations than in adults (Fels et al., 1998).

Additional evidence derived from several U.S. and European general population studies substantiates further that low-level Pb exposures affect the above, or other indicators of altered renal function, as discussed in Gonick (2002) and EPA (2006). EPA (2006) noted that the Belgian Cadmibel study (1) was the first large environmental study to adjust for multiple renal risk factors, (2) evaluated several renal outcome measures among a general adult European population, and (3) found decreased creatinine clearance to be related to blood Pb and Zn protoporphyrin concentrations in both men (blood Pb mean of 11.4 μg/dL and range of 2.3–72.5 μg/dL) and women (blood lead mean of 7.5 μg/dL and range of 1.7–60.3 μg/dL), thus raising concerns that the exposure/dose threshold for adverse Pb effects on renal function among the general population might be much lower than earlier thought based on studies of occupationally exposed workers. EPA (2006) also highlighted several other published analyses (Payton et al., 1994; Kim et al., 1996; Wu et al., 2003; Tsaih et al., 2004) of data from the Normative Aging Study (a long-term study of Boston area adult men, aged 21–80 years, with participants initially having been recruited in the 1960s and undergoing periodic follow-up evaluations), which demonstrated relationships between blood and/or bone Pb and various indicators of reductions in measured or estimated creatinine clearance, including among men with peak blood Pb <10 μg/dL in some analyses. Also highlighted by EPA (2006) were the results of analyses by Muntner et al. (2003) of associations between blood Pb and renal outcomes among ~15,000 NHANES III adult participants during 1988–1994. The analyses were stratified because of an interaction between blood Pb and the hypertension (HTN) (as per Pb-related cardiovascular effects noted later), with (1) mean blood Pb being <4.2 μg/dL in hypertensive and 3.3 μg/dL in normotensive subjects, (2) the prevalence of elevated serum creatinine levels (indicative of reduced renal clearance of creatinine) being about 10 times higher in hypertensive (11.8%) than in normotensive (1.8%) subjects, and (3) higher blood Pb levels being associated with a higher prevalence of chronic kidney disease in diabetics among the nonhypertensive subjects. EPA (2006) also noted that blood Pb was reported (Åkesson et al., 2005; Åkesson, 2006) to be significantly related to indications of altered renal function (e.g., reductions in estimated creatinine clearance) among 820 women, aged 53–64 years, in the Lund area of Sweden, with the association being apparent over the entire blood Pb range (mean blood lead of 2.2 μg/dL).

Pb is recognized as exerting notable effects, including at relatively low exposure levels, on a number of organ systems besides those (neurologic, renal, etc.) classically recognized key target organs. Among the most important classes of demonstrated types of effects are cardiovascular impacts of Pb exposure. EPA (2006) assesses key information on Pb-related cardiovascular effects.

Epidemiological studies have examined relationships between human blood Pb levels and blood pressure have found positive associations, even after controlling for confounding factors such as tobacco smoking, exercise, body weight, alcohol consumption, and socioeconomic status. Meta-analyses of such studies found robust, statistically significant, though small, effect sizes and associations between blood Pb concentrations and blood pressure. The Nawrot et al. (2002) meta-analysis found that a doubling of blood Pb corresponded to a 1 mmHg increase in systolic blood pressure, and while not necessarily being clinically meaningful for a given individual, a population shift in blood pressure of 1 mmHg is likely of concern in terms of associated increased risk for more serious cardiovascular outcomes (heart attack, cerebrovascular events, etc.). EPA (2006) further noted that the majority of studies evaluating relationships between the bone Pb and the cardiovascular effects found strong associations between the long-term Pb exposure (indexed by bone Pb concentrations) and the arterial blood pressure. Also noted, too, was supportive evidence from numerous animal studies showing that low-level Pb exposures for extended periods of time resulted in the eventual onset of arterial hypertension, which persisted long after exposure cessation, with both *in vivo* and *in vitro* toxicology studies (Gonick et al., 1997; Vaziri et al., 1997), providing strong evidence that oxidative stress, at least in part, can play a key role in mediating Pb-related HTN. A review of pertinent literature on this subject was provided by Vaziri (2002).

Impacts on the immune system have also emerged for another important class of Pb-related toxic effects including notable Pb impacts seen with rather low-level exposures. Pb-related immune system effects were assessed by EPA (2006) and Dietert and Piepenbrink (2006). Pb effects on nonhuman animal immune systems include the targeting of T cells and macrophages, with Pb-induced alterations being typified by (1) an increased inflammatory profile for macrophages (e.g., elevated tumor necrosis factor alpha, oxygen radical and prostaglandin production) and (2) skewing of the T-cell response away from T-helper 1 (Th1)-dependent functions toward T-helper 2 (Th2)-dependent functions. Resulting impacts on immune system function, as noted by EPA (2006), include increased production of Th2 cytokines (e.g., IL-4, IL-10) and certain immunoglobulins (e.g., IgE) and decreases in Th1-associated cytokines (interferon gamma and IL-12) and Th1 functions [e.g., the delayed-type hypersensitivity (DTH) response], but not major immune cell population changes (complicating interpretation of human epidemiological study results). Also noted was an approximate order-of-magnitude age-related difference in immune system sensitivity to Pb between the perinatal period and adulthood, with Pb effects on immune function being seen at blood Pb levels <10 μg/dL following gestational or perinatal exposures. A key point noted by Dietert and Piepenbrink (2006) was that the most informative sources of functionally reactive immune cells (e.g., antigen-reactive ones in lymphoid organs and lymph nodes) are not readily accessible in humans. The circulating lymphocytes and serum or plasma immunoglobulin (e.g., IgE) levels serve as readily accessible surrogate indices of immune status in human studies. Similar immune system effects were observed in both humans and laboratory animals in terms of positive associations between the circulating IgE levels and the blood Pb concentrations following early-life Pb exposures, even at blood Pb levels <10 μg/dL.

The reproductive effects of Pb focused on male reproductive toxicity were reviewed by Apostoli et al. (1998): 32 experimental animal studies, 22 human epidemiological studies, and 1 case report in humans. They concluded that when blood Pb exceeded 40 μg/dL, there were associated decreases in sperm count, volume, motility, and morphological alterations (with a possible effect also on endocrine profile), but dose–response relationships, particularly possible thresholds for effects, remained poorly understood. Bellinger (2003) and EPA (2006) provided additional overviews of Pb-related reproductive effects.

Pb and its compounds were recognized as being carcinogenic to animals (IARC, 1987). Possible Pb carcinogenicity among humans was discussed in a review of the by Vainio (1997) following publication of two cohort studies among smelter workers by Lundstrom et al. (1997) and by Cocco et al. (1997). Overall, Vainio (1997) concluded that a long-term, high-level exposure to Pb compounds was associated with an increased risk of cancer and that the "weight of evidence was beginning to be convincing enough concerning kidney and even lung cancer" for humans. In addition, more recent extensive reviews published by both Silbergeld (2003) and EPA (2006) evaluated available human and/or animal evidence concerning carcinogenicity effects of Pb.

The broad Pb assessment by EPA (2006) covered available information on all of the types of Pb health effects discussed above, but also other classes of Pb effects are not addressed to any great extent here due to space limitations. It is recommended that the reader consult the discussion by Hu et al. (1998) of Pb effects on bone and teeth.

The most recent authoritative review of the literature on evidence on causation of human health effects that have been associated with environmental Pb exposures was provided in the latest EPA Pb ISA (EPA, 2013). It contains the judgments of a wide range of EPA's own in-house experts and consultants and gained the endorsement of its external Clean Air Scientific Advisory Committee (CASAC).

17.9 MECHANISMS UNDERLYING LEAD TOXICITY

Early investigations into Pb toxicity focused on mechanisms underlying gross effects observed at very high exposure levels. The animal and human studies of kidney pathology demonstrated Pb deposition in cells of the proximal tubule, especially in nuclear material, and mitochondrial degeneration (Goyer and Rhyne, 1973). Scott et al. (1971) noted that such effects are consistent with proximal tubular dysfunction seen in Pb toxicity, but they did not clarify the mechanism or the heme biosynthetic pathway. Pb inhibits enzymes in this pathway, such as porphobilinogen synthetase and heme synthetase. Heme synthetase is of interest as a mitochondrial-bound enzyme, with Pb inhibiting this it by altering mitochondrial function.

The importance of Pb effects on Ca-dependent systems comes close to being an explanation for Pb toxicity being related to Ca transport or to other mechanisms of Ca-dependent function. Also, Pb accumulates preferentially in areas very metabolically active and rich in mitochondria and is a potent inhibitor of mitochondrial function. Pb has nervous system targets (Bondy, 1988), and some of these targets might be of environmental concern, especially inorganic Pb compounds. Some properties of organo-Pb can also help to clarify biochemical mechanisms of neurotoxicity. The chemistry of organo-Pb compounds differ from ionic compounds (Grandjean and Grandjean, 1984). For example, the toxicity of tetraethyl Pb results from its breakdown in the organism to a salt of triethyl Pb. The triorgano-Pb compounds actions are due to two chemical properties: (1) the lipophilic (rather

amphoteric) nature of their chlorides and hydroxides and their affinity constants that allow dissociation at biological concentrations of hydroxyl and chloride ions and (2) the potential for five-coordinate binding. Thus, the behavioral toxicity effects of alkyl PBs do not resemble those of inorganic Pb. Similarities exist, such the degradation of the alkyl Pb to divalent Pb in the organism. A small amount of metabolism of this type could be significant because of the altered tissue distribution of Pb as a result of the lipophilic properties of organo-Pb compounds. More attention should be paid to the possible complexes of inorganic Pb with hydrophobic ligands such as hemic acid. The general lack of involvement/importance of the divalent Pb ion in organo-Pb toxicity is supported by the observation that the usual chelators have little effect on intoxication from organo-Pb compounds.

The study of chemical mechanisms of inorganic Pb compound toxicity is complicated by the amount of Pb absorbed after oral administration varies significantly with animal species, age, diet, and chemical and physical form. In the absence of food in the gut (as during fasting), Pb compounds can be more readily acidified and solubilized for tissue uptake. Because of ubiquity of Pb in the environment and its relatively high toxicity, there is still concern about Pb in the diet. Separation of the influence of nutritional status on biokinetics of Pb from specific neurotoxic events needs more research.

There are only a few triggering events in biological systems that could account for the potent neurotoxic effects of Pb that, in turn, could result in a range of secondary effects. The important initiating events depend on the distinctive chemical properties of divalent Pb, since all other divalent metals produce a toxic syndrome that is qualitatively or quantitatively different.

The relatively low affinity of Pb for S suggests that interaction with specific S-containing proteins, such as metallothionein, would not be critical. Carboxyl-oxygen-containing amino acids are more likely points of interactions with proteins, and this possibility is supported by Pb-containing nuclear inclusion bodies being in tissues rich in acidic amino acids (Choie and Richter, 1972). Since divalent Pb is a stable oxidation state, it seems likely that the divalent Pb ion mediates the range of effects seen and that metabolic redox pathways involving tetravalent Pb are not important.

The toxic effects of divalent Pb may result of either physical or chemical change in the biochemical systems. Pb may damage membranes by changing the ultrastructure of cellular components or by initiating oxidative damage. Membrane damage may be a significant factor in Pb toxicity, and myelin-containing membranes may be especially sensitive. Membrane damage by peroxidative processes may involve change in Ca homeostasis. Effects of Pb on energy production could be related to direct interaction with mitochondrial membranes, altering ion transport, or changes in Ca homeostasis within the cell. Pb accumulates in mitochondria and is associated with the inhibition of oxidative phosphorylation. Endothelial cells in brain capillaries contain three to five times more mitochondria than endothelial cells in the vessels of other organs (Oldendorf and Brown, 1975). This difference may reflect the high rate of metabolic activity necessary to maintain the active transport of ions across the blood–brain barrier and may explain susceptibility to a wide variety of toxic compounds that cause brain edema. Mitochondria may be a critical subcellular target for the toxic effects of Pb.

Inorganic Pb also competes with essential divalent metals at several different levels (Chisolm, 1980) including Ca, Zn, Cu, and Fe. These interactions can occur at several levels, including absorption from the gut and transport across the blood–brain barrier, and at the synapse. For example, active Ca uptake by mitochondria is a critical process required to maintain Ca at very low concentration in the cytosol of cells. Inhibition of Ca accumulation

by mitochondria may involve a direct blockage of the Ca pumps, but this may also be attributed to depletion of ATP or key intermediates involved in its synthesis, such as inorganic phosphate. Pb chemistry suggests that a variety of metals can show altered distribution in biological systems in the presence of Pb, with some associated biochemical changes. Dietary supplements of various minerals and vitamins do not completely protect against the toxic effects of Pb, suggesting also that there are more critical biochemical changes associated with the potent Pb neurotoxic effects.

Other biochemical interactions may be important in the toxic responses of Pb, including inhibitory effects on the cholinergic system and activation of catecholaminergic function, which may be related to the Ca agonist property of Pb. Pb may be equipotent with Ca in binding to calmodulin. Pb may also be involved in direct reactions carried out by mixed-function oxidases. Complex secondary interactions between organ systems are also a possible factor determining the overall pattern of toxicity. A possible interrelated sequence of events by which Pb compounds could cause neurotoxicity, as expressed by behavioral change, was suggested by Bondy (1988). What aspects of Pb chemistry may throw light on the important biochemical/molecular mechanisms of toxicity?

The relative binding strengths of the various endogenous Pb ligands determines the fate and distribution of Pb *in vivo* and its competition with Ca. Once Pb is inside cells, its eventual fate will be to bind to sites that are stronger than cytosolic pool chelators, such as citrate. At slightly acidic pH, at which divalent Pb ionic species are likely to be present, phosphate ligands are prime candidates because of the very large association binding constants. The extreme tenacity for phosphate may be the most distinctive feature of Pb chemistry relative to other divalent metal ions. Several types of phosphate ligands must be considered, including inorganic orthophosphates, ATP, and the phosphate groups of membrane lipophosphates and phosphorylated proteins. This primary mechanism underlying Pb toxicity is compatible with the major reproducible biochemical findings, particularly the suggestion that mitochondria (where oxidative phosphorylation takes place) may be a target site in cells. It is also compatible with the ultimate localization of the Pb in bone as insoluble phosphate salts.

Pb may function as a phosphate scavenger and siphon off minority phosphate species crucial to developing/proliferating cells, especially in neural tissues. If one of the functions of Ca is to store phosphate in the form of calcium phosphate, then Pb would be an efficient antagonist for this process. It is not clear if the effects of Pb on Ca homeostasis are the result of direct competition between Pb and Ca for binding sites and/or of their differential affinity for phosphate ligands, particularly inorganic phosphate. For example, Markovac and Goldstein (1988) indicate that Pb is a potent activator of protein kinase C, which could be interpreted as a direct effect of Pb on sequences of protein phosphorylating–dephosphorylating that do not involve the enzyme at all. Phosphorylating–dephosphorylating sequences are of critical importance to energy transformation in cells and tissues, and this is particularly true of those in the nervous system during rapid growth and maturation.

Direct phosphate-deleting effects of Pb resemble those seen in poisoning by nitrophenols (Clayton and Clayton, 1981–1982), which are known to uncouple oxidative phosphorylation (presumably also reducing the body's reservoirs of high-energy phosphate compounds). Such uncoupling apparently stimulates oxidative metabolism and, in turn, heat production of the body. Oxygen consumption, body temperature, respiration, and heart rate are all increased. Some similar effects are also associated with the hyperthyroid state (Dratman, 1978). With regard to mechanisms of Pb neurotoxicity, there is some evidence that hyperthyroidism is associated with reduced catecholamine production rates in

both the peripheral nerve tissue and the brain. In studies on rat, McIntosh et al. (1989) reported effects of Pb on catecholaminergic and cholinergic transmission being regionally specific within the brain, the midbrain, and the diencephalon, showing the greatest degree of change in concentrations of neurotransmitters (dopamine concentrations usually decreased) and activities of rate-limiting enzymes. See also Cory-Slechta (1997) for information on relationships between Pb effects on neurotransmitter systems and behavioral toxicity.

Although Pb neurotoxicity, poisoning by nitrophenols, and hyperthyroidism all involve different triggering mechanisms, these responses may have in common secondary effects related to the maintenance of cellular homeostasis and metabolism. The remarkable affinity of Pb for phosphate may be the most sensitive primary event to explain the neurotoxic effects of Pb being evident at low concentrations and after only a brief exposure period. This suggests that thyroid status and metabolic state might be important factors to consider in examining correlations between Pb exposure and a causal relationship to increased oxidative metabolism.

Since the above overview of hypotheses/information related to Pb toxicity (especially neurotoxicity), reviews by EPA (2006) and White et al. (2007) included further advances regarding mechanisms. The White et al. (2007) review highlighted four major areas of key advances: (1) experimental studies showing that stress markedly influences Pb effects, possibly mediated by interactions of corticosteroid hormones with components of the mesocorticolimbic dopamine system of the brain (with heightened stress causing consequent elevations in circulating corticosteroid levels hypothesized to contribute to increased vulnerability to many diseases and other dysfunctions among lower socioeconomic status populations); (2) cellular models of learning and memory used to evaluate possible mechanisms of Pb-related cognitive deficits (with studies of long-term potentiation in the rodent hippocampus having shown Pb-related increased thresholds, decreases of magnitude, and shorter retention times for neural plasticity and alterations in the form of adult neurogenesis in the hippocampus, which may contribute to learning impairment); (3) *in vitro* evidence for strong binding of Pb to glucose-related protein (GRP-78), induction of GRP aggregation, and blocking of secretion of interleukin-6 (IL-6) by astroglial cells (implicating Pb in "chaperone deficiency" processes, which, in the long term, could underlie protein confrontational diseases, e.g., Alzheimer's disease); and (4) implication of Pb exposure in the early development and in the later progression of amyloidogenesis in rodent brains during old age (thereby contributing to increased proteins associated with Alzheimer's disease pathology). Overall, as noted by White et al. (2007), such findings provide evidence for Pb exposures having adverse effects on the nervous system that environmental factors increase nervous system susceptibility to Pb and that Pb exposures early in life may contribute to neurodegeneration later in old age.

17.10 TREATMENT OF LEAD TOXICITY

Metal chelation therapy has been used with some success to treat Pb poisoning (Bondy, 1988). However, chelators used for this purpose can also remove essential elements resulting in kidney damage; most of these drugs tend to have unpleasant side effects, they are most generally useful for acute rather than sustained therapy, and the benefits derived are usually only transitory, since blood Pb can be rapidly replaced from bone stores. In view of the nature of Pb chemistry, and the possibility that Pb is basically functioning as a phosphate

scavenger, it is unlikely that a complexing/chelating agent of the usual variety could be found that could compete effectively with phosphate in a ligand exchange reaction, but it may be possible to develop a derivative of phosphate that is sufficiently reactive and excretable to be useful in therapy. On the other hand, soluble forms of phosphate itself might offer some protection against toxic effects, perhaps until Pb is deposited in bone. This approach does not reduce the Pb body burden, but it may buy time during the process of deposition of Pb in bone, which at least offers transient sequestering.

The prospects for successful chemical treatment of long-term, low-dose Pb toxicity have not been promising. Chelating agents have been used mainly to treat the short-term, high-dose exposures. For children, CDC (1991) recommended the use of chelation therapy only when blood Pb concentrations exceed 45 μg/dL (>2.17 μmol/L). It remains unclear whether chelation has sustained benefits for children with blood Pb of 25–44 μg/dL. Criteria such as the persistence of elevated blood Pb levels despite environmental intervention have also been offered as justifications for chelation (American Academy of Pediatrics, 1993). Deciding which children may respond to chelation therapy is complex. Children with changes in erythrocyte protoporphyrin and hematological index are more likely to respond to chelators with markedly enhanced urinary Pb excretion. At least some chelators (i.e., succimer) are more effective in removing Pb from blood than Pb from brain (Cory-Slechta, 1988; Pappas et al., 1995). Smith et al. (1998) cautioned with regard to basing judgments on changes in blood Pb concentration on predictions of the impact of chelators on brain Pb concentrations. The ratios of change in brain Pb and change in blood Pb differed over the duration of chelation therapy in rodents.

The usefulness of nutritional therapy depends on the timing of its introduction, the severity of Pb exposure, and underlying nutritional status. It is clear that marginal nutritional status is associated with increased prevalence of elevated blood Pb concentrations. Data from the NHANES, conducted in the United States during the 1970s through the l990s, demonstrated that young children from socially disadvantaged, low-income, minority families are more likely to have a greater prevalence of elevated blood Pb levels (Mahaffey et al., 1982; Pirkle et al., 1994) and of marginal nutritional status (Mahaffey et al., 1986; Life Science Research Office, 1996). The consumption of a higher Ca diet is inversely related to bone Pb among women living in Mexico City (Hernandez-Avila et al., 1996).

The physiological changes that accompany poor nutritional status for Ca and Fe result in enhanced absorption of these required elements from the GI tract (Mahaffey, 1995; Fullmer, 1997). Because Pb absorption also increases with these physiological changes, improving nutritional status results in reduced future Pb absorption. Some reports indicate reduced blood Pb following Fe treatment (Granado et al., 1994). Short-term correction of low Ca intake has not been shown to alter blood Pb, but it is clear that skeletal mineral can be mobilized for Ca under conditions of physiological stress and that Pb will be released along with Ca during this mineral mobilization (Gulson et al., 1997, 1998a).

Among recommended "treatment" activities is identification through screening of cases for environmental/nutritional/pharmaceutical intervention. In 1991, the "case" definition was lowered to define childhood Pb poisoning as a blood Pb >10 μg/dL (0.48 μmol/L) (CDC, 1991; American Academy of Pediatrics, 1993). Although the 10 μg/dL action level remains in effect, in a move away from universal screening (CDC, 1997), emphasis is now on geographic areas with higher prevalence of older housing (defined as where 27% or more housing was built before 1950), or presence of other risk factors (e.g., child receiving services from public assistance programs for the poor, or the child having a sibling or

playmate who has had Pb poisoning). Part of this change reflects the overall decline in blood Pb concentrations in the United States.

17.11 SUMMATION

Lead (Pb) dispersion into the global environment has diminished in recent decade due to more stringent emission controls in most developed countries. However, Pb that was widely dispersed before the start of the twenty-first century into environmental media will remain in soil, surface dusts inside and outside homes, stream-, lake-, and riverbeds, and oceans and be capable of resuspension into airs and waters leading to inhalation and ingestion exposures of human populations.

There is clear evidence that past environmental exposures to Pb have been causally associated with adverse human health effects. These include cognitive function decrements in both children and adults, auditory and visual function decrements in adults, and attention, impulsivity and hyperactivity in children.

Also in adults, these included hypertension, coronary heart disease, decreased RBC survival and function, and altered heme synthesis and developmental male reproductive function effects.

Other adverse effects were considered likely to be causal. For children, these included conduct disorders, auditory and motor function decrements. For adults, they included cognitive function decrements and psychopathological effects, atopic and inflammatory responses, decreased host resistance, and cancer.

In a more recent review of the toxicology of Pb and its damage to mammalian organs, Caito et al. (2017) concluded that Pb is a highly toxic metal that is regulated to prevent exposures in the workplace. Environmental exposures to Pb remain a continued problem in communities with old homes and water systems. The recent mass exposure to Pb in drinking water in Flint, MI (USA), illustrates the importance of monitoring environmental levels.

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18

MERCURY

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18.1 INTRODUCTION

Mercury (Hg) is a global pollutant that poses risks to human and ecological health (Driscoll et al., 2013). Though a naturally occurring element, large quantities of Hg have been mobilized by human activities such as mining and fossil fuel combustion and have subsequently been released into the biosphere (Streets et al., 2017). Methylmercury (MeHg) is the most toxic and the only form of Hg that biomagnifies in food webs (Clarkson and Magos, 2006). Toxicological effects of MeHg exposure were highlighted in the poisoning incident in Minamata, Japan, in 1956 (Harada, 1995). There, more than 2000 residents consumed fish and shellfish contaminated by MeHg discharged in wastewater from a local chemical plant and suffered from severe neurological disorders known as the Minamata disease. The devastating effects of MeHg poisoning also caused long-term psychiatric symptoms (e.g., impairment of intelligence and mood and behavioral dysfunction) among the general population in Minamata who did not have Minamata disease (Yorifuji et al., 2011).

Globally, exposures to MeHg at the levels of the Minamata incident are rare. However, worldwide exposure to MeHg occurs at levels associated with neurocognitive deficits and other adverse health effects and results in billions of dollars of lost earnings in the European Union (EU) and the United States (Grandjean et al., 2010; Bellanger et al., 2013; Sheehan et al., 2014). Most of the Hg emitted from human sources is transported long distances in the atmosphere and mixed hemispherically to globally before being deposited to aquatic and terrestrial ecosystems (Corbitt et al., 2011). Once deposited in an aquatic environment, some of this Hg is converted by microorganisms to MeHg and can subsequently biomagnify in food webs, reaching the highest concentrations in top predators (Gilmour et al., 2013; Sunderland et al., 2018). Worldwide, more than 3.1 billon people rely on fish as their primary source of animal protein (FAO, 2016). Indigenous and subsidence populations are especially vulnerable to MeHg exposure because they consume large quantities of fish and marine mammals. Artisanal and small-scale miners that use large quantities of elemental

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Hg as an amalgam for gold, mostly in low- and middle-income countries (LMIC), are another impacted population (Gibb and O'Leary, 2014). Global concerns related to Hg contamination of ecosystems and exposures of humans and wildlife has led to international efforts to reduce anthropogenic emissions. Most notably, the first global treaty aimed at reducing anthropogenic Hg emissions, the Minamata Convention, was signed in 2013 and entered into force in 2017 (United Nations Environment Programme, 2013).

18.2 CHEMISTRY

Mercury has three primary oxidation states: Hg^0 (metallic mercury or gaseous elemental mercury), Hg^+ (mercurous), and Hg^{2+} (mercuric) mercury, which is normally covalently bonded to a variety of ligands in the atmosphere and in solution (Hg^{II}). Hg^0 in liquid form is a dense, silvery-white, shiny metal and is the only liquid metal at room temperature (Calvo et al., 2013). At 20 °C, the vapor pressure of Hg^0 is 0.17 Pa (0.0013 mmHg). A saturated atmosphere at this temperature contains 14 mgHg^0/m^3, which is more than 100 times the occupational limit established by the World Health Organization (WHO) of 1.0 μg Hg/m^3. In organometallic derivatives, Hg^{2+} is covalently bound to one or two carbon atoms, and the organic part of the molecule is often an alkyl group or an alkoxyalkyl group. The former compounds are more toxic, because they are more easily absorbed and more slowly metabolized. Hg compounds differ greatly in their solubility. At 25 °C, the solubility in water is 60 μg/L for Hg^0, 2000 μg/L for mercurous chloride, and 69,000,000 μg/L for mercuric chloride. Certain Hg species are soluble in nonpolar solvents. These include Hg^0 and the halide compounds of alkylmercurials.

There are seven naturally occurring stable isotopes of Hg: ^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, and ^{204}Hg. Recent advances in the sensitivity of multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) have allowed for quantification of the fractionation of Hg isotopes in natural samples. Both mass-dependent fractionation (MDF) of Hg isotopes and mass-independent fractionation (MIF) of the odd mass isotopes occur during the photochemical reduction of Hg^{II} by natural sunlight (Blum et al., 2014). The composition of different Hg isotopes in environmental samples provides a useful tracer for many different biogeochemical processes such as methylation/demethylation, vaporization/condensation, or oxidation/reduction (Bergquist and Blum, 2007; Rodríguez-González et al., 2009; Ghosh et al., 2013; Sun et al., 2016). Many recent studies have proposed new applications of Hg stable isotopes for characterizing the origin of MeHg taken up by food webs, including humans (Laffont et al., 2011; Li et al., 2014, 2016; Du et al., 2018).

18.3 SOURCES

Natural emission sources of Hg to the atmosphere include crustal degassing and volcanoes and are estimated to be approximately 76 ± 30 Mg/year (Amos et al., 2015). Anthropogenic emissions in 2010 were approximately 2270 Mg/year, and the two largest sources globally were artisanal and small-scale gold mining (ASGM) and coal-fired utilities (UNEP, 2013; Streets et al., 2017). Evasion of Hg^0 from soils and the ocean is also substantial. Amos et al. (2013) estimated gross emissions of Hg^0 to the atmosphere from soils each year to be 2200 and 4900 Mg/year from the oceans. Most of these emissions are from previously deposited anthropogenic Hg from legacy sources because cumulative releases of Hg from human

sources since 1850 are about 78 times larger than natural sources (Streets et al., 2017). This means humans are exposed to Hg from both present-day emission sources and legacy sources that cycle through the environment over decades to centuries (Amos et al., 2013).

Use of Hg^0 has escalated over the last few decades in ASGM (Veiga et al., 2006). Alluvial deposits of fine gold particles are often extracted using liquid Hg^0 (quicksilver). The gold particles are dissolved in the Hg^0 as amalgam, and the Hg^0 is subsequently removed by heating with a gas torch. This use therefore exposes the gold miner and also leads to extensive release of Hg^0 into confined and sometimes ecologically sensitive areas (Kocman et al., 2017). The annual emission of Hg^0 to air in such mining operations is 410–1040 Mg/year, mainly in Asia, Central Africa, and Latin America (UNEP, 2013; Streets et al., 2017).

Hg is also released directly to aquatic and terrestrial ecosystems either through direct wastewater effluent, industrial pollution, or leachate from disposal waste sites. Kocman et al. (2017) developed the first spatially resolved estimates of aquatic Hg releases to freshwater environments and estimated that total releases to rivers and lakes are at least 1000 Mg/year, with the majority of releases from ASGM. Soil Hg contamination may remain for many decades. Previous gold mining operations in the United States (e.g., Carson River, Nevada) are now recognized as being heavily contaminated with Hg, with estimated Hg reservoirs exceeding 6000 Mg. Deposition of sewage sludge and contamination from other industrial activities often involve mercuric salts with low solubility (i.e., sulfides). Ecological and health implications of different Hg releases depend on the extent of Hg methylation and uptake in food webs.

The stable elemental form of Hg in the atmosphere (Hg^0) is oxidized by bromine and variety of second-stage oxidants (ClO, NO_2, HO_2) and then deposited as Hg^{II} species to terrestrial and aquatic ecosystems (Horowitz et al., 2017). A fraction of the Hg^{II} that is deposited is reduced photochemically, or by microbes, and returned to the atmosphere (Soerensen et al., 2010). The remaining pool of Hg^{II} forms the substrate potentially available to microbes for conversion to MeHg (Benoit et al., 2003). MeHg is accumulated by fish and marine mammals and attains its highest concentrations in large predatory species at the top of the aquatic food chain (Lavoie et al., 2013). By this means, it enters the human diet. Diverse communities of microorganisms demethylate MeHg back to Hg^{II} or Hg^0 (Marvin-Dipasquale et al., 2000; Lu et al., 2016). Thus, microorganisms play an important role in the fate of Hg in the environment and affect human exposure.

18.4 ENVIRONMENTAL EXPOSURES

18.4.1 Air

Gaseous Hg^0 comprises more than 90% of the Hg reservoir in the atmosphere (Horowitz et al., 2017). Gaseous oxidized Hg^{II} species and Hg^{II} bound to particulate matter are less common because they are highly water soluble and scavenged quickly from the atmosphere (Amos et al., 2012). Hg^0 has a lifetime of between 6 and 12 months in the atmosphere and therefore can be transported to remote areas (Corbitt et al., 2011). Rapid deposition of oxidized and particulate Hg^{II} mean such emissions can drive regional pollution signals next to point sources.

Between 1990 and 2010, co-benefits from emission controls for traditional air pollutants (SO_x, NO_x) on coal-fired utilities led to a reduction in atmospheric Hg concentrations

by an estimated 30% in North America and Europe (Zhang et al., 2016). Average atmospheric Hg^0 concentrations decreased from 1.8 to 1.3 ng/m^3 in North American between 1994 and 2014 and decreased from 3.0 to 1.5 ng/m^3 in Western Europe from 1990 to 2011 (Zhang et al., 2016). Strong seasonality in atmospheric Hg^0 has been widely observed in the Northern Hemisphere, but is less pronounced in the Southern Hemisphere. In addition to the seasonal variation in the strength of human energy consumption and associated Hg releases, recent research has suggested that terrestrial vegetation can act as a Hg^0 pump and contribute to the seasonal variation of atmospheric Hg^0 (Jiskra et al., 2018).

Mean Hg concentrations in urban air are usually three- to fourfold higher than in rural areas. "Hot spots" of atmospheric Hg exceeding 10,000 ng/m^3 have been reported close to mining regions or above areas where Hg fungicides have been used extensively. In Palu, Indonesia, Hg^0 concentration was 12,782 ng/m^3 in the gold-processing area and ranged from 2096 to 3299 ng/m^3 in the city area (Nakazawa et al., 2016).

Limited data are available on average indoor Hg air pollution. Fatalities and severe poisonings have resulted from heating Hg^0 and Hg-containing objects at home. Hg^0 is sometimes used for certain cultural and religious practices that may involve sprinkling Hg inside, burning it in a candle, or mixing it with perfume. Such practices can create exposures that greatly exceed currently permitted occupational exposures (Shen et al., 2017). Release of Hg from dental amalgam fillings is otherwise the predominant source of human exposure to inorganic Hg in the general population (Homme et al., 2014).

From the atmosphere, the WHO estimates that the daily amount absorbed as a result of respiratory exposure into the bloodstream in adults is approximately 32 ng Hg in rural areas and about 160 ng Hg in urban areas, assuming rural concentrations of 2 ng/m^3 and urban concentrations of 10 ng/m^3 (absorption rate of 80%). Depending upon the number of amalgam fillings, Hg concentrations in inhaled air have ranged up to several thousand ng/m^3, and the estimated average daily absorption is thought to vary between 1000 and 22,000 ng/day, with most people having a dose of less than 5000 ng/day (World Health Organization, 2003).

18.4.2 Diet (Drinking Water and Food)

Fish, shellfish, and marine mammals constitute the dominant sources of methylmercury exposure in most populations (Sunderland et al., 2018). Methylmercury concentrations in edible tissues of various species of fish span approximately two orders of magnitude depending on environmental pollution, species, age, and size of the fish (Mahaffey et al., 2011). Large predatory fish, such as pike, swordfish, and tuna, as well as shark, seals, and toothed whales contain the highest average concentrations (Karimi et al., 2012). Hg is generally found at low concentrations in drinking water, and this is not an important pathway for exposure. Concentrations of Hg in terrestrial foods are often below the detection limit and likely to be inconsequential.

A variety of personal care products containing Hg have been linked to elevated levels of exposure. These include Hg-containing skin lightening creams and other pharmaceuticals, in particular thimerosal, widely applied as a preservative of vaccines and immunoglobulins (up to 100 µg mercury per injection). Skin lightening creams sold and used in Arabian and African countries often contain Hg concentrations of about 1000 ppm, and some products may reach concentrations in the percent range. Consumers are usually not warned about the toxic contents. For example, a number of highly exposed individuals in New York City were linked to the use of skin whiteners containing high concentrations of Hg (McKelvey et al., 2011).

18.4.3 Relative Significance of Different Routes of Environmental Exposure

Human exposure to the three major forms of Hg present in the environment (Hg^0, Hg^{II}, and MeHg) is summarized in Table 18.1 (World Health Organization, 2003). Although the choice of values given is associated with some uncertainty, the numbers provide a perspective on the relative magnitude of the contributions from various media.

Humans may be exposed to additional quantities of Hg occupationally, from living in heavily polluted areas, or through use of skin lightening lotions. The intake from drinking water is about 50 ng Hg per day, mainly as Hg^{II}, but only a small fraction is absorbed. The main determinant of exposure is the intake of fish and seafood products, mainly in the form of MeHg. Very high exposures occur in Arctic populations, who eat marine mammals. Increased levels also occur in Japanese and Mediterranean populations, who frequently eat fish high in the food chain. Exposures are lower in countries, such as the United States, where NHANES 2001–2014 data suggest that childbearing-age women, including pregnant women, only consumed an average of 0.44 oz. eq. of seafood per day (approximately 3.08 oz. eq. per week), much lower than the recommendation level of 8–12 oz (Zhang et al., 2018). Because fish and seafood are recommended as an essential part of a varied diet, advisories often focus on identifying the types of fish that are low in Hg. Hair Hg assessment can be used as a useful instrument when advising pregnant women about healthy seafood diets and avoidance of elevated MeHg exposure (Kirk et al., 2017).

Total dietary Hg intake has usually been measured as part of market basket surveys or as part of specific monitoring. Probabilistic analyses based on dietary questionnaire data and fish analyses suggest that small children, on a body weight basis, may receive a higher exposure than adults. Significant variability exists among different demographic groups, and populations who consume considerable amounts of fish are more susceptible to Hg contamination. Recent development includes a nationally representative survey of

TABLE 18.1 Estimated Average Daily Intake (Retention) of Mercury Compounds

		Intake (Retention)[a] (ng)		
Media		Mercury Vapor (Hg^0)	Inorganic Mercury Compounds (Hg^{II})	Methylmercury (MeHg)
Air		40–200[b] (30–160)	0[c]	0[c]
Food				
	Marine	0	600[d] (60)	2400[d] (2300)
	Non-marine	0	3600 (360)	Uncertain
Drinking water		0	50 (5)	0
Dental amalgam		1200–27,000 (1000–21,600)	0	0
Total		1200–27,000 (1000–2200)	4300 (430)	2400 (2300)

[a]Figures in parentheses are the amounts retained that were estimated from the pharmacokinetic parameters (i.e., 80% of inhaled vapor, 95% of ingested MeHg, and 10% of inorganic Hg are retained).
[b]Assumes an air concentration of 2–10 ng/m^3 and a daily respiratory volume of 20 m^3.
[c]For the purposes of comparison, it is assumed that in the atmospheric concentrations of species of Hg other than Hg^0 vapor are negligible.
[d]It is assumed that 80% of the total Hg in edible fish tissues is MeHg and 20% in the form of inorganic Hg compounds. Marine food intake may vary considerably between individuals and across populations.

high-frequency fish consumers (defined as individuals consuming three or more fish meals per week) in the United States (von Stackelberg et al., 2017).

Due to the uncertain nature of dietary survey data and well-known recall bias, there is a need for an empirical tool to better characterize exposure sources. Emerging evidence suggests Hg ratios in human hair can be used as a new method for discerning MeHg exposure sources. The isotopic composition of Hg in human biomarkers, such as hair and urine, has been proposed as a tool for distinguishing exposure to Hg^0 from dental amalgam and exposure to MeHg from seafood (Sherman et al., 2013). Similarly, different isotopic signatures of coastal and oceanic fish mean that stable Hg isotopes can be used as a tracer for such exposure sources in human hair (Li et al., 2014).

18.4.4 Occupational Exposures

Occupational exposures are almost exclusive to inorganic Hg and occur at chlor-alkali plants, Hg mines, thermometer factories, Hg refineries, and in dental clinics. Some 70,000 workers in the United States were considered exposed to Hg, primarily Hg^0. The number has likely decreased as Hg has been phased out in most industrial and commercial applications. High Hg concentrations have been described for all these situations, with considerable variations depending on the working conditions.

Serious Hg exposures may occur in connection with ASGM, especially when gold amalgam is heated. In developing countries, this process is often carried out under field conditions or in small gold vending shops lacking or with insufficient ventilation. An estimated 15 million workers in Africa, Latin America, and Asia work in ASGM, and 100 million people worldwide depend on this activity for their livelihood (Spiegel and Veiga, 2006). Gibb and O'Leary conducted a comprehensive review in 2014 and summarized more than 60 studies conducted in 19 different countries (Gibb and O'Leary, 2014). Eight studies reported urinary Hg concentrations well above 100 μg Hg/g-creatinine, which is the level considered by the WHO to have high risks of neurological intoxication. Other reviews also provide evidence that artisanal gold miners and residents of the mining sites are exposed to Hg at levels close to cause acute and long-term toxic effects. Interventions aimed at reducing exposure and emission of Hg from ASGM are needed (Kristensen et al., 2014; Basu et al., 2015).

18.5 KINETICS AND METABOLISM

The bioavailability, kinetics, and biotransformation of Hg depend upon its chemical and physical form.

18.6 ABSORPTION

18.6.1 Hg^0

Approximately 80% of the inhaled Hg^0 is absorbed via the lungs and retained in the body. It can pass through the blood–brain barrier and cause damage to the brain once oxidized to Hg^{II}. Liquid Hg^0 is poorly absorbed in the gastrointestinal tract (less than 0.01% in rats), though increased blood Hg concentrations have been measured in humans after accidental ingestion of several grams of liquid Hg^0.

18.6.2 Inorganic Mercurous (Hg^{+}) and Mercuric (Hg^{2+}) Mercury

The absorption of inhaled aerosols of inorganic Hg is generally thought to be low and no data have been reported for humans. In dogs, 45% of deposited particulate Hg^{II} were cleared in less than 24 h and the remainder cleared with a half-life of 33 days. Ten to 15% of an oral, nontoxic dose of Hg^{II} may be absorbed from the gastrointestinal tract in adults and retained in body tissues, but considerable individual variations may exist. In children, the gastrointestinal absorption is probably greater.

18.6.3 Organic Mercury

MeHg was assumed to be almost 100% bioavailable in risk assessment practices. However, recent evidence suggests not all of the ingested MeHg is absorbed into the systemic circulation. A review of 45 studies on fish and humans concluded that MeHg bioavailability and assimilation is less than 100%. Assimilation efficiencies in fish ranged from 10 to 100% for MeHg in fish and 12 to 79% for humans (Bradley et al., 2017). Factors influencing bioavailability include source, cooking methods, and co-ingested nutrients (Siedlikowski et al., 2016). A significant data gap exists for various species of fish so that a consistent correction factor cannot yet be developed.

18.7 DISTRIBUTION

18.7.1 Hg^{0}

After exposure, Hg^{0} vapor is found in blood as physically dissolved Hg^{0}. Within a few minutes, it is oxidized to Hg^{2+} in the erythrocytes, a reaction catalyzed by the enzyme catalase via the catalase–hydrogen peroxide pathway. This reaction occurs throughout the human body. Thus, following short-term exposure to Hg^{0} vapor, the maximum concentration of Hg in erythrocytes is seen after less than 1 h, whereas plasma levels peak after about 10 h. Before oxidation, Hg^{0} readily crosses cell membranes, including the blood–brain barrier and the placental barrier. After oxidation, the Hg^{2+} ions (or complexes) are distributed in the body via the blood. The kidneys and the brain are the main deposits of Hg after exposure to Hg^{0} vapor, whereas absorbed inorganic Hg^{II} mainly accumulates in the kidneys.

The uptake and/or elimination of Hg after exposure to Hg^{0} vapor can be altered by a moderate intake of alcohol, possibly due to inhibition of catalase. Thus, the amount of Hg in red blood cells of humans exposed to Hg^{0} vapor was significantly reduced in the humans given alcohol before Hg exposure (Hursh et al., 1980).

18.7.2 Hg^{II} Species

The kidney is the predominant site of Hg^{II} accumulation. However, after oral exposure, accumulation also occurs in the cells of the mucous membranes of the gastrointestinal tract, though a significant part of this accumulation is later eliminated due to cell shedding and therefore never reaches the general circulation. Hg^{II} in blood is divided between erythrocytes and plasma in about equal amounts. In erythrocytes, Hg is probably to a large extent bound to sulfhydryl groups on the hemoglobin molecule and possibly also to glutathione (GSH). The distribution between different plasma-protein fractions varies with dose and time after exposure.

Hg^{II} species do not cross the blood–brain and placental barriers. However, Hg^{II} does accumulate in the placenta, fetal membranes, and amniotic fluid. The rate of uptake from blood and different organs varies widely; so does the rate of elimination from different organs. Thus, the distribution of Hg within the body and within organs varies widely with dose and time lapse after absorption. However, under all conditions, the dominating Hg pool in the body after exposure to Hg^{II} is the kidney. Inorganic divalent Hg can induce metallothionein (MT) and a large proportion of the Hg in the kidneys is soluble and bound to MT.

18.7.3 Organic Mercury

MeHg is distributed via the bloodstream to all tissues in the body. The pattern of tissue distribution is much more uniform than after Hg^{II} exposure, except in red cells, where the concentration is 10–20 times greater than the plasma concentration. MeHg readily crosses the blood–brain and placental barriers. In the fetus, MeHg is accumulated and concentrated, especially in the brain. As with other forms of Hg, the kidneys retain the highest tissue concentration, but the brain still contains about fivefold higher concentrations than blood. This is because after crossing the blood–brain barrier, MeHg can be demethylated to Hg^{II} within the brain, where it cannot readily cross the blood–brain barrier and be eliminated. The half-life of Hg^{II} in the brain can thus be very long at 27.4 years (Rooney, 2014). MeHg accumulates in hair during hair strand formation, with average concentrations being about 250-fold higher than in blood. However, this ratio has been varies between individuals, which can add uncertainty to exposure estimates (Yaginuma-Sakurai et al., 2012; Liberda et al., 2014).

18.8 ELIMINATION

18.8.1 Hg^0

After short-term exposure to Hg^0 vapor, about one-third of the absorbed Hg will be eliminated in an unchanged form through exhalation, whereas the remaining Hg will be eliminated as Hg^{II} mainly through feces. Assuming first-order kinetics for the clearance of urinary Hg after exposure to Hg^0 vapor, the median half-life was found to be 41 days. Blood concentrations can serve as indicators of recent Hg^0 vapor exposure, though speciation must be carried out in order to eliminate possible influence of dietary intake of Hg from seafood.

18.8.2 Hg^+ and Hg^{II}

Excretion of absorbed inorganic Hg is mainly via urine and feces, the rates by each pathway being roughly equal. The whole-body half-life in adults is also about 40 days. The elimination of inorganic Hg follows a complicated pattern with biological half-lives that differ according to the tissue and the time after exposure. Hence, there are, at present, no general or suitable indicator media that will reflect concentrations of inorganic Hg in the critical organs, the brain or kidney, under different exposure conditions. One important consequence is that concentrations of Hg in urine or blood may be low soon after exposure has ceased, despite the fact that concentrations in the critical organs may still be high.

18.8.3 Organic Mercury

Hg excretion after MeHg exposure is predominantly via the feces. MeHg is slowly demethylated in the gut, and enterohepatic recirculation of MeHg explains that most, if not all, of the Hg excreted is in the demethylated inorganic form (Hg^{II}). Some elimination also occurs via urine. The whole-body half-life of MeHg is generally about 70 days. Laboratory animal studies have shown that, following acute dosage with MeHg, blood Hg concentrations will initially reflect organ concentrations reasonably well, but, with time, an increasing fraction of the body burden will be in the brain, muscles, and kidney. Interindividual variability exists in people's ability to demethylate MeHg. A recent development using laser ablation inductively coupled plasma mass spectrometry has made it possible to obtain individual MeHg elimination rate via longitudinal hair Hg analysis and to further investigate genetic and dietary factors (Rand et al., 2016).

The blood concentration might be a useful indicator of the body burden of Hg, although the erythrocyte Hg concentration is more specific for MeHg exposure. Thus, if exposure to Hg^0 vapor or other inorganic Hg compounds is suspected, Hg should be speciated or a serum sample analyzed. Hg in hair, when measured along the length of a hair strand, has also been used as an indicator of past blood levels. Prenatal exposure to MeHg is best determined as the Hg concentration in cord blood (Ha et al., 2017; Sakamoto et al., 2018).

18.9 HEALTH EFFECTS

18.9.1 Acute and Local Effects

Acute poisoning with Hg^0 vapor may cause a severe airway irritation, chemical pneumonitis, and pulmonary edema, in severe cases. Ingestion of inorganic Hg compounds may cause gastrointestinal corrosion and irritation, such as vomiting, bloody diarrhea, and stomach pains. Subsequently, shock and acute kidney dysfunction with uremia may ensue. Cutaneous exposure to Hg compounds may result in local irritation, and Hg compounds are among the most common allergens in patients with contact dermatitis.

18.9.2 Chronic and Systemic Effects

Chronic intoxication may develop as soon as a few weeks after the onset of a Hg exposure. More commonly, however, the exposure has lasted for several months or years, and an insidious onset may complicate early diagnosis. The symptoms depend on the degree of exposure and the kind of Hg in question. The symptoms mainly involve the oral cavity, the peripheral and central nervous system, and the kidneys. As the Hg^0 present in vapor is oxidized to Hg^{2+} in the blood, the non-neurotoxic effects of absorbed Hg^0 vapor and other inorganic Hg compounds will be similar.

18.9.2.1 Hg^0 Severe exposure to inorganic mercury causes an inflammation of gingiva and oral mucosa, which become tender and bleed easily. Salivation is increased, most obviously so in subacute cases. Often the patient complains of a metallic taste in the mouth. Especially when oral hygiene is bad, a gray border is formed on the gingival edges.

In exposures to Hg^0 vapor, the classic triad of symptoms includes erethism, intention tremor, and the gingivitis described above. The fine intention tremor of fingers, eyelids, lips, and tongue may progress to spasms of arms and legs. A jerky micrographia

is typical as well. The changes in the central nervous system result in psychological effects known as erethism: restlessness, irritability, insomnia, concentration difficulties, decreased memory, and depression, sometimes in combination with shyness, unusual psychological vulnerability, and anxiety. Newer studies suggest that early stages of erethism, dubbed "micro-mercurialism," may occur. The main symptoms appear to be decreased memory, dizziness, and irritability. Similar nonspecific symptoms are described by patients who attribute their ill health to Hg from their dental fillings. Two randomized controlled clinical trials on dental amalgam, the Children's Amalgam Trials, in New England and Portugal, found null results in neurobehavioral outcomes between the amalgam group and the control group (Bellinger et al., 2006; DeRouen et al., 2006). However, recent reanalysis suggest otherwise. Dental amalgams are found to be a chronic contributor to Hg body burden (Geier et al., 2011). In some genetically susceptible subpopulations, significant associations between amalgam and neurobehavioral deficits and biomarkers of kidney damage were found (Geier et al., 2013; Woods et al., 2013). Induction of minimal tremor by Hg^0 vapor has been reported at urinary excretion levels of 50 µg/L (0.25 µmol/L) and above.

Limited information is available on effects of Hg^0 vapor on early stages of the human life cycle. Effects on pregnancy and birth in women occupationally exposed to Hg^0 vapor have been reported, but insufficient details were available to evaluate dose–response relationships. In children, "pink disease" may occur, as described below.

18.9.2.2 Inorganic Mercurous (Hg^+) and Mercuric (Hg^{2+}) Mercury The target organ following long-term exposure causing no acute toxicity is the kidneys. In general, the early renal effects of Hg appear to be reversible after cessation of exposure.

Nephrotoxic effects include proximal tubular damage, as indicated by an increased excretion of small proteins in the urine (e.g., β_2-microglobulin). Glomerular damage seems to be caused by an autoimmune reaction to Hg complexes in the basal membrane, as demonstrated in experimental studies, although human evidence is inconclusive in this regard.

In children, a different syndrome is seen, the so-called "pink disease" or acrodynia, diagnosed most frequently in children treated with teething powders, which contained calomel, and also occasionally seen in children who had inhaled Hg^0 vapor (e.g., from broken thermometers) (Bose-O'Reilly et al., 2010). A generalized eruption develops, and the hands and feet show a characteristic, scaly, reddish appearance. In addition, the children are irritable, sleep badly, fail to thrive, sweat profusely, and have photophobia. This condition was extremely common until 40 years ago, when the etiology was finally found and teething powders were phased out.

18.9.2.3 Organic Mercury Intoxications with alkoxyalkyl or aryl compounds are similar to intoxications with inorganic Hg compounds, because these organomercurials are relatively unstable. Alkyl Hg compounds, such as MeHg, result in a different syndrome. The earliest symptoms in adults are paresthesias in the fingers, the tongue, and the face, particularly around the mouth. Later on, disturbances occur in the motor functions, resulting in ataxia and dysphasia. The visual field is decreased, and, in severe cases, the result may be total blindness. Similarly, impaired hearing may progress to complete deafness. This syndrome appeared as Minamata disease in Japan as a result of MeHg contamination from a local factory. Epidemics also occurred when MeHg-treated seed grain was used for baking or animal feed in Iraq, Guatemala, Pakistan, and elsewhere (Amin-Zaki et al., 1976; Marsh et al., 1981; Grandjean and Herz, 2011).

Children and the fetus are more susceptible to the toxic effects of MeHg than adults, and congenital MeHg poisoning may result in a cerebral palsy syndrome, even though the mother remains healthy or suffers only minor symptoms due to the exposure. In populations with a high consumption of fish or marine mammals, MeHg intakes may approach the levels that resulted in such serious disease in Japan and Iraq. Prenatal exposure to MeHg has been found to associate with low birth weight, delayed neurodevelopment, and growth and development of children (Grandjean et al., 2010; Karagas et al., 2012). Evidence from a long-term follow-up of a Faroese birth cohort has reported that a doubling of the prenatal exposure to MeHg results in a developmental delay of 1–2 months when the child is aged 7 years (Grandjean et al., 1997). A recent development from the same cohort suggested that the observed neurocognitive impact persists into adulthood at age 22 years, with an approximate deficit that corresponds to about 2.2 IQ points at a 10-fold increased prenatal MeHg exposure (Debes et al., 2016).

In adults, the earliest effects, such as paresthesia, appear to occur when blood concentrations are above 200 μg/L (1 μmol/L). Epidemiological studies from more than 20 years ago suggest that adverse cardiovascular effects may occur at much lower exposures than those that are prevalent among people regularly eating seafood (Salonen et al., 1995; Virtanen et al., 2005). Inconsistent results are still being reported (Houston, 2014; Choi et al., 2015). These findings may suggest that the relationship between cardiovascular disease and MeHg exposure is negatively confounded by the co-intake of other nutrients that protect cardiovascular health in seafood, such as long-chain *n*-3 polyunsaturated fatty acids (*n*-3 PUFA). Two recent studies provide some emerging evidence on Hg exposure and metabolic syndrome, such as body mass index, waist circumference, blood pressure, blood lipid profile, and diabetes (He et al., 2013; Eom et al., 2014). Seafood consumption was found to elevate brain Hg levels, but higher Hg brain levels were not associated with increased Alzheimer's disease or dementia in a group of deceased participants (Morris et al., 2016).

Sufficient evidence exists that MeHg chloride is carcinogenic to experimental animals. In the absence of comprehensive epidemiological data, MeHg is considered a possible human carcinogen (class 2B) (International Agency for Research on Cancer, 1994). The U.S. Environmental Protection Agency (USEPA) has classified both inorganic Hg compounds and MeHg as possible human carcinogens.

MeHg toxicity is modulated by several nutrients and genetic factors. Selenium (Se) is a nutritional trace mineral that has a U-shaped link to health effects (Rayman, 2012). Its potential interaction with Hg has been the focus of a longtime debate. Selenium (Se) may decrease MeHg toxicity in animal experiments, such as those with rats and zebrafish, by decreasing MeHg bioavailability to organisms and reversing oxidative damage in brain and neuroendocrine tissues (Ralston and Raymond, 2010; Amlund et al., 2015). However, no evidence has been found in human epidemiological studies indicating Se as an important protective factor against MeHg neurotoxicity or cardiovascular effects (Choi et al., 2008; Mozaffarian et al., 2011). Other nutrients such as soluble dietary fiber, iodine, tea polyphenols, and antioxidants in tomatoes have been associated with modulating MeHg toxicity (Ha et al., 2017).

Genetic polymorphisms in a number of environmentally responsive genes can explain the interindividual variability in Hg toxicokinetics and toxicodynamics. Genes involved in the toxicokinetics of Hg include GSH function [e.g., glutathione *S*-transferases (GSTs)], proteins that bind and transport Hg (e.g., selenoproteins, MTs), and xenobiotic transporters [e.g., multidrug resistance proteins (MRPs)] (Wang et al., 2012). These genes influence the

absorption, distribution, metabolism, and elimination of Hg and body burdens. Other genetic polymorphisms may explain the variability in Hg-associated adverse health outcomes. For example, in a cohort of male dentists and female dental assistants with occupational Hg^0 exposure, a nonsynonymous single nucleotide polymorphism (SNP) in BDNF(rs6265) was found to modify the relationship between Hg^0 exposure and performance on several neurobehavioral tests including hand steadiness and finger tapping (Echeverria et al., 2005). The same SNP also showed a significant main effect on general cognitive function in a U.K. birth cohort (Julvez et al., 2013). Not only do genetic factors influence Hg toxicity, but Hg exposure also affects epigenetic endpoints and modulates gene expression and may be the underlying mechanism of how early-life exposure can lead to adverse health outcomes later in life (Basu et al., 2014).

18.10 PREVENTION

The adverse health effects of Hg pollution and the international efforts to control the releases of Hg to reduce human exposures are well recognized. For example, the EU stopped production of Hg in 2003 and banned exports of Hg and certain Hg compounds since 2011. The economic benefits of preventing developmental neurotoxicity through controlling MeHg exposures across Europe have been estimated to be between €8000 and €9000 million per year (Bellanger et al., 2013), while the health benefits of prevention have been bolstered by the ratification of the Minamata Convention. This international treaty is expected to reduce global emissions of Hg by covering the whole Hg life cycle, from primary mining to waste proposal. Also, the best available techniques to control industrial Hg emissions at point sources such as coal-fired power plants and incinerators could provide added global benefits to public health. Minimizing Hg exposures from dental amalgam fillings, as suitable alternative restorative materials are now available for most purposes, also will benefit public health globally. Some countries discourage the use of amalgam fillings during pregnancy and in small children. Finally, the Minamata Convention addresses ASGM, which, currently, is one of the most predominant sources of Hg worldwide, affecting around 15 million people mostly in lower- and middle-income countries.

The USEPA established a reference dose (RfD) of 0.1 μg/kg body weight per day based on developmental neurologic abnormalities in human infants (EPA, 2001). This corresponds to a hair Hg level of about 1 μg/g hair. However, updated calculations accounting for imprecision of exposure parameters suggested an adjusted biological limit at about half the original recommendation, 0.58 μg/g hair (Grandjean and Budtz-Jørgensen, 2007). Data on blood Hg concentrations in the EU general population suggest that every year more than 1.8 million children are born with MeHg exposure above the limit of 0.58 μg/g hair (Bellanger et al., 2013).

For now, public health interventions such as dietary seafood advisories may be used to manage the health risks of Hg exposure. For example, in almost every U.S. state, increased Hg concentrations in freshwater fish have prompted state authorities to issue fish advisories as a guide to sport fishers. On the other hand, fish advisories may have unintended consequences, such as encouraging the general population to lower their fish consumption. In some areas, fish advisories have led to a 17% reduction in Hg exposure at the cost of a 21% reduction in cardioprotective *n*-3 PUFA (Shimshack and Ward, 2010). While important to acknowledge the health benefits of consuming fish and balancing the risk of MeHg with the benefit of *n*-3 PUFA intake (Rheinberger and Hammitt, 2012), consumers need better

information on fish consumption to optimize the potential associated health benefit while lowering risk of MeHg exposure. One way to do this is to promote fishing at lower trophic levels and catching smaller and younger fish and harvests from relatively unpolluted ecosystems (Sunderland and Selin, 2013; Karimi et al., 2014).

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19

CARDIOPULMONARY EFFECTS OF NANOMATERIALS

Eric Saunders, Lung-Chi Chen, Terry Gordon, and Morton Lippmann

19.1 INTRODUCTION

Nanoparticles (NPs) are particulate matter (PM) ranging in size from 1 to 100 nanometers (nm) in at least one dimension, usually diameter (ASTM, 2006). For many years, NPs were grouped with ultrafine particles (UFPs) due to the limitations of equipment used for quantification (Donaldson et al., 2004). The imaging of nano-sized PM has been achievable since the mid-1930s, when field emission microscopes first made it possible to reach such high magnification with a resolution clear enough to detect these small particles (Initiative UST, 2018, nano.gov). In 1986 Gerd Binnig and Heinrich Rohrer were awarded the Nobel Prize in Physics for inventing the scanning tunneling microscope, which was one of the first instruments capable of viewing and handling of NPs with precision. In the late 1950s, physicist Richard Feynman postulated in his lectures that precise control and manipulation of substances at the molecular level would lead to great innovations (Feynman, 1959), thus envisioning the "world of tomorrow" as a place where one would be able to manipulate things at the atomic level. Achieving this vision of tomorrow through controlled manipulation and intentional targeted use of these very small particles in consumer goods on a large scale is a practice that has only recently been achievable. NPs have come to be highly valued for their seemingly endless possibilities associated with specific parameters (e.g., surface area, surface-to-mass ratio, surface charge). The number of uses that generate potential for human exposure has far outpaced the published research on exposure and outcomes. The purpose of this chapter is to provide an overview of the cardiovascular health effects associated with the inhalation of engineered NPs.

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19.2 NANOPARTICLES: SCOPE AND TOXICITY

As interest in intentional manipulation of materials at the atomic or nanolevel gained momentum throughout the later part of the twentieth century, Taniguchi (1974) lumped together the uses and study of nano-sized materials into one term, "nanotechnology." Nanotechnology is now defined as a wide range of technologies that measure, manipulate, or incorporate materials and/or physical features with at least one dimension between approximately 1 and 100 nm (ASTM, 2006). The nanotechnology marketplace is a constantly evolving stream of old products being modified, new materials being created, and a seemingly endless process of innovation bringing Feynman's world of tomorrow to today. The current number of NP-based technologies is unknown in the United States. There are at least 400 documented NP products using silver (Ag) alone in the European Union (EU) (Braakhuis et al., 2014). While the size and scope of the market are constantly in flux, it is estimated that by 2022, the global nanomaterial market will generate $16.8 billion U.S. dollars (USD) annually (Zion Market Research, 2017). Major use sectors include the chemical, medical device, pharmaceutical, and personal care product industries. While this is not an exhaustive list, each of these industries is heavily invested in nanotechnology for use in everyday products. Over the last few decades, there has been a growing interest in use of NPs to improve diagnostics and therapies for many diseases. Nanotechnology is widely used in practically every branch of medicine such as imaging, biosensing, drug delivery, tissue engineering, implants, and microsurgery (Card et al., 2008; Jain, 2008; Wang et al., 2009). The relatively large surface area of NPs provides a great medium for functionalization or to attach peptides and antibodies that precisely target cell types and tissues (Card et al., 2008). Based on their abundance of surface area, NPs have the potential to revolutionize (1) biological imaging at the cellular level, (2) cancer detection and treatment, (3) radio- and chemosensitizing agents, and (4) targeted drug delivery (Rzigalinski and Strobl, 2009). In addition, protein nanochips are being developed to detect traces of proteins in biological fluids that have much higher sensitivities than conventional bioassays and nano-biosensors that can provide platforms to develop portable or implantable detection and monitoring devices (Jain, 2008).

Properties that are easy to manipulate like size, shape, surface chemistry, surface area, and surface charge have proven to be of great use in industrial and consumer products. As innocuous as it may seem to alter any of these properties, research suggests that each of them can potentially alter specific toxicities affecting multiple endpoints. For example, various *in vitro* and *in vivo* studies have shown that when chemical composition and mass of exposures are held equal, NPs induce stronger inflammatory or toxic responses, per unit of mass, than their larger-sized counterparts (Zhang et al., 2003; Oberdörster et al., 2005; de Haar et al., 2006; Nurkiewicz et al., 2008). One reason is that NPs have a much higher surface-to-volume ratio than larger particles. One way to visualize this is to compare a jar of marbles weighing 100 g (~20 marbles) to a jar of sand weighing 100 g (~9100 grains of 2 mm quartz sand). For evenly spread marbles on a table, one could easily count the number of contact points between the marbles and the table or notice that a large portion of the marbles mass (atoms) are concealed within the marble's interiors. However, the same mass of sand evenly spread onto a table would not only cover more of the table, but it would have substantially more contact points. Additionally, a higher proportion of the NP's atoms would be on the surface, allowing more ready and completely reactions with adjacent atoms and substances (Powell and Kanarek, 2006), including other particles or surrounding biological media. However, it should be noted that the extent to which this holds true is

greatly influenced not only by particle size, but the porosity or topographical structure of the NP as well (Ruenraroengsak and Tetley, 2015). Additionally, it is theorized that a reduction in particle size may alter electrostatic properties of the material by increasing the number of structural defects and disrupting the well-structured electronic configuration (Jiang et al., 2017a; Klaassen et al., 2017). One such example can be demonstrated by the testing of biological responses to titanium dioxide (TiO_2) particles that are varied by size and crystal phase. When tested in a cell-free system for their potential to generate reactive oxygen species (ROS), the results strongly suggested that the inherent oxidant capacity of these NPs was related to its crystalline phase, which directly translates to an availability of surface defect sites (Jiang et al., 2008). Thus, it is not likely that one specific parameter can explain the toxic effects of NPs. The varying toxic potential of many NPs has been linked to multiple factors, none of which can be considered the sole predictive indicator of toxicity (Alexis et al., 2008; Madl and Pinkerton, 2009).

19.3 LESSONS LEARNED

There have been many lessons learned in the study of fine particles ($PM_{2.5}$) and UFPs ($PM_{0.1}$) that can be applied to NPs (Stone et al., 2017). It has long been known that particle size affects respiratory tract deposition (Oberdörster et al., 2005; Peng et al., 2017) and cellular uptake (Saikia et al., 2016). The lung has extensive, location-specific defense systems including mucociliary clearance in the conducting airways and macrophage clearance in the gas exchange airways and at natural airway bifurcations that will influence whether a particle is deposited along an airway or impacted onto a surface downstream of a bifurcation. Given the relationships between particle size and deposition efficiency, it is easy to see why the small sizes of NPs play an important role in its toxicity (Borm et al., 2006). An additional factor to consider in NP toxicity is the rate of dissolution of NPs in the proteinaceous surfactant of the conducting airways, which affects free ion availability systemically (Peretyazhko et al., 2014; Theodorou et al., 2016; Falconer and Grainger, 2018). Lastly, it has been shown that the size of a particle may affect its ingestion by different cell types, with some smaller NPs being too small to be recognized at all by airway macrophages (Limbach et al., 2005; Rothen-Rutishauser et al., 2007; Geiser et al., 2008; Shang et al., 2014). For example, it has been shown that inhaled NPs <10 nm (Geiser et al., 2008) are not recognized as efficiently as larger particles by alveolar macrophages (AM), leading to increased contact with epithelial cells and/or airborne penetration into deeper regions of the lung (Semmler-Behnke et al., 2007; Unfried et al., 2007; Geiser et al., 2008), potentially causing direct particle contact-induced effects (Oberdörster et al., 2005).

Another unique mechanism regarding NP-induced toxicity is transmembrane translocation. It has long been theorized that inhaled NPs can escape pulmonary cell junctions and translocate into the bloodstream, and therefore reach the liver, heart and brain (Elder et al., 2006; Yu et al., 2007; Geiser et al., 2008). This phenomenon may provide an explanation for epidemiological findings of cardiovascular effects associated with inhaled ambient NPs as a consequence of direct particle impacts on the cardiovascular system (Oberdörster et al., 2005). There is also substantial evidence of systemic availability of intact NPs in rodents that have had NPs orally administered. Research has shown that NPs are captured by phagocytic cells in the spleen, liver, kidney, and bone marrow (Sadauskas et al., 2007; Fabian et al., 2008; Wang et al., 2009). While it has been experimentally verified that translocation across the air–blood barrier is possible, it is likely that this phenomenon occurs in

very small numbers and is highly dependent upon particle size (surface area) and surface charge (Kreyling et al., 2014). Additionally, it has been found that a major factor affecting the ability of an inhaled NP to translocate is the amount of albumin available in the NP protein corona, a factor highly influenced by the base material of the NP (Konduru et al., 2017). Taken together, the literature suggests that translocation of inhaled NPs is both infrequent and highly variable depending on several biological and physicochemical NP and host properties. This suggests that a majority of the systemic availability of NPs is the result of ions generated by NP dissolution or ion adsorption to the surface of particles. However, the literature does show that intact NPs can and do pass directly into the circulatory system, but at what is likely to be an insignificant rate (Geiser and Kreyling, 2010; Kreyling et al., 2014).

19.4 PARTICLE CHARACTERIZATION

It has been suggested that numerous physicochemical parameters can influence NP toxicity, such as chemical composition, dissolution rate, particle size, size distribution, shape, and surface area (Warheit et al., 2007). Thus, NPs for toxicological studies need to be characterized, in terms of physicochemistry, as completely as is practical (Powers et al., 2007). We also recommended that purchased NPs, which usually come with listed physicochemical properties provided by the manufacturer, be independently characterized because of batch-to-batch variations, difficulties with quality control for the large-scale production (Pettibone et al., 2008), and the possibility of further changes due to transport or storage in transit or in the laboratory. A thorough characterization of NPs will not only help in establishing a causal relationship between physicochemical properties and toxic potential but will also be critical in producing reproducible and reliable results in toxicology studies.

Without sufficient characterization, it is extremely challenging to interpret the results of individual studies and virtually impossible to adequately compare the results of different studies. Although there is not yet a universally accepted set of parameters that are deemed necessary for NP characterization, several key physicochemical elements strongly recommended to be reported include method of synthesis, particle size and distribution, shape, composition, crystal structure, aggregation and agglomeration status, dissolution rate, purity, surface area, and other surface characteristics (OECD, 2017) and characterization of NPs in the context of the experimental exposure media (cell culture media, dosing solution, aerosol, etc.). Also of considerable importance are some physicochemical parameters that are likely to differ depending on whether they are determined in the experimental media or in the bulk (i.e., “as received”) state (Card et al., 2008).

19.5 RELEVANT EXPOSURE SCENARIO

As heavy usage of intentionally engineered NPs has become commonplace, new exposure avenues for the induction of adverse human health effects have been created. Assessing NP exposures has become a challenge for both exposure scientists and toxicologists, as they become important much more frequently and in terms of chronic effects (Oberdörster et al., 2005). In response to the growing body of knowledge highlighting the uncertainty of the health effects associated with NP exposures, the Organization for Economic Cooperation and Development (OECD) began addressing both the human and environmental impacts of

NPs in 2006. In 2013, the OECD recommended that current international and national regulatory frameworks assessing the safety and use of chemicals should be applied to NPs. By extending existing regulatory guidelines to NPs, the risks associated with manufacture, use, and environmental fate of NPs can be more closely evaluated and placed into context for use in risk assessment. Additionally, the OECD recognized, as do the vast majority of researchers, that the techniques that apply to bulk materials will not always be the most effective method for characterization or study of NPs. A number of projects are currently underway to evaluate the best approaches for specific endpoints (OECD, 2017). As regulators and occupational health experts struggle to deal with current challenges, the next major phase of nanotechnology is being developed. The continued push for innovation far outpaces health research abilities, highlighting the need for coordinated testing strategies and enforcement of safety protocols.

Currently, most toxicology tests of NPs face critical limitations that must be taken into account when assessing adverse outcomes. They have often used extraordinarily high doses, or have delivered the NPs as a single bolus dose. Such study designs provide little to no relevancy to anticipated human exposure scenarios (Oberdörster et al., 2007). Additionally, studies that do not include recovery time, and/or focus only on acute results, do not take into consideration natural clearance or metabolic transformations that may play a role in inducing NP-specific toxicity. In addition, studies utilizing *in vitr*o exposure methods are very important for determining the mechanistic steps involved in an already established toxicity value. However, *in vitro* tests do not necessarily provide insight into the complete biologic reaction.

When studying NPs, there is an additional potential confounder that must be considered; a majority of methods have administered NPs as aqueous suspensions not likely to be relevant to real-world inhalation exposures. When NPs are stored in suspension, they tend to agglomerate, being held together by relatively weak forces, for example, van der Waals or capillary forces. Such agglomerates may, or may not, break apart into smaller particles or clusters, and the end result is likely to be aggregate particles of greater diameter than the singlet NP originally intended for use in the study. Thus, since many aspects of NP-induced toxicity are considered size dependent, delivering heavily agglomerated NPs is likely to lead to inaccurate assessments of NP toxicity (Sager et al., 2007), making it difficult to perform reproducible and reliable NP toxicological studies. Due to lack of standardization in NP research, many researchers have used a variety of different approaches to suspend NPs, which might be one of the reasons for contradictory results when compared with those of similar studies (Buford et al., 2007). Recent studies have clearly shown that dispersion pattern and state of agglomeration of the NPs can vary significantly, depending on dispersing media and efficiency (Buford et al., 2007; Sager et al., 2007), thereby greatly affecting biological reactivity of the same material (Buford et al., 2007; Mercer et al., 2008; Shvedova et al., 2008).

19.6 NP EXPOSURE

Human exposure to NPs is possible via six principle routes: intravenous (IV), dermal, subcutaneous, inhalation (most common; Oberdörster et al., 2005), intraperitoneal (IP), and oral (Ryman-Rasmussen et al., 2007). Numerous studies have shown potential toxicity to NP exposure, both systemically and molecularly (Oberdörster et al., 2005; de Haar et al., 2006; Card et al., 2008; Nurkiewicz et al., 2008; Murray et al., 2009). Though many organs

are likely targets, cardiac toxicity is one of the major concerns regarding NP-induced toxicity, because (1) targeted use of NPs for cardiac and hemolytic therapies is increasingly expanding and (2) systemic distribution of xenobiotics and their metabolic by-products occurs via the cardiovascular system and carries with it the potential for toxicity from secondary effects from NP exposure occurring via other routes.

19.6.1 Direct Cardiac Exposure to NPs

Cardiovascular research is one of the major fields of use and study for NPs. There is great clinical relevance in noninvasively assessing the development of atherosclerotic plaque, which has been a topic of active research using ligand-conjugated NPs to recognize the stage-specific markers of atherosclerosis (Douma et al., 2009). More than 10 types of NPs have been used in clinical and preclinical studies for stage-specific visualization of plaque progression, including quantum dots (QD) and iron oxide NPs (Douma et al., 2009). One study reported their development of polymer-based nanomaterial for a synthetic heart valve based on its superior biocompatibility and *in vivo* bio-stability (Kidane et al., 2009). Another found that polyelectrolyte-coated gold nanorods had potential to modulate cell-mediated matrix remodeling in cardiac fibroblasts, suggesting that these NPs could be applied for antifibrotic therapies (Sisco et al., 2008).

Most of these applications require direct administration of nanomaterials into the target cardiovascular tissues to be effective, which means that exposures to relatively high concentrations of NPs are likely to occur in those target tissues. This poses emerging health concerns, since accumulating evidence suggests that NPs may exert adverse effects on the cardiovascular system.

19.7 CARDIOVASCULAR EFFECTS FOLLOWING PULMONARY NP EXPOSURE

In addition to cardiac effects resulting from direct exposure to NPs, it is possible that the cardiovascular system may experience secondary effects from NP exposures in distant organs. Among the six principal routes, inhalation is considered the major route of occupational exposure for NPs (Oberdörster et al., 2005; Medina et al., 2007). Epidemiological studies have suggested associations between inhalation exposures to ambient air UFPs and increased risk of cardiopulmonary disease and also increased mortality (Pope et al., 2004). Recent studies have also shown that exposure to ambient air particles (including air ambient NPs) for 6 months could enhance development of atherosclerosis in hyperlipidemic mice (Chen and Nadziejko, 2005; Sun et al., 2005). Since UFPs and NPs have several physical characteristics and toxicological properties in common (Oberdörster et al., 2007), it has been suggested that inhaled NPs might have potential to induce systemic cardiovascular toxicity as well.

To explain how pulmonary NP exposure can elicit cardiovascular responses, several pathways and mechanisms have been proposed. One hypothesis is that NPs deposited in the lung act upon the central nervous system to alter cardiac autonomic function (Nurkiewicz et al., 2006). A second hypothesis is that NPs deposited in the lung initiate local inflammatory responses via oxidative stress, leading to systemic oxidative stress/inflammation (Yamawaki and Iwai, 2006a). Pulmonary oxidative stress and inflammation after exposure to NPs or PM are well documented (Oberdörster et al., 2005; Brook, 2008), and systemic

pro-oxidative and pro-inflammatory reactions after PM exposure have also been widely studied (Brook, 2008). More recently, a third hypothesis was proposed whereby NPs deposited in the lung could translocate into the systemic circulation and directly interact with target tissues, including vascular endothelial cells (ECs), inducing injury, inflammation, and destabilization of plaques (Yamawaki and Iwai, 2006b). It should be noted that there is evidence to support all three mechanisms, and they are not mutually exclusive. Some of the recent studies supporting these mechanisms are discussed in the following sections.

There are reportedly 1300 nanomaterials that were either being currently used or were being considered for use in industrial or commercial applications (Papp et al., 2008). Considering the rapid increase of publications in nanotechnology and nanotoxicology, we concluded that reviewing every publication related to NP toxicity was not feasible, and was beyond the scope of this chapter. Therefore, in this section, studies investigating the most widely used nanomaterials are reviewed by categories, that is, the four key NP classes used in many commercial applications. These are (1) fullerenes, (2) carbon nanotubes (CNTs), (3) QDs, and (4) metallic and metal oxide-based NPs (Papp et al., 2008).

The purpose of this section is to cover studies investigating biological effects of NP exposure on the cardiovascular system. Studies mainly focused on toxicokinetics or biodistribution of NPs were not considered, unless they were considered particularly relevant to cardiac toxicity.

19.8 TYPES OF NP IN COMMON USAGE

19.8.1 Fullerenes

Fullerenes (C_x) are novel allotropes of carbon consisting of carbon atoms joined to form hollow spheres with a cage-like structure (Nielsen et al., 2008). The most popular form of fullerene is composed of 60 carbon atoms (C_{60}). The structure of fullerenes resembles that of the geometric pattern first pioneered by Buckminster Fuller in his geodesic dome structures. This resemblance has earned them the nickname Buckminsterfullerene's or "Buckyballs" (Poma and Di Giorgio, 2008).

Fullerenes are characterized by having numerous points of attachment resembling the pattern of lines commonly seen on a soccer ball, the most stable of which have an even number of carbon atoms. Such strong organic structures provide great platforms for functionalization and carboxylation, attributes that are readily taken advantage of in modern medical research (Medina et al., 2007). Beyond their uses for potential medical delivery devices, there is great potential for industrial usage as components in a variety of plastics, including filtration membranes (Gelderman et al., 2008). These organic structures are valued for many reasons in the pharmaceutical industry, most notably the following: (1) they have hollow structures capable of carrying compounds to precise locations, and (2) the diameter of C_{60} is about 0.7 nm, which is roughly the size of many small pharmaceutical molecules (Nielsen et al., 2008). Fullerenes and their hydroxylated derivatives [i.e., $C_{60}(OH)_{24}$] are also reported to have potent antioxidant and antibacterial properties that have been extensively studied (Gelderman et al., 2008).

While fullerene usage in medical and industrial applications continues to show promise, safety concerns have been raised about the cytotoxic effects of fullerenes and their derivatives in multiple studies (Sayes et al., 2005; Zhu et al., 2006; Gelderman et al., 2008).

As the field of nanotechnology has advanced, more data have become available regarding potential toxicity. The following studies, while not exhaustive, are representative of what is currently known regarding the cardiac toxicity of fullerenes.

Bosi et al. (2004) investigated the hemolytic effect of various water-soluble C_{60} derivatives on human red blood cells *in vitro*. After 30-min incubation, C_{60} fullerenes with two cationic chains resulted in hemolysis of 40–50% of the cells at concentrations ranging from 20 to 60 μM, whereas compounds with bis-functionalized chains, bearing carboxylic functions or only one cationic chain, did not show any hemolytic effects up to 80 μM. These results suggest that the hemolytic potentials of fullerenes are dependent on numbers and positions of cationic chains on the fullerene.

Another well-known *in vitro* study regarding the acute toxicity of a polyhydroxylated fullerene derivatives was conducted by Yamawaki and Iwai (2006b) using human umbilical vein endothelial cells (HUVECs) that were subjected to a 24-h exposure to 1–100 mg/L of fullerenol [$C_{60}(OH)_{24}$]. The fullerenol induced cytotoxic morphological changes in HUVECs, such as vacuole formation in the cytosol and decreased cell density in a dose-dependent manner. Fullerene aggregates were accumulated in numerous autophagosomes in the cells after 24-h exposure, and after 10 days exposure to 100 mg/L, vacuoles and spindles cells were observed along with decreased growth rates.

An additional study by Gelderman et al. (2008) also using HUVECs to determine the adverse effects of C_{60} and $C_{60}(OH)_{24}$ on endothelial cells showed that, after 24-h exposure to a C_{60} water suspension (nC_{60}; 4 μg/mL) or $C_{60}(OH)_{24}$ (10, 50, and 100 μg/mL), both materials caused cell cycle arrests at G1 and acute Ca^{2+} influx. At a high concentration (100 μg/mL) of $C_{60}(OH)_{24}$, cell surface expression of intercellular adhesion molecule 1 (ICAM-1) (also called CD54) and tissue factor (CD142) on HUVECs were significantly elevated. In addition, increased apoptosis was observed in the cells exposed to 100 μg/mL of $C_{60}(OH)_{24}$. However, when brown adipose-derived stem cells (BADSCs), a cell line capable of differentiating into cardiomyocytes are exposed to C_{60} at levels up to 100 μg/mL an increased expression of mitogen-activated protein kinase (MAPK) was observed, along with improved expression of cardiomyocyte-specific proteins (cTnT and α-sarcomeric actinin) (Hao et al., 2016). Additional research into drug delivery has found that C_{60} potentially can play a protective role against superoxide dismutase and glutathione peroxidase inhibition induced by doxorubicin, a powerful cancer drug known for high cardiac toxicity (Prylutska et al., 2014). Additional *in vitro* exposures to various cell lines such as HeLa and human embryonic kidney 293 (HEK293) cells have shown that exposure to fullerenes for 33 days increased proliferation while not affecting lactate dehydrogenase activity. However, when duration of exposure was extended to 80 days, Chinese hamster ovary, HeLa, and HEK 293 cells all displayed genotoxic effects in the micronucleus assay (Niwa and Iwai, 2006). Additionally, exposure to fullerene C_{60} was shown to exhibit a very low toxicity and inhibit nitric oxide (NO) production in human macrophage cells (Fiorito et al., 2006). While this is not directly related to cardiac toxicity, it is worth noting that the effects found in one *in vitro* assay are not to be considered universal for systemic toxicity.

In addition to cytotoxicity concerns, there is evidence that fullerenes may contribute to ischemia–reperfusion injury by altering critical mechanisms of cardiac function. Satoh et al. (1997a, 1997b) investigated the effects of water-soluble C_{60} derivatives (mono- or dimalonic acid C_{60}) on vasomotor dysfunction, specifically on endothelium-dependent relaxation, using isolated rabbit aorta. After incubation with the C_{60} derivatives (10 μM), potent and selective inhibitory effects were observed on the endothelium-dependent relaxation induced either by agonists (i.e., acetylcholine) or by endogenous NO. Since these

inhibitory effects of the malonic acid C_{60} derivatives were masked in the presence of superoxide dismutase, it was suggested that these C_{60} derivatives inhibit endothelium-dependent agonist-induced relaxation of the aorta through the production of superoxide. While the *in vitro* research suggested impaired cardiac function, research by Thompson et al. (2014) verified these findings *in vivo*. It was found that IT instillation of fullerene C_{60} can cause augmented vasocontraction in response to endothelin-1 attenuated with indomethacin in male rats and a depressed vasorelaxation in response to sodium nitroprusside in female rats. Additionally they found that IV injection of fullerene C_{60} impaired acetylcholine relaxation response in male rats. Additional research into mechanism disruption has shown the association between IP injection of pristine C_{60} fullerenes and vasomotor dysfunction. The $ApoE^{-/-}$ mouse model is widely used in cardiac research due to the poor clearance of lipoproteins, which promotes accumulation of cholesterol in the blood, ultimately leading to cardiac plaque formation. In this research the aorta of $ApoE^{-/-}$ mice of different ages (11–13 vs. 40–42 weeks old) was studied, and vasomotor dysfunction was measured 1 hour post IP injection of 0.05 or 0.5 mg/kg of pristine C_{60}. Measurements were taken postmortem with aorta segments mounted in myographs. In general, IP injection of pristine C_{60} affected mainly the vasorelaxation response in young $ApoE^{-/-}$ mice, whereas the vasomotor dysfunction in older $ApoE^{-/-}$ mice was less affected. Both endothelium-dependent and endothelium-independent vasorelaxation responses were slightly decreased in the young $ApoE^{-/-}$ mice post C_{60} treatment. These findings indicated that IP administration is associated with a moderate decrease in the vascular function of mice with atherosclerosis (Vesterdal et al., 2009). Combined, these studies show that there is significant cause for concern beyond cytotoxicity. Both ischemia–reperfusion injury and those with elevated lipoprotein profiles may experience serious cardiac dysfunction as a result of exposure to C_{60} fullerenes.

When interpreting the fullerene toxicity data, especially from some of the earlier studies, special concern needs to be paid regarding the purity of the test materials, or any functionalized conjugates that may be used for testing. It has been demonstrated that residual tetrahydrofuran (THF) used for C_{60} solubilization substantially influenced the observed toxicity in the Gelderman et al. (2008) study, according to Andrievsky et al. (2005). The toxic effects observed by Oberdörster (2004) were associated with the presence of a large quantity of impurities (~10% of organic impurities). Additionally, it was shown that with functionalized with carboxylic acid or a hydroxyl group, selective organ uptake and persistence is greatly influenced (Wang et al., 2016).

19.8.2 Carbon Nanotubes

CNTs are one of the most extensively used types of carbon NPs. They are valued for their strength and for being light in weight. The production of CNTs has reached into the hundreds of metric tons per year, with new uses constantly being developed (Poma and Di Giorgio, 2008). CNTs are allotropes of carbon with nanostructures that resemble a sheet of graphite (a hexagonal network of carbon atom) rolled into a hollow cylinder, which diameters as small as 0.7 nm that can reach up to several millimeters in length. There are two main forms of manufactured CNTs: single-walled carbon nanotubes (SWCNTs) composed of a single layer of cylindrical framework and multi-walled carbon nanotubes (MWCNTs) with several layers of concentric SWCNTs (Ju-Nam and Lead, 2008).

CNTs have many unique properties that make them highly desirable for use in a variety of products beyond being ultralight in weight, including having very high mechanical

strength and electrical conductivity. In particular, the use of CNTs has allowed for great advances in electronics, polymers, composites, and biomedical devices (Medina et al., 2007; Poma and Di Giorgio, 2008). However, as is often the case, the properties that make a nanomaterial like CNTs desirable are also likely to contribute significantly to their toxicity. The length/width ratio (aspect ratio), surface chemistry, residual elemental composition (purity), and poor solubility have led to safety concerns that apply to mineral fibers, such as those if asbestos, and to crystalline silica (Mitchell et al., 2007; Shvedova et al., 2009; Walker et al., 2009).

A substantial number of *in vitro* studies were conducted to examine the cellular uptake, cytotoxicity, and stimulatory effects of CNTs (reviewed by Shvedova et al., 2009) as well as several dozen animal studies. These *in vivo* studies showed that pulmonary exposure to SWCNTs and MWCNTs has caused acute pulmonary inflammation, as well as chronic responses such as granulomas and interstitial fibrosis (Shvedova et al., 2009; Walker et al., 2009). Studies investigating cardiovascular effects have shown a variety of outcomes, ranging from oxidative protein modification to endothelial dysfunction (Zhiqing et al., 2010; Shvedova et al., 2014)

To investigate the role SWCNTs play on cardiac specific endpoints, Raja et al. (2007) exposed rat aortic smooth muscle cells (SMC) to SWCNTs and monitored the cell growth rates for 3.5 days. They showed a significant dose-dependent inhibition of cell growth post SWCNT exposure. Additionally, finely dispersed non-aggregated CNTs were significantly associated with the cell growth inhibition, despite their low (<1%, v/v) concentration in the media. Further investigations using *in vitro* methods described the effects that SWCNTs have on rat aortic endothelial cells (RAECs). These SWCNT exposures induced expression of the stress protein heme oxygenase 1 (HO-1) and altered expression of adhesion molecules VCAM-1 and ICAM-1 (Zhiqing et al., 2010; Cheng et al., 2012). These studies also indicated that exposure to SWCNTs induced cardiac stress and played a role in inducing the inflammatory cascade that could contribute to CNT-induced endothelial damage (Zhiqing et al., 2010; Cheng et al., 2012). Further investigation in human aortic endothelial cells (HAEC) showed marked disruption in actin filaments and vascular endothelial (VE) cadherin, along with cytotoxicity and reduced tubule formation 24-h post-exposure (Walker et al., 2009). Additional *in vitro* research into the systemic effects of SWCNTs found that human peripheral blood lymphocytes (HPBL) treated with SWCNTs showed significant decreases in cell growth. Upon further investigation, it was found that these decreases were triggered by a marked decrease in metabolic activity and not the result of cytotoxicity (Zeni et al., 2008). While many studies have focused on metabolism, apoptosis, or oxidative stress, at least one *in vitro* study, which examined neonatal rat ventricular cardiomyocytes, found that exposure to SWCNTs created a stimulating effect that triggered an increase in impulse conduction velocity and action potential (Helfenstein et al., 2008), indicating that SWCNTs can directly affect the function of the cardiac cells. However, it should be noted that SWCNTs showed the mildest effects compared with other particle types included in this study, that is, diesel engine exhaust particles and titanium dioxide. Taken together the *in vitro* data suggest that SWCNTs can trigger inflammation, inhibit cellular growth, activate stress mechanisms, alter expression of adhesion molecules, and increase impulse conductivity in cardiac specific cells.

Several *in vivo* studies have investigated the systemic effects of CNTs. The cardiac effects of pulmonary exposure to SWCNTs induced oxidative stress in the aorta and also exacerbated plaque formation in hyperlipidemic mice ($ApoE^{-/-}$) (Li et al., 2007). Additionally, pulmonary SWCNT exposure resulted in increased mitochondrial DNA

damage, decreased mitochondrial glutathione, and increased protein carbonyl formation in the aorta, at 7, 28, 60 days' post-exposure. Repeated exposures led to a significant increase in plaque lesions in the brachiocephalic arteries, suggesting that pulmonary exposure to SWCNTs can induce cardiovascular effects in mice (Li et al., 2007). Studies investigating the effects of SWCNT exposure in $ApoE^{-/-}$ mice, via intrapharyngeal instillation, found both mtDNA damage and accelerated plaque formation; however, exposure did not alter the lipid profile and was not accompanied by significant increases in inflammation (Li et al., 2007). Further investigations into the base strain of the $ApoE^{-/-}$ mouse, the C57BL/6 mouse, showed an increase in oxidized proteins, lipid peroxidation, depletion of antioxidants, and inflammation in the heart and liver as far out as 7 days' post inhalation exposure (Shvedova et al., 2008, 2009, 2014). Much of the literature has focused on acute endpoints. However, at least one study, investigating the long-term effects of inhalation exposure to SWCNTs, showed significant genotoxicity with micronuclei formation and nuclear protrusions 1 year after 4 days of 5-h exposure to 5 mg/m^3 in C57BL/6 mice (Shvedova et al., 2014). Additional studies in occupationally relevant doses show rapid and progressive alveolar fibrosis (Castranova et al., 2013). Additionally, it was found that when Wistar-Kyoto rats were exposed IT to SWCNTs, they displayed a decrease in heart rate, as well as a drop in baroreflex sequences, indicating that SWCNTs are capable of altering the autonomic response of the cardiovascular system (Legramante et al., 2009).

Nemmar et al. (2007) investigated thrombogenic effects induced by pulmonary exposure to MWCNTs. They introduced 200 and 400 μg of MWCNTs IT to Swiss mice. At 24-h post-exposure, MWCNT-exposed mice showed substantial neutrophil influx in the lung, elevated plasma coagulant microvascular tissue factor activity, and enhanced peripheral thrombogenicity. Circulating platelet–leukocyte conjugates were detected exclusively at 6-h post-exposure, indicating that there was early transient activation of platelets induced by pulmonary MWCNT exposure. When they neutralized P-selectin *in vivo* using blocking antibodies, the MWCNT-induced thrombogenesis was mostly abrogated. Based on this result, the authors suggested that the mild lung inflammation induced by MWCNT exposure can translate into P-selectin-mediated systemic inflammation via platelet activation, which may lead to inflammation-induced procoagulant activity and prothrombotic risk. Additional studies verified this pathway by verifying prothrombrotic effects at low doses (0.1 mg/kg^{-1}/body weight) *in vivo* and induction of P-selectin as well as formation of platelet granulocyte complexes *in vitro* (Holzer et al., 2014).

However, it is important to consider that, due to the manufacturing process, CNTs like fullerenes may contain substantial amount of various impurities, including toxic metals (i.e., Co, Fe, Ni) (Donaldson et al., 2006; Lam et al., 2006; Shvedova et al., 2009). For example, Shvedova (2007) reported that SWCNTs synthesized by certain methods could have significant amount of metal impurities (i.e., 30% of Fe or 20% of Ni) in content, and they could induce marked oxidative stress and decrease in viability in BEAS-2B cells. Ge et al. (2012) showed that the coexistence of various metal residues can affect the severity or likelihood of adverse events.

When taken together, the *in vitro* and *in vivo* data showed a very real possibility that the heart may be a target organ for toxicity of SWCNTs. While animal data do not always translate to human effects, the literature shows a clear pathology that is ultimately manifested in both mice and rats. However, it should be pointed out that due to confounding factors, like inadequate dispersion and purity, a full understanding of CNTs, and especially SWCNTs, remained incomplete (Lam et al., 2006; Shvedova et al., 2009).

19.8.3 Quantum Dots

QDs are heterogeneous NPs consisting of a colloidal nucleus surrounded by one or more surface coatings, with sizes ranging from 2 to 100 nm (Ryman-Rasmussen et al., 2007; Rzigalinski and Strobl, 2009). The nuclei of QDs may be composed of metals (i.e., Cd, Co, and Fe) or semiconductors, and it was suggested that due to their diversity in chemical composition, QDs could not be considered as a single uniform group of materials (Hardman, 2006). QDs have unique optical and electronic properties, including high stability and "size-tunable" fluorescence, which make them particularly attractive for imaging and diagnostic purposes (Card et al., 2008; Rzigalinski and Strobl, 2009). As the application of QDs in biomedical devices advanced, considerable concern was raised concerning that certain types of QDs have inherently toxic elements (i.e., Cd) in their core (Douma et al., 2009). Since cardiomyocytes are reported to be more sensitive to Cd-induced toxicity compared with other cell types, there is even greater significance in investigating potential cardiovascular toxicity following QD exposure (Limaye and Shaikh, 1999).

While interest in using QDs for imaging and diagnostics continued to grow, very little work examined their cardiovascular toxicity. In one study, amine- and carboxyl-coated QDs (CdSe/ZnS core) were administered IV at high doses (144, 720, and 3600 pmol/mouse) (Geys et al., 2008). Both types caused pulmonary vascular thrombosis, but the carboxyl QDs were more potent inducers of this effect than to amine QDs. This was not the only study that found adverse side effects on the pulmonary system. Additional pulmonary research found adverse effects including inflammation, attenuated vasodilation, and endothelial apoptosis as a result of QD exposure (Yan et al., 2011; Shukur et al., 2013; Stan et al., 2015). Geys et al. (2008) found that after pretreatment with heparin, pulmonary thrombosis induced by carboxyl QDs was mitigated, indicating that negatively charged QDs activated the coagulation process via contact activation rather than platelet activation. However, research into the effects of QDs [CdSe/ZnS-mercaptopropionic acid, (CdSe/CdZnS)ZnS-polyT, and CdSeCdSZnS/polyT/SiO_2-NH_2] on erythrocyte's showed changes in cell morphology and size due to QD exposure (Pleskova et al., 2014). Mechanistic research in the zebrafish model showed that amino-functionalized QDs cleared more rapidly than carboxyl-functionalized QDs, highlighting the role charge plays on vascular clearance (Jiang et al., 2017b). In addition to providing a better understanding of clearance, the zebrafish model has shown that QDs preferentially accumulate in smaller low blood flow veins and capillaries, a property that is often exploited in medical imaging (Chen et al., 2017; Jiang et al., 2017b). Given all of the concerns presented with QDs in the bloodstream, there is little evidence to suggest direct cardiac toxicity. Studies exploring the long-term toxicity (3 months) of high-dose (25 mg/kg) exposure to InP/ZnS QDs in the BALB/C mice concluded with no evidence of specific toxicity attributable to exposure (Lin et al., 2015).

19.8.4 Metallic and Metal Oxide NPs

Many metallic and metal oxide-based NPs have been developed or are in use for various applications. For example, gold NPs are used extensively in electron microscopy, and they are also approved for treatment of rheumatoid arthritis (Douma et al., 2009). Research applications of superparamagnetic iron oxide NPs for cardiac magnetic resonance imaging (MRI) are providing never-before-seen clarity to the functioning heart (Au et al., 2009). While many metals including Fe, Cd, and Ni are widely used in various products, titanium (Ti), silica (Si), and Ag make up the largest volume of commercially used NPs (Vance

et al., 2015). There is little that can be said that universally applies to effects generated by each of these particle types. Effects ranging from DNA damage, cytotoxicity, and lipid peroxidation have been observed from both direct NP interaction and the result of downstream events triggered by induction of ROS. An increase in ROS has been shown to induce the MAPK pathway, which increases the production of Nrf2 and NF-κB. These pro-inflammatory molecules are important as they are directly involved in the pathogenesis of multiple inflammatory diseases such as atherosclerosis (Guo et al., 2015). Each of these effects plays a different role in cardiac toxicity. The following is a breakdown of the current understanding of the most widely used NPs in consumer and industrial goods.

19.8.5 Titanium Dioxide

TiO_2 has long been widely used as a white pigment for a wide range of products including paints, food colorants, cosmetics, and disinfectants, and it was once considered physiologically inert, posing little risk to humans (Chen et al., 2009; Park et al., 2009). However, some studies have shown that TiO_2 NPs (or ultrafine TiO_2) could induce significant cytotoxicity, genotoxicity, and inflammatory responses (Oberdörster et al., 2005; Chen et al., 2009). Hong et al. (2015, 2016) published two articles detailing the triggering and expression of Nrf2, NF-κB, tumor necrosis factor (TNF)-α, C-reactive protein (CRP), and ICAM-1, all of which are known to play a pronounced role in cardiovascular disease. Research in rat hearts showed an increase in mitochondrial permeability and cytochrome C activation as well as direct DNA damage evidenced by an increased tail length of DNA in the comet assay. All of these factors can lead to an increase in apoptosis of cardiac cells (Faddah et al., 2013). Additional research in mice has shown that production of ROS post TiO_2 exposure induced augmented DNA peroxidation in the heart and attenuated antioxidant enzyme activity, specifically superoxide dismutase and glutathione transferases (Sheng et al., 2013). Taken together these studies showed a clear inflammatory pathway that is directly and indirectly caused by exposure to TiO_2. However, the route of exposure should be taken into account for all of these studies. The physical result of these pathways manifests itself acutely in the research published by Nurkiewicz et al. (2008). This research group published two articles regarding microvascular dysfunction induced by inhaled TiO_2. They exposed rats to fine or ultrafine TiO_2 aerosols (primary particle diameters: ~1 μm vs. ~21 nm) in a whole-body inhalation chamber. Their results suggested that on equivalent-pulmonary-deposition basis, ultrafine TiO_2 inhalation induced greater remote microvascular dysfunction than fine TiO_2. Nurkiewicz et al. (2009) identified potential mechanisms for the remote microvascular dysfunction induced by inhaled ultrafine TiO_2 in the previous study. Ultrafine TiO_2 exposure significantly increased microvascular oxidative stress by approximately 60% and also produced an elevated nitrosative stress response (~fourfold). Additionally, NO production was decreased in a dose-dependent manner, indicating that inhaled ultrafine TiO_2 can affect the remote vascular system by not only inducing microvascular dysfunction but also decreased NO bioavailability. In addition to microvascular dysfunction, IT instillation of TiO_2 was shown to increase cardiac conduction velocity as well as tissue excitability, which ultimately increased the likelihood of arrhythmia or cardiac dysfunction (Savi et al., 2014).

19.8.6 Zinc

Zinc oxide (ZnO) is primarily used for its antimicrobial properties and as the main ingredient in sunscreens. Additionally, ZnO is widely used in glass, plastic, and ceramic production.

Exposure to ZnO produces a significant amount of ROS, which generates multiple inflammatory biomarkers including TNF-α, CRP, and interleukin-6 (IL-6), VEGF, and cardiac calcium (Suematsu et al., 2003; de Ferranti and Rifai, 2007; Baky et al., 2013). Additionally, it was shown that post-ZnO exposure ROS production can induce significant damage to both the DNA backbone and base pairs (Huang et al., 2001; Martinez et al., 2003; Sharma et al., 2009). Inhalation studies designed to mimic occupational exposure levels (1.1 and 4.9 mg/m^3) showed that repeated exposure (2 weeks) to ZnO can cause cardiac inflammation and fibrosis that could, in turn, cause cardiac impairment, even among a healthy population of workers (Chuang et al., 2014). Additional research into the pathological effects of ZnO exposure has shown that oral exposure can cause DNA damage, inflammation, cardiac apoptosis, and elevated creatine kinase-muscle/brain (CPK-MB) and myoglobin (Baky et al., 2013). Taken together, these findings suggested that exposure to ZnO can trigger an inflammatory pathway capable of inducing cardiac damage and fibrosis.

19.8.7 Silica (SiO_2)

Crystalline silica (SiO_2) is abundant in nature in the form of quartz. As a nanomaterial, silica is highly desirable for its porosity, biocompatibility, and thermal stability. As a raw material, silica is available in both crystalline and amorphous forms. Crystalline silica is known to cause chronic obstructive pulmonary disorder and lung cancer, whereas amorphous silica is known to be cytotoxic (Yu et al., 2009). However, when administered to mice at high doses, up to 10 mg/kg/bw, increases in inflammatory markers IL-6, TNF-α, and interleukin-1 beta (IL-1β) ICAM-1 and vascular cell adhesion molecule-1 (Vcam-1) were observed, indicating that for high-dose exposures, oxidative stress and inflammatory reactions could lead to endothelial dysfunction (Du et al., 2013). When compared with other materials that are widely used in commercial products, amorphous silica displays limited toxicity at low to moderate doses. However, research in rats has shown an age-dependent association with inhalation exposure and myocardial ischemic events leading to altered cardiac rhythm (Chen et al., 2008). Given the limited amount of research available on this particular nanomaterial, it is difficult to fully assess the toxicity of amorphous nano-SiO_2. This is not to say that a lack of data implies a level of safety, only that more research is needed to make any conclusions on its cardiotoxicity.

19.8.8 Silver

Silver is widely used in commercially available metallic NPs (Vance et al., 2015). One of the main reasons that it is so popular is that it serves as a powerful antimicrobial agent. AgNPs are commonly found in many everyday products such as deodorants, toothpastes, bandages, clothing, and food contact surfaces. The mechanism accounting for Ag use as an antimicrobial agent is as a catalyst between O_2 and H within a cell, altering key enzymatic functions (Davies and Etris, 1997). The cleaving of O_2–H bonds internally generates ROS to act as an antimicrobial agent. However, as a possible result of this interaction, AgNPs have been shown to be cytotoxic (Kim et al., 2012), damage mitochondria, causing DNA damage (Carlson et al., 2008; AshaRani et al., 2009; Taju et al., 2014), and interfering with the action potential of neurons in the brain (Liu et al., 2009).

When examining acute cardiac effects of Ag, no increases in inflammatory cytokines have been observed after IT instillation (Holland et al., 2016). However, AgNPs have been shown to directly affect the electrochemistry of the heart, altering heart rate, QT cycle,

myocardial force production, and inducing lethal bradyarrhythmias (Holland et al., 2016; Lin et al., 2017; Callaghan et al., 2018). The lack of significant pro-inflammatory markers in these acute studies indicates that these results are due to Ag itself, acting directly upon ion channels as opposed to having a downstream effect caused by Ag+, or ROS (Lin et al., 2017). Very little information is available regarding chronic or subchronic cardiac effects of AgNP exposure; however, kinetic studies have shown that Ag accumulates in heart tissue and causes deformities in cardiomyocytes (Korani et al., 2013). Given the prevalence of AgNPs in the marketplace, there is a real need for additional chronic studies.

19.9 CASE STUDY: SUBCHRONIC EFFECTS OF INHALED NICKEL NANOPARTICLES ON THE PROGRESSION OF ATHEROSCLEROSIS IN A HYPERLIPIDEMIC MOUSE MODEL

Since most nanotoxicology studies investigate acute exposures, our group at NYU explored the effects of subchronic exposure to inhaled NiNPs on the cardiovascular system. The hypothesis was that inhaled NPs could induce oxidative stress and inflammatory responses, not only in the lung but also in the cardiovascular system, and, in the long term, these effects could exacerbate progression of atherosclerosis in a hyperlipidemic apoprotein E knockout ($ApoE^{-/-}$) mouse model.

Ni was selected as a test material due to its popular use in industry and its potential to induce cardiovascular effects (Campen et al., 2001; Lippmann et al., 2006). The use of NiNPs is also growing in usage in catalytic converters, solar cells, battery anodes, and protective coatings. As a bulk material, Ni is a known carcinogen (IARC Class 1), and there was every reason to suspect that, at the nanolevel, the same concerns apply. It has been documented that NiNPs affect vascular reactivity (Cuevas et al., 2010), pulmonary inflammation (Gillespie et al., 2010), and production of endothelial progenitor cells (Liberda et al., 2010). NiNPs were generated in the laboratory using a Palas spark generator, and chemical composition of the generated particles was confirmed as nickel hydroxide [$Ni(OH)_2$] (nano-NH) via X-ray photoelectron spectroscopy (XPS).

For subacute and subchronic exposure, 5-month-old male $ApoE^{-/-}$ mice were exposed either to filtered air or nano-NH (diameter of primary particle: 5 nm, count median diameter of agglomerates: ~40 nm) at ~80 μg Ni/m^3, less than 10% of the current permissible exposure limit (PEL) for $Ni(OH)_2$ set by the Occupational Safety and Health Administration (OSHA), for 5 h/day, 5days/week, for either 1 week or 5 months. Various indicators of oxidative stress and inflammation were measured in the lung and in cardiovascular tissue, and plaque formation on the ascending aorta was determined after 5 months of exposure.

The assays using bronchoalveolar lavage fluid (BALF) revealed significant oxidative stress and pulmonary inflammation at both time points, consistent with gene expression analyses in the lung showing upregulation of genes like heme oxygenase-1 (Ho-1), IL-6, and monocyte chemotactic protein-1 (Mcp-1). These three genes were also upregulated in the heart tissue after 5 months of exposure, indicating systemic effects induced by inhaled nano-NH. Furthermore, Mcp-1 was over-expressed in the aorta tissue, along with Cd68 and Vcam-1, after the 5-month exposure. This phenomenon coincides with increased plaque formation in the ascending aorta, providing a molecular rationale for the exacerbated atherosclerosis, suggesting that inhaled nano-NH, at occupationally relevant levels, can induce significant chronic effects on the cardiovascular system, including exacerbation of atherosclerosis. These findings will contribute to further understanding of potential risks,

mechanisms of NP-induced toxicity, and the establishment of a database for NP-specific regulations in occupational and environmental settings.

19.10 HUMAN DATA

Currently, there is little conclusive evidence of human health effects directly associated with exposure to NPs in the literature. One inhalation study (Nemmar et al., 2002) that specifically dealt with human NP exposure was both insightful and contentious. The results of this study are not yet clear and have not yet been verified or replicated (Nemmar et al., 2002; Geiser et al., 2006). Earlier studies by Fine et al. (1997, 2000) examined the health effects of inhaled ZnO ultrafine PM (i.e., NPs) and determined that metal fume fever occurred after a single exposure at an airborne concentration relevant to occupational exposures and was accompanied by elevations in blood cytokines (e.g., IL-6 and TNF). These studies also showed that adaptation to metal fume fever and cytokine elevations occurred after repeated inhalation in human subjects.

19.11 FUTURE STUDIES

Significant progress has been made in nanotoxicology research in recent years, both quantitatively and qualitatively. However, several important research topics such as subchronic and chronic exposure need to be addressed to better understand potential health risk of NPs. Most importantly, researchers need to use relevant routes of exposure with real-world NPs. The majority of inhalation studies to date, for example, have used nebulization to generate NP exposure atmospheres despite the fact that the nebulization of NPs in an aqueous media (and any subsequent drying of the airborne particles) results in agglomerated NPs. Not only will these larger agglomerated particles deposit in different regions of the respiratory tract (compared with singlet NPs), but the rate of de-aggregation of these agglomerations is not known. Similarly, the translational relevance of *in vitro* studies is compromised by the delivery of NPs in a wide variety of dispersion media that may or may not be comparable their occurrence *in vivo*. These NP delivery issues are of critical importance and must be optimized in future studies.

19.12 SUMMARY

In the past decade, engineered NPs became an important and unique class of new materials (Medina et al., 2007). With a growing interest in the research and development of new nanotechnology, it is important to address concerns about the potential adverse health effects caused by NP exposures.

The cardiovascular system is one of the major targets for NP-induced toxicity, because it can come into direct contact with NPs, especially those developed to diagnose or treat cardiovascular diseases. Also, there can be secondary effects from NP exposures occurring via other routes. The purpose of this chapter was to review and discuss the lessons learned from recent publications pertaining to cardiac toxicity induced by engineered NPs in which various classes of NPs have been covered including fullerenes, CNTs, QDs, metals, or metal oxides. As summarized here, a considerable amount of data from *in vitro* and *in vivo*

studies that illustrate that various types of engineered NPs has the capacity to exert adverse cardiac effects. Continuous research in this field will lead to a better understanding of the potential hazards associated with NP exposure and ultimately to the development of safe and effective production and applications of NPs.

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NITROGEN OXIDES

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Oxides of nitrogen (NO_x), so called because they consist of various nitrogenous chemical species, many of which are interconvertible within the atmosphere, can exist as either gases/vapors or particles. The former includes nitric oxide (NO), nitrogen dioxide (NO_2), nitrous oxide (N_2O), and occasionally, nitrogen trioxide (NO_3), dinitrogen trioxide (N_2O_3), dinitrogen tetroxide (N_2O_4), and dinitrogen pentoxide (N_2O_5), while the latter includes nitrate (NO_3^-) salts. Species that may exist either within particulate droplets or gaseous molecules are nitric acid (HNO_3) and nitrous acid (HONO). NO and NO_2 are considered to be the most important of the NO_x in terms of public health concern since they are often present in the atmosphere in significant concentrations and are chemically reactive. N_2O is also ubiquitous, being released due to natural biological processes in soil, but is not involved in chemical reactions in ambient air. Most other NO_x, if found at all, are present at much lower concentrations. A possible exception, from a health standpoint, is HNO_3.

20.1 INTRODUCTION

This chapter identifies, evaluates, and characterizes risk factors that address the question of what populations and life stages are at increased risk of health effects associated with exposure to NO_x along with the other gas-phase and particulate matter (PM) components the complex and varying pollutant mixtures in ambient air. Individuals, and ultimately populations, have been shown to be at increased risk of an air pollution-related health effect for various reasons. Very young individuals and adults with preexisting disease are at greater risk for some air pollution-related health effects depending on the

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disease and severity. Effect modifications by preexisting disease include asthma, chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD), diabetes, and obesity, as well as the influences of genetic and sociodynamic factors. The epidemiologic evidence, summarized in this chapter, collected across diverse global regions (North America, Europe, Asia, Australia), has consistently demonstrated associations between short-term increases in ambient NO_2 concentration with increases in asthma-related hospital admissions, emergency department (ED) visits, or outpatient visits among children and adults (Anderson et al., 1998; Atkinson et al., 1999; Peel et al., 2005; Hinwood et al., 2006; Villeneuve et al., 2007; Ko et al., 2007b; Sinclair et al., 2010; Son et al., 2013). Most results based on comparisons between children ages 0–14 years and people ages 15–64 years showed NO_2-associated increases in asthma hospital admissions 1.8–3.4-fold greater in children (Anderson et al., 1998; Atkinson et al., 1999; Ko et al., 2007b; Son et al., 2013). Some studies of asthma hospital admissions, outpatient visits, and medication sales showing no difference in association with health outcomes and NO_2 between children and adults or no association in either group (Migliaretti et al., 2005; Burra et al., 2009; Laurent et al., 2009). A few pointed to larger NO_2-related increases in asthma hospital admissions or ED visits among younger children (e.g., age 0–4 years vs. 2–4 years) than older children ages 5–14 years (Villeneuve et al., 2007; Samoli et al., 2011). However, inference from these findings is limited because of the questionable reliability of asthma diagnosis in children below the age of 5 years. Overall, the epidemiologic evidence generally demonstrated greater NO_2-associated asthma exacerbation is in children compared with adults, with general consistency for asthma hospital admissions and ED visits and for similar age comparisons (ages 0–14 years vs. 15–64 years).

One big, and as yet unresolved, question is whether exposure to NO_x in ambient air is responsible for causing observed health effects and, if so, which effects can be attributed to its major components, that is, NO or NO_2. Alternatively, could the effects be attributed to other biologically active chemical components of the ambient air mixture, such as HNO_3, O_3, SO_2, or H^+, soot, or trace-level metals and polycyclic aromatic hydrocarbons (PAHs), and/or their interactions. A second, and related, question is whether we should continue to rely on either NO_2 or NO_x, the most commonly monitored components of the toxic ambient air mixture as useful indicators of health risk. Dominici (2010) suggested that greater public health protection from air pollution could be achieved by shifting from a single pollutant approach to a multi-pollutant approach. They noted the case of PM, which was first treated as a single pollutant through studies of total mass, then as masses within a set of different particle size ranges, looking forward to the possibility of controls based on one or more single pollutants or of separate complex mixtures of particles from specific sources. They acknowledged that actual implementation is daunting, since each aspect of a multi-pollutant approach (scientific assessment of health risk, the setting of regulations, and compliance with regulations) has to recognize the involvement of multiple pollutants. Most importantly, the development of a multi-pollutant approach requires scientific knowledge of how pollutant mixtures affect health and the underlying mechanisms and potential synergism among pollutants. Unfortunately, the state of the science on how simultaneous exposure to multiple pollutants affects human health in real-world settings is still incomplete, including risk based on validated statistical models that can estimate the total health effect associated with the simultaneous exposure to multiple pollutants, including their potential interactions.

20.2 SOURCES OF NO_X

Ambient atmospheric NO_x derive from both natural sources, such as forest fires, organic decay, and lightning, and mobile and stationary anthropogenic activities that involve high-temperature combustion processes. The major mobile source category is motor vehicles, while the major stationary source category is electric power generation using fossil fuels, with industrial combustion processes being a close second. From a global perspective, however, the total mass of emissions released from natural sources is much greater than that from human activities. NO_x, primarily NO_2, can also be an important indoor pollutant. The main indoor NO_x sources are unvented or improperly vented natural gas or other fossil fuel-fired appliances, such as stoves and heaters.

During combustion processes, nitrogen derived from the combustion air and/or the fuel being consumed reacts with atmospheric oxygen. Although most of the resulting NO_x produced is initially in the form of NO, this is generally rapidly oxidized to NO_2, with the conversion rate depending upon a number of factors, including the concentration of NO, temperature of the combustion process, and distance from the emission zone. Several reaction pathways are possible. While simple oxidation involving molecular oxygen (O_2) is the primary one for NO_2 production in combustion gas effluents, it does not play a major role in the ambient atmosphere since transformations via other pathways, for example, reaction with free radicals and ozone (O_3), occur at faster rates. Thus, in air containing other reactive chemical species, irradiation by sunlight can catalyze photochemical reactions, leading to a very rapid formation of NO_2.

HNO_3 is also a product of the photooxidation cycle of polluted air but, along with HONO (HNO_2), can additionally derive from primary emissions released by mobile sources. The major production pathway involves reaction between the hydroxyl radical (OH), formed within the smog cycle, and NO_2. Other routes, which are potentially important at night, involve reactions between N_2O_5 with water or nitrate radicals with volatile organics or production in droplets containing both hydrogen ion (H^+) and nitrate (NO_3^-). Because of its high saturation vapor pressure, HNO_3 generally exists as a vapor under most ambient conditions, for example, within photochemical smog, where levels generally peak during daytime hours, although within acidic fogs, HNO_3 may be found in the particulate state. Similarly, HONO can be found in ambient air both as a primary product from combustion sources and as a secondary product of photochemical smog reactions. HNO_3 may also be produced indoors via reaction of O_3 with NO_2, water vapor, and volatile organics. Water on indoor surfaces can react with NO_2 to form HONO, which can then be released into indoor air as gas-phase acid.

Nitrate salts may be formed in the atmosphere via various pathways, many of which involve gaseous HNO_3. For example, ammonium nitrate (NH_4NO_3) results from the homogeneous reaction between nitric acid and atmospheric ammonia (NH_3). Nitrates may also be formed by heterogeneous reactions involving NO_2 or NO and water droplets or HNO_3 vapor and dust or sea salt particles.

Outdoor levels of NO_x are often directly related to motor vehicle emissions and traffic density around busy roadways, and, along with PM and various organics, NO_2 is considered to be a useful indicator of the complex particulate–gaseous mixture attributable to vehicular traffic. However, temporal trends are affected not only by sources but also by atmospheric transformations and meteorological conditions. Ambient NO_2 levels tend to show seasonal variation, being generally higher in winter than in summer. Outdoor

concentrations in urban areas are generally characterized by two daily peaks related to morning- and afternoon-traffic peaks. In areas having significant stationary and regional sources, the pattern is characterized by a baseline NO_2 level and superimposed higher spikes. In those areas not impacted by significant local sources, levels have little variation on an hourly basis throughout the day unless there is transport into the region.

Within the United States, there are wide variations in ambient NO_2 levels. The mean daily l-h maximum outdoor concentration of NO_2 across hundreds of sites in the United States for the period 2011–2013 was 0.019 ppm, with a 5th–99th percentile range of 0.002–0.055; average levels in urban areas would tend toward the higher level, while levels in nonmetropolitan or rural areas tend to be lower. The mean annual average concentration was 0.0086, with a 5th–99th percentile range of 0.0014–0.0225 (U.S. EPA, 2016). While, as noted, natural emissions far outweigh those from anthropogenic sources on a total mass basis, the former are distributed over a wider area; this results in generally very low background levels due to natural sources.

NO_2 also has indoor combustion sources, such as gas-fired ranges, kerosene heaters, and improperly or unvented gas space heaters. NO is also a major component of smoke derived from the burning of tobacco products. Cigarette smoke contains high levels of NO, which is oxidized to NO_2 as the smoke ages. In any case, indoor levels vary widely depending upon the strengths of the specific sources and the degree of ventilation. Furthermore, because combustion from indoor sources tends to be episodic, depending upon the specific sources, fairly high short-term peaks are possible. Daily (24-h average) levels of NO_2 in homes using gas-fired ranges or heaters have ranged between 0.008 and 0.209 ppm (U.S. EPA, 2016), with short-term (15 min) peaks ranging from 0.029 to 0.955 ppm.

From a health standpoint, the only relevant route of exposure to NO_x is via inhalation, and such exposures can occur in numerous settings, including residential areas, transportation vehicles, and outdoors. The integrated exposure is the sum of the individual exposures over all possible time intervals and for all of these different microenvironments. Such exposure can be assessed either by direct methods, which include biomarkers and personal monitoring, or by indirect estimation methods, which involve measurement of concentrations at monitoring sites and the use of mathematical models to estimate actual individual or population mean exposures.

There is currently no accepted biomarker for exposure to NO_2, although some suggested ones have included urinary hydroxyproline excretion, the NO–heme protein complex in bronchial lavage, and 3-nitrotyrosine in urine. However, because of their lack of sensitivity and/or specificity, they have not been shown to be practical for assessing environmental NO_2 exposures. In addition, inhaled NO_2 may also be involved in the production of mutagenic and/or carcinogenic nitroderivatives under conditions of co-exposure to certain organics via nitration reactions.

Outdoor measures of NO_2 levels, while related to and contributing to total exposure, are poor predictors of total personal exposures for most people, because indoor concentrations are often greater than those outdoors. In such instances, indoor exposures can be the main contributors to total exposure, and actual personal exposures to NO_2 may differ from what would be presumed based upon ambient outdoor air measures. Of course, there are likely to be selected groups of people for which indoor levels are not a good predictor of total exposure due to greater percentages of time spent in other significant NO_x-containing environments, especially for those occupationally exposed or those living near heavily traveled roadways.

There are various ambient and occupational exposure limits for NO_x. Some of the major ones are listed in Table 20.1.

TABLE 20.1 Exposure Limits for Nitrogen Oxides

Nitric oxide (NO)	
TLV[a]	25 ppm
PEL[b]	25 ppm
REL[c]	25 ppm
IDLH[d]	100 ppm
Nitrogen dioxide (NO_2)	
NAAQS[e]	0.053 ppm
PEL[f]	5 ppm
REL[g]	1 ppm
TLV[a]	3 ppm
STE[h]	5 ppm
IDLH[d]	20 ppm

[a]Threshold limit value (ACGIH; time-weighted average for an 8-h workday and a 40-h workweek).
[b]Permissible exposure limit (OSHA; time-weighted average for an 8-h workday).
[c]Recommended exposure limit (NIOSH; time-weighted average for an 8-h workday).
[d]Immediately dangerous to life and health (NIOSH; 30-min average).
[e]National ambient air quality standard (USEPA; annual average).
[f]Permissible exposure limit for general industry (OSHA; ceiling for 15 min).
[g]Recommended exposure limit (NIOSH; ceiling for 15-min exposure).
[h]Short-term exposure limit (ACGIH; ceiling for 15-min exposure).

20.3 NITROGEN DIOXIDE

20.3.1 Dosimetry

Up to 90% of the NO_2 inspired during normal respiration can be removed within the human respiratory tract (Wagner, 1970). Estimates of regional uptake for the upper respiratory tract (i.e., airways proximal to the trachea) based upon laboratory animal studies range from 28 to 90% of the amount inhaled (Dalhamn and Sjoholm, 1963; Yokoyama, 1968; Vaughan et al., 1969; Cavanagh and Morris, 1987), while that for the lungs range from 36 to 90% of the amount entering the trachea (Postlethwait and Mustafa, 1981; Kleinman and Mautz, 1989).

Specific ventilatory factors influence uptake. Thus, more NO_2 will be absorbed in the upper respiratory tract during nasal breathing than during oral breathing (Kleinman and Mautz, 1989), with the latter allowing a greater percentage of inhaled NO_2 to reach the lungs. Increased ventilation such as due to exercise reduces NO_2 uptake in the upper respiratory tract and tracheobronchial tree and increases the amount delivered to and absorbed in the respiratory (alveolar) region of the lungs (Wagner, 1970; Miller et al., 1982; Overton, 1984; Kleinman and Mautz, 1989; Mohsenin, 1994).

Within the lungs, inhaled NO_2 is absorbed throughout the entire tracheobronchial tree and respiratory region, with the major dose to tissue being delivered at the junction between the conducting and respiratory airways (Miller et al., 1982; Overton, 1984). Regardless of the site of initial contact with airway surfaces, a primary determinant of NO_2 uptake is surface reactivity, that is, direct interaction with airway lining fluid and/or cellular components (Postlethwait and Bidani, 1990). While inhaled NO_2 is likely to react primarily with water molecules in the fluid to form HNO_2 and HNO_3, absorption is also linked to

reactive substrates in the fluid, with production of nitrite ion and perhaps various radicals. Some of the potential substrates include oxidizable chemical species, such as amino acids, proteins, and unsaturated fatty acids (Hood et al., 1993), resulting in the production of nitrite ion or various radicals (Postlethwait and Mustafa, 1981; Saul and Archer, 1983; Postlethwait and Bidani, 1989, 1990), which can then interact with the epithelium or rapidly pass into the bloodstream and undergo other chemical reactions in extrapulmonary sites, for example, oxidation to nitrate by interaction with hemoglobin in red blood cells (Case et al., 1979; Kosaka et al., 1979; Oda et al., 1981; Parks et al., 1981). Antioxidants present within airway lining fluid can react with deposited NO_2, potentially modulating its toxicological impact. It is likely that both oxidative and non-oxidative mechanisms are involved in toxicity from inhaled NO_2.

20.3.2 Health Effects: Epidemiology

Many epidemiological studies have assessed the potential roles of exposure to either NO_2 or NO_x ($NO + NO_2$) in producing adverse human health effects. Many studies have related health endpoints to outdoor NO_2 concentrations at central monitoring sites, but the current trend is to provide better indices of actual personal exposures, which, as noted, can reflect strong indoor sources. While some studies have used NO_2 as the sole pollutant, others have used it as a general marker for pollution attributable to motor vehicle emissions. A major problem, however, is the close association between NO_2 and other pollutants, especially NO and PM, derived from the same combustion sources, making it difficult to determine whether any of the effects are due solely to either NO or NO_2.

The robustness of some epidemiological findings are affected by a lack of reliable estimates of actual NO_x exposure conditions, inadequate sample size, inadequate compensation for the effects of covariates, and/or misclassification of health endpoints. Still, they do provide linkages between controlled exposure (toxicology) studies and "real-world" exposure of humans for both acute exposures and effects, as well as for chronic responses to long-term exposure.

Ambient NO_2 has long been associated with increased daily mortality (e.g., Anderson et al., 1996; Sunyer et al., 1996; Wietlisbach et al., 1996; Touloumi et al., 1997; Katsouyanni et al., 2001; Stieb et al., 2002, 2003; Dominici et al., 2003). Some studies have indicated that chronic exposure to NO_2 is associated with increased risk of all-cause mortality (Hoek et al., 2002; Nafstad et al., 2004; Filleul et al., 2005) and suggested a specific association of NO_2 with cardiopulmonary mortality as well. However, other early studies in the United States (Dockery et al., 1993; Pope et al., 2002) did not seem to provide similar evidence for such an effect of chronic NO_2 exposure and all-cause mortality. However, Fig. 20.1 (U.S. EPA, 2016) shows that the inclusion of more recent studies from the United States and elsewhere provides support for some of the earlier work.

Figure 20.2 shows that the significant association of overall daily mortality with NO_2 increases with multiday exposures and that the largest increment is attributable to cardiac causes. There is a further significant increment for respiratory causes and further increment that is not quite significant from cerebrovascular causes. Figure 20.3 shows that many early and recent multicity studies demonstrate that both cardiac and respiratory causes account for significant increases in daily mortality associated with NO_2.

Many studies of the associations of acute responses to NO_2 used indices of respiratory illness, such as hospital admissions and ED visits to assess health consequences of NO_2 exposure. A strong association was found between NO_2 and asthma admissions to hospital

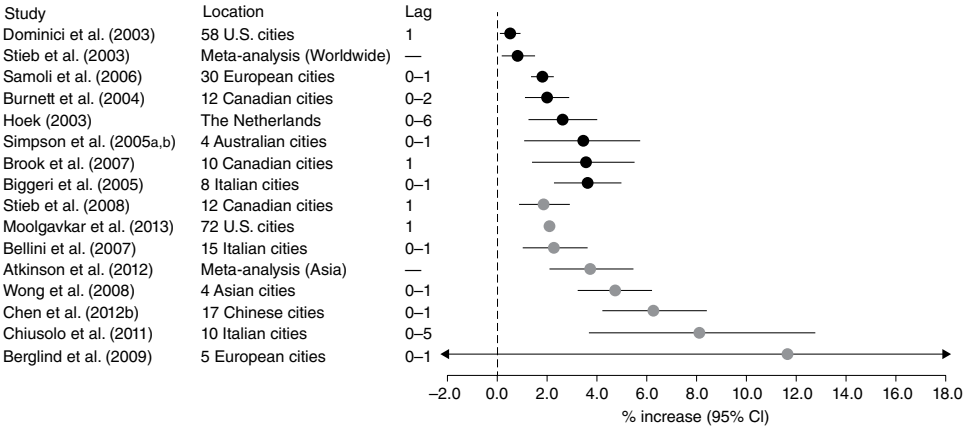

FIGURE 20.1 Summary of multicity studies that examined the association between short-term nitrogen dioxide exposure and total mortality. *Notes*: CI, confidence interval; black, studies from the 2008 Integrated Science Assessment for Oxides of Nitrogen; red (grey in print), recent studies. Results are presented per a 20-ppb increase in 24-h average nitrogen dioxide concentration or a 30-ppb increase in 1-h max nitrogen dioxide concentrations.

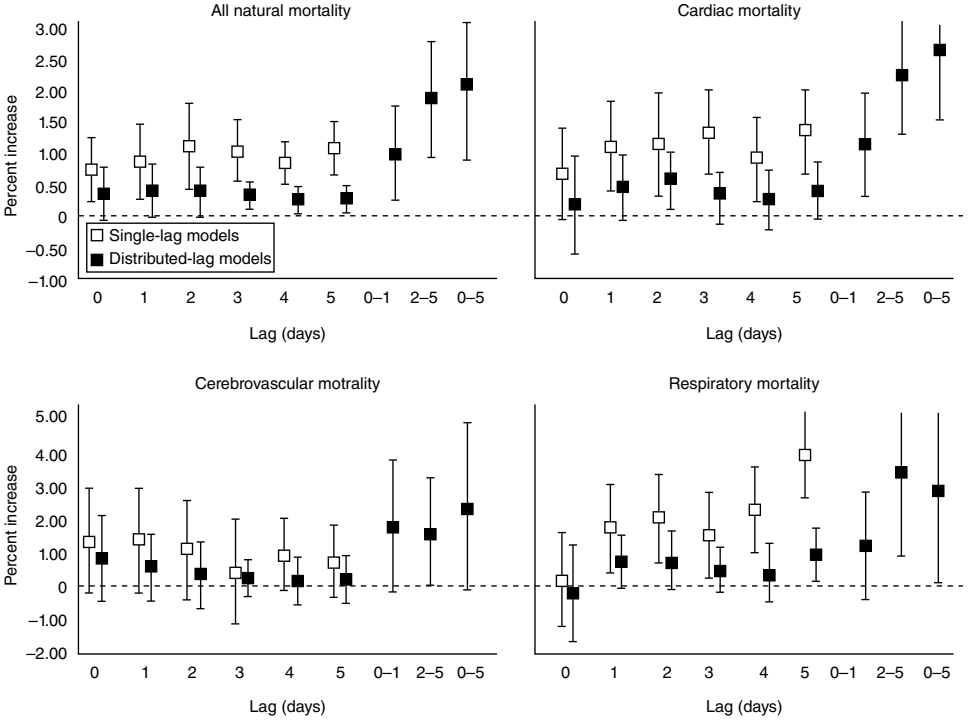

FIGURE 20.2 Percentage increase in total and cause-specific mortality associated with short-term increases in ambient NO_2 concentration at single-day lags, individual lag days of a constrained polynomial distributed lag model, and multiday lags of an unconstrained distributed lag model. *Source*: Reproduced from Environmental Health Perspectives (Chiusolo et al., 2011).

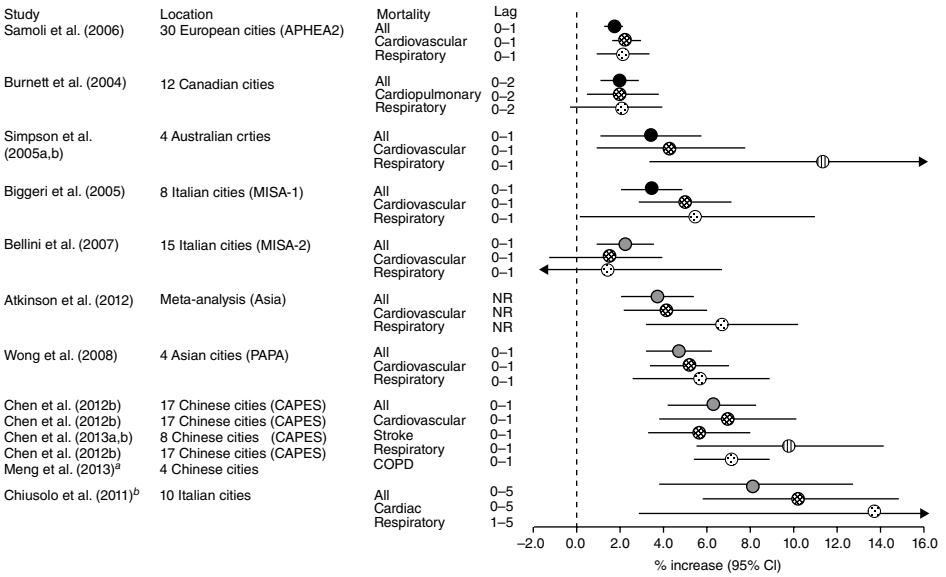

FIGURE 20.3 Percentage increase in total, cardiovascular, and respiratory mortality from multicity studies in relation to ambient NO_2 concentrations. *Notes*: APHEA2, Air Pollution and Health: A European Approach 2; CAPES, China Air Pollution and Health Effects Study; CI, confidence interval; MISA, meta-analysis of the Italian studies on short-term effects of air pollution; NR, not reported; PAPA, Public Health and Air Pollution in Asia; ppb, parts per billion. Black, studies from the 2008 Integrated Science Assessment for Oxides of Nitrogen; red (grey in print) symbols, recent studies; filled circles, total mortality; crosshatch, cardiovascular mortality; dots, respiratory mortality. [a]Although the study was not part of the CAPES study, it included four of the cities also included in CAPES. [b]Study examined individuals ≥35 years of age, while the other studies examined all ages. Results are presented per a 20-ppb increase in 24-h average nitrogen dioxide concentrations or a 30-ppb increase in 1-h max nitrogen dioxide concentrations.

for children under 14 years of age (Sunyer et al., 1997), while significant effects of NO_2 on doctor visits or hospital admissions for asthma were found in children in various areas of the world (Medina et al., 1997; Anderson et al., 1998; Morgan et al., 1998; Lee et al., 2002; Lin et al., 2002). A stronger association was found to exist between NO_2 and asthma symptoms in children in London than for adults in the same area (Hajat et al., 1999).

In a study examining respiratory symptoms in adult women and children aged 13 years and younger (Berwick et al., 1984), indoor NO_2 levels were measured in homes. Children under the age of 7 years exposed to 0.016 ppm were at an increased risk of upper and lower respiratory tract symptoms compared with those who were not so exposed. No increased risk was found in older children or adults. In a later study (Samet et al., 1993), no association was found between indoor levels of NO_2 and either the incidence or duration of respiratory illness in infants examined during their first 18 months of life. A meta-analysis (Hasselblad et al., 1992) of studies using indoor NO_2 levels suggested a relationship between incidence of lower respiratory tract symptoms and chronic exposure in children less than 12 years of age, while no effect on lower respiratory tract illness during the first year of life was seen in relation to indoor NO_2 in another study (Sunyer et al., 2004).

A meta-analysis using data from Australia and New Zealand (Barnett et al., 2005) noted an association between all respiratory-related hospital admissions and admissions specifically for asthma, pneumonia, and acute bronchitis in children grouped into various age ranges. While both PM and NO_2 were noted to be associated with total respiratory admissions in children in the 1–4-year age group, the largest effect was noted for NO_2. In older children, aged 5–14 years, an association was also found with PM and NO_2, but with the latter showing a larger effect. However, the greatest association was noted for asthma admissions increase, related to an increase of about 5 ppm in the 24-h mean concentration of NO_2. However, when PM was controlled for, the effect of NO_2 in the younger children was attenuated, but that in the older age group was not. Finally, a number of studies have noted associations between NO_2 and various symptoms, such as cough, wheeze, and shortness of breath, in children with asthma (e.g., Quackenboss et al., 1991; Segala et al., 1998; Just et al., 2002; Mortimer et al., 2002).

In an examination of the relationship between ambient air pollutants and emergency room visits in Atlanta, GA (Peel et al., 2005), the effect estimate for respiratory admissions due to a 1-h exposure to 0.020 ppm was found to be 1.6% [95% confidence interval (CI): 0.6–2.7] for all respiratory admissions and 1.9% (95% CI: 0.6–3.1) for upper respiratory infections only. While the effects were attenuated in multi-pollutant models that also considered PM and CO, the effect of NO_2 on emergency room visits specifically for asthma was not, and NO_2 showed the strongest association with these visits of all pollutants in the model.

Asthma may not be the only disease that can predispose to enhanced effects from exposure. Some studies (e.g., Burnett et al., 1997; Wong et al., 1999; D'Ippoliti et al., 2003) noted an association between NO_2 and admissions for cardiovascular disease but, as above, effect estimates were often modulated when other pollutants, especially PM, were considered in the model. Some studies strongly suggested (Mann et al., 2002; Metzger et al., 2004) that individuals with ischemic heart disease and accompanying congestive heart failure and/or arrhythmia may be especially sensitive to effects of motor vehicle-derived pollution. In an examination of air pollution and emergency room visits for cardiovascular causes in Atlanta, GA, Metzger et al. (2004) found a significant association between NO_2 and cardiovascular emergency room visits that remained after adjusting for PM. In an examination of air pollutants and congestive heart failure

(Wellenius et al., 2005), effects with NO_2 were modulated when considering CO and PM, but there was a conclusion that the general mix of pollution from motor vehicles was responsible for the observed effects. Finally, daily outdoor concentrations of NO_2 were associated with emergency room admissions due to cerebrovascular disease and short-term ischemic attacks (Ponka and Virtanen, 1996).

Figure 20.4 summarizes study findings for asthma diagnoses, while Fig. 20.5 shows corresponding study findings for COPD. Other studies have focused on symptomatic responses to short-term exposures to NO_2 and other components of the ambient air pollution mixture. Figure 20.6, from the Children's Health Study of cohorts studied from second grade through high school in 12 Southern California communities (McConnell et al., 2003) found elevations in bronchitic symptoms that were significantly associated with peak concentrations of NO_2, but also with $PM_{10-2.5}$, inorganic acid (HNO_3), and soot (EC).

A relationship between outdoor levels of NO_2 and common respiratory symptoms (e.g., cough, sore throat, etc.) in children up to 5 years of age was noted by Gnehm et al. (1988), while another found an association between NO_2 exposure and wheeze in females, but not in males, aged 4 months to 4 years (Pershagen et al., 1995). Braun-Fahrlander et al. (1992) examined symptoms in children in relation to outdoor and indoor levels of NO_2. While the incidence of symptoms was not associated with either indoor or outdoor levels, the duration of increased symptoms was associated with outdoor NO_2 concentration.

The association of indoor exposure to NO_x on the respiratory health of asthmatic schoolchildren in Australia was studied in terms of difficulty breathing during the day and night, chest tightness during the day, daytime asthma attacks, FEV_1, and PD_{20}. The intervention eliminated the use of unvented gas-fired heaters but not in the matched nonintervention schools. These indices of short-term effects were significantly lower in the intervention schools than in the matched nonintervention schools (Pilotto et al., 2003).

In studies of the effect of NO_2 on respiratory health in 6–9-year-old children, personal exposures to NO_2 were measured, as were indoor levels in the home (Brunekreef et al., 1987; Houthuijs et al., 1987). The prevalence of lung disease was found to be associated with the presence of unvented gas water heaters, with weekly average exposures estimated at 0.021 ppm. However, Dijkstra et al. (1990) found no association between respiratory symptoms with indoor NO_2 measurements in homes. Koo et al. (1990) used personal samplers to monitor NO_2 exposure in children aged 7–13 years in Hong Kong. No association was noted between exposure levels (means ranged from 0.013 to 0.023 ppm for a 1-week period) and respiratory symptoms, such as wheeze, running nose, or cough.

The effects of both indoor and outdoor air pollution on respiratory illness in a cohort of primary schoolchildren indicated a gradient of increased respiratory symptoms with increasing indoor levels of NO_2 in homes with gas stoves (Melia et al., 1977). A later assessment also indicated some increase in relative risk in homes with gas stoves, but this was not a consistent finding (Melia et al., 1979). In this case, levels of NO_2 measured in bedrooms of homes having gas stoves ranged from 0.003 to 0.017 ppm. In another study of children aged 5–6 years, no significant relationship was noted between levels of NO_2 and the prevalence of respiratory illness (Melia et al., 1982); levels of NO_2 in the bedrooms of homes with gas stoves were 0.005–0.029 ppm. Other attempts to relate gas stove use in homes to acute respiratory illness, respiratory symptoms, or indices of reduced lung function for various age populations have had mixed results, with some studies reporting no association and others reporting some relationship (Keller et al., 1979; Comstock et al., 1981; Dodge, 1982; Ekwo et al., 1983; Schenker et al., 1983).

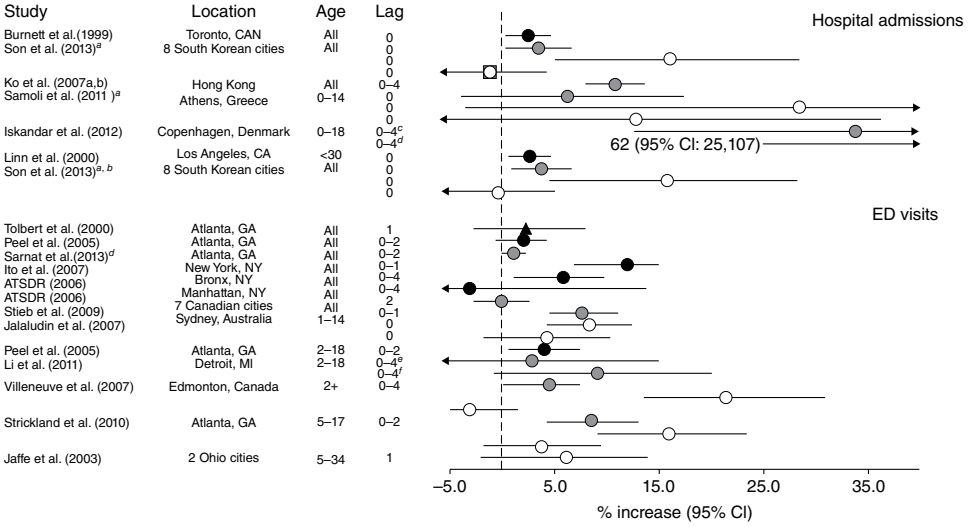

FIGURE 20.4 Percentage increase in asthma hospital admissions and emergency department visits in relation to short-term increases in ambient NO_2 concentrations. *Notes*: CA, California; CI, confidence interval; ED, emergency department; GA, Georgia; MI, Michigan; NO_2, nitrogen dioxide; NO_x, sum of NO_2 and nitric oxide; NY, New York; black, studies from the 2008 ISA for Oxides of Nitrogen; red (grey in print), recent studies; circles, NO_2; triangles, NO_x; solid symbols, all year; horizontal stripes, warm/summer months; vertical stripes, cool/winter months. Results are standardized to a 20-ppb increase in 24-h average NO_2, a 30-ppb increase in 1-h max NO_2, a 40-ppb increase in 24-h average NO_x, and a 100-ppb increase in 1-h max NO_x. [a]Results were presented for four seasons; however the summer and winter estimates represented the largest and smallest estimates across seasons. [b]This estimate is for allergic disease, which includes asthma. [c]Risk estimate for NO_2. [d]Risk estimate for NO_x. [e]Time-series results. [f]Case-crossover results.

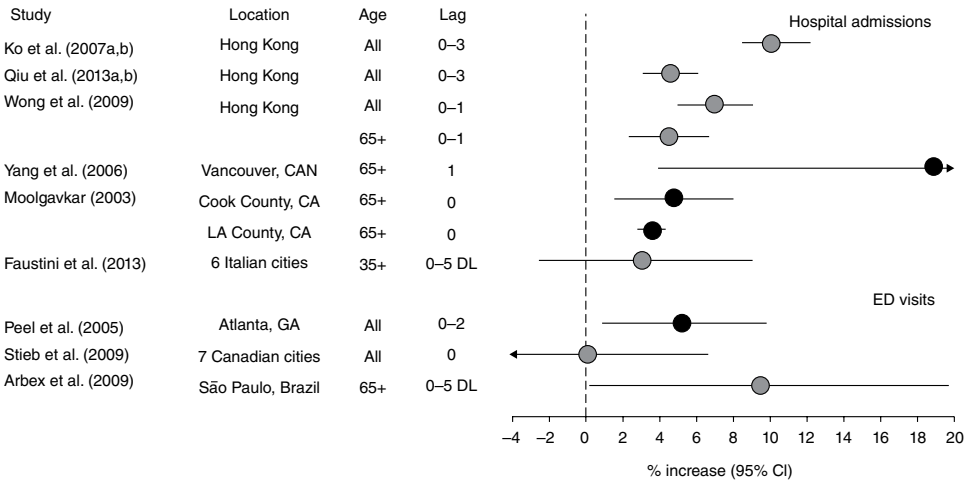

FIGURE 20.5 Percentage increase in chronic obstructive pulmonary disease hospital admissions and emergency department visits in relation to NO_2 concentrations. *Notes*: CA, California; CAN, Canada; CI, confidence interval; DL, distributed lag; ED, emergency department; GA, Georgia; LA, Los Angeles; black, studies from the 2008 Integrated Science Assessment for Oxides of Nitrogen; red (grey in print), recent studies. Effect estimates are standardized to a 20-ppb increase in 24-h average nitrogen dioxide and 30-ppb increase in 1-h max nitrogren dioxide.

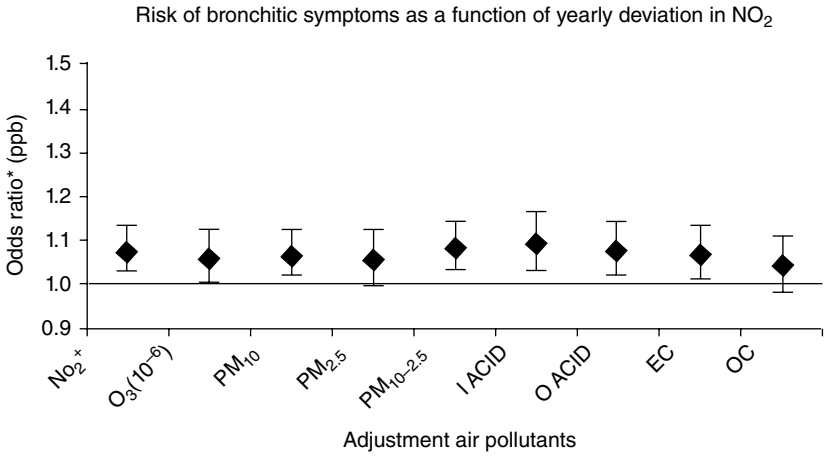

FIGURE 20.6 Within-community odds ratios for bronchitis symptoms associated with NO_2 adjusted for a co-pollutant in the 12 communities of the Children's Health Study. *Notes*: EC, elemental carbon; I ACID, inorganic acid; NO_2, nitrogen dioxide; O_3, ozone; O ACID, organic acid; OC, organic carbon; $PM_{2.5}$, particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 μm; PM_{10}, particulate matter with a nominal mean aerodynamic diameter less than or equal to 10 μm; $PM_{10-2.5}$, particulate matter with a nominal mean aerodynamic diameter less than or equal to 10 μm and greater than a nominal mean of 2.5 μm; ppb, parts per billion. * 95% CI.

Numerous studies have looked for associations of ambient air pollutants with pulmonary function decrements. In a classic series of surveys conducted on cohorts of adults and schoolchildren in six U.S. cities that were selected to represent a range of outdoor air quality (Harvard Six Cities Air Pollution Health Study), children within each community were followed for multiple years with the collection of questionnaire data and by annual measurements of pulmonary function in relation to outdoor pollutant levels that were measured at various sites within each community, as well as to indoor levels that were measured in selected households. Results of this study from 1974 to 1977 on over 8000 children aged 6–10 years indicated a significant increase in the rate of respiratory illness before age 2 in homes with gas-fired stoves compared with those with electric stoves (Speizer et al., 1980). However, a later examination by the same team in the same communities over a longer time period did not show any NO_2-related increase in respiratory illness (Ware et al., 1984). A further analysis of over 5000 children aged 7–11 years during the period 1983–1986 noted marginal significance for physician-diagnosed respiratory illness prior to age 2 in homes using gas-fired stoves compared with those using electric stoves (Dockery et al., 1989). When pulmonary mechanical indices in children were evaluated (Ware et al., 1984), gas stove use was associated with significant reductions in parameters of expiratory flow (FEV_1, FVC) in a first examination, but not in a subsequent evaluation.

Certain subpopulations, based upon age or preexisting disease state, or both, may be more susceptible to effects of NO_2 than others. A borderline significant effect was noted between a peak expiratory flow reduction in healthy children residing in homes having gas stoves, while a much stronger association was noted in asthmatics (Lebowitz et al., 1985). Children with asthmatic symptoms appeared to be more susceptible to reduced lung function when outdoor average NO_2 concentrations 0.02 ppm, but no such effect was found

with children having no asthmatic symptoms (Moseler et al., 1994). A meta-analysis that included mostly European studies (Weinmayr et al., 2010) noted a relationship between NO_2 exposure and peak expiratory flow (PEF) in children with asthma. Figure 20.7 shows that chronic exposure to ambient air NO_2 was associated with a significantly reduced pulmonary capacity in young adulthood.

In the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA), an important prospective cohort study of the possible cumulative effects of ambient air pollution on the cardiopulmonary system, Kaufman et al. (2016) noted a relationship between coronary calcium progression as measured by CT scanning against the long-term mean airborne effect of outdoor NO_2 exposure on decreasing lung function in children with asthma, but other studies did not support this finding (e.g., Spira-Cohen et al., 2011; Greenwald et al., 2013; Smargiassi et al., 2014). Similarly, among studies that measured ambient NO_2 at central sites, some found associations with lung function decrements in child asthmatics (e.g., Dales et al., 2009; Yamazaki et al., 2011), while others found no such association (e.g., Odajima et al., 2008; Wiwatanadate and Trakultivakorn, 2010). A study of adults in Europe (Schindler et al., 1998) indicated a relationship between short-term NO_2 exposure and changes in FVC. The Pilotto et al. (2003) intervention indoor pollution study cited earlier, in terms of eliminating the use of unvented gas-fired heaters from schools, showed that indices of short-term respiratory effects were significantly lower in the intervention schools than in the matched nonintervention schools.

The discussion above involved numerous studies of acute responses to short-term NO_x exposures. Fewer studies have examined the associations of long-term exposure to NO_2 on health effects. Faustini et al. (2014) conducted a review and meta-analysis of 23 epidemiological papers published from 2004 through 2013 that examined associations between long-term mean ambient air concentrations and annual mortality for overall, respiratory, and cardiovascular causes. For an increase of 10 μg/m^3, the risk for NO_2 was 1.04 (1.02–1.06), while for $PM_{2.5}$, it was 1.05 (1.01–1.09). For respiratory mortality, the corresponding risk estimates were 1.03 (1.02–1.03), while for cardiovascular, they were 1.13 (1.09–1.18).

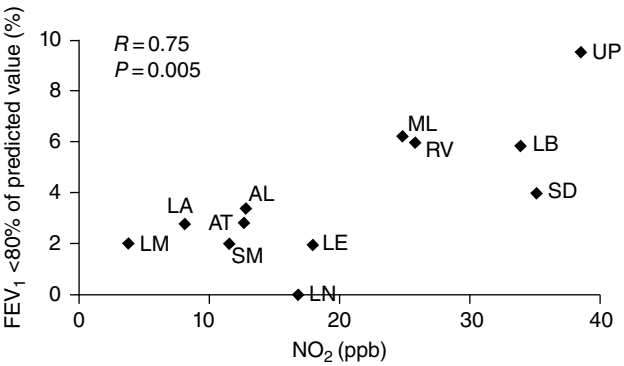

FIGURE 20.7 Community-specific proportion of 18-year-olds with an FEV_1 below 80% of the predicted value, plotted against the average concentrations of NO_2 from 1994 through 2000. *Notes*: AL, Alpine; AT, Atascadero; FEV_1, forced expiratory volume in 1 second; LE, Lake Elsinore; LA, Lake Arrowhead; LN, Lancaster; LM, Lompoc; LB, Long Beach; ML, Mira Loma; NO_2, nitrogen dioxide; P, *p* value; ppb, parts per billion; R, correlation coefficient; RV, Riverside; SD, San Dimas; SM, Santa Maria; UP, Upland.

As reported in WHO-EURO (2013), European cohort studies provide evidence that NO_2 effects on overall and cause-specific mortality were similar to, if not larger than, those estimated for PM. The registry cohort study from Italy (Cesaroni et al., 2013) and the North America (Hart et al., 2011; Jerrett et al., 2011) and Canadian (Gan et al., 2011) studies have attempted multi-pollutant models, and they provided support for an effect of NO_2 independent from particle mass metrics. In three of these mortality studies with multi-pollutant models, the major fraction of the populations studied was exposed to NO_2 levels lower than 40 μg/m^3; in one of them, nearly all participants were exposed to levels lower than 40 μg/m^3 (21.3 ppb) (Jerrett et al., 2011). Four of the six European analyses were centered around 40 μg NO_2/m^3. In the French study, areas with (possibly nonrepresentative) monitor averages above 32 μg NO_2/m^3 (17 ppb) were excluded. The study by Naess et al. (2007) looked at nonlinear exposure–response functions and found a possible threshold around 40 μg/m^3 for NO_2 and also a threshold for particles in the age group of 51–70-year-old people, especially for cardiovascular mortality. In contrast, they found no thresholds in the age group of 71–90-year-old people for all-cause and cardiovascular mortality and none for COPD mortality in either age group. The Italian study found some evidence for nonlinearity in the association between NO_2 and ischemic heart mortality, but not for cardiovascular or non-accidental mortality. All other investigators applied linear exposure–response functions.

Atkinson et al. (2018) also reviewed literature on the associations of long-term mean concentrations of NO_2 with increased annual mortality and reported similar NO_2-associated risks. They used hazard ratios (HRs) from 48 cohort studies to assess population health impact and burden. They undertook meta-analyses to derive concentration–response functions suitable for such evaluations and assessed their sensitivity to study selection based on the 48 articles analyzing 28 cohorts. Meta-analysis of HRs found positive associations between NO_2 and all-cause mortality [1.02 (95% CI: 1.01, 1.03); prediction interval (PI): (0.99, 1.06) per 10 μg/m^3 [5.3 ppb] increment in NO_2), cardiovascular [1.03 (95% CI: 1.02, 1.05); PI: (0.98, 1.08)], respiratory [1.03 (95% CI: 1.01, 1.05); PI: (0.97, 1.10)], and lung cancer mortality [1.05 (95% CI: 1.02, 1.08); PI: (0.94, 1.17)] with evidence of substantial heterogeneity between studies. In subgroup analysis, summary HRs varied by age at cohort entry, spatial resolution of pollution estimates, and adjustment for smoking and body mass index at the individual level; for some subgroups, the HR was close to unity, with lower confidence limits below 1. They concluded that given the many uncertainties inherent in the assessment of this evidence base and the sensitivity of health impact calculations to small changes in the magnitude of the HRs, calculation of the impact on health of policies to reduce long-term exposure to NO_2 should use PI and report ranges of impact rather than focusing upon point estimates. In some of the earlier reports, the prevalence and/or incidence of asthma or allergic airway disease related to NO_2 showed differing results (Dockery et al., 1989; Braun-Fahrlander et al., 1997; Studnicka et al., 1997; McConnell et al., 1999, 2003; Peters et al., 1999b; Shima and Adachi, 2000). However, Fig. 20.8 shows results from a later study of children (Gehring et al., 2010) that indicate significant associations of chronic exposures to NO_2, $PM_{2.5}$, and soot with incident asthma.

There does, however, appear to be an association between long-term exposure to NO_2 and decreased lung function growth with age in children, based upon studies in Southern California (Peters et al., 1999a, 1999b; Gauderman et al., 2000, 2002, 2004), but NO_2 was correlated with other motor vehicle-related pollutants, again implicating motor vehicle-derived concentrations of $PM_{2.5}$, NO_2, NO, and soot over 10 years for six U.S. communities, as shown in Fig. 20.9. There were significant associations for $PM_{2.5}$ and NO_x, but not for soot, while the association for NO_2 was just short of being significant. All four indices of exposure, as well as proximity to heavy traffic, have been used as surrogates of exposure to

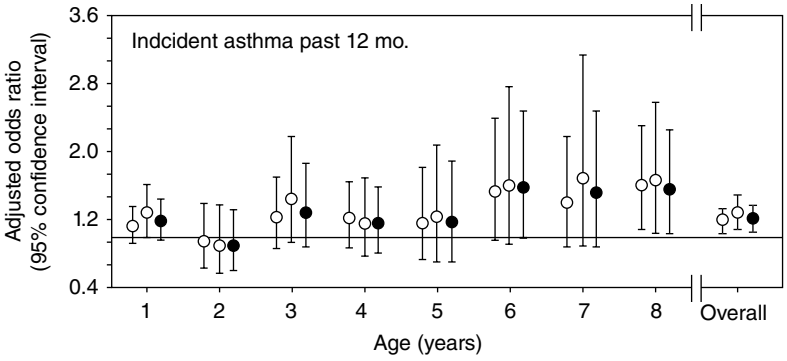

FIGURE 20.8 Overall and age-specific associations between annual average air pollutant concentrations at the birth residence and asthma during the first 8 years of life. *Notes*: mo., months. Blank circle, nitrogen dioxide (NO_2); gray circle, particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 μm; black circle, soot. Results are not adjusted for study region. Study regions a key determinant of air pollutant concentrations in the land use regression models used to estimate exposures, and adjustment may partly remove the influence of the pollutant. Odds ratios were calculated for an interquartile range increase in air pollutant (5.5 ppb for NO_2).

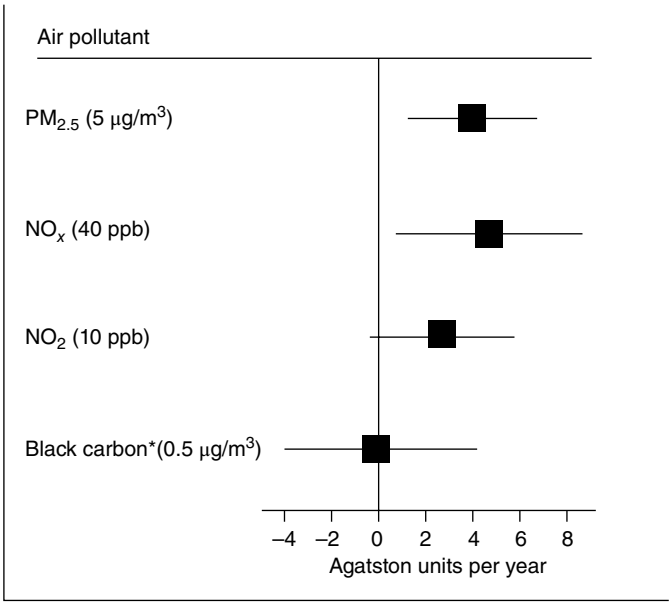

FIGURE 20.9 Long-term average air pollutant concentrations and coronary artery calcium progression. The linear longitudinal association of $PM_{2.5}$, NO_x, NO_2, and black carbon (soot) with coronary artery calcium progression (Agatston units per year), from linear mixed models adjusted for age, sex, ethnicity, city, income, employment outside the home, smoking status, second-hand smoke exposure, physical activity, adiposity, cholesterol, statin use, neighborhood socioeconomic index, income, education and scanner type. * 95% CI

motor vehicle effluents. These findings provide support for the hypothesis that traffic-related pollutants warrant continued, and perhaps more stringent, emission limits. These findings suggest that the exposure metric that could provide the strongest associations in future epidemiological studies is NO, rather than BC, NO_2, or proximity. The ratio of NO to NO_2 is

greater at street level than further downwind of the roadway; thus this ratio could account for the utility of proximity for studies without the capacity for multi-pollutant concentration measurements. The known ability of NO as an O_3 scavenger may also be of interest.

In summary, given the available epidemiological evidence, there has been a variety of both positive and negative findings associated with various levels of NO_x exposure, accompanied by various degrees of precision in measuring actual exposure levels. With the growing number of well-conceived studies, there is an increasing recognition that significant relationships do exist between exposure and increased mortality for all-cause, cardiovascular, and respiratory effects, although the effect estimates are generally reduced when adjustments are made for the co-presence of other ambient air pollutants, specifically PM and O_3. Some results are also suggestive that an increase in acute respiratory illness, especially in younger children, may be associated with chronic exposure. A number of studies indicate there to be a fairly strong association between NO_2 and hospital admissions or emergency room visits for asthma in children where NO_2 was the only associated pollutant or where adjusting for other pollutants did not affect the association with NO_2. Finally, there is fairly strong evidence that chronic exposure to NO_2 adversely affects lung function growth in children that could be reflected in reduced function as adults and that exposure to NO_x affects coronary artery calcium progression. Thus, while acute and chronic exposure to NO_2 has been associated with adverse health outcomes, it is often unclear as to whether there are independent effects of one specific traffic-related pollutant. However, there is growing evidence for traffic-related air pollution, which contains both NO and NO_2, having adverse health effects.

20.3.3 Health Effects: Toxicology and Controlled Human Exposoures

Toxicological studies can be helpful in providing biological plausibility for health outcomes noted in epidemiological studies. However, a significant fraction of the early NO_2 toxicological database involves experimental exposures to concentrations >5 ppm. While such studies can elucidate mechanisms of toxicity that influence responses to high concentrations, they are often of limited use in attempts to determine the public health significance from actual, much lower concentration, ambient air exposures. Thus, in this chapter, generally only studies using <5 ppm will be discussed. However, when necessary to help elucidate certain mechanisms, effects at higher levels will be presented as well. It should be noted that there are very few recent (since the last edition of this book) toxicological or controlled human studies on nitrogen dioxide exposure.

20.3.3.1 Studies in Animals The largest database concerning the biological effects of NO_2 is that derived from controlled exposures of laboratory animals. Since the mechanisms underlying many responses are usually similar across species, effects in these animals may have implications for humans. It should be borne in mind, however, that the exposure concentrations needed for comparable response likely differ between species. In any case, NO_x compounds have been shown to be significantly associated with a wide range of biological effects, mostly within the cardiopulmonary system.

Respiratory Tract Defenses Mucociliary clearance provides a first line of defense against prolonged retention of deposited particles in the tracheobronchial tree. Acute (1–2 h) exposures to NO_2 at levels <10 ppm did not alter mucociliary transport rate from the tracheobronchial tree of laboratory animals (Schlesinger, 1989). Rats exposed for 6 weeks

to 6 ppm NO_2 showed a transient depression in mucociliary activity (Giordano and Morrow, 1972), while rabbits exposed for 2 h/day for 14 days to 0.3 or 1 ppm did not show altered tracheobronchial mucociliary transport (Schlesinger et al., 1987). Thus, the available data suggests that with either single or short-term repeated exposures, higher than ambient levels are needed to alter tracheobronchial mucociliary transport.

Particle clearance from the gas exchange region of the lungs has also been assessed following exposures to NO_2. Rats exposed to 1, 15, and 24 ppm showed a decrease in macrophage-mediated clearance after 22 daily exposures to 15 and 24 ppm, but accelerated clearance after exposures to 1 ppm (Ferin and Leach, 1977). Rabbits exposed for 2 h to 0.3, 1, 3, or 10 ppm showed accelerated particle clearance at all concentrations, while repeated 2 h/day exposures for 14 days to 1 or 10 ppm NO_2 resulted in clearance patterns similar to those with single exposures at the same concentration (Vollmuth et al., 1986). Ferrets exposed to either 0.5 or 10 ppm NO_2 for 4 h/day, 5 days/week for 8 or 15 weeks showed a reduction in clearance rate measured 12 weeks after the start of either exposure regime (Rasmussen et al., 1994).

Since alveolar macrophages play a central role in the defense of the lungs, and alterations in numbers and functional properties of these cells may affect susceptibility to disease or injury. Macrophage numbers increased with continuous exposure of rats to 17 ppm, but not with 2 ppm (Stephens et al., 1972), and after 7 days of continuous exposure of rats to 4 ppm (Mochitate et al., 1986). However, no change in cell number was found following exposure of rabbits to 0.3 or 1 ppm NO_2 for 2 h/day for 13 days (Schlesinger, 1987). A subpleural accumulation of alveolar macrophages was found in rats exposed for 7 h/day, 5 days/week for 15 weeks to 5 ppm NO_2, but not to 1 ppm (Gregory et al., 1983). Rombout et al. (1986) noted some increase, by 2 days, in the number of macrophages in terminal bronchioles and adjacent alveoli in the lungs of rats exposed continuously to 5 ppm NO_2; this was not seen with 1 or 2.5 ppm. Others have noted concentration-related increases in macrophage numbers with exposure to 5–40 ppm for 2 days to 15 weeks (Busey et al., 1974; DeNicola et al., 1981; Kleinerman et al., 1982; Wright et al., 1982; Foster et al., 1985).

Various functional properties of macrophages essential to adequate defense, for example, surface attachment, mobility, and phagocytosis, have been assessed following exposure to NO_2. Schlesinger (1987) exposed rabbits to 0.3 or 1 ppm for 2 h/day and found no effect on attachment, but a depression of mobility at day 3 in the 0.3 ppm group. Macrophages obtained from baboons exposed to 2 ppm for 8 h/day, 5 days/week for 6 months showed reduced responsiveness to migration inhibitory factor, a lymphokine that mediates cell movement (Greene and Schneider, 1978).

Suzuki et al. (1986) found depressed phagocytic activity in macrophages obtained from rats exposed for 10 days to 4 or 8 ppm NO_2, while Lefkowitz et al. (1986) noted no change in such activity in macrophages from mice exposed for 7 days to 5 ppm. The phagocytic activity of rabbit macrophages was reduced by *in vivo* exposure to 0.3 ppm, but was enhanced with exposure to 1 ppm by 3 days, and returned to control values by 7 days and remained there through 13 days of exposure (Schlesinger, 1987). An exposure-concentration-dependent difference in the direction of phagocytic response seems to be a characteristic of NO_2. Thus, Schlesinger (1989) found a reduction in phagocytic activity of macrophages recovered immediately after a 2-h exposure of rabbits to 1 ppm NO_2; with 10 ppm, no change was seen immediately after exposure, but activity was increased 24-h post-exposure. Ehrlich et al. (1979) found exposure of mice to 0.5 ppm NO_2 for 3 h/day, 5 days/week for 2 months to depress phagocytosis, while Sone et al. (1983) showed

enhanced phagocytosis in macrophages obtained from rats exposed to 40 ppm for 4 h/day for 7 days. The reasons for such differences in direction of response are unknown.

Macrophages are a source of various biological mediators, and their ability to produce these may be compromised by pollutant exposure. The eicosanoids are a class of mediators produced in response to a wide variety of cellular perturbation and have various effects on airway physiology and the immune system. Alveolar macrophages obtained from rats exposed to 0.5 ppm NO_2 for 0.5–10 days exhibited complex responses related to the production of eicosanoids (Robison et al., 1993). An initial depression of production was followed by recovery for some of these mediators, but not others, with increasing exposure duration. The complexity of response was also noted in a study of rat alveolar macrophages acutely exposed *in vitro* to 0.1–20 ppm NO_2 (Robison and Forman, 1993). Low concentrations (up to 5 ppm) had small effects on basal synthesis of eicosanoids but amplified response to stimulated production of eicosanoids, while high concentrations (20 ppm) showed the reverse pattern of response. Finally, rats continually exposed to 10 ppm for 1, 3, or 20 days showed reduced levels of tumor necrosis factor in lavage and suppression of cytokine signaling 3 mRNA, both of which were likely due to NO_2-induced changes in activation state of the macrophages (Garn et al., 2003).

NO_2 may impair the ability to resist infectious agents; this is suggested by some epidemiological studies noted above. Mice exposed continuously to 0.5 ppm NO_2 showed increased mortality to *Klebsiella pneumoniae* after 3 months of exposure (Ehrlich and Henry, 1968), while 0.05 ppm for 24 h/day for 15 days did not change bacterial resistance (Gardner et al., 1982). The finding of increased susceptibility does, however, depend upon the specific organism being used. Thus, while exposure of mice for 3 h/day for 3 months to 0.5 ppm increased mortality to *Streptococcus* sp. (Ehrlich et al., 1979), exposure to 0.5–1.5 ppm NO_2 continuously for 3 months produced no effect on mortality due to *K. pneumoniae* (McGrath and Oyervides, 1985); on the contrary, exposure to 5 ppm for 3 days did result in enhanced mortality.

It may be that peak exposure and exposure pattern are important modulators of response to NO_2. A number of infectivity studies involved exposure to a baseline NO_2 concentrations upon which spikes to a higher level were superimposed to mimic ambient exposures. The relative effect of such spikes is not always clear, but seems to depend upon both spike duration and time between spikes. Miller et al. (1987) noted that mortality due to infection was greater in a spike regimen (to 0.8 ppm) than in the baseline-exposed group (0.2 ppm). Others have found that both the number and amplitude of spikes are of importance in increasing mortality (Gardner et al., 1979; Graham et al., 1987). In fact, effects from such exposure excursions may approach those due to more continuous exposure to a lower concentration. This is consistent with the notion that, in general, brief exposures to high NO_2 levels are more hazardous than longer-duration exposures to lower concentrations (Lehnert et al., 1994).

The effect of NO_2 on mortality due to bacterial infection appears to increase with both exposure duration (T) and peak concentration (C), although the latter seems to have more influence than the former for fixed $C \times T$ values (Gardner et al., 1979). Any differences between intermittent and continuous exposure also seem to disappear as the number of days of exposure increases (Gardner et al., 1979). Other studies suggest that as concentration increases, a shorter exposure time is needed for intermittent and continuous exposure regimes to produce similar degrees of effect (Ehrlich and Henry, 1968; Ehrlich, 1979). Mortality is also proportional to exposure duration if the bacterial challenge is given immediately after exposure, but may not be when the challenge is given much later (Gardner

et al., 1982). For example, no effect of 3.5 ppm NO_2 for 2 h was seen in mice when bacteria were administered 27 h after exposure, while increased mortality was evident when administration was immediately after NO_2 inhalation (Ehrlich, 1980). Effects of 25 ppm for 2 h on mice were seen only when the microbial challenge was given within 72 h after NO_2 exposure (Purvis and Ehrlich, 1963). These results suggest that a critical time frame exists between exposure and bacterial challenge after which NO_2 will not affect resistance.

The mechanisms underlying any NO_2-induced change in host resistance to bacteria are not yet known. However, since exposure levels that alter resistance do not affect physical clearance processes, the response to NO_2 may be due to impaired intracellular killing of microbes, perhaps reflecting macrophage dysfunction. For example, macrophages are a source of numerous biochemical mediators that are directly involved in antibacterial action, for example, superoxide anion, and a depression in superoxide production has been noted following NO_2 exposure in some studies (Amoruso et al., 1981; Suzuki et al., 1986; Robison et al., 1993), although at higher than ambient levels. However, human alveolar macrophages exposed *in vitro* to 0.1–0.5 ppm NO_2 for 30–120 min showed increased reactive oxygen intermediate production in a dose-dependent fashion (Kienast et al., 1994). The effects of NO_2 on viral infectivity have also been examined. Exposures to 0.5 ppm or greater on a continuous basis likely increase susceptibility, while at higher concentrations the exposure duration needed for any effect is lowered (Ito, 1971; Rose et al., 1988). Furthermore, environmental stresses may enhance the lethality of infectious agents over and above that due solely to NO_2 exposure. These may include exercise (Illing et al., 1980), elevated temperature (Gardner et al., 1982), and the presence of other pollutants.

Exposure to NO_2 may affect allergic response. Rats exposed to 5 ppm for 3 h after sensitization with house dust mite antigen had higher levels of serum IgE and local respiratory tract IgA, IgG, and IgE antibodies than controls (Gilmour, 1995). The exposed animals also had increased lymphocyte activity in the spleen and local lymph nodes and showed an increase in respiratory tract inflammatory cells. This suggests that NO_2 may enhance immune responsiveness and increase the severity of pulmonary inflammation in sensitized lungs and may, thus, play some role in the exacerbation of immune-mediated respiratory disease.

A number of studies have examined the effects of NO_2 on specific parameters of respiratory and/or systemic humoral and cellular immunity. While immune suppression and/or enhancement of factors involved in airway hyperreactivity has clearly been shown to follow exposure to levels of NO_2 above 5 ppm, as evidenced by various endpoints including the response of T cells, antibodies, or production of interferon or other inflammatory mediators (e.g., Valand et al., 1970; Campbell and Hilsenroth, 1976; Holt et al., 1979; Fujimaki and Shimizu, 1981; Ayyagari et al., 2004), there are only a few reports of response to lower levels. These studies suggest that short-term repeated exposures may result in reductions in counts of lymphocytes in the lungs or spleen or a depression in antibody responsivity to particular antigens.

Mice exposed for 7 h/day, 5 days/week for 7 weeks to 0.25 ppm NO_2 showed reduced total T-lymphocyte numbers in the spleen, with concomitant reductions in certain subpopulations of these cells, for example, helper cells (Richters and Damji, 1988). Exposure of mice for 3 months to 0.5 ppm NO_2 resulted in a depressed responsiveness of both T- and B-lymphocytes in spleen (Maigetter et al., 1978). No effect on the cell-mediated immune system was found either in mice exposed for 24 h to 5 ppm NO_2 (Lefkowitz et al., 1986) or in those exposed to 1.6 ppm NO_2 for 4 weeks (Fujimaki et al., 1982). Mice exposed to 0.4 or 1.6 ppm NO_2 for 4 weeks showed depressed primary antibodyresponsivity to sheep red

blood cells *in vitro* (Fujimaki et al., 1982), while mice exposed to 4 ppm NO_2 continuously for up to 56 days showed no change in the antibody response to T-cell-dependent and T-cell-independent antigens in spleen (Fujimaki, 1989). In another study, mice were vaccinated with influenza virus after they had undergone 3 months of continuous exposure to 0.5 or 2 ppm NO_2 with daily spikes (1 h) of 2 ppm for 5 days/week. Both concentrations resulted in a reduction in mean serum neutralizing antibody titers (Ehrlich et al., 1975). Guinea pigs exposed to 1 ppm for 6 months showed a reduction in all immunoglobulin fractions (Kosmider et al., 1973). On the contrary, Balchum et al. (1965) noted an increase in serum antibody titers against lung tissue in guinea pigs exposed to 5 ppm NO_2 for 4 h/day after 160 h of exposure and further increases as exposure duration increased. Enhanced immune function may be just as detrimental as suppressed function through overstimulation of response and hypersensitivity.

As with other endpoints, the effects of NO_2 upon the immune system appear to be related to various exposure parameters. While some studies show no effects, others show enhancement or depression of immune parameters, depending upon the exposure concentration, the length of exposure, and the animal species used. In addition, the direction of change appears to depend upon exposure concentration. For example, humoral response in monkeys chronically exposed to NO_2 was enhanced at a low concentration (1 ppm), but suppressed at a higher level (5 ppm) (Fenters et al., 1971, 1973).

Respiratory Tract Structure Exposure to NO_2 may produce structural alterations in the respiratory tract. As noted, the anatomic region most sensitive to NO_2 is the area encompassing the terminal and respiratory bronchioles and adjacent alveolar ducts and alveoli. The primary cellular targets within this region are ciliated cells of the bronchiolar epithelium and type 1 cells of the alveolar epithelium. Acute exposure to NO_2 can result in hypertrophy and hyperplasia of alveolar type 1 cells, followed by cell death and desquamation and proliferation of and replacement by type 2 cells. The end result can be a thickened air–blood barrier. The bronchiolar response is characterized by hypertrophy and hyperplasia of epithelial cells, loss of secretory granules and surface protrusions of Clara cells, and loss of ciliated cells, or of cilia. With chronic exposure, many of these same changes are seen, but there is increased cilia loss over larger areas of epithelium and in more proximal airways, and the structure of the remaining cilia may be altered.

The temporal progression of NO_2-induced lesions has best been described for the rat. The earliest alterations resulting from concentrations >2 ppm occur within 24–72 h of continuous exposure, with repair of injured tissue and replacement of destroyed cells beginning within 24–48 h of continuous exposure. Division of type 2 cells is observed within 12 h after initial NO_2 exposure, the rate becoming maximal by about 48 h and then decreasing to pre-exposure levels by about 6 days, even with continued exposure. In some cases, the resolution of NO_2-induced morphologic changes may be complete after exposures end; on the contrary, some lesions may resolve, while others remain, even when exposure continues (DeNicola et al., 1981; Rombout et al., 1986; Kubota et al., 1987).

Chronic exposure to NO_x may result in alterations in lung architecture resembling emphysema-like disease, for example, enlargement of airspaces, increase in mean linear intercept (a measure of the distance between alveolar walls), and reduction in the internal surface area of the alveolar region. However, the relationship between exposure and the development of emphysema remains unclear. A problem in evaluating reported emphysematic changes in animal models is the definition of the disease, which has changed over the years and which has been defined differently by various professional groups (NIH, 1985).

While long-term exposure to high NO_2 concentrations (>10 ppm) are required to produce clearly definable emphysema-like changes (e.g., Barth et al., 1995), there is evidence that lower NO_2 levels may result in emphysema, emphysema-like changes, or altered alveolar dimensions if present in complex mixtures of NO_x (Hyde et al., 1978) or when administered during lung development (Rasmussen and McClure, 1992). However, clear evidence of changes characteristic of human emphysema, that is, alveolar septal degeneration, enlarged airspaces, and associated functional changes, is absent with exposure at low levels. There is, however, some evidence for changes similar to those seen in human emphysema with exposure to high concentrations. These involved exposures to levels ranging from 8 to 20 ppm for up to 33 months (Haydon et al., 1967; Freeman et al., 1972). Another study that involved exposure of dogs for 5.5 years to a mixture of NO_2 at 0.64 ppm and NO at 0.25 ppm followed by a post-exposure period of 2.5 years noted structural changes similar to human centrilobular emphysema that were noted after the post-exposure period had ended (Hyde et al., 1978).

While the extent and degree of structural alterations induced by NO_x appear to be related to exposure concentration, little is known about effects of other modifying factors, for example, exposure duration or the temporal pattern of exposure. The contribution of exposure time in the histopathologic response to acute inhalation was examined in rats (Stavert and Lehnert, 1988). The most pronounced effects were found with the highest NO_2 concentration in any particular set of exposures where the product of concentration and time was equivalent, indicating that concentration played a more important role than exposure time in tissue injury. This is consistent with the relative roles of C and T in infectivity, discussed previously. Another study (Rombout et al., 1986) assessed the concentration–time response relation for intermittent and continuous exposures and likewise concluded that concentration played a more important role in inducing morphologic lesions than exposure duration, as long as the product of $C \times T$ was constant. The effect of concentration was found to be greater with intermittent than with continuous exposure, and the onset of response was also delayed with intermittent compared with continuous exposure. The morphological effects of exposure patterns involving transient spikes were examined in a number of studies (Gregory et al., 1983; Crapo et al., 1984; Chang et al., 1986; Miller et al., 1987). Results are equivocal, and it is not clear whether these peaks significantly contributed to morphological damage in excess of that due to integrated exposure.

In spite of the fact that there is a fairly extensive database concerning morphologic effects of NO_x in animal models, it is still quite difficult to establish a threshold exposure condition for these endpoints. This is due to the great complexity of changes occurring with exposure, as well as to large interspecies differences in response. For example, the rat appears to be less sensitive to NO_2 compared with other species, such as the guinea pig or monkey. Furthermore, different cell types show differential sensitivity to NO_2. In general, morphological alterations, some of which may be persistent, are found with chronic exposure to concentrations <1 ppm. However, long-term exposures to levels >2 ppm are generally required to produce more extensive or permanent changes.

An added complication in evaluating morphologic effects is that they may depend upon the age of the animals at the time of exposure (Kyono and Kawai, 1982; Stephens et al., 1982; Azoulay-Dupuis et al., 1983; Chang et al., 1986). Neonates, specifically prior to weaning, seem to be relatively resistant to NO_2, with sensitivity increasing with age until adulthood. However, the response in animals of different ages is similar in terms of the cell types affected, the nature of the damage incurred, and repair capacity. Age-related

differences occur in the extent of damage and in the time required for repair, this latter taking longer in older animals. The reasons for age differences in sensitivity are not known, but may reflect diet and variable sensitivity of target cells during different growth phases (Hahn, 1979; Stephens et al., 1982). In any case, age-related differences in response are also observed in epidemiological studies.

Of importance in assessing the morphological effects of NO_x is consideration of individuals with compromised lung function, for example, those with respiratory disease. There is, however, a very limited database in laboratory animals with preexisting chronic disease. No effect of NO_2 exposure upon pneumoconiosis development in guinea pigs was found in one study (Gross et al., 1968). Two studies assessed whether prolonged exposure to NO_2 would alter the progression or severity of preexisting emphysema (produced by elastase instillation). Nitrogen dioxide did not potentiate preexisting disease in rats (Stavert et al., 1986), but may have done so in hamsters (Lafuma et al., 1987).

It is also possible that acute lung disease may affect NO_2 toxicity. Fenters et al. (1973) challenged squirrel monkeys with an influenza virus at various times during continuous exposure to 1 ppm NO_2 for 16 months and compared the response to that seen in animals not challenged but similarly exposed. Only the virus-challenged animals showed effects of NO_2, namely, slight emphysema-like changes and thickening of the bronchial and bronchiolar epithelium. This suggests that the presence of acute lung disease may have affected NO_2 toxicity.

Respiratory Tract Biochemistry NO_2 is, as noted, quite reactive. Exposure can result in damage to the cell membrane, and fairly low exposure levels have been associated with alterations to specific membrane components. For example, lipid peroxidation was noted in rats exposed to 0.04 ppm for 9 months (Sagai et al., 1984). However, extended exposures at low levels may be needed for such effects, since rats and guinea pigs exposed to 0.4 ppm NO_2 for 24 h/day for only 2 weeks showed no change in the level of lipid peroxides in the lungs (Ichinose and Sagai, 1982). With the exception of effects on lipids, most studies of biochemical pulmonary alterations show significant effects with acute or short-term repeated exposures generally only at levels above about 2 ppm. Such effects include oxidation of protein or protein components, such as elastin and collagen. Thickened collagen fibrils were noted in the lungs of monkeys exposed to 3 ppm for 4 h/day for 4 days (Bils, 1976), while increased rates of lung collagen synthesis, a possible marker for development of fibrosis, has been noted in NO_2-exposed rats (Last and Warren, 1987; Last et al., 1993). Exposures to high concentrations (Kleinerman and Ip, 1979; Kleinerman et al., 1985) suggest that NO_2 may reduce elastin content via an increase in the activity of neutrophil elastase, the enzyme responsible for elastin breakdown.

Other NO_2-induced biochemical effects related to proteins involve changes in activity of various pulmonary enzymes. For example, glutathione (GSH) is a reducing compound found in the lungs, and NO_2 exposure has been reported to alter the activity of enzymes that regulate its levels or to affect the lung content of GSH itself. Suppressed GSH peroxidase activity has been noted, for example, in mice exposed continuously for 17 months to 1 ppm, but not to 0.5 ppm (Ayaz and Csallany, 1978), while an increase in GSH reductase activity was noted in mice exposed to 6 ppm for 4 h/day for 30 days (Csallany, 1975) and in rats exposed to 6.2 (but not 1 or 2.3 ppm) for 4 days (Chow et al., 1974). High NO_2 exposure levels are, thus, apparently needed for changes in GSH metabolism.

The biochemical response to NO_2 may be modulated by dietary factors, in particular, levels of certain antioxidant vitamins. Increases in the lavage content of proteins and lipids

were noted in vitamin C-depleted guinea pigs exposed to 1 ppm NO_2 for 72 h, but not in normal controls (Selgrade et al., 1981); similarly depleted guinea pigs exposed for 1 week to 0.4 ppm showed an increase in lavage protein content, an indicator of serum transudation and possible membrane damage (Sherwin and Carlson, 1973). Another possible modulator of effect is vitamin E. Changes in protein content and enzyme activity in lung homogenates from rats exposed for 7 days to 3 ppm were found to be more severe in those animals that were deficient in this vitamin (Elsayed and Mustafa, 1982).

Respiratory tract tissue can metabolize various xenobiotics, and it is possible that inhaled pollutants may alter the ability to handle these materials. However, if NO_2 does alter xenobiotic metabolism, it is only at high exposure levels. Thus, exposure of rats continuously to 4 ppm NO_2 for up to 2 months produced no change in the cytochrome P450 content of the lungs, although some other xenobiotic-metabolizing enzymes were decreased (Takahashi and Miura, 1989).

Respiratory Mechanics The effects of NO_2 on respiratory mechanics have been studied in laboratory animals using standard indices of function, with mixed results. Hamsters exposed to 2 ppm for 8 h/day, 5 days/week for 8 weeks exhibited an increase in tidal volume, but no change in compliance or vital capacity (Lafuma et al., 1987). Exposure of mice to 0.2 ppm NO_2 for 23 h/day, 7 days/week for up to 52 weeks resulted in no change in pulmonary mechanics (Miller et al., 1987); however, when 1-h spikes to 0.2 ppm twice daily for 5 days/week were superimposed upon this baseline, a significant decrease in end-expiratory volume and vital capacity, as well as a trend toward increased residual volume, was found. Stevens et al. (1988) exposed neonate and 7-week-old mice continuously for 1, 3, or 6 weeks to baseline levels of 0.5, 1, or 2 ppm NO_2 upon which were superimposed twice- daily 1-h spikes (5 days/week) to 1.5, 3, or 6 ppm, respectively. The two higher levels produced increased vital capacity and compliance by 3 weeks only in the neonates, but the effect resolved by 6 weeks. Furthermore, adult animals showed a reduction in compliance after 6 weeks of exposure to 2 ppm. Suzuki et al. (1982) noted a concentration-related increase in respiratory rate in mice exposed to 5–20 ppm NO_2 for 24 h; exposure to 5 ppm also resulted in a decrease in arterial CO_2 tension ($PaCO_2$), suggesting hyperventilation.

Bronchoprovocation challenge testing is often used to assess nonspecific airway hyperresponsivity. Silbaugh et al. (1981) examined the effects of histamine aerosol on guinea pigs exposed to NO_2 for 1 h at 7–146 ppm. While a concentration-related increase in sensitivity to histamine was noted when the latter was inhaled 10 min after NO_2 exposure (but not 2 or 19 h after exposure), the response became significant only when NO_2 levels were >25 ppm. A study involving long-term exposures (Kobayashi and Miura, 1995) involved exposure of guinea pigs to 0.06, 0.5, 1, 2, or 4 ppm NO_2 for 24 h/day for 6 or 12 weeks. Airway responsiveness to histamine and specific airway resistance was assessed on the last day of each exposure. Exposure to 2 and 4 ppm by 6 weeks of exposure resulted in increased airway responsiveness, while exposure to the same concentration resulted in increased resistance by 12 weeks of exposure.

Tepper et al. (1993) performed a long-term exposure of rats to NO_2. Animals were exposed to NO_2 having a 0.5 ppm background with 1.5 ppm peaks (2 h) for up to 78 weeks. No exposure-related changes in nitrogen washout, compliance, lung volume, or CO diffusion capacity were noted, but at 78 weeks there was some reduction in a measure of forced expiratory flow rate. However, the authors indicated that the change was borderline and suggested that long-term exposure to high ambient urban levels did not lead to any dysfunction suggestive of degenerative lung disease. The overall database suggests that

NO_2 at realistic levels in terms of ambient exposure has not been shown to significantly alter pulmonary mechanics or bronchial responsivity in animal models, consistent with results of controlled clinical studies in humans.

Extrapulmonary Effects Exposure to NO_x may affect target sites beyond the respiratory tract. Endpoints that have been shown to be altered include body weight, blood cell counts, blood cell membrane and serum chemistry, liver and kidney function, brain protein enzymes, and neuromotor function (e.g., Wagner et al., 1965; Freeman et al., 1966; Kosmider et al., 1973; Sherwin and Layfield, 1974; Case et al., 1979; Kaya et al., 1980; Miller et al., 1980; Graham et al., 1982; Kaya and Miura, 1982; Mochitate et al., 1984; Tabacova et al., 1985; Takahashi et al., 1986). More recently, Li et al. (2011) exposed rats to 2.66 or 5.32 ppm for 7 days and noted a small increase in the activity of the antioxidant enzyme Cu/Zn-SOD at both concentrations and an increase in malondialdehyde, an indicator of lipid peroxidation, only at the higher concentration. Campen et al. (2010) noted that exposure to 2 or 0.2 ppm in ApoE knockout mice resulted in a concentration-related decrease in the expression of the antioxidant enzyme HO-1 in the aorta. Overall, however, the ability to relate reported changes to human health effects is severely limited from these studies.

Carcinogenicity/Reproductive Toxicity Exposure to NO_2 even at high levels does not seem to be genotoxic or teratogenic in appropriate assay systems (Gooch et al., 1977; Kripke and Sherwin, 1984). While one study did note an increase in the rate of DNA strand breaks in hamster cells exposed *in vitro* to 10 ppm for 20 min, exposure to 5 ppm for up to 30 min had no such effect (Görsdorf et al., 1990), and *in vivo* exposure of mice to 20 ppm for up to 23 h did not result in any genotoxicity (Victorin et al., 1990). This apparent conflict in response may be due to repair mechanisms operating *in vivo* that are not operative in *in vitro* assays.

The ability of NO_2 to act as a carcinogen, or co-carcinogen, is unclear, but there is no direct evidence that NO_2 exposure results in the development of tumors. Some concern is, however, based upon the fact that exposure can result in nitrite in blood, and this, in turn, may produce carcinogens, such as nitrosamines, after further reaction in the body. Although there have been no long-term carcinogenesis bioassays performed with NO_2, one chronic inhalation study, in which mice were exposed to 1, 5, and 10 ppm NO_2 for 6 h/day, 5 days/week for 6 months, suggested a small increase in tumor (pulmonary adenoma) frequency and incidence in the highest dose group (Adkins et al., 1986). However, such data must be interpreted with caution, and the relationship between cancer development in mice and that in humans is not clear.

Although not likely a carcinogen itself, NO_2 may modulate tumorigenic processes in the lungs (Witschi, 1988). For example, in conjunction with a specific carcinogen, NO_2 exposure may be involved in the pathogenesis of small cell carcinoma (Witschi, 1988), especially since it has been shown to modulate the number of neuroendocrine cells, the precursor cells for this disease (Palisano and Kleinerman, 1980; Kleinerman et al., 1981). As another example, an enhancement of tumor colonization in the lungs of mice injected (IV) with melanoma cells was noted after exposure to NO_2 at 0.4 or 0.8 ppm for 8 h/day,5 days/week for 10–12 weeks (Richters and Kuraitis, 1981). This could be due to injury of lung capillary endothelium by NO_2, facilitating metastases of blood-borne cancer cells to the lungs (Richters and Richters, 1989), or to the suppression of immune system components. However, as with other endpoints, the database

regarding the role of NO_2 in carcinogenic processes is conflicting. For example, NO_2 has been shown to actually enhance the cytotoxic response of macrophages (Sone et al., 1983), which implies greater antitumor defense capabilities. Thus, any role for NO_2 in cancer etiology requires further evaluation.

Is there any epidemiological evidence that NO_2 is involved in the etiology of cancer? Some studies do suggest a relationship between NO_2 and lung cancer (e.g., Hoek et al., 2002; Nafstad et al., 2004). However, NO_2 is generally associated with other pollutants from the same source, many of which are known carcinogens, so all results related directly to NO_2 must be interpreted with great caution.

There have been some epidemiological studies suggesting that exposures to mixtures containing NO_2 during pregnancy may be associated with fetal/reproductive effects, such as low birth weight and perinatal mortality, but, again, any independent effect from NO_2 is unclear (Liu et al., 2003; Wilhelm and Ritz, 2003).

20.3.3.2 Controlled Studies with Humans By their nature, studies with human volunteers can only be used to evaluate transient effects of acute exposure. They have generally used changes in standard respiratory mechanical indices as markers of response; a few studies, however, have employed other endpoints, which include bronchoprovocation challenge testing, clearance of inhaled aerosols, and analysis of biochemical and cellular components of bronchopulmonary lavage. Various subject groups have been examined. These include healthy individuals with no history of respiratory disease, allergy, and so on, as well as people with allergies or a history of asthma or COPD.

Acute exposures (up to about 2 h) to NO_2 at levels <1 ppm have not resulted in any consistent, significant changes in respiratory mechanics in normal, healthy adult subjects at rest (Beil and Ulmer, 1976; Hazucha et al., 1982; Bylin et al., 1985; Koenig et al., 1985; Bascom et al., 1996). Regarding higher levels, the study of Beil and Ulmer (1976) involving 2-h exposures to 1, 2.5, 5, and 7.5 ppm indicated a change in total respiratory resistance occurring at >2.5 ppm, although the effects were quite small. Von Nieding et al. (1973) noted a decrease in diffusing capacity (DLco) with a 15-min exposure to 5 ppm. Finally, no changes in lung mechanical function were found with exposure to 2 ppm for 2 h or for 2 h/day for 3 days (Mohsenin, 1988; Goings et al., 1989).

Exposure to <1 ppm NO_2 in conjunction with various degrees of exercise have also resulted in inconsistent effects on respiratory mechanics in healthy people; most of the studies showed no effects that could be unequivocally attributed to NO_2 (Folinsbee et al., 1978; Hackney et al., 1978; Kerr et al., 1979; Frampton et al., 1989a; Morrow and Utell, 1989). Reduced compliance was noted following exposure for 2 h at 0.5 ppm (Kulle, 1982), but the meaning of this with a lack of other lung mechanical changes was not clear. Linn et al. (1985a) found no change in resistance or spirometry with exposure at 4 ppm for 75 min.

Increased airway responsivity in healthy subjects has been noted following a 2-h exposure to 7.5 ppm (Beil and Ulmer, 1976), with 1 h to 2 ppm (Mohsenin, 1988) and with 3 h (with intermittent exercise) to 1.5 ppm (Frampton et al., 1989a). Again, however, the results are not consistent, with other studies at similar concentrations and exposure durations finding no change (e.g., Kulle and Clements, 1987). Exposures at <0.6 ppm have not produced any change in responsivity at all (Hazucha et al., 1983; Bylin et al., 1985; Frampton et al., 1989a; Morrow and Utell, 1989).

Particular subsegments of the population may be especially susceptible to the effects of NO_2. As noted in epidemiological studies, one such group is asthmatics. A number of studies have been performed with exposure levels ranging from 0.1 to 4 ppm for durations

ranging up to 4 h, usually with exercise; effects on various aspects of mechanical function, such as spirometry or airway resistance, have ranged from none to slight and all with much inconsistency (e.g., Kerr et al., 1979; Ahmed et al., 1982; Hazucha et al., 1982; Hazucha et al., 1983; Kleinman et al., 1983; Bylin et al., 1985; Koenig et al., 1985, 1987; Linn et al., 1985a; Bauer et al., 1986; Mohsenin, 1987; Avol et al., 1988; Morrow and Utell, 1989; Roger et al., 1990; Rubinstein et al., 1990; Morrow et al., 1992; Salome et al., 1996). A study that measured pulmonary function in adult asthmatics in their home and also monitored indoor NO_2 levels noted that average exposures to >0.3 ppm produced a decline in certain pulmonary function measures, but inconsistent effects were seen at lower exposure levels (Goldstein et al., 1988). Finally, when there is any response, it may only occur with exercise. Exposure to 0.3 ppm for 30 min produced no change in pulmonary mechanics indices in resting asthmatics, but effects were noted when exercise was incorporated into the exposure protocol (Bauer et al., 1986).

The most sensitive pulmonary mechanical response to NO_2 in people with airway disease appears to involve changes in airway responsiveness. However, there is variability in results from different studies and also an apparent lack of a dose–response relationship. While some studies have indicated increased responsiveness due to NO_2 exposures at 0.14–0.5 ppm (Kleinman et al., 1983; Bauer et al., 1986; Mohsenin, 1987; Bylin et al., 1988; Salome et al., 1996; Strand et al., 1996), others have indicated no such effects at similar levels (Orehek et al., 1981; Hazucha et al., 1983; Linn et al., 1986; Avol et al., 1988; Bylin et al., 1988; Roger et al., 1990), and exposure to a much higher level (3 ppm) has also produced no effect (Linn et al., 1986). Any NO_2-induced increased responsiveness may occur with a several-hour delay following exposure in asthmatics (Strand et al., 1996). Potentiation of cold-induced airway constriction and airway responsiveness to histamine in asthmatics was enhanced by exposure to 0.3 or 0.26 ppm, respectively (Bauer et al., 1986; Strand et al., 1996). Riedl et al. (2012) found an increase in respiratory symptoms in adult asthmatics during, but not after, exposure to 0.35 ppm for 2 h with alternating periods of exercise and methacholine challenge.

A meta-analysis of a number of studies involving both asthmatics and normals indicated that acute exposure to NO_2 would enhance responsiveness to various stimuli with exposure to at least 0.11 ppm in asthmatics, but at least 1 ppm in normals (Folinsbee, 1992). On the other hand, Goodman et al. (2009) using meta-analysis evaluated the effects of NO_2 exposure on airway responsiveness in subjects with asthma. They concluded that exposure was not associated with clinically relevant effects on airway responsivity at exposures up to 0.6 ppm.

While the mechanism of any NO_2-induced hyperresponsiveness is not known, it may involve alterations in the metabolism of endogenous bronchoconstrictors (Hoshi et al., 1996) or activation of specific cells within the airways (Ohashi et al., 1993). Mild asthmatics and normals were exposed to 1 ppm NO_2 (with intermittent exercise) for 3 h, followed by bronchopulmonary lavage 1-h post-exposure. While no change in differential cell counts was noted in either group, the asthmatics showed changes in lung eicosanoids not seen in normals, suggesting that NO_2 could activate cells compatible with airway inflammation (Jorres et al., 1995).

Even if asthmatics or allergic individuals may not show any enhanced response directly to NO_2, exposure may alter their response to antigens. A number of studies have examined response to NO_2 in terms of enhancement of the response to inhaled allergens in sensitized individuals. Humans having a history of allergic rhinitis were exposed to 0.4 ppm NO_2 for 6 h, followed by challenge with an allergen (Wang et al., 1995). There was some evidence

that NO_2 primed eosinophils for subsequent activation by the allergen. Acute exposure to 0.43 ppm NO_2 enhanced airway constriction in mild asthmatics in response to inhaled house dust mite antigen (Tunnicliffe et al., 1994). Similarly, airway constriction to pollen was enhanced in allergic asthmatics acutely exposed to 0.27 ppm NO_2 (Strand et al., 1997) and following repeated exposure at 0.27 ppm (Strand et al., 1998). Finally, allergic asthmatics were exposed in a roadway tunnel to NO_2 at a median level of 0.17 ppm, but ranging from0.11 to 0.25 ppm, for 30 min; subsequent inhalation of an allergen resulted in greater early asthmatic reaction and more symptoms during the later phase asthmatic response when compared with air control exposed individuals (Svartengren et al., 2000). However, one must realize that roadway exposure involves more than just NO_2, so effects may not have been due to NO_2 alone, or at all. NO_2-induced modulation of response to antigens may be due to recruitment of eosinophils (Barck et al., 2002). Thus, for example, subjects with allergic asthma were exposed to 0.27 ppm for 15 min on 1 day and for two 15-min intervals the next day; they were noted to have increased levels of eosinophil cationic protein, a component found in eosinophil granules, in both systemic blood and sputum when subsequently exposed to allergen.

Another possibly sensitive subsegment of the population is people with COPD, that is, chronic bronchitis and emphysema. Increased airway resistance has been found in individuals with COPD after exposure to 1.6 ppm in conjunction with exercise (von Nieding and Wagner, 1979), while a decrease in FVC was noted following exposure to 0.3 ppm for 4 h with intermittent exercise (Morrow and Utell, 1989), and a decrease in FEV_1 was noted following exposure for 1 h to 0.3 ppm (Vagaggini et al., 1996). On the contrary, no changes in airway resistance in chronic bronchitics exposed to 0.5 ppm for 2 h with exercise, or in spirometry of COPD patients exposed to 0.5–2 ppm for 1 h also with exercise, have been noted (Kerr et al., 1979; Linn et al., 1985b).

Thus, the database is currently not sufficiently robust to allow determination of the specific exposure conditions, that is, concentration, duration, and ventilation, for threshold effects on lung function in healthy humans with acute exposure. Lung mechanics may, in fact, not provide very sensitive indices of response in such people. On the contrary, functional changes may occur in individuals with asthma and/or COPD following exposure to lower levels of NO_2 than those affecting normals. Again, however, the results are inconsistent. Results of one study examining pulmonary functional indices with asthmatics have not been confirmed by a subsequent one, or responses of a particular subject group are not always reproducible (Orehek et al., 1976; Hazucha et al., 1983; Bauer et al., 1985; Bromberg, 1988). There is, however, some evidence that especially sensitive subgroup(s) may exist within the asthmatic population (Bauer et al., 1986; Morrow and Utell, 1989). That is, the variability in responses noted above may be the result of differences in the severity or type of asthma in the subjects examined within one study or between different studies. Asthmatics also exhibit a wide range of response to external stimuli, so some variability may merely be due to an interindividual variation in response to NO_2. The lowest concentration that does result in observed effects on airway responsivity in exercising asthmatics is in the 0.2–0.5 ppm range; in normals, levels of 5 ppm may cause bronchoconstriction, but minimum levels of at least 1–2 ppm are generally needed for changes in pulmonary functional parameters. Most mild asthmatics are not sensitive to NO_2 at less than or equal to 0.6 ppm, at least in terms of changes in respiratory mechanics, while nonspecific airway responsiveness in mild asthmatics may be increased at levels >0.1 ppm.

Controlled clinical studies have examined other aspects of pulmonary biology after exposure to NO_2. Humans exposed for 20 min to 1.5–3.5 ppm NO_2 did show a reduction of

mucociliary activity measured 45 min following exposure (Helleday et al., 1995). The effects, upon infectivity, of an attenuated influenza virus in healthy humans were assessed by Kulle and Clements (1987); NO_2 exposure levels were 1–3 ppm. There were no overall statistically significant changes in infectivity rates, although they were elevated in some of the NO_2-exposed groups. In another study (Goings et al., 1989), there was suggestive evidence that exposure for 2 h/day for 3 days to 1 or 2 ppm NO_2 increased susceptibility to respiratory viruses in healthy adults. Frampton et al. (1989b) examined the effect of NO_2 exposure *in vivo* on the ability of alveolar macrophages to inactivate influenza virus *in vitro*. Healthy humans were exposed either to 0.6 ppm for 3 h or to 0.05 ppm for 3 h with three15-min spikes to 2 ppm. There appeared to be less effective inactivation of the virus by macrophages harvested from the humans exposed to 0.6 ppm, but the results just missed statistical significance. No effects were noted in the individuals exposed to the lower concentration with the 2 ppm spikes. There also seemed to be a trend of increased production of interleukin-1 (IL-1) by macrophages from some individuals, namely, those whose cells tended to have reduced viral inactivation activity. Effects on IL-1 were also examined by Pinkston et al. (1988), with exposure of macrophages harvested by lavage to 5–15 ppm NO_2 for 3 h. No change in cell viability nor in release of IL-1 was noted. In any case, increased infectivity in NO_2-exposed laboratory animals together with the above suggestive findings in humans indicates that NO_2 may indeed alter host defense in humans. Healthy subjects exposed to 1–3 ppm NO_2 for 2 h/day for 3 days and then exposed to attenuated influenza virus showed a slight trend toward increased infectivity (Goings et al., 1989). However, exposure for 3.5 h to 0.6 ppm NO_2 resulted in a decreased inactivation of the influenza virus by alveolar macrophages (Frampton et al., 1989b).

Healthy subjects exposed to 0.6 ppm for 2 h on 4 days showed a small increase in the percentage of NK lymphocytes (Rubinstein et al., 1991), but repeated exposures to 1.5 or 4 ppm for 20 min every other day for a total of 6 days reduced numbers of both B-lymphocyte and NK lymphocyte and altered the ratio of $CD4^+/CD8^+$ cells (Sandström et al., 1992a, 1992b). Healthy subjects exposed to 1.92 ppm NO_2 for 4 h on 4 days showed upregulation of the expression of IL-5, IL-10, IL-13, and ICAM-1 (Pathmanathan et al., 2003); the effects on the interleukins suggest that repeated exposure may exert a proallergic effect on the airway epithelium, while the effect on ICAM suggests a mechanism for neutrophil influx into the epithelium during an inflammatory response. Riedl et al. (2012) did not find enhanced airway inflammatory response following exposure to 0.35 ppm for 2 h in asthmatic adults.

Some other biochemical effects of inhaled NO_2 have been examined in controlled clinical studies. *In vitro* exposure of human blood to high levels (>6 ppm) of NO_2 has been shown to result in production of methemoglobin (metHb) (Chiodi et al., 1983), but Chaney et al. (1981) found no such change in normal humans exposed for 2 h to 0.2 ppm. A reported elevation of GSH in these exposed subjects was not supported by the results of Posin et al. (1978), who found no effect following exposure to 1 ppm. The results of some of these studies are clouded by the lack of any consistent dose–response relationship.

Exposure to 4 ppm NO_2 for 20 min resulted in an inflammatory response in healthy individuals, as evidenced by changes in lymphocyte counts in lavage fluid obtained 4–24 h after exposure (Sandström et al., 1990). This is, however, not a consistent finding in humans (e.g., Mohsenin and Gee, 1987), possibly due to differences in experimental protocols, such as the times at which lavage was performed after exposure. Thus, exposure to 0.3 ppm for 1 h with exercise produced no acute inflammation in the proximal airways of normals, asthmatics, or people with COPD (Vagaggini et al., 1996). It should be noted that

exposures of laboratory animals to NO_2 at levels up to 8 ppm for up to 10 days did not produce evidence of acute inflammation (Gregory et al., 1983; Mochitate et al., 1986; Suzuki et al., 1986; Schlesinger et al., 1987). Perhaps NO_2 is not very effective in eliciting an inflammatory response at ambient levels with short-term exposure.

NO_2 exposure has been associated with development of emphysema in animal models. A component of the lungs' defense against proteolysis is alpha 1-protease inhibitor. Mohsenin and Gee (1987) noted a decrease in levels of this enzyme in the lavage fluid of subjects exposed to 3–4 ppm for 3 h. However, the investigators noted that the extent of the decrease was not associated with any increased risk of emphysema. On the contrary, exposure of normal humans for 3 h (with intermittent exercise) to 1.5 ppm, or for 3 h to0.05 ppm with three 2 ppm peaks, did not result in any change in activity of alpha 1-protease inhibitor in lavage fluid (Johnson et al., 1990). A 3-h exposure to 0.6 ppm resulted in an increase in levels of another antiprotease (alpha 2-macroglobulin) in lung lavage (Frampton et al., 1989c). In another study of potential lung damage, normal humans exposed to 0.6 ppm NO_2 for 4 h/day for 3 days showed no effect on the excretion of hydroxyproline, a marker for connective tissue injury (Muelenaer et al., 1987). Effects of repeated exposures, which would more likely to be involved in disease development, on these endpoints are unknown.

Recent controlled studies concentrated on effects of NO_2 on cardiovascular endpoints. Exposure to healthy adults to 0.5 ppm for 2 h with some exercise resulted in no change in circulating IL-8 or coagulation factors (Huang et al., 2012). Similarly, Langrish et al. (2010) noted no effect on the fibrinolytic pathway from exposure for 1 h with intermittent exercise of healthy adults to 4 ppm. Riedl et al. (2012) found no effect of exposure for 2 h with intermittent exercise of healthy adults and those with asthma to 0.35 ppm when examining various blood coagulation factors or IL-6. Channell et al. (2012) collected plasma immediately after and 24 h after exposure to 0.5 ppm for 2 h with intermittent exercise; the 24-h sample showed an increase in a protein that plays a role in the pathogenesis of atherosclerosis.

20.3.4 Health Effects of NO_2: Summary and Conclusions

A large database exists concerning biological responses resulting from the inhalation of NO_2. While there have been a significant number of epidemiology studies conducted over the past 25 years, there are fewer new toxicology studies aimed at assessing mechanisms of response to NO or NO_2. In any case, comparisons between animal studies, controlled human exposures, or epidemiologic studies are difficult, since the assays used in these different types of evaluations are not always directly comparable. One type of response index that has been examined in all of these studies is respiratory mechanics. However, changes in pulmonary function may not be very sensitive to NO_2 due to the tendency of such tests to reflect changes in the large airways, while the major targets for NO_2 are the smaller conducting airways and respiratory region. In any case, there is little evidence that exposure of normal humans or laboratory animals to <1 ppm NO_2 affects standard pulmonary mechanics responses. Even exposure to higher levels has resulted in inconsistent results. Airway responsiveness may be increased in normal human subjects, but generally only with exposures at >1 ppm NO_2. Epidemiological data suggest that there may be long-term effects of NO_2 on pulmonary function in children.

Asthmatics may represent a population subgroup showing susceptibility to NO_2. However, even among asthmatics, responses were not always consistent or reproducible,

and those that have occurred involved increased airway responsiveness rather than changes in standard respiratory function indices. Surprisingly, effects noted in some studies at <1 ppm NO_2 have not always been found with higher levels (up to 3 ppm), and this apparent lack of dose–response complicates any evaluation of the health significance of NO_2 exposure. While it is possible that differences in the degree of asthma severity in the subjects used in the various studies may have accounted for some of this discrepancy, it does seem that mild asthmatics are not generally sensitive to NO_2 concentrations <0.6 ppm when respiratory function and airway responsiveness are examined. The database for pulmonary function effects in COPD patients is also conflicting, with some studies showing effects and others none at NO_2 exposure levels <2 ppm. Nevertheless, while controlled clinical studies do not unequivocally indicate any enhanced susceptibility to NO_2 among mild asthmatics or people with COPD, there is indication that some asthmatics may respond at lower exposure concentrations than healthy individuals. Airway responsiveness in people with asthma can be increased by brief exposures to NO_2, generally in the range of 0.1–0.3 ppm depending upon the length of exposure, but exercise does not exacerbate the response following exposure.

Various biological responses not generally examined in humans have been assessed in animal models, and these indicate effects due to NO_2 that may have potential health significance. This includes NO_2-related alterations in various host defense parameters, such as mucociliary clearance, pulmonary macrophage and immunologic function, and susceptibility to respiratory infection. Tracheobronchial mucus transport rates remain unaltered by single exposures up to 10 ppm or short-term repeated exposures to <1 ppm, while respiratory region clearance may be affected by short-term repeated at <1 ppm. Morphological changes in macrophages begin to occur at 0.5 ppm, while functional activity has been affected by short-term repeated exposures to 0.3 ppm. As a likely consequence of altered defenses, animals may be less able to cope with respiratory infection. Nitrogen dioxide levels as low as 0.5 ppm will increase bacterial infectivity if exposure is prolonged. Clear, direct suppressive effects on humoral or cellular immunity have been noted only with exposure to >5 ppm NO_2, with lower levels possibly resulting in some depression or activation of immune system components. Limited results from controlled clinical studies suggest that some similar responses may be occurring in humans.

Additional evidence for human health effects resulting from NO_2 exposure comes from epidemiological examinations of acute respiratory illness, especially since this is supported by toxicological studies on host defense mechanisms and a limited number of controlled clinical studies. Those that provide relatively reliable estimates of NO_2, either by direct measure or suitable surrogate, are somewhat suggestive of increased risk or susceptibility to lower and/or upper respiratory tract infection in young children associated with long-term NO_2 exposure.

Animal models have been extensively used in studying effects of NO_2 on pulmonary morphology. The target site is consistently the area around the terminal/respiratory bronchiolar junction and associated alveoli, and the cells that are most sensitive are the ciliated cells of the bronchiolar epithelium and the type 1 cells of the alveolar epithelium. Neonates seem to be more resistant than adults to these morphological effects. Acute exposure to <5 ppm can produce hyperplasia and hypertrophy of bronchiolar and alveolar cells and proliferation of type 2 cells. Long-term exposure to 0.3–0.5 ppm can result in similar lesions, although chronic exposures to >2 ppm are generally needed to produce extensive and permanent pulmonary structural changes. Some changes may resolve even with continued exposure. Although the database does not currently allow for determination of the lowest

NO_2 level and shortest exposure duration that will produce clear and permanent morphological effects, the concentrations that seem to result in such changes are well above those currently found in most outdoor or indoor environments.

The primary target organ for NO_2 is the respiratory tract, but there may be some extrapulmonary effects of exposure as well. However, conclusions as to the possible health significance of these cannot, as yet, be made. Furthermore, there is no support for any teratogenic or genotoxic potential for NO_2, nor for any direct carcinogenic action. Although NO_2 may modulate pulmonary cancer originating elsewhere, the database is weak in this regard as well.

Quite a few questions regarding health effects from exposure to NO_2 remain unanswered. For example, there is no complete picture of the transition between acute and chronic effects, nor is the extent of reversibility of effects resolved, especially with short-term peak exposures. High concentrations of NO_2 (>5 ppm) are associated with structural and physiological changes in the respiratory tract. However, the extent to which these may occur at levels more relevant to either outdoor or indoor exposures is not clear. Furthermore, the relationship between biological responses and specific exposure patterns, that is, constant low-level versus low baseline plus higher spikes, is also not clear; the latter scenario may be more relevant to indoor exposure, the former to most outdoor exposures. The contribution of differing biochemical mechanisms, that is, acid versus oxidative, in the expression of NO_2 toxicity is not fully understood. Observations that the direction of change in various biological endpoints seems to depend upon exposure concentration may reflect differences in underlying mechanisms of action.

Thus, while toxicologic studies may provide indications of possible mechanisms of action leading to adverse short-term health effects, controlled clinical and epidemiologic studies have not as yet resulted in a consistent pattern of responses. The epidemiology studies often cannot separate effects of NO_2 from those due to co-pollutants released by the same source, and effects of NO_2 could very well be due to or modulated by one of more of these co-pollutants. Thus, determination of independent effects of NO_2 is difficult. However, what is emerging seems to be an independent effect from NO_2 primarily on hospital admissions or emergency room visits for cardiopulmonary disease or on lung functional development in children.

20.4 NITRIC OXIDE

20.4.1 Exposure

There are far fewer data on ambient levels of NO than for NO_2. Maximum hourly average NO concentrations can range from 0.17 to 1 ppm in metropolitan areas, while annual averages are in the range of 0.01–0.06 ppm (U.S. EPA, 2008). The overall 50th percentile concentration in the United States between 2011 and 2013 was 0.0029 ppm. Rural areas show maximum hourly averages of 0.01–0.4 ppm and annual averages of 0.005–0.009 ppm. Monitoring of various regions in Southern California indicated an 8-year (1994–2001) mean concentration range of 0.001–0.039 ppm. However, there is a wide variability in regional NO concentrations. For example, hourly average concentrations in California were found to range from 0 to 0.87 ppm during 1 year (1999), while maximum hourly average concentrations during the same time period were noted to range from 0.50 to 0.79 ppm in various areas in the Midwest to Northeast (Lazarus, 2001). NO levels near or on roadways tend to be higher than

in other areas, and can range from 0.095 to 0.361 ppm, depending on the type and extent of traffic, for 2-h averages of 1-min data (Fujita et al., 2011).

Indoor concentrations are not commonly measured and, thus, data are limited. While it seems that most of the NO found indoors derives from penetration from outdoor air (Lazarus, 2001), direct indoor emission can result from combustion appliances, such as natural gas-fired cooking stoves and gas or kerosene heaters. Indoor concentrations, thus, are strongly influenced by utilization of such devices. Indoor levels that ranged from 0.001 to 0.386 ppm were noted in one home in Southern California during a 1-year period (Weschler et al., 1994).

20.4.2 Dosimetry and Toxicology

The lower aqueous solubility of NO compared with NO_2 may result in greater amounts of the former reaching the respiratory region (Yoshida and Kasama, 1987). This NO will then diffuse rapidly, at a somewhat faster rate than would NO_2 (Chiodi and Mohler, 1985), through pulmonary tissue with little reaction, but it is not transported to any great extent through the vasculature due to its rapid interaction with oxyhemoglobin, as discussed further below. While the direct toxicity of NO is low, indirect toxicity can result from its reaction with superoxide to produce peroxynitrite, a potent oxidant.

NO entering the bloodstream binds to hemoglobin (Hb), producing nitrosylhemoglobin (NOHb). The affinity of Hb for NO is very high, much higher even than that for O_2. The NOHb formed is rapidly oxidized to MetHb in the presence of O_2. The MetHb is subsequently reduced into ferrous Hb by MetHb reductase, an enzyme present in red blood cells. In spite of the affinity for hemoglobin, *in vivo* exposures to NO at levels ranging from 2 to 10 ppm have shown that the amount of NOHb in blood was such that any reduction of O_2 transport was not lethal nor damaging to organs sensitive to O_2 depletion (e.g., Oda et al., 1976, 1980; Azoulay et al., 1977). Exposure of humans to 40 ppm for 2 h resulted in a small increase in MetHb in peripheral blood that was considered as not clinically significant (Luhr et al., 1998). Thus, it appears that as long as the activity of MetHb reductase is maintained, the conversion of NOHb to MetHb should mitigate any potential toxicity due to NO-related O_2 transport effects. However, it should be borne in mind that some groups, especially neonates, have less capacity than adults to remove MetHb from their circulation and may, thus, show greater effects at lower exposure concentrations.

NO is synthesized endogenously in the cells of many tissues from arginine and molecular oxygen via various nitric oxide synthase (NOS) enzymes. Human tissues contain three such enzymes: nNOS in neurons, iNOS in macrophages and eNOS in endothelial cells. Because NO diffuses freely across cell membranes and there are many molecules with which it can react, it is consumed very rapidly near the site of synthesis. Since endogenous NO is involved in numerous physiological processes, such as nervous system signaling, regulation of pulmonary and systemic vascular resistance, and mediation of immune defenses, the impact of inhaled, exogenous NO, especially at low concentrations, is often difficult to evaluate.

NO can react with thiol-associated iron in enzymes, which is a mechanism for cytotoxicity. It can also react with superoxide, producing peroxynitrite that can then react with proteins (Ischiropoulos et al., 1992). Many of these effects have been noted *in vitro* and offer potential explanations for effects of NO on host defenses. Whether they can explain any effects of NO inhalation exposure is not clear. There is, however, indication that, at least for some endpoints, effects of endogenous NO can be mimicked by exposure to

exogenous NO (Gustafsson, 1993). It has been suggested, for example, that the vasodilatory response of the bronchial and pulmonary vascular systems to cigarette smoke is due to NO in the smoke (Alving et al., 1992). Furthermore, individuals with depressed endogenous NO may be more sensitive to inhaled NO.

The specific substrates and reactions that mediate NO toxicity are not clear. Some studies indicate that the toxic effects of NO are different from the membrane damage due to NO_2. For example, NO may target fibroblasts that are responsible for the maintenance and repair of the alveolar interstitium (Mercer et al., 1995). Any respiratory tract morphologic responses to NO are similar to those found with NO_2, except that NO levels needed to produce them in most studies were higher, that is, greater or equal to 2 ppm with continuous exposure (Oda et al., 1976; Azoulay et al., 1977; Holt et al., 1979; Hugod, 1979; Oda et al., 1980). While little NO appears to react with lung tissue at exposure concentrations found in ambient outdoor or indoor air, with most diffusing into the blood, chronic exposure of rats to 0.5 ppm (with spikes to 1.5 ppm) produced interstitial lung damage (Mercer et al., 1995). While this may suggest that NO is more potent than NO_2 for certain types of morphological injury, there was found to be no structural change in the alveoli in rats exposed continuously to 2 or 6 ppm NO for 6 weeks (Mercer, 1999).

Studies of physiological effects of inhaled NO are sparse, and exposure levels used were quite high. Murphy (1964) found no change in pulmonary mechanical function of guinea pigs exposed for 4 h to NO at 16 or 50 ppm. Holt et al. (1979) examined immunological endpoints in mice exposed to 10 ppm NO for 2 h/day, 5 days/week up to 30 weeks. Leukocytosis was evident by 5 weeks of exposure, while a decrease in mean hemoglobin content of red blood cells was found by 30 weeks. The ability of spleen cells to mount a graft versus host reaction was stimulated by 20 weeks of exposure, but suppressed by 26 weeks. When the ability of mice to reject virus-induced tumors was assessed, less of the NO-exposed animals survived tumor challenge compared with control; this suggests that NO, at high levels, may have affected immunologic competence. In this regard, mice were exposed continuously to 2 ppm NO for 6 h up to 4 weeks to assess the effect on resistance to bacterial infection (Azoulay et al., 1981). There was some indication that NO-exposed females, but not males, showed a significant increase in mortality and a significant decrease in survival time. Exposure to 20 ppm NO for 2 h was noted to reduce the viability and production of superoxide by neutrophils (Daher et al., 1997). Finally, NO does not appear to be genotoxic (Görsdorf et al., 1990).

There are very few data on controlled exposures of NO in humans. Inhalation of 30 ppm for 0.5 h in normal subjects did not affect platelet function (Albert et al., 1999). On the contrary, exposure for 40 min at up to 40 ppm resulted in increased bleeding time at the highest concentration (Gries et al., 1997). Healthy males inhaling 50 ppm NO for 0.5 h showed no effect on systemic blood pressure or heart rate (Krejcy et al., 1995). Following a 2-h exposure to 1 ppm NO (Kagawa, 1982), there appeared to be some variability in pulmonary function response among subjects, but only one of a large battery of tests showed statistical significance; it is likely that this effect, if not due to chance, has little biological significance. On the contrary, vasomotor tone is a sensitive target for NO, and pulmonary vasodilation has been noted with acute exposure to 5–10 ppm in normal animals and humans (Gustafsson, 1993). Normal adults and those with airway hyperresponsivity, asthma, or COPD were exposed to 80 ppm NO for 10 min with measurements of specific airway conductance and functional residual capacity (Hogman et al., 1993). Normal subjects and those with hyperactive airways showed no effect, while asthmatics actually showed some improvement in conductance with exposure. The interpretation of responses

to exogenous NO is complicated by the presence, as noted, of endogenous NO, which can act as a bronchodilator.

In summary, a large fraction of inhaled NO reaches the respiratory region of the lungs, where it rapidly diffuses into blood and reacts with hemoglobin; little NO directly interacts with lung tissue, especially at ambient concentrations. In spite of any binding with hemoglobin, anoxia of O_2-sensitive organs does not seem to occur, at least with NO exposure levels <10 ppm.

20.5 NITRIC/NITROUS ACID

20.5.1 Exposure

There are few data for ambient HNO_3 levels, and those that are available suggest much variability. Levels in various cities in mid- to Southern California were found to range from 0.075 to 5.6 mg/m^3 (0.003–0.22 ppm), averaging about 0.07 ppm (Munger et al., 1990; Fischer et al., 2003); concentrations in a relatively urban area in Southern California were noted to range as high as 0.5 ppm. Indoor levels of HNO_3 have been reported to range up to 0.001 ppm (Brauer et al., 1991). Indoor levels of HNO_2 can reach 0.1 ppm when gas stoves and unvented kerosene heaters are used (Beckett et al., 1995), while outdoor (short-term) levels of 0.007–0.016 ppm have been reported in Southern California (Winer and Biermann, 1991).

20.5.2 Dosimetry and Toxicology

The dosimetry of inhaled HNO_3 is unknown. However, because of its very high water solubility and vapor state, inhaled HNO_3 should undergo significant removal in the upper respiratory tract. It has also been suggested that inhaled vapor-phase HNO_3 may be converted into or deposited onto small particles within the humid atmosphere of the respiratory tract, thus facilitating its transport to and deposition within the deep lung. By contrast, HNO_3 inhaled in fog droplets would deposit in large airways.

The database concerning potential health effects from short-term exposure to HNO_3 is limited. In one study, both normal and allergic sheep were exposed for 4 h to 1.6 ppm (42.7 μg/m^3) HNO_3 vapor (Abraham et al., 1982). A decrease in specific pulmonary flow resistance, compared with pre-exposure control values, in both groups of sheep was noted. However, allergic sheep showed increased airway responsiveness, both immediately and 24 h after HNO_3 exposure. Although there was no significant change in responsiveness in the normal group as a whole, two of the animals showed an increase in responsiveness to bronchoconstrictor challenge (carbachol) after HNO_3 exposure. According to the investigators, this suggested that some individuals in a normal population may be more sensitive than others.

Allergic adolescent asthmatic human subjects were exposed for 40 min during rest and moderate exercise to 0.05 ppm (1.3 μg/m^3) HNO_3. An increase in total respiratory resistance and a decrease in forced expiratory volume were noted (Koenig et al., 1989). In another report, Koenig (1989) examined exercising adolescent asthmatics exposed to 0.057 (1.5 μg/m^3) ppm HNO_3 vapor for 45 min. Small, but not statistically significant, decreases in forced expiratory volume and expiratory flow rates were found. Particulate HNO_3 has also been found to enhance bronchoconstriction in humans produced by exposure to hypoosmolar aerosols (Balmes et al., 1988).

Aris et al. (1991) measured pulmonary functional parameters (specific airway resistance, SRaw, FEV_1, FVC) and lavage indices (total and differential cell counts, LDH, fibronectin, total protein) in healthy subjects exposed for 4 h (including moderated exercise) to 500 μg/m^3 HNO_3 vapor. Lavage was performed 18-h post-exposure. No HNO_3-related effects on any of the measured endpoints were found. Healthy adults exposed to 500 μg/m^3 HNO_3 for 4 h with exercise showed no change in measures of pulmonary mechanics (CARB, 1996).

Heat shock proteins (HSP) have been correlated with environmental stress and pathophysiological conditions. Stress-induced HSP 70 in rat lungs was examined following inhalation exposure 4 h/day, 3 days/week for 40 weeks to 50 μg/m^3 HNO_3 (Wong et al., 1996). HNO_3 was found to elevate lung stress-inducible HSP above baseline control levels.

Schlesinger et al. (1994) exposed rabbits for 4 h/day, 3 days/week for 4 weeks to HNO_3 vapor at 0, 50, 150, and 450 μg/m^3. Exposure was followed by assays of biochemical markers in lavage fluid, pulmonary macrophage function, and *in vitro* bronchial responsivity to smooth muscle constrictor challenge. HNO_3 had no effect either on viability or numbers of cells recovered or on lactate dehydrogenase or total protein in lavage. All acid concentrations reduced both basal levels and stimulated production of superoxide anion by macrophages, while the release/activity of tumor necrosis factor by stimulated macrophages was reduced following exposure to >150 μg/m^3 HNO_3. Bronchi from rabbits exposed to >150 μg/m^3 HNO_3 exhibited reduced smooth muscle responsivity *in vitro* compared with control.

HNO_3-induced alterations in both conducting and respiratory airways were also noted by Mautz et al. (1993), who observed changes in breathing pattern, alveolar macrophage receptor binding capacity, and alveolar morphometry in rats exposed to 50, 170, and 470 μg/m^3 HNO_3 for 4 h/day, 3 days/week for 4 weeks. Further evidence for penetration of HNO_3 into the deep lung was provided by Nadziejko et al. (1992), who noted reduced production of superoxide anion by macrophages harvested from rats exposed to 250 μg/m^3 for 4 h/day for 4 days. Similar to results of Schlesinger et al. (1994), Nadziejko et al. (1992) found no effects of acid exposure either on total numbers of cells recovered by lavage, on differential counts, or in total soluble protein in lavage fluid.

Healthy adults exposed, with some exercise, to HNO_2 for 3.5 h at 77 and 395 ppb showed a decrease in specific airway conductance compared with air exposure (Rasmussen et al., 1995). Mildly asthmatic adults exposed to 650 ppb HNO_2 for 3 h with exercise periods showed a decrease in FVC, which began during the exposure period. Respiratory symptoms indicative of irritation were also associated with the acid exposure (Beckett et al., 1995).

20.6 INORGANIC NITRATES

There are limited data on ambient levels of particulate inorganic nitrates (NO_3^-). Maximum (24-h average) ambient concentrations are generally well below 10 μg/m^3, although certain regions having persistent smog, for example, Southern California, may show peaks between 20 and 35 mg/m^3 (Shaw et al., 1982; Ellestad and Knapp, 1988; Pierson and Brachaczek, 1988). The annual averages for fine particulate nitrate over an 8-year period (1994–2001) in a number of rural areas in California were noted to range from around 1 to 5 μg/m^3, while 8-year averages over a number of rural, suburban, and urban areas ranged from 0.76 to 11.5 μg/m^3 (Peters, 2004). In the United States, nitrates generally account for 1–5% or 8–15% of the total $PM_{2.5}$ mass in the eastern or western parts of the country, respectively (U.S. EPA, 1993), although in parts of California it may account for up to 40% (Peters, 2004).

The toxicologic database supporting any health effects from inhaled nitrates is sparse. Anesthetized dogs exposed to sodium nitrate ($NaNO_3$) at up to 10,000 μg/m^3 for 7.5 min showed no effect on pulmonary function endpoints, while exposure for 4 h to 5000 μg/m^3 produced no alterations in pulmonary function, pulmonary or systemic arterial blood pressures, cardiac output, or heart rate (Sackner et al., 1979). Conscious sheet similarly exposed for 4 h to 5000 μg/m^3 demonstrated no alteration in tracheal mucous velocity (Sackner et al., 1979). Both normal rats and guinea pigs or those with elastase-induced emphysema were exposed to 1000 mg/m^3 ammonium nitrate for 6 h/day, 5 days/week for 5 or 20 days; the guinea pigs showed no exposure-related effect on lung volumes or lung compliance, while rats showed only minor changes in pulmonary function and there was no additional effect related to the emphysema state (Loscutoff et al., 1985). Furthermore, morphological analysis of those animals exposed for 20 days showed no effect of exposure.

Normal mice and those sensitized to ovalbumin were exposed to ammonium nitrate at either 140 μg/m^3 (0.58 μm) or 250 mg/m^3 (0.22 μm) for 4 h/day for 3 days (Cassee et al., 1998). There were no effects on protein or lactate dehydrogenase levels in lung lavage fluid, but the level of *N*-acetylglucosaminidase was increased in the sensitized mice exposed to the smaller particles. Mice in both groups exposed to the larger particles showed increased airway responsiveness, suggesting that ammonium nitrate did not exacerbate airway responsiveness differentially in atopic animals.

In a similar study (Cassee et al., 1999), normal rats and those treated with monocrotaline exposed to ammonium nitrate for 4 h/day for 3 days at 418 μg/m^3 (0.087 μm) or 361 μg/m^3 (0.643 μm) showed no effect on various enzymes in lavage, but did show an increased number and severity of lesions in the lungs that were determined to be due to a background bacterial infection, suggesting some effect on resistance to infection. Ehrlich (1979) examined the effect of 3-h exposures to various nitrate salts (1290–4500 μg/m^3) on resistance to respiratory bacterial infection in mice. Only zinc nitrate ($Zn(NO_3)_2$) resulted in any significant mortality increase, the extent of which seemed to be exposure concentration related. However, since the response was similar to that seen with zinc sulfate ($ZnSO_4$), the effect was likely due to the zinc ion (Zn^2) rather than to the NO_3^-. Charles and Menzel (1975) examined the effects of nitrate upon the release of histamine by guinea pig lung fragments; response to some pollutants may be a function of their ability to elicit biologic mediators. Histamine was released in proportion to the concentration of salt present. However, the response was not totally due to NO_3^-; ammonium (NH_4^+) ion was also a possible contributor. The relation of this to actual *in vivo* exposures is, however, not clear. Other *in vitro* studies suggest that NO_3^- may affect red blood cells by altering the transport of calcium across the cell membrane (Kunimoto et al., 1984).

Some controlled clinical studies have been conducted with NO_3^- aerosols (Sackner et al., 1979; Utell et al., 1979, 1980; Kleinman et al., 1980; Stacy et al., 1983). Concentrations ranged from 200 to 7000 μg/m^3, and pulmonary function was the endpoint. The only effects noted were decreases in airway conductance and partial expiratory flow volume curves in subjects with influenza exposed for 16 min to 7000 μg/m^3 of $NaNO_3$ aerosol (Utell et al., 1980). This was not seen in normals or asthmatics (Utell et al., 1979).

The concentrations used in most studies with nitrates are well above ambient levels. The results suggest that there are likely to be no adverse effects, as far as cardiopulmonary function is concerned, from current levels of NO_3 aerosols, even in presumably more sensitive asthmatics. However, some potentially sensitive measures of cardiopulmonary function, such as heart rate variability, have not been assessed in controlled studies with nitrate particles, and these measures have been shown to be altered by exposure to various types of ambient PM (Schlesinger et al., 2006).

20.7 SUMMARY AND CONCLUSIONS

This chapter presents a wealth of findings on the associations of exposures to nitrogen oxides and on short- and long-term health-related effects in human populations. It also summarizes observed associations between controlled exposures to humans and laboratory animals and short-term responses to NO and NO_2 alone, usually at much higher concentrations than those in ambient air. It is abundantly clear that there are many statistically significant associations between both short-and long-term exposures to NO_x components in the ambient air and health-related responses in human populations. What is not at all clear are the individual and/or the combined contributions of NO, NO_2, and other nitrogen oxides as causal factors for the effects and whether the manifestation of effects requires the co-presence of other ambient air pollutants.

WHO-EURO (2013) concluded that it is possible that NO_2 has no direct effect itself but is, instead, only acting as a marker for primary PM, such as ultrafine PM, and such PM constituents as metals, PAHs or other OC carried on PM to particular locations in the lung. NO_2 could also act as a marker for CO or NO near roads or, as it is a secondary as well as primary pollutant, for regional pollutants such as O_3. Whether this is the case or whether NO_2 has a direct effect is a crucially important policy question. The implementation of filter traps in diesel vehicles to meet the Euro 5 emission standards and lowering sulfur in fuel, coupled with the fact that NO_2 levels have not been reduced in real-life driving conditions in the EU, may have led to important increases in the NO_2/ultrafine particle, NO_2/BC, and NO_2/EC plus OC ratios. For example, at a London roadside site, the ratio of NO_x as NO_2 ($\mu g/m^3$) to particle number (N/cm^3) changed more than twofold over years 2008–2010 (Jones et al., 2001). Comparison of data from the ESCAPE Project in Europe, suggests that the traffic–urban background contrast for NO_2 increased more over time than for $PM_{2.5}$ absorbance (by about 10%). The rural–urban background contrasts showed a change of less than 10%. The changes in ratios are likely to be specific to location, site type, and time period and could have been better defined if there had been more widespread and robust measurements of the relevant PM metrics over time. Given the change in these ratios, now and in the future, and between and within cities, there are clearly policies and behaviors that are changing NO_2 independently of other traffic pollution constituents. Unfortunately, there are no means in observational studies to fully test the hypothesis of a direct effect of NO_2. Adjustment of NO_2 associations for PM_{10} or $PM_{2.5}$ may not be sufficient, as there is often a closer correlation between NO_2 and traffic pollutants, such as primary PM and its constituents. Correlations with regional pollutants, such as O_3 and secondary particles, are usually not as close.

Much more research is needed to determine the roles of individual chemical species within the ambient pollutant mixture in the observed human health impacts of NO and NO_2. Some still evidence does point to specific effects of NO and NO_2, the two major NO_x components. Some examples include the following:

1. Crouse et al. (2015) indicated that the significant associations of NO_2 with excess cardiovascular, ischemic heart disease, and respiratory mortality in Canadian cities were due more to within-city than intercity variations, suggesting that increased levels of NO_2 within the cities resulted in a more toxic mixture.
2. Kaufman et al. (2004), reported that while mean NO_2 in six U.S. communities was correlated with other motor vehicle-related pollutants, implicating motor vehicle-derived concentrations of $PM_{2.5}$, NO_2, NO, and soot over 10 years for six U.S. communities, there were significant associations of coronary artery calcium progression

for $PM_{2.5}$ and NO_x, but not for soot, while the association for NO_2 was just short of being significant. These findings provided support for the hypothesis that traffic-related pollutants warrant continued, and perhaps more stringent, emission limits, but suggest that the exposure metric that could provide the strongest associations in future epidemiological studies is NO, rather than BC, NO_2, or proximity. The ratio of NO to NO_2 is greater at street level than further downwind of the roadway; thus, this ratio could account for the utility of proximity for studies without the capacity for multi-pollutant concentration measurements. The known ability of NO as an O_3 scavenger may also be of interest.

3. Fischer et al. (2014) used existing Dutch national databases on mortality, individual characteristics, residence history, neighborhood characteristics, and national air pollution maps to establish a cohort of 7.1 million individuals ≥30 years of age and followed them for 7 years. After adjustment for individual and area-specific confounders, for each 10 μg/m^3 (5.3 ppb) increase, PM_{10} and NO_2 were associated with total non-accidental, respiratory, and lung cancer mortality. Furthermore, PM_{10} was associated with circulatory disease mortality, while NO_2 was not. PM associations were robust to adjustment for NO; NO associations remained for non-accidental mortality and lung cancer mortality after adjustment for PM.

In looking at the overall body of evidence related to health effects from NO_x, the following conclusions may be made:

- There appears to be a causal relationship between short-term exposure and various respiratory system effects, especially exacerbation of asthma symptoms and overall exacerbation of the disease in asthmatics resulting in visits to the ED. Any relationship between long-term exposure and respiratory system effects is less certain, but the database suggests the potential for such relationships, which may include development of asthma in children.
- WHO-EURO (2013) emphasized that it is difficult to extrapolate quantitatively from animal toxicological studies. Modeling (with its many assumptions) suggests about a 10-fold higher local NO_2 concentration in the bronchi of humans than in those of rats, at the same concentration in the air that is being inhaled, and vice versa (much lower) in the alveoli. Several of the respiratory effects in animals occur in the bronchi, and it is plausible that some of the epidemiological results in human beings (such as asthma admissions) are primarily the result of effects on the bronchi. There may, therefore, be some evidence that supports application of the 10-fold safety factors used in traditional toxicology for extrapolation from animals (mainly rats) to human beings. This, together with the distribution of personal exposures and the wide range of susceptibility in the human population, suggests that the epidemiological findings are not necessarily incompatible with the toxicological evidence on NO_2 itself. There are too few studies that examine NO_2 and PM from defined sources in the same experimental system to directly compare the toxicological importance of these two pollutants, and their relative toxicological importance may vary by biological endpoint.
- The apparent mismatch between the time-series evidence and the lack of apparent responses in the chamber studies at background concentrations may be a consequence of only a small proportion of the population responding at particular times, an effect that could be picked up only in the much larger samples used in time-series studies.

Specifically, more sensitive groups, such as severe asthmatics, are not studied in chamber studies because of the risks involved. Thus, the lack of robust effects and/or the lack of evidence at lower doses in chamber studies is insufficient to rule out the reported associations with NO_2 in the time-series studies found at the concentrations present in the wider environment. Considering the presence of more sensitive subgroups in the population together with the higher concentrations at microenvironments (such as the curbsides described above) could explain some of the apparent mismatch.

- There is suggestive evidence for associations between both short- and long-term exposures and various effects in the cardiovascular system. However, the epidemiological studies in this chapter have not adequately accounted for potential confounding by co-pollutants, and the toxicology and controlled clinical study database is not, as yet, sufficiently robust to permit conclusions on the consistency of the biological mechanisms underlying causality in humans and laboratory animals. Similarly, while there is suggestive evidence for a role of long-term exposure with total mortality and birth defect outcomes, the same caveats apply.

Thus, the database does support that people with asthma, children, and older adults appear to be at increased risk for NO_x-related health effects. The evidence is strongest for respiratory system effects, especially for short-term exposures, and perhaps for long-term exposures as well. As noted in Health Canada (2016), adverse health effects associated with NO_2 have been observed in epidemiological studies in countries at NO_2 concentrations well below existing ambient air quality standards. For those health outcomes for which the weight of evidence and statistical power are greatest (i.e., mortality, respiratory/asthma hospitalizations and asthma-related ED visits for short-term ambient NO_2 exposure, and respiratory morbidity for long-term ambient NO_2 exposure), the mean or median ambient levels at which effects are observed overlap those measured at remote site types, ranging from non-urban to transportation- and potentially industrial source-influenced sites. Therefore, adverse health effects in epidemiological studies are occurring at ambient NO_2 concentrations that are commonly experienced in Canada.

Since the epidemiological effects that have been associated with NO_2 and /or NO_x concentrations in ambient air have exhibited non-threshold relationships, it is clear that reducing NO_x emissions will reduce public health impacts. Still, it is not clear how the inhalation of NO_x in ambient air contributes to the various adverse short- and long-term health effects that have been associated with elevations in the ambient air concentrations of NO_2 and or NO_x. Thus, it is important to determine their individual contributions to help guide the development of NO_x ambient air quality standards and guidelines.

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21

OZONE

Morton Lippmann

21.1 INTRODUCTION

21.1.1 Historical Overview of Ozone as a Component of Ambient Air and a Marker for the Photochemical Air Pollution Standard

In 1851, soon after its initial laboratory synthesis, Schonbein recognized ozone (O_3) as a powerful lung irritant (see Bates, 1989). O_3 is the strongest, but not the only oxidant in the ambient air. It has been used as the indicator chemical for the National Ambient Air Quality Standard (NAAQS) for photochemical oxidants, one of six pollutants for which the U.S. Environmental Protection Agency (EPA) sets a NAAQS to protect public health with an adequate margin of safety, as required by the U.S. Clean Air Act (CAA). The current O_3-based NAAQS, 70 ppb averaged over 8 h, was promulgated in October 2015 (U.S. EPA, 2015), relying, in part, on the U.S. EPA's Integrated Science Assessment (U.S. EPA, 2013).

O_3 is almost entirely a secondary air pollutant, formed in the atmosphere through a complex photochemical reaction sequence requiring reactive hydrocarbons, nitrogen dioxide (NO_2), and sunlight. Reduction of ambient O_3 concentrations requires reducing the emissions of hydrocarbon vapors, NO_2, or both. Both nitric oxide (NO) and NO_2, known collectively as NO_x, are primary pollutants resulting from fixation of atmospheric nitrogen and oxygen during high-temperature fuel combustion. In the atmosphere, NO is gradually converted to NO_2. Motor vehicles, which are the largest sources of hydrocarbons and NO_x, have been the target of control efforts, and major reductions (>90%) have been achieved in hydrocarbon emissions per vehicle-mile. Reductions in NO_x from motor vehicles and stationary source combustion have been somewhat smaller. However, while vehicle-miles driven have continued to increase, there has been a substantial net reduction in O_3 exposure in the United States, with the greatest reductions in areas with more stringent controls, such as the northeastern states and California, as indicated in Fig. 21.1.

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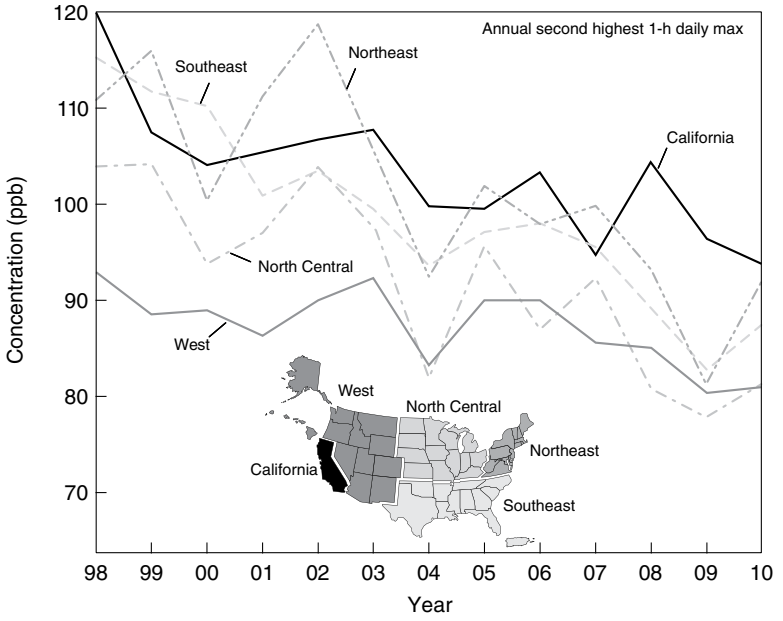

FIGURE 21.1 Trend in mean 8-h daily max O_3 by region, 1998–2010 (mean of annual fourth highest 8-h daily max O_3 in ppm). *Source*: From U.S. EPA (2013).

A great deal is known about O_3 chemistry and EPA has developed highly sophisticated O_3 air quality models (U.S. EPA, 2013). We also know a great deal about some of the health effects of photochemical oxidant inhalation. However, much of what we know best has been limited to transient, apparently reversible effects of O_3 on respiratory tract responses in healthy young adults that follow acute inhalation in laboratory exposures lasting from 5 min to 6.6 h. These effects include changes in lung capacity, flow resistance, epithelial permeability, neutrophilic airway inflammation, and hyperreactivity to bronchoactive challenges; such effects can be observed within the first few hours after the start of the exposure and may persist for many hours or days after the exposure ceases. Repetitive daily exposures over several days or weeks can exacerbate and prolong these transient effects. There has been a great deal of controversy about the health significance of such effects and whether such effects are sufficiently adverse to serve as a basis for the NAAQS indexed by O_3 (Lippmann, 1988, 1991, 1993).

Decrements in respiratory function such as forced vital capacity (FVC) and forced expiratory volume in the first second of a vital capacity maneuver (FEV_1) fall into the category in which adversity begins at some specific level of pollutant-associated change. However, there are clear differences of opinion on what the threshold of adversity ought to be. The 2006 O_3 Staff Paper (U.S. EPA, 2006) focused the discussion of thresholds for adversity on persons with impaired respiratory symptoms, as well as on healthy people, because NAAQS are generally set to protect sensitive subgroups of the population. The gradations are presented in Tables 21.1 and 21.2 for healthy persons and persons with impaired respiratory symptoms, respectively.

With respect to adversity, the 1996 EPA Staff Paper (U.S. EPA, 2006) concluded that pulmonary responses designated as large or severe were clearly adverse. For responses listed as moderate, it was concluded that they could be considered adverse if there were repetitive exposures.

TABLE 21.1 (a–c) Gradation of Individual Responses to Short-Term Ozone Exposure in Healthy Persons

Functional Response	None	Small	Moderate	Large
(a)				
FEV_1	Within normal range (±3%)	Decrements of 3–≤10%	Decrements of >10% but <20%	Decrements of ≥20%
Nonspecific bronchial responsiveness	Within normal range	Increases of <0%	Increases of ≤300%	Increases of >300%
Duration of response	None	<4 h	>4 but ≤24 h	>24 h
(b)				
Symptomatic response	Normal	Mild	Moderate	Severe
Cough	Infrequent cough	Cough with deep breath	Frequent spontaneous cough	Persistent uncontrollable cough
Chest pain	None	Discomfort just noticeable on exercise or deep breath	Marked discomfort on exercise deep	Severe discomfort on exercise or deep breath
Duration of response	None	<4 h	>4 but ≤24 h	>24 h
(c)				
Impact of various functional and/or symptomatic responses	Normal functional and/or symptomatic responses	Small functional and/or mild symptomatic responses	Moderate functional and/or symptomatic responses	Large functional and/or severe symptomatic responses
Interference with normal activity	None	None	A few sensitive individuals likely to limit activity	Many sensitive individuals likely to limit activity

Source: U.S. EPA (1996b).

Recent short-term inhalation exposure studies have measured both pulmonary and cardiovascular functional responses to short-term inhalation exposures to O_3 at 70 and 120 ppb of healthy elderly volunteers for 3 h who were engaged to an experimental protocol with intermittent mild exercise (Frampton et al., 2017). These recent studies, and their findings, which will be discussed in some detail later in this chapter, did not find pulmonary effects that differed much from those for healthy younger adults, nor did they find significant cardiovascular effects of the exposures.

Although we know a great deal about the transient pulmonary effects following single exposures to O_3 resulting from controlled laboratory exposures and short-term responses in populations associated with peak ambient air concentrations, our current knowledge about the chronic health effects of O_3 inhalation is much less complete. What we know about the chronic effects derives from epidemiological studies of populations with both short- and long-term exposures to O_3 in ambient air. Such ambient air mixtures also contain other

TABLE 21.2 (a–c) Gradation of Individual Responses to Short-Term Ozone Exposure in Persons with Impaired Respiratory Systems

Functional Response	None	Small	Moderate	Large
(a)				
FEV_1 change	Decrements of <3%	Decrements of 3–≤10%	Decrements of >10% but <20%	Decrements of ≥20%
Nonspecific bronchial responsiveness	Within normal range	Increases of <100%	Increases of ≤300%	Increases of >300%
Airway resistance (SR_{aw})	Within normal range (±20%)	SR_{aw} increased <100%	SR_{aw} increased up to 200% or up to 15 cm H_2O/s	SR_{aw} increased >200% or more than 15 cm H_2O/s
Duration of response	None	<4 h	>4 but ≤24 h	>24 h
(b)				
Symptomatic response	Normal	Mild	Moderate	Severe
Wheeze	None	With otherwise normal breathing	With shortness of breath	Persistent with shortness of breath
Cough	Infrequent cough	Cough with deep breath	Frequent spontaneous cough	Persistent uncontrollable cough
Chest pain	None	Discomfort just noticeable on exercise or deep breath	Marked discomfort on exercise or deep breath	Severe discomfort on exercise or deep breath
Duration of response	None	<4 h	>4 but ≤24 h	>24 h
(c)				
Impact of various functional and/ or symptomatic responses	Normal functional and/or symptomatic responses	Small functional and/or mild symptomatic responses	Moderate functional and/or symptomatic responses	Large functional and/or severe symptomatic responses
Interference with normal activity	None	Few individuals likely to limit activity	Many individuals likely to limit activity	Most individuals likely to limit activity
Medical treatment/ self-medication	No change	Normal medication as needed	Increased frequency or additional medication use	Increased likelihood of physician or ER visit

Source: U.S. EPA (1996b).

strong oxidants, such as peroxides, as well as weaker oxidants, such as nitrogen oxides; strong acids, such as nitric acid vapor (HNO_3) and sulfuric acid droplets (H_2SO_4); and organic vapor irritants. As discussed in later part of this chapter, the known chronic effects associated with such exposures include alterations in lung function or structure. Such effects may result from cumulative damage and/or from the side effects of adaptive responses to repetitive daily or intermittent exposures to both O_3 and the other irritants in the ambient air mixture. This chapter is limited to a critical review of the health effects data and their significance to public health in relation to the populations exposed. The judgments made herein are my own and have been influenced by my participation in public CASAC reviews of EPA documents. In cases, they differ from those of the EPA and of other members of the CASAC panels.

21.2 BACKGROUND ON EXPOSURES AND HEALTH-RELATED EFFECTS

21.2.1 Sources and Distribution of O_3 in Ambient Air

Some of the O_3 in ambient air is attributable to sources other than fuel combustion and photochemistry, such as the intrusion of stratospheric O_3, especially in the spring when the stratospheric–tropospheric air exchange is greatest. The background concentration of methane has been rising over the last hundred years as a result of increasingly intensive agriculture and animal husbandry. The coincident increase in continental background O_3, from 10 to 20 ppb (Altshuller, 1987) to the current O_3 background level near 40 ppb, may be due to the rising background of both methane and NO_x. However, the largest source of ambient air O_3 is photochemistry that is driven by the complex reaction sequences requiring input of organic vapors, NO_x, and actinic radiation. Reactive organic vapors such as olefinic hydrocarbons, formaldehyde, and *m*-xylene, which are largely products of anthropogenic activities, are highly efficient contributors to O_3 formation. NO_x concentrations have grown as fossil fuel usage has increased. The increase in NO_x may also account for a greater rate of O_3 formation by photochemical reactions with isoprene and terpenes emitted by trees. The role of NO_x in tropospheric O_3 formation is especially critical. Unless NO_x concentrations exceed about 0.02–0.03 ppb, photochemical O_3 loss exceeds photochemical O_3 production. In some remote regions of the troposphere, the NO_x concentrations may be below such values.

The peak concentrations of O_3 reached during a specific day at a specific location are determined largely by the background level in the air aloft, the photochemical production rate during the day, and the concentration of O_3 scavenging chemicals such as NO and ethylene. It depends on the ambient ratio of reactive organic gases (ROG) to NO_x concentrations. When [ROG]/[NO_x] is approximately 5–6, the two species have about an equal chance of reacting with hydroxy radical ($OH^{\cdot}$). When this ratio is larger than 5, there is a shortage of NO that can be oxidized to NO_2, and O_3 production is then controlled by the amount of NO_x available. In this region, decreasing NO_x leads to a decrease in the peak O_3. On the other hand, when [ROG]/[NO_x] is on the order of 5 or less, the ready availability of NO_x makes O_3 formation dependent on ROG. NO scavenges O_3 faster than it reacts with RO_2, and NO_2 reacts with OH to form nitric acid. Decreasing NO_x can lead to an increase in peak O_3 as the efficiency of O_3 formation increases.

The daily formation of O_3, in the absence of a substantial background level from the air aloft or upwind, leads to a relatively sharp daily peak in concentration, with a major part of the effective exposure taking place over a relatively few hours. In heavily populated

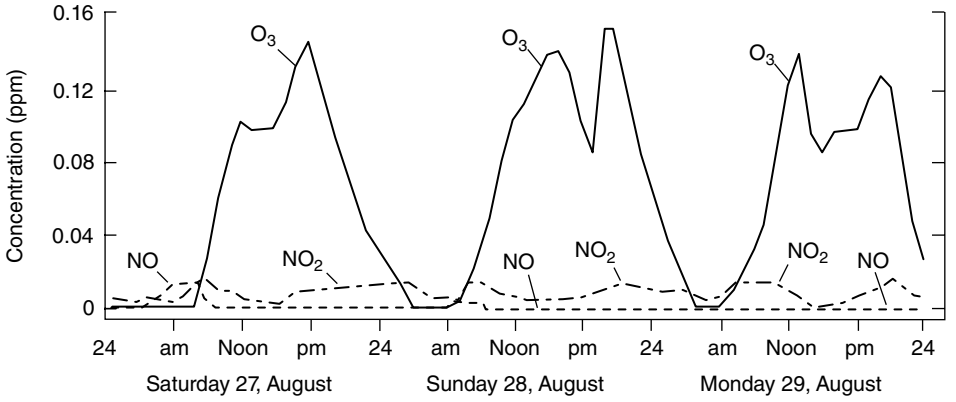

FIGURE 21.2 Three-day sequence of hourly O_3 concentrations at Montague, MA, SURE station showing locally generated midday peaks and long-range transport late peaks. *Source*: From U.S. EPA (1986).

regions, such as the Eastern United States and Western Europe, a typical daily plateau of exposure occurs after 10 a.m., and the maximum 8-h exposure is ~90% of the maximum 1-h exposure (Rombout et al., 1986). Relatively little of the exposure on a typical high exposure summer day is attributable to local sources or is amenable to local source control. Rather, the local generation of O_3 represents a bump on a broad daily hump arising from a series of upwind sources and photochemistry. The size of the bump depends on the concentration of precursor reactants in the incoming air and the local increments of reactants. The broad humps can be attributed to the sum of the contributions of stratospheric O_3 injections and O_3 formed upwind and retained aloft for one or many days.

Since O_3 is highly reactive with ground level surfaces, it drops markedly in the evening. On the other hand, it can remain at elevated concentrations in the ambient air above the mixing layer. This elevated reservoir of O_3 can then contribute to elevated ground level O_3 on the following day as air mixing increases. This contributes to multiday summer episode exposures. The likelihood of O_3 >80 ppb continuing for 3 days or longer, once it was in existence for 1 day, is high in the Northeastern United States (Rao, 1988).

The nature of contemporary O_3 exposure is illustrated for a rural area of western Massachusetts in Fig. 21.2, showing both locally generated midday peaks and late afternoon peaks from upwind population centers superimposed on a broad daily plateau (Lioy and Dyba, 1989). It clearly illustrates that the O_3 exposure problem affects broad areas of the country and is not only an urban problem.

21.2.2 Ozone Exposures and Dosimetry

For O_3, the only significant exposure route is inhalation, with the exposure being the concentration at the nose and mouth. It is generally assumed that the concentrations that we breathe are the same as those measured at central monitoring sites, but this assumption has limited validity. For outdoor exposures, local concentrations are reduced in the vicinity of heavy vehicular traffic due to O_3 scavenging by NO. By contrast, less trafficked areas downwind of the monitor may have a higher O_3 concentration because of the enrichment of the air mass with motor vehicle exhaust precursor chemicals and active photochemistry.

Thus, outdoor O_3 concentrations can be either substantially higher or lower than those measured at fixed monitoring sites.

Indoor O_3 concentrations are usually much lower than those outdoors because of (1) efficient scavenging by indoor surfaces and (2) the lack of indoor sources. The only common indoor O_3 sources are copying machines and electrostatic air cleaners. Since most people spend more than 80% of their time indoors, their O_3 exposures are much lower than estimates based on outdoor concentrations. Weschler (2006) estimated that daily inhalation intake of O_3 while indoors can be from 25 to 60% of total daily O_3 intake and that O_3-initiated chemical reactions are responsible for exposures to irritating O_3 reaction products, such as aldehydes, hydroperoxides, and ultrafine particles.

Ambient O_3 concentration is only one determinant of O_3 dose. Dose is also determined by the inhaled volumes and by the pattern of uptake of O_3 molecules along the respiratory tract. When people work or exercise outdoors, and thereby increase their ventilation rate, the contribution of outdoor O_3 exposure to total O_3 dose becomes even greater, and the dose to target tissues in the respiratory acini, the region from the terminal bronchioles through the alveolar ducts, increases even more with exercise than total respiratory tract dose, since O_3 penetration increases with increased tidal volume and flow rate.

Gerrity et al. (1988) measured the efficiency of O_3 removal from inspired air by the extrathoracic and intrathoracic airways in healthy, nonsmoking, young male volunteers. Removal efficiencies were measured at O_3 concentrations of 100, 200, and 400 ppb for nose only, mouth only, and oronasal breathing at 12 and 24 breaths/min. The mean extrathoracic removal efficiency for all measurements was $39.6 \pm 0.7\%$, and the mean intrathoracic removal efficiency was $91.0 \pm 0.5\%$. The effects of concentration, breathing frequency, and mode of breathing on removal efficiency were relatively small. On inspiration, the O_3 uptakes by structures between the mouth and each location were $17.6 \pm 3.7\%$ (SE), $27.1 \pm 2.4\%$, $35.5 \pm 3.0\%$, and $32.5 \pm 3.1\%$ for above the vocal cords, upper trachea, conductive airways, and respiratory airways, respectively.

Rigas et al. (2000) studied the O_3 uptake within the human respiratory tract during tidal breathing. Adults inhaled 200 or 400 ppb O_3 while exercising at 20 L/min for 60 min or 40 L/min for 30 min. Absorption ranged from 5.6 to 9.8%, with an intersubject variability of ~10%. Asplund et al. (1996) used a continuous O_3 exposure followed by an O_3 bolus, and Rigas and Ben-JebriaUltman (1997) used O_3 boli following continuous NO_2 and SO_2 exposures. With continuous O_3 exposure, the absorbed fraction decreased, suggesting that biochemical substances on the airways were being depleted, while with continuous NO_2 and SO_2 exposures, the absorbed fraction increased, suggesting that the NO_2 and SO_2 exposures were increasing the availability of the biochemical substances that absorb O_3.

The tissues within the respiratory acini of humans, rabbits, guinea pigs, and rats receive the highest local doses from inhaled O_3 (Miller et al., 1978b; Overton and Miller, 1987; Hatch et al., 1989). The dose in humans is about twice that in rats for the same exposure (Gerrity and Wiester, 1987), with children having somewhat greater doses than adults (Overton and Graham, 1989), consistent with the greater effects of O_3 on lung function seen in humans than in rats (Costa et al., 1989).

21.2.3 Populations of Concern for Health Effects

In general, the NAAQS have been established to protect against adverse health effects in the most sensitive subpopulations, such as cardiovascular patients, who were of paramount concern in establishing the NAAQS for carbon monoxide (CO). Asthmatics were of special

concern in establishing the sulfur dioxide (SO_2) NAAQS because the concentrations required to produce comparable levels of bronchoconstriction were about an order of magnitude lower than for normal people and because of the potential for a disabling or fatal bronchospasm being initiated by a transient high concentration of SO_2.

In the case of O_3, no special functional responsiveness has yet been clearly demonstrated among the potentially more sensitive groups with preexisting disease (Lippmann, 1989, 1993). Thus, healthy people who exercise regularly outdoors were the primary population of concern on the basis of their higher O_3 exposures and doses. The 2006 EPA Ozone Staff Paper (U.S. EPA, 2006) also identified people with asthma as a population of concern on the basis of reports of symptomatic responses, increased rates of visits to clinics, and hospital admissions at very low ambient concentrations.

21.2.4 Health-Related Responses of Concern

O_3 in ambient air has been associated with a variety of transient effects on the respiratory airways. These include dose-related decrements in indices of forced expiratory flow capacity, which are reproducible in individuals, but highly variable within populations. Increased rates of symptoms, clinic visits, hospital admissions, and excess daily mortality are other responses of concern with respect to peak short-term exposures.

More persistent physiological decrements associated with structural alterations of lung airways could be adverse effects when they occur in humans as a result of repetitive O_3 exposures. Such effects have been produced in laboratory animals following chronic exposures, but human evidence is currently limited.

21.3 EFFECTS OF SHORT-TERM EXPOSURES TO OZONE IN HUMANS

Historically, there was a very large, and perhaps excessive, focus on the health effects of a single day's maximum hourly exposure to ambient O_3. The 1971 and 1979 NAAQS for photochemical oxidants were based on the maximum 1-h concentrations as the most relevant index of exposure, and most of the earlier clinical research involved either 1- or 2- h exposures. However, effects can be produced with exposures as short as 5 min, and effects can become progressively larger as exposures at a given concentration are extended out to 6.6 h (Folinsbee et al., 1988, 1994; Horstman et al., 1990). The results of these longer exposures in chambers provided key data leading to the NAAQS adoption of an 8-h averaging time in 1997. This section examines the complex of effects that result from single exposures of <8- h duration. The effects that successive days of exposure or long-term chronic exposures produce are discussed later in this chapter.

21.3.1 Respiratory Mechanical Function Responses

21.3.1.1 One- and Two-Hour Chamber Exposure Studies There have been many more studies on respiratory function responses than on any of the other functional responses to short-term O_3 inhalation.

It is well established that the inhalation of O_3 causes concentration-dependent mean decrements in exhaled volumes and flow rates during forced expiratory maneuvers and that the mean decrements increase with increasing depth of breathing (Hazucha, 1987). There are wide ranges of reproducible responsiveness among healthy adults (McDonnell et al.,

1985a; Weinmann et al., 1995; Frampton et al., 1997, 2017), and functional responsiveness to O_3 is no greater, and usually lower, among cigarette smokers (Shephard et al., 1983; Kagawa, 1984; Frampton et al., 1997), older adults (Drechsler-Parks et al., 1987; Reisenauer et al., 1988; McDonnell et al., 1993, 1995), asthmatics (Linn et al., 1980; Koenig et al., 1987), and patients with chronic obstructive pulmonary disease (COPD) (Solic et al., 1982; Linn et al., 1983). An exception is that patients with allergic rhinitis had greater changes in airway resistance (McDonnell et al., 1987). Emmons and Foster (1991) reported reduced responsiveness to 400 ppb O_3 for 2 h among asymptomatic cigarette smokers before and after 2 h of O_3 at 400 ppb with light exercise: (1) before they stopped smoking and (2) again after 6 months of not smoking. None were responsive to O_3 exposure before smoking cessation. During smoking cessation, their mean baseline $FEF_{25–75}$ was raised from 3.02 to 4.08 L/s. For the subjects who were re-exposed to O_3 6 months later, the exposure reduced their mean $FEF_{25–75}$ from 3.86 to 2.99 L/s. The subjects with the greatest improvement in $FEF_{25–75}$ after withdrawal had the largest acute decrements after O_3 exposure.

Weinmann et al. (1995) showed that O_3-induced changes in $FEF_{25–75}$ were unexplained by and followed a different time course than O_3-induced changes in FVC, indicating that intrinsic narrowing of the small airways may be a significant indicator of the functional response.

While the results of some laboratory studies have indicated that responses in young females were greater than those in young males (Messineo and Adams, 1990), the largest study of both males and females did not find gender-related differences in responsiveness to O_3 among either African-American or Caucasian adults (Seal et al., 1993).

The effects of O_3 on respiratory function accumulate over more than 1 h in chamber exposures to O_3 in purified air for 2 h with the volunteer subjects engaged in vigorous intermittent exercise. Significant function decrements after 2 h of exposure were not present after 1 h (McDonnell et al., 1983; Kulle et al., 1985).

21.3.1.2 Field Studies Spektor et al. (1988a) noted that children at summer camps with active outdoor recreation programs had greater decrements in lung function than children exposed to O_3 at comparable concentrations in chambers for 1 or 2 h (McDonnell et al., 1983; Kulle et al., 1985). Furthermore, the activity levels of the children, although not measured, were considerably lower than those exposed in the chamber studies while performing more vigorous exercise. Since functional responses to O_3 increase with levels of physical activity and ventilation (Hazucha, 1987), the greater responses in the camp children had to be caused by other factors, such as greater cumulative exposure, or by the potentiation of the response to O_3 by other pollutants in the ambient air. Cumulative daily exposures to O_3 were greater for the camp children, who were exposed all day than for a 1- or 2-h period preceded and followed by clean air exposure.

Kinney et al. (1988) and Hoek et al. (1993) studied schoolchildren. In the Kinney et al. study in Kingston and Harriman, TN, lung function was measured in school on up to six occasions during a 2-month period in the late winter and early spring. Child-specific regressions of function versus maximum 1-h O_3 during the previous day indicated significant associations between O_3 and decreased lung function, with coefficients similar to those seen in the summer camp studies of Lippmann et al. (1983), Spektor et al. (1988a, 1991), Higgins et al. (1990), and Hoek et al. (1993). Since children in school have relatively low activity levels, the relatively high response coefficients may be related to co-exposure to other atmospheric pollutants or to a low level of seasonal acclimation to repeated daily exposures. As shown by Spengler et al. (1989), Kingston and Harriman had higher annual

average and higher peak acid aerosol concentrations than other cities studied, that is, Steubenville, OH; St. Louis, MO; and Portage, WI. Alternatively, the relatively high response coefficients could have been caused by the fact that the measurements were made in the late winter and early spring. Linn et al. (1988) provided evidence for a seasonal adaptation, and children studied during the summer may not be as responsive as children measured earlier in the year. The Linn et al. study is discussed further under Section 21.9.

In a study of children with moderate to severe asthma at a summer camp in the Connecticut River Valley (Thurston et al., 1997), decrements in peak expiratory flow rates (PEFR) associated with ambient O_3 concentrations were similar in magnitude to those reported by the same group of investigators for healthy children at other Northeastern U.S. summer camps (Spektor et al., 1988a, 1991). However, the level of physical activity of the asthmatic children and hence their O_3 intake was much lower. Thus, the level of health concern for such comparable functional decrements is greater.

Other studies of the effects of O_3 on lung function in children in natural settings reported O_3-related functional decrements. Braun-Fahrländer et al. (1994) found O_3-related reductions in PEFR among 9–11-year-old Swiss children following 10 min of heavy exercise at peak O_3 concentrations below 80 ppb. Neas et al. (1995) reported O_3-related reductions in PEFR between morning and evening in fourth and fifth grade children in Uniontown, PA, in relation to 12-h av. O_3 below 88 ppb. Castillejos et al. (1995) studied the change in lung function following exercise out of doors for 7–11-year-old children in Mexico City repeatedly exposed to high ambient levels of O_3 and PM. They had O_3-related decrements in FVC, $FEV_{1.0}$, $FEF_{25–75}$, and $FEV_{1.0}$/FVC when peak 1-h O_3 exceeded 150 ppb.

Spektor et al. (1988b) performed field studies of functional responses of young adults engaged in recreational activities outdoors for about one-half hour per day in an area with regional summer containing varying levels of O_3. They made pre- and post-exercise respiratory function measurements whose magnitudes per unit of ambient O_3 concentration were similar to those observed in volunteers exposed while exercising vigorously for either 1 or 2 h in controlled chamber exposure studies. Functional decrements in proportion to relatively low ambient O_3 concentrations were also reported for joggers in Houston, TX (Selwyn et al., 1985), competitive cyclists in the Netherlands (Brunekreef et al., 1994), hikers on Mount Washington in NH (Korrick et al., 1998), and agricultural workers in British Columbia (Brauer et al., 1996).

21.3.1.3 Prolonged Daily Exposures in Chambers The observations from the field studies in the children's camps stimulated the EPA Clinical Studies Laboratory in Chapel Hill, NC, to undertake a chamber exposure study of 10 adult male volunteers involving 6.6 h of O_3 exposure at 120 ppb (Folinsbee et al., 1988). Moderate exercise was performed for 50 min/h for 3 h in the morning and again in the afternoon. The functional decrements become progressively greater after each hour of exposure, reaching average values of ~400 mL for FVC and −540 mL for FEV_1 by the end of the exposure day, but no residual functional decrements on the following day. The decrements in FEV_1 after 6.6 h of exposure at 120 ppb averaged 13.6% and were comparable to those seen previously in the same laboratory on similar subjects following 2 h of intermittent heavier exercise (68 L inhaled per minute for a total exercise time of 60 min) at an interpolated concentration of ~220 ppb. Assuming ventilation at 10 L/min between exercise periods, the total amount of O_3 inhaled during 2 h of intermittent heavy exercise at 220 ppb (430 $\mu g/m^3$) would have been [60 min × 0.068 m^3/min + 60 min × 0.010 m^3/min] × 430 $\mu g/m^3$ = 2.01 mg O_3. The corresponding amount of O_3 inhaled during 6.6 h of intermittent moderate exercise at 120 ppb [300 min × 0.040 m^3/min

+ 100 min × 0.010 m^3/min] × 235 μg/m^3 = 3.06 mg O_3. Thus, the effect accumulated with time, but with a temporal decay of effect going on at the same time. Follow-up studies by Horstman et al. (1990) were done on 21 adult males with 6.6-h exposures at 80, 100, and 120 ppb. The exposures at 120 ppb produced very similar responses, that is, a mean FEV_1 decline of 12.3%, whereas those at 80 and 100 ppb showed lesser changes that also became progressively greater after each hour of exposure (Fig. 21.3).

A further follow-up study using the same exposure protocol on 38 additional healthy young men was done by McDonnell et al. (1991) at 80 ppb. The mean FEV_1 decline of 8.4% was similar to that seen by Horstman et al. (1990) at that concentration.

The time scale for effective O_3 dose in relation to functional response was explored further by Hazucha et al. (1992) in exposures of healthy young adults lasting 8 h, with 30 min of exercise (at 40 L/min) at the beginning of each hour. The O_3 concentration rose from 0 to 240 ppb over the first 4 h and dropped back to 0 over the second 4 h. The functional responses were compared to both sham exposures and constant 120 ppb exposures in the same subjects. By 4 h, the FEV_1 changes from both O_3 exposures were similar, and the largest decrement in FEV_1, which occurred after 6 h of exposure, was about twice that after 5–8 h of constant exposure at 120 ppb. The peak response faded by the end of 8 h and was not significantly greater than that produced by the constant 120 ppb exposure at the 8 h.

Another study examined the integral effects of temporally varying exposures with the same integral exposure was performed by McKittrick and Adams (1995). Aerobically trained young adult men were exposed while exercising at 60 L/min to either 1 h at 300 ppb O_3 followed by 1 h of clean air: ½ h at 300 and 0 ppb or ¼ h intermittent at 300 and 0 ppb. The FEV_1 decrements at the end of O_3 exposure were essentially the same, that is, −17.6, −17.0, and −17.9%.

Larsen et al. (1991) modeled the data of Horstman et al. (1990) using multiple linear regression on the mean responses at each hour for all three concentrations, but excluding those with FEV_1 decreases of less than 0.5%. With O_3 concentration and duration of exposure as the only two independent variables, the model explained 95% of the variance

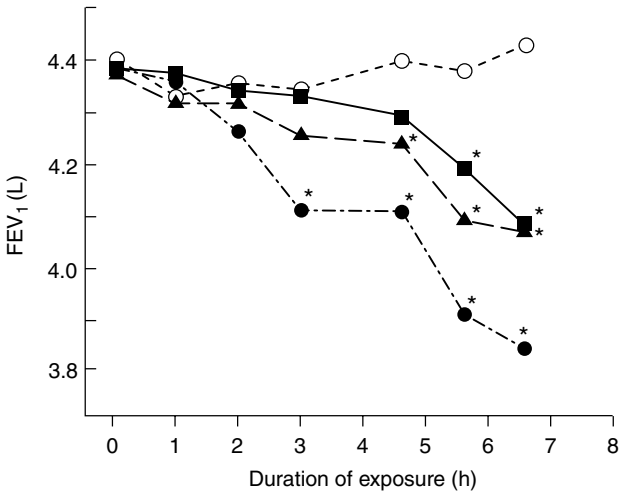

FIGURE 21.3 Mean FEV_1 after each 50 min of exercise during exposure to O_3 at 0 (open circles), 80 ppb (squares), 100 ppb (triangles), and 120 ppb (solid circles). Asterisks indicate significant reduction in FEV_1 from corresponding values at 0 ppb. *Source*: From Horstman et al. (1990).

of the dependent variable *Z*, a Gaussian transform of the percentage decrease in FEV_1. In this model, the exponent of the exposure duration is 0.754. This further demonstrates that exposure time is almost equally important to exposure concentration in cumulative response when concentrations are in the range of normal peak ambient levels.

Further evidence of the time scale for the biological integration of O_3 exposure can be deduced from the rate at which the effects dissipate were produced by Folinsbee and Hazucha (1989) in a study in which young adult females were exposed to 350 ppb O_3 for 70 min, including two 30- min periods of treadmill exercise at 40 L/min. Their mean decrement in FEV_1 at the end of the exposure was 21%. After 18 h, their mean decrement was 4%, whereas at 42 h, it was 2%.

There was large interindividual variability of O_3-induced functional responses, illustrated in Fig. 21.4, and functional responses in individuals did not correlate well with the other responses, as discussed below. Using the large EPA database, McDonnell et al. (1993) found that O_3 concentration explained 31% of the variance in FEV_1 responses and subject age explained another 4%. The modeled influence of age is illustrated in Fig. 21.5.

Upon further modeling of this large data set, McDonnell et al. (1997) reported that a sigmoid-shaped model was consistent with previous observations of O_3 exposure–response (E-R) characteristics and could accurately predict the mean response with independent data. They did not find that response was more sensitive to changes in C than in V_E, nor did they find convincing evidence of an effect of body size upon response, but response to O_3 decreased with age.

In recent studies named the Multicenter Ozone Study in oldEr Subjects (MOSES) of both respiratory and cardiovascular responses to short-term O_3 inhalations that were sponsored by the Health Effects Institute (HEI), there was coordination with three research centers, led by John Balmes at the University of California–San Francisco (UCSF), Philip

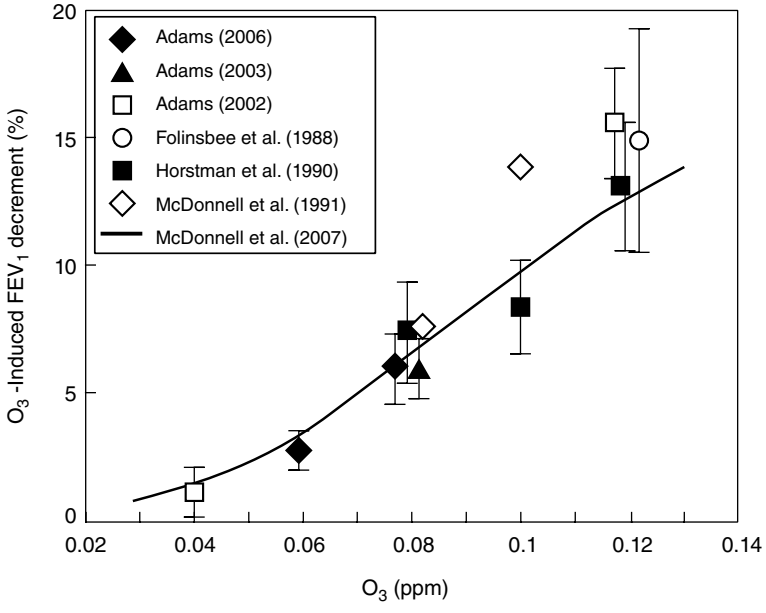

FIGURE 21.4 Comparison of mean O_3-induced FEV_1 decrements due to 6.6- h exposures with mild exercise in the various studies cited in the inset.

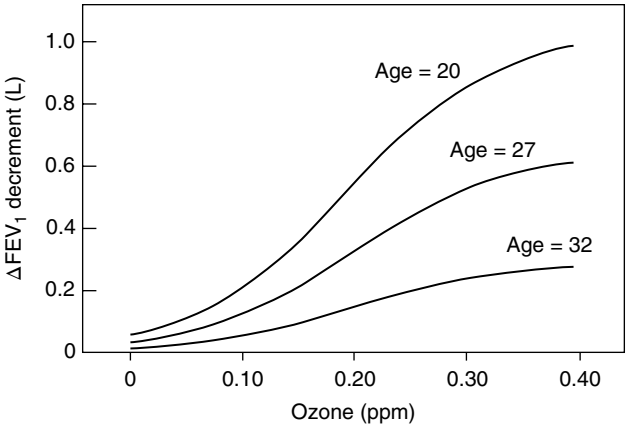

FIGURE 21.5 Predicted mean decrements in forced expiratory volume in $L(\Delta FEV_1)$ following 2- h exposures to ozone while undergoing heavy intermittent exercise for three ages. (Note: To convert ΔFEV_1 to $\%\Delta FEV_1$, multiply by 22.2%.). *Source*: From McDonnell et al. (1993).

Bromberg at the University of North Carolina–Chapel Hill (UNC), and Mark Frampton at the University of Rochester Medical Center (URMC) in Rochester (Frampton et al., 2017). They studied the effects of short-term O_3 exposure on the respiratory and cardiovascular systems. It focused on relatively low O_3 concentrations (70 and 120 parts per billion [ppb]), relevant to those observed in ambient air in the United States. Each of the three MOSES teams recruited and tested about 30 participants, resulting in a total of 87. Exposures took place from mid-2012 to mid-2015. The studies involved three exposure sessions (randomized at 0, 70, and 120 ppb O_3) that lasted 3 h, during which the participants exercised on a stationary bicycle, alternating 15 min of exercise and rest. For these healthy older adults, short-term exposures at these O_3 concentrations did produce moderate pulmonary effects, with results similar to those found in previous studies in younger adults.

The MOSES study (Frampton et al., 2017) confirmed O_3 effects on the respiratory system quite low concentrations in older volunteers. Moderate exercise during clean air exposure (0 ppb) led to an increased FVC and FEV_1 at 15 min after exposure compared with pre-exposure, and they remained significantly higher after 22 h. These improvements in lung function were attenuated after O_3 exposure in a dose–response manner at 70 and 120 ppb. The respiratory effects observed after O_3 exposure were consistent with the results of other studies finding such effects at current ambient O_3 concentrations. However, because the subjects were all healthy elderly volunteers, the results may not be generalizable to the overall adult population.

We need more investigation of the mechanisms underlying the pulmonary responses to inhaled O_3. One mechanism was summarized by Bates (1989, 1995), who noted that after O_3 exposure, the inspiratory capacity is initially reduced on taking a full inspiration due to a lower maximal negative intrapleural pressure, while maximal inspiratory and expiratory mouth pressures are not affected. Also, the FEV_1/FVC ratio is not initially affected after O_3 exposure, that is, the FVC and FEV_1 initially fall together. He postulated that stimulation of the C-fiber system in the lung must lead to a "braking" effect on the inspiratory muscles as a first consequence of O_3 exposure, and this probably occurred as a result of induced inflammation. The increased respiratory rate after O_3 exposure, the increased lung permeability, the increased airway reactivity, and the fact that β-blockers do not prevent the

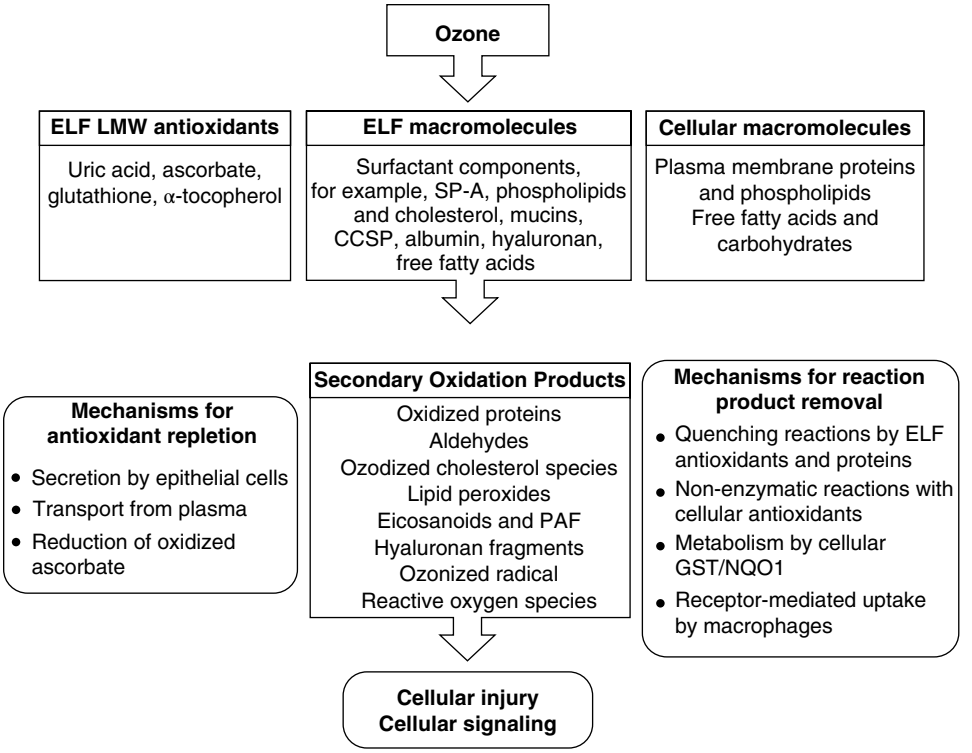

FIGURE 21.6 Ozone interactions with airway fluids to form secondary oxidation products. *Source*: From U.S. EPA (2015).

changes induced by O_3 all support this hypothesis. Frampton et al. (2017) outlined mechanisms related to the formation of secondary oxidation products formed when O_3 deposits on and reacts with cells and liquids on the respiratory tract airways as indicated in Fig. 21.6.

21.3.2 Effects on Athletic Performance

It has been five decades since epidemiological evidence suggested that the percentage of high school track team members failing to improve performance increased with increasing oxidant concentrations the hour before a race (Wayne et al., 1967). The effects may have been related to increased airway resistance or to associated discomfort, which may have limited motivation to run at maximal levels. Controlled exposure studies of heavily exercising competitive runners have demonstrated decreased function at 200–300 ppb (Savin and Adams, 1979; Adams and Schelegle, 1983). At 210 ppb O_3, Folinsbee et al. (1984) reported symptoms as well in seven distance cyclists exercising heavily ($V_E = 81$ L/min).

Some studies demonstrated reduced performance at lower O_3 concentrations by Schelegle and Adams (1986) and Kim et al. (2011) who exposed young male adult endurance athletes to 120, 180, and 240 ppb O_3 while the exercised at a mean minute volume of 54 L/min for 30 min, followed by a mean of 120 L/min for an additional 30 min. Although they all completed the protocol for filtered air (FA) exposure, some of them could not complete it for the 120-, 180-, and 240- ppb exposures. Linder et al. (1988) also found that maximum performance time was reduced for their 16–28- min progressive maximum

exercise for V_E of 30–120 L/min in young adults when O_3 was present. For example, performance was reduced 11% in females exposed to 130 ppb O_3.

21.3.3 Cardiovascular and Nervous System Function Responses

Gong et al. (1998) hypothesized that O_3 exposure could acutely affect cardiovascular hemodynamics in humans and, in particular, in subjects with essential hypertension. They studied 10 non-medicated hypertensive and 6 healthy male adults. Each subject, after catheterization of the right heart and a radial artery, was exposed to FA on one day and to 300 ppb O_3 on the following day for 3 h with intermittent exercise. Relative to FA exposure, O_3 exposure induced no statistically significant changes in cardiac index, ventricular performance, pulmonary artery pressure, pulmonary and systemic vascular resistances, ECG, serum cardiac enzymes, plasma catecholamines and atrial natriuretic factor, or SaO_2. While the overall results did not indicate major acute cardiovascular effects of O_3 in either the hypertensive or the control subjects, mean pre- to post-exposure changes were significantly ($p < 0.02$) larger with O_3 than with FA for rate-pressure product (1,353 beats/min/mmHg) and for HR (8 beats/min); these responses were not significantly different between the hypertensive and the control subjects. Significant O_3 effects were observed for mean FEV_1 (−6%) and an O_2 partial pressure (>10 mmHg increase), which were not significantly different between the two groups, suggesting that O_3 exposure can increase myocardial work and impair pulmonary gas exchange to a degree that might be clinically important in persons with significant preexisting cardiovascular impairment, with or without concomitant lung disease.

Urch et al. (2005) exposed healthy nonsmoking adults for 2 h to a mixture of 120 ppb O_3 and concentrated ambient $PM_{2.5}$ at an average concentration of 147 μg/m^3. There was an exposure-associated increase in diastolic blood pressure (BP).

Park et al. (2005) studied the associations of heart rate variability (HRV) with ambient air pollutants in the Boston, MA, area in adult men. Decreases in HRV were associated with O_3 and $PM_{2.5}$ and were stronger in men with ischemic heart disease (IHD) and hypertension.

Sarnat et al. (2006) studied the association between ambient air pollutants in a panel of older adults in Steubenville, OH, and ventricular arrhythmias and reported that increased levels of ambient O_3 and sulfate (SO_4^{2-}) may increase the risk of supraventricular arrhythmia in the elderly.

Rich et al. (2006) studied a panel of cardiac patients in the Boston area having implanted cardioverter defibrillators (ICDs) for the associations between air pollutants and defibrillator discharges. They found that concentration was significantly associated with the risk of episodes of rapid ventricular response due to paroxysmal atrial fibrillation.

Jia et al. (2011) measured the effects of very brief (5- min) exposures to O_3 on high-frequency (HF) and low-frequency (LF) HRV on 20 elderly subjects in Beijing in both summer and winter. After adjusting for co-exposure to $PM_{2.5}$ and NO_2, the greatest effect was a decrease in HF, that is, −4.9% per 10 ppb O_3 (CI: −8.6 to −1.0%).

In more recent controlled exposure studies that included cardiovascular endpoints (Devlin et al., 2012; Arjomandi et al., 2015) in young healthy subjects, at O_3 concentrations sufficient to elicit changes in pulmonary function (100 ppb for 4 h and 300 ppb for 2 h, respectively, with intermittent exercise), effects were found for frequency-domain HRV in terms of decreases in the HF power band (0.15–0.40 Hz) and in increased systemic inflammation. However, Barath et al. (2013) found no effects on HRV of 300 ppb O_3 for 75 min in 36 healthy men undergoing intermittent exercise. Frampton et al. (2015) found no effects

of 3- h exposures to 100 or 200 ppb O_3, with intermittent exercise, on measures of systemic and pulmonary vascular function, impedance cardiography, blood MPs, or blood platelet activation. Thus, clinical studies findings on the cardiovascular effects of O_3 have been inconsistent, and they were limited to studies on short-term responses.

The MOSES study investigators (Frampton et al., 2017) measured changes in heart rhythm, BP, and markers of endothelial function, thrombosis, lung injury, and both systemic and lung inflammation. Some cardiovascular endpoints were considered to be of primary interest; all other endpoints were considered to be secondary. Most of these outcomes were assessed at HEI-designated central laboratories that handled samples or electrocardiographic recordings from all three clinical centers to standardize outcome assessment. Study participants were also genotyped for glutathione S-transferase mu 1 (GSTM1), a gene involved in antioxidant defenses. Individuals who lack the GSTM1 gene may be at increased risk for acute health effects. The investigators measured each participant's exposure to O_3 and NO_2 using a personal sampler for 72 h before the pre-exposure visit. They also collected air quality data for O_3, $PM_{2.5}$, NO_2, SO_2, and carbon monoxide (CO) from central monitors closest to each clinical center.

21.3.4 Symptomatic Responses

Respiratory symptoms have been closely associated with group mean pulmonary function changes in adults acutely exposed in controlled exposures to O_3 and in ambient air containing O_3 as the predominant pollutant. However, Hayes et al. (1987) found only a weak to moderate correlation between FEV_1 changes and symptoms severity when the analysis is conducted using individual data.

In controlled 2- h O_3 exposures, McDonnell et al. (1983) reported that some heavily exercising adult subjects experienced cough, shortness of breath, and pain on deep inspiration at 120 ppb O_3, although the group mean response was statistically significant for cough only. Above 120 ppb O_3, respiratory and non-respiratory symptoms have included throat dryness, chest tightness, substernal pain, cough, wheeze, pain on deep inspiration, shortness of breath, dyspnea, lassitude, malaise, headache, and nausea.

The prolonged exposure studies involving 6.6 h of exposure at concentrations between 80 and 120 ppb also produced significant increases in cough and pain on deep inspiration (Linn, 1980; Koenig et al., 1987). Linder et al. (1988) reported that brief exposures (16–28 min) to 120–130 ppb O_3 at high ventilatory rates (30–120 L/min) produced symptoms of irritation and cough in young adults.

Although O_3 causes symptomatic responses in adults at current peak levels, there have been studies indicating that such responses do not occur in healthy children (Avol et al., 1985, 1987). Children (ages 8–11) exposed for 2.5 h at 120 ppb O_3 while intermittently exercising ($V_E = 39$ L/min) showed small but statistically significant decreases in FEV_1 but showed no changes in frequency or severity of cough compared with controls (McDonnell et al., 1985a, 1985b). Similarly, adolescents (ages 12–15) continuously exercising ($V_E = 31$–33 L/min) during exposure to 144 ppb mean O_3 in ambient air showed no changes in symptoms, despite statistically significant decrements in group mean FEV_1 (4%), which persisted at least 1 h during post-exposure resting (Avol et al., 1985).

These laboratory results are consistent with the results obtained in field studies of healthy children at summer camps, which failed to find any symptomatic responses despite the occurrence of relatively large decrements in function that were proportional to the ambient O_3 concentrations (Spektor et al., 1988a; Hoek and Brunekreef, 1995). In panels

of 300 healthy children in the Harvard Six Cities Study, there were significant associations between O_3 and the incidence of cough that was independent of other measured pollutants (Schwartz, 1994a, 1994b, 1994c).

For 7–9-year-old children in Mexico City repeatedly exposed to high concentrations of O_3 and PM, Castillejos et al. (1995) reported that mean O_3 in the previous 48 h was associated with a child's report of cough or phlegm, while mean O_3 in the previous day or week was not. For 71 asthmatic 5–7-year-old children in Mexico City, respiratory symptoms (coughing, phlegm production, wheezing, and difficulty breathing) and the frequency of lower respiratory illness on the same day were associated with both O_3 and particulate matter 10 μm or less (PM_{10}) (Romieu et al., 1996). In the study of children with moderate to severe asthma in the Connecticut River Valley, where O_3 exposures were much lower than those in Mexico City, Thurston et al. (1997) found that respiratory symptoms were significantly associated with O_3.

Other epidemiology studies have provided evidence of qualitative associations between ambient oxidant levels >0.10 ppm and symptoms in children and young adults, such as throat irritation, chest discomfort, cough, and headache (Hammer et al., 1974; Makino and Mizoguchi, 1975). Thus, symptoms reported in individuals exposed to O_3 are similar to those found for ambient air exposures except for eye irritation, a common symptom associated with exposure to photochemical oxidants, which has not been reported for controlled exposures to O_3 alone. Other oxidants, such as aldehydes and peroxyacetyl nitrate (PAN), are primarily responsible for eye irritation and are generally found in atmospheres containing higher ambient O_3 levels (Altshuller, 1977; National Research Council, 1977).

Several studies reported associations between ambient photochemical oxidant pollution and exacerbation of asthma (Schoettlin and Landau, 1961; Whittemore and Korn, 1980; Holguin et al., 1985), but the role of O_3 specifically and the nature of the exposure–response relationships remain poorly defined.

Respiratory symptoms in healthy young adult females (student nurses) in Los Angeles, in relation to ambient pollution levels, were reported by Hammer et al. (1974). Schwartz and Zeger (1990) reexamined the original diaries from that study, which contained smoking and allergy histories as well as symptom reports that had not been analyzed. Diaries were completed daily and collected weekly for as long as 3 years. Air pollution was measured at a monitoring location within 2.5 miles of the school. Incidence and duration of a system were modeled separately. Photochemical oxidants (74 ppb) were associated with increased risk of chest discomfort (OR = 1.17 $p < 0.001$) and eye irritation (OR = 1.20 $p < 0.001$).

Ostro et al. (1993) recorded the respiratory symptoms in nonsmoking adults residing in Southern California. Participants recorded their daily incidences of several respiratory symptoms over a 6-month period between 1978 and 1979. Ambient concentrations of O_3, SO_4^{2-}, and other air pollutants were measured. There was a significant association between the incidence of lower respiratory tract symptoms and 7-h O_3 (OR = 1.32, 95% CI: 1.14–1.52, for a 100 ppb change) and SO_4^{2-} (OR = 1.30, 95% CI: 1.09–1.54, for a 10-μg/m^3 change), but no association was found with coefficient of haze (CoH), a more general measure of PM. The existence of a gas stove in the home was also associated with lower respiratory tract symptoms (OR = 1.23, 95% CI: 1.03–1.47). The effects of O_3 were greater in the subpopulation without a residential air conditioner. In addition, O_3 had a greater effect among individuals with a preexisting respiratory infection.

Desqueyroux et al. (2002) studied symptomatic responses to community air pollutants among patients with COPD. During a 14-month period, Parisian adults with severe COPD were monitored by their physicians. Daily levels of four air pollutants were provided by an

urban air quality network. Exacerbation of COPD was associated only with O_3 (odds ratio [OR] = 1.44 for a 5- ppb increase in O_3; (95% CI = 1.14, 1.82), with a lag of 2–3 days. The effect of O_3 was greater in patients' partial pressure of carbon dioxide in arterial blood ($PaCO_2$) was higher than 43 mmHg (OR = 1.83; 95% CI = 1.36, 2.47).

Symptomatic effects of ambient air O_3 at 8-h average concentrations near and below the NAAQS on infants were studied by Triche et al. (2006) in a population of newborns in southwestern Virginia. There were statistically significant associations between ambient O_3 and respiratory symptoms, most notably for infants whose mothers had physician-diagnosed asthma.

21.3.5 Effects on Airway Reactivity

Exposure to O_3 can also alter the responsiveness of the airways to other bronchoconstrictive challenges as measured by changes in respiratory mechanics. Folinsbee et al. (1988) reported that airway reactivity to the bronchoconstrictive drug methacholine (MCh) was approximately doubled following 6.6-h exposures to 120 ppb O_3. Airway hyperresponsiveness (to histamine) had previously been demonstrated, but only at O_3 concentrations ≥400 ppb (Holtzman et al., 1979; Seltzer et al., 1986). Folinsbee et al. (1988) found no apparent relationship between the O_3-associated changes in MCh reactivity and those in FVC or FEV_1. On the other hand, Aris et al. (1991) reported a closer relationship, more similar to reported responses to inhaled H_2SO_4 aerosol, where changes in function correlated closely to changes in reactivity to carbachol aerosol, a bronchonconstrictive drug (Utell et al., 1983). The O_3-associated changes in bronchial reactivity may predispose individuals to bronchospasm from other environmental agents, such as acid aerosol and naturally occurring aeroallergens.

The tests by Horstman et al. (1990), involving 6.6-h exposures to 80, 100, and 120 ppb, produced 56, 89, and 121% increases in MCh responsiveness, respectively. Increased responsiveness to MCh was also found in the Folinsbee and Hazucha (1989) study with 1 h at 350 ppb. Gong et al. (1988) reported an increased responsiveness to histamine in one of 17 competitive cyclists exposed at 120 ppb for 1 h at V_E of 89 L/min followed by 3–4 min at 150 L/min. At 200 ppb, responsiveness increased in 9 of the 17 subjects. McDonnell et al. (1987) found increased histamine responsiveness in 26 young adult males with allergic rhinitis after O_3 at 180 ppb during 2 h of exercise at 64 L/min. Jorres et al. (1996) exposed 24 subjects with mild stable allergic asthma, 12 subjects with allergic rhinitis without asthma, and 10 healthy subjects to 250 ppb O_3 or FA for 3 h with intermittent exercise. They determined the concentration of MCh and the dose of allergen producing a 20% fall in FEV_1 ($PD_{20}FEV_1$). In the subjects with asthma, FEV_1 decreased by $12.5 \pm 2.2\%$, $PC_{20}FEV_1$ of MCh by 0.91 ± 0.19 doubling concentrations, and $PD_{20}FEV_1$ of allergen by 1.74 ± 0.25 doubling doses after O_3 compared with sham exposure to FA. The changes in lung function, MCh, and allergen responsiveness did not correlate with each other. In the subjects with rhinitis, mean FEV_1 decreased by 7.8 and 1.3% when O_3 and FA, respectively, were followed by allergen inhalation.

21.3.6 Effects on Airway Permeability

Kehrl et al. (1987) studied the effects of inhaled O_3 on respiratory epithelial permeability in healthy, nonsmoking young men exposed for 2 h to purified air and 400 ppb O_3 while performing intermittent treadmill exercise at 67 L/min. Specific airway resistance (SR_{aw}) and

FVC were measured before and at the end of exposures. At 75 min after the exposures, the pulmonary clearance of a radioisotope-labeled organic molecule, that is, ^{99m}Tc-labeled diethylenetriaminepentaacetic acid (DTPA), was measured as an index of epithelial permeability. O_3 exposure caused respiratory symptoms in all eight subjects and was associated with a $14 \pm 2.8\%$ decrement in FVC ($p < 0.001$) and a $71 \pm 22\%$ increase in SR_{aw} ($p = 0.04$). Compared with the air exposure day, seven of the eight subjects showed increased ^{99m}Tc-DTPA clearance after the O_3 exposure, with the mean value increasing from 0.59 ± 0.08 to $1.75 \pm 0.43\%$/min ($p = 0.03$). Thus, O_3 exposure sufficient to produce decrements in the respiratory function of human subjects also caused an increase in permeability. An increased permeability could facilitate the uptake of other inhaled toxicants and/or the release of inflammatory cells such as polymorphonuclear leukocytes (PMNs) onto the airway surfaces.

Foster and Stetkiewicz (1996) studied the influence of O_3 on lung permeability in healthy subjects at 18–20 h after 2- h exposures at 150 and 350 ppb. Permeability was measured in terms of the clearance rate of a water-soluble aerosol containing ^{99m}Tc-DTPA. Based on a sequence of γ-camera measurements of ^{99m}Tc clearance from the lungs, they concluded that ^{99m}Tc-DTPA clearance from the lung periphery and apexes was significantly increased by O_3 exposure, but changes in clearance for the base of the lung were not significant. The FEV_1 at the late time after O_3 was slightly, but significantly, reduced (−2.1%) from pre-exposure levels. Functional changes observed acutely after O_3 exposure were independent of subsequent changes in ^{99m}Tc-DTPA clearance or FEV_1 observed at the late period. These results suggested that epithelial permeability of the lung is altered 18–20- h post-O_3, that the injury was regional, and that the lung base appeared to have a different time course of response or was in an adapted state with respect to O_3 exposure.

21.3.7 Effects on Airway Inflammation

Seltzer et al. (1986) showed that O_3-induced airway reactivity to MCh was associated with PMN influx into the airways and with changes in cyclooxygenase metabolites of arachidonic acid. For 2-h exposures to O_3 at 400 ppb with intermittent exercise, the bronchoalveolar lavage fluid (BALF) had increased prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ and thromboxane B_2 3 h after the O_3 exposure.

Koren et al. (1989) and Devlin et al. (1991) also described inflammatory and biochemical changes in the airways following O_3 exposure. In initial studies, subjects were exposed to 400 ppb for 2 h while performing intermittent exercise at a ventilation of 70 L/min to examine cellular and biochemical responses in the airways. The BALF was collected 18 h after the O_3 exposure. An 8.2-fold increase in PMNs was observed after O_3 exposure, confirming the observations of Seltzer et al. (1986). Twofold increases in protein, albumin, and IgG were indicative of increased epithelial permeability, as previously suggested by the ^{99m}Tc-DTPA clearance studies of Kehrl et al. (1987). In addition to confirmation of the Kehrl et al. findings, Koren et al. (1989) provided evidence of stimulation of fibrogenic processes including increases in fibronectin (6.4×), tissue factor (2.1×), factor VII (1.8×), and urokinase plasminogen activator (3.6×). There were a twofold increase PGE_2 and a similar elevation of the complement component C3a. Levels of leukotrienes C_4 and B_4 were not affected.

Devlin et al. (1991) reported that a significant inflammatory response, as indicated by increased PMNs, was also observed in BALF from subjects exposed to either 80 or 100 ppb O_3 for 6.6 h. The 6.6 h at 100 ppb O_3 produced a 3.8-fold increase in PMNs at 18 h after the exposure, whereas the 6.6 h at 80 ppb produced a 2.1-fold increase. The amounts of O_3

inhaled in the 80 and 100 ppb protocols were ~2.0 and ~2.5 μg and ~3.6 μg in the 400- ppb protocol. Thus, the effect of concentration was apparently somewhat greater than that of exposure duration. The significant increase in PMNs at a concentration as low as 80 ppb suggests that lung inflammation from inhaled O_3 has no threshold down to ambient background O_3 levels.

The inflammatory process caused by O_3 exposure was promptly initiated (Seltzer et al., 1986) and persisted for at least 18 h (Koren et al., 1989). The time course and the O_3 exposures necessary to initiate it were, however, not fully elucidated. Furthermore, these studies demonstrated that cells and enzymes capable of causing damage to pulmonary tissues were increased and the proteins that play a role in the fibrotic and fibrinolytic processes were elevated as a result of O_3 exposure.

Scannell et al. (1996) studied a group of asthmatic subjects to O_3 using the same exposure protocol previously used for 81 healthy subjects and reported no significant differences in lung function responses and a trend toward higher airway resistance ($p < 0.13$). By contrast, the asthmatic subjects had significantly greater ($p < 0.05$) O_3-induced increases in inflammatory endpoints (PMN percent and total protein) in BALF, as compared with 20 of the normal subjects who also underwent bronchoscopy.

Prolonged inflammatory processes following repetitive exposures to O_3 in ambient air were reported by Kinney et al. (1996) in terms of reduced release of reactive oxygen species (ROS), increased BALF levels of PGE_2, lactic dehydrogenase (LDH), a marker of cell death, and interleukin-8 (IL-8), a neutrophil chemotactic factor.

Interpretation of the nature and significance of the inflammatory responses following short-term O_3 exposures is difficult without knowledge of the cumulative effects that may be triggered by repetitive episodes of lung inflammation. The relation of the inflammatory responses, if any, to the well-studied respiratory function responses also remains unknown. We do know that these responses are poorly correlated. Balmes et al. (1996) tested the hypothesis that changes in lung function induced by O_3 are correlated with indices of respiratory tract/injury inflammation. They exposed healthy subjects, on separate days, to 0.2 ppm O_3 and FA for 4 h during exercise. Symptom questionnaires were administered before and after exposure, and pulmonary function tests (FEV_1, FVC, and SR_{aw}) were performed before, during, and immediately after each exposure. Fiber-optic bronchoscopy, with isolated left main bronchus proximal airway lavage (PAL) and BALF (bronchial fraction, the first 10 mL of fluid recovered) of the right middle lobe, was performed 18 h after each exposure. The PAL, bronchial fraction, and BALF were analyzed for the following: total and differential cell counts and total protein, fibronectin, IL-8, and colony-stimulating factor 2 (granulocyte-macrophage) (CSF2, aka GM-CSF) concentrations. The study population was divided into least sensitive ($n = 12$; mean O_3-induced change in FEV_1 = −7.0%) and most sensitive ($n = 8$; mean O_3-induced change in FEV_1 = −36.0%). There was a highly significant O_3 effect on SR_{aw} and lower respiratory symptoms for all subjects combined, but no significant differences between the least and most sensitive groups. O_3 exposure increased significantly PMN percent in PAL; PMN percent, total protein, and IL-8 in bronchial fraction ($p < 0.001$, $p < 0.001$, and $p < 0.01$, respectively); and PMN percent, total protein, fibronectin, and CSF2 in BALF for all subjects combined; there were no significant differences, however, between least and most sensitive groups. Thus, levels of O_3-induced symptoms and respiratory tract injury/inflammation were not correlated with the magnitude of decrements in FEV_1 and FVC.

A similar conclusion was drawn by Torres et al. (1997), who studied whether individuals who differed in lung function responsiveness to O_3, or in smoking status, also differed

in susceptibility to airway inflammation. Healthy subjects were exposed to 220 ppb O_3 for 4 h with exercise (responders, with a decrease in $FEV_1 > 15\%$, and nonresponders, with a decrease in $FEV_1 < 5\%$). Three groups were studied: nonsmoker-nonresponders ($n = 12$), nonsmoker-responders ($n = 13$), and smokers ($n = 13$, 11 nonresponders and two responders). Each subject underwent two O_3 exposures and one to air, separated by at least 3 weeks; BALF and nasal lavage (NL) were collected on three occasions: immediately (early) and 18 h (late) after O_3 exposure and either early or late after air exposure. Recovery of PMNs increased progressively in all groups and by up to sixfold late after O_3 exposure. IL-8 and IL-6 increased early (by up to 2-fold and up to 10-fold, respectively) and correlated with the late increase in PMNs. Lymphocytes, mast cells, and eosinophils also increased late after exposure. Thus O_3-induced airway inflammation was independent of smoking status or airway responsiveness to O_3.

Alexis et al. (2000) used indomethacin pretreatment prior to O_3 exposure to investigate the role that cyclooxygenase (COX) metabolites of arachidonic acid, including prostaglandins, might play. They reported that COX metabolites contribute to restrictive-type changes in normals and obstructive-type changes in small airways in asthmatics.

Grievink et al. (1999) reported that 100 or 500 mg vitamin E provided partial protection against O_3-related function decrements in adult Dutch cyclists for O_3 concentrations ranging up to 93 ppb during the exercise. For 9-year-old children with moderate to severe asthma in Mexico City, with 8-h average O_3 concentrations ranging up to 184 ppb, daily supplements of 50 mg vitamin E and 250 mg vitamin C modulated the pulmonary effects of O_3 (Romieu et al., 2002).

On the other hand, 2 weeks of pretreatment of 800 μg budesonide (a corticosteroid) inhaled twice/day provided no protection against inhaled O_3 in terms of pulmonary function, MCh reactivity, or PMN recruitment (Nightingale et al., 2000).

Holz et al. (1999) reported that respiratory function and O_3-induced airway inflammatory changes differed between individuals, both for healthy and asthmatic subjects, were reproducible, but were not related to each other, and that therefore the two kinds of responses are independent of each other.

Vagaggini et al. (2002) studied subjects with mild asthma, as indicated by an MCh challenge. They exposed them to an allergen 24 h before inhaling O_3. The O_3 exposure increased the percentage of eosinophils, but not PMNs in induced sputum above that associated with the allergen challenge alone.

Samet et al. (2001) studied the pulmonary effects of O_3 on healthy adults with and without dietary supplementation of antioxidants and found that the antioxidants reduced the O_3-induced functional decrements, but not its effect on increasing PMNs and IL-6 in lavage fluid.

Inflammatory reactions occur in the nasal passages as well as in the lungs. Graham et al. (1988) exposed 41 subjects to either FA or 500 ppb O_3 for 4 h on 2 consecutive days. NL were collected before and immediately following each exposure (post-1 and post-2) and 22 h after the last exposure (pre-2). Lavage PMN counts increased significantly ($p = 0.005$) in the O_3-exposed group, with 3.5-, 6.5-, and 3.9-fold increases over the air-exposed group when measured at different days post-1, pre-2, and post-2, respectively. Graham and Koren (1990) compared the cellular changes detected in NL with those detected in BALF from the same individual. Subjects were exposed to either FA or 400 ppb O_3, with exercise, for 2 h. The NL were done prior to, immediately following, and 18- h post-exposure; the BALF was collected only at 18- h post-exposure. A significant increase in PMNs was detected in the NL immediately post-exposure to O_3

(7.7-fold increase; $p = 0.003$) and remained elevated in the 8- h post-O_3 NL (6.1-fold increase; $p < 0.001$). A similar increase in PMNs was detected in the BALF 18 h after O_3 exposure (6.0-fold increase; $p < 0.001$). The albumin levels in the NL and BALF were also similarly increased 18 h after O_3 (3.9- and 2.2-fold, respectively). Although a qualitative correlation in the mean number of PMNs existed between the upper and lower respiratory tract after O_3, comparison of the NL and BALF PMNs from each individual showed a significant quantitative correlation for the air data ($r = 0.74$; $p = 0.01$) but not for the O_3 data ($r = 0.41$; $p = 0.24$).

The utility of NL at low ambient levels of O_3 was demonstrated by Frischer et al. (1993). They studied nasal airway inflammation after O_3 exposure in children by repeated NL from May to October 1991. During this time 5–8 NL were collected for each child. On 14 days following "high" O_3 (>90 ppb), 148 NL were performed, and on 10 days following "low" O_3 (<70 ppb), 106 NL were performed, and a significant increase of PMNs from low to high-O_3 days was observed. Concomitant with a decrease of O_3 in the fall, mean PMNs showed a decreasing trend. Humoral markers of inflammation were also measured, and eosinophilic cationic protein and myeloperoxidase increased, indicating that O_3 at ambient concentrations initiated a reversible inflammatory response of the upper airways in normal children.

Peden et al. (1995) studied the role of O_3 in the exacerbation of airway responses in asthmatics, either by priming the airway mucosa, such that cellular responses to allergen are enhanced, or by exerting an intrinsic effect on airway inflammation. The effect of exposure to 400 ppb O_3 on nasal inflammation was examined in allergic asthmatics sensitive to house dust mite (*Dermatophagoides farinae*) allergen using a study design that emphasized the effect of O_3 exposure on the late-phase reaction to allergen based on eosinophil influx and changes in eosinophil cationic protein. By employing a "split-nose" design, in which allergen was applied to only one side of the nose while saline was applied to the contralateral side, both the effect of O_3 on nasal inflammation due to allergen challenge and its direct action on non-allergen-challenged nasal tissues were examined. O_3 exposure had both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways.

In the MOSES study (Frampton et al. (2017), O_3 exposure at 120 ppb increased PMN percent in sputum as well as secretoglobin 1A1 (SCGB1A1, aka CCSP, a marker of airway epithelial cell injury) in blood 22 h later, compared with FA exposure. In contrast, changes in sputum concentrations of the inflammatory markers IL-6, IL-8, and tumor necrosis factor (TNF) were not statistically significant. There was no evidence of statistically significant interactions between sex, age, or GSTM1 status and the observed changes in lung function, sputum PMN leukocytes, or plasma SCGB1A1 after O_3 exposure.

21.3.8 Effects on Particle Clearance

Foster et al. (1987) studied the effect of 2-h exposures to 200 or 400 ppb O_3 with intermittent light exercise on the rates of tracheobronchial mucociliary particle clearance in healthy adult males. The 400 ppb O_3 exposure produced a marked acceleration in particle clearance from both central and peripheral airways, as well as a 12% drop in FVC. The 200 ppb O_3 exposure produced a significant acceleration of particle clearance in peripheral airways, but failed to produce a significant reduction in FVC, suggesting that significant changes in the ability of the deep lung to clear deposited particles take place before significant changes in respiratory function take place.

21.3.9 Effects on Aerosol Dispersion

To study the potential effects of O_3 on small airways in humans, Keefe et al. (1991) employed a test of aerosol dispersion. Healthy nonsmoking male volunteers were exposed to 400 ppb O_3 for 1 h while exercising at 20 L/min/m^2 body surface area (BSA). Prior to and immediately following exposure, tests of spirometry (FVC, FEV_1, and $FEF_{25–75}$) and plethysmography (R_{aw} and SR_{aw}) were performed. Subjects also performed an aerosol dispersion test before and after exposure. Each test involved a subject inhaling five to seven breaths of a 300 mL bolus of a 0.5 μm triphenyl phosphate (TPP) aerosol injected into a 2-L tidal volume. The bolus was injected into the tidal breath at three different depths: at depth A after 1.6 L of clean air from functional residual capacity (FRC), at depth B after 1.2 L, and at depth C after 1.2 L but with inhalation beginning from residual volume (RV). The primary measure of bolus dispersion was the expired concentration half-width (HW). Changes in pulmonary function following O_3 exposure were consistent with previous findings. When corrected for exercise, FVC, FEV_1, and $FEF_{25–75}$ decreased, while there were nonsignificant increases in R_{aw} and SR_{aw}. The HW increased following O_3 exposure relative to air exposure at all depths (17 mL at depth A, 56 mL at depth B, and 53 mL at depth C). The HW was only weakly correlated with spirometric measures, accounting for less than 25% of the variance. HW was not correlated with R_{aw} or SR_{aw}. They concluded that the changes in aerosol dispersion seen with O_3 exposure were related to changes in turbulent mixing and/or regional time constants in the small airways, thus suggesting a possible O_3 effect in that region of the lung as well as effects in the larger airways that produce the respiratory function decrements.

21.3.10 Effects on the Nervous System

Rivas-Arancibia et al. (1998) studied the effects of O_3 exposures on memory and correlated them with pulmonary and brain superoxide dismutase 1 (SOD1) levels. Male Wistar rats were exposed for 4 h to O_3 at 0, 100, 200, 500, or 1000 ppb and tested in a passive avoidance conditioning protocol to measure short- and long-term memory. Motor activity was determined 1 and 24 h after O_3 exposure. SOD1 levels in the brain and pulmonary tissue were measured. Rats exposed for 4 h to 200, 500, and 1000 ppb O_3 showed long-term memory deterioration and decreased motor activity, which were reversed 24 h later. Brain and pulmonary SOD1 increased in animals exposed to 100, 200, and 500 ppb O_3 doses, but decreased in animals exposed to 1000 ppb O_3, suggesting that O_3 exposure affects long-term memory, possibly in association with oxidative stress.

21.4 FACTORS AFFECTING RESPONSIVENESS IN HUMANS

O_3 is an oxidant gas that readily reacts with extracellular and cellular macromolecules. While O_3 is a ROS, it is not generated by cell metabolism and must be inhaled to have lung and systemic effects. Despite its low aqueous solubility, about 80% of O_3 inhaled by humans undergoes irreversible chemical reaction with substrates and cells at the airway surface. Some of the O_3-reactive substances (e.g., urate, ascorbate) act as scavengers (Behndig et al., 2009). After inhalation, O_3 reacts with constituents of the nasal or lung-lining fluid to generate ROS that can cause localized oxidative stress in the lung, leading to lung irritation. With repeated exposure, oxidative stress may lead to lung injury and chronic

lung disease. O_3 may have effects on the cardiovascular and other organ systems through systemic inflammation, oxidative stress, or changes in activity of the autonomic nervous system, which could lead to changes in heart rhythm, endothelial dysfunction, constriction of arteries, and blood clotting. While a great deal is known about O_3 exposure–respiratory function response in humans, very little is known about the mechanisms responsible for the responses. Other irritants, such as SO_2, NO_2, and H_2SO_4, produce responses at lower concentrations among asthmatics than among healthy human subjects, but this may not be true for O_3. For other irritants, functional responses correlate with responsiveness to bronchoconstrictor challenge. For example, Utell et al. (1983) found a high correlation between reactivity to inhaled carbachol and responsiveness to inhaled H_2SO_4 in asthmatics ($r = 0.90$, $p < 0.001$), whereas Horstman et al. (1986) reported that MCh reactivity and SO_2 response were significantly but weakly correlated ($r = 0.31$). Although both functional decrements and bronchial responsiveness are produced by O_3 exposure, Folinsbee et al. (1988) and Horstman et al. (1990) found no apparent relationship between these responses for individual subjects. On the other hand, Aris et al. (1991) screened healthy, nonsmoking volunteers for their functional responsiveness to 3 h of exposure to 200 ppb O_3 at a ventilatory rate of 40 L/min and found that the MCh responsiveness of 10 O_3-sensitive subjects ($PC_{100} = 3.0 \pm 0.8$) was significantly greater than that of 10 O_3-nonsensitive subjects ($PC_{100} = 18.7 \pm 4.5$).

Beckett et al. (1985) examined the effect of atropine, a parasympathetic muscarinic receptor antagonist, on responses to exposure to 400 ppb O_3. Atropine pretreatment prevented O_3-induced increased R_{aw} and partially prevented decreased FEV_1. Atropine did not prevent O_3-induced decreased FVC, changes in respiratory frequency and tidal volume, or the frequency of reported respiratory symptoms. These results suggest that the increase in R_{aw} during O_3 exposure is mediated by a parasympathetic mechanism and that changes in other measured variables are mediated, at least partially, by mechanisms not dependent on muscarinic cholinergic receptors.

Gong et al. (1988) studied β-adrenergic mechanisms to the acute airway responses in which symptoms, pulmonary function, exercise performance, and post-exposure histamine bronchoprovocation were measured in non-asthmatic athletes exposed to 210 ppb O_3 during heavy continuous exercise, with mean $V_E \geq 80$ L/min for 60 min, followed by a maximal sprint (peak $V_E > 140$ L/min) until exhaustion. Each subject was exposed randomly to either 210 ppb O_3 or FA during the four single-blinded exposure sessions. Albuterol, a β-adrenergic agonist, pretreatment resulted in modest but significant bronchodilation as compared with placebo. However, albuterol did not prevent O_3-induced respiratory symptoms; decrements in FVC, FEV_1, and maximum mid-expiratory flow rate (FEF_{25-75}); and positive histamine challenges as compared with that with placebo and O_3. There were no statistically significant differences in the metabolic data or ride times across all drugs and exposures, although the peak V_E was significantly lower with O_3 than FA regardless of drug. The results indicate that acute pretreatment with inhaled albuterol is unable to prevent or ameliorate O_3-induced symptoms and alterations in pulmonary function and exercise performance. The contribution of β-adrenergic mechanisms in the acute airway responses to O_3 appears to be minimal.

In their study on bronchial hyperresponsiveness to O_3 exposure, Seltzer et al. (1986) found increased PGE_2, PGF_{2a}, and thromboxane B_2 in BALF. PGE_2 and PGF_{2a} stimulate pulmonary neural afferents that initiate several responses characteristic of acute O_3 exposure (Coleridge et al., 1976; Roberts et al., 1985), suggesting that the release of prostaglandins in the lung may be involved in routinely observed pulmonary function

decrements and perhaps in altered exercise ventilatory pattern and reported subjective symptomatology.

Schelegle et al. (1987) studied whether O_3-induced pulmonary function decrements could be inhibited by indomethacin, a cyclooxygenase inhibitor that diminishes prostaglandin formation, in healthy human subjects. College-age males completed six 1-h exposure protocols with workloads set to elicit a V_E of 60 L/min, with no drug, placebo, or indomethacin pretreatments, with FA and O_3 (350 ppb) exposures within each pretreatment. Exposures consisted of 1-h exercise on a bicycle ergometer. There were significant differences for no drug versus indomethacin and for placebo versus indomethacin, suggesting that cyclooxygenase products of arachidonic acid, which are sensitive to indomethacin inhibition, play a role in the development of pulmonary function decrements following short-term O_3 exposure. In a similar study, Ying et al. (1990) administered indomethacin for 4 days to young adult male nonsmokers prior to 2-h O_3 exposures with intermittent exercise at 400 ppb to determine if it would alter their O_3 responsiveness as well as their lung function. For subjects who had detectable serum levels of indomethacin and significant responses to MCh on the sham exposure day, indomethacin attenuated the O_3-induced decrements in lung function but did not attenuate the O_3-induced augmented MCh responsiveness. They concluded that the O_3-induced decrements in respiratory function are mediated by cyclooxygenase products, but that the O_3-induced increase in airway reactivity occurs by some other mechanism.

Available data indicate that O_3-induced pulmonary function decrements and ventilatory pattern changes are neurally mediated (Lee et al., 1979; Hazucha et al., 1989). Hazucha et al. (1989) concluded that O_3 inhalation stimulates airway receptors, which leads to an involuntary inhibition of full inspiration, reduction in FVC, and a concomitant decrease in maximal expiratory flow rates in humans. The observation that cyclooxygenase products stimulate neural afferents in the lung (Coleridge et al., 1976; Roberts et al., 1985), combined with the observation of reduced O_3-induced pulmonary function decrements after indomethacin pretreatment, suggests that cyclooxygenase products released consequent to O_3-induced tissue damage stimulate neural afferents in the lung, which results in the observed pulmonary function decrements.

21.5 MECHANISTIC STUDIES IN LABORATORY ANIMALS

Studies in rodents have provided some evidence for acute effects of O_3 on markers of cardiovascular function and systemic stress responses. When concentrations exceed 400–500 ppb, rodents are relatively resistant to O_3 respiratory effects, in part because of effective nasal scrubbing. Chuang et al. (2009) found that in 6-week-old C57Bl/6J mice, 500 ppb O_3 exposure for 8 h increased HR about 6%, without changes in BP. Consecutive daily exposures for 5 days increased mean and diastolic BP as well as HR.

Hamade et al. (2008) examined cardiac effects in three strains of mice following acute O_3 at 600 ppb and $PM_{2.5}$ black carbon (BC) exposures. The sequence of 2 h of O_3 followed by 3 h of BC had the largest effect on HRV (in the time domain) and on HR—although HR in this study decreased rather than increased as was reported by Chuang et al. (2009). The two C3H mouse strains (/HeJ and /OuJ) reacted similarly, but the C57Bl/6J strain was relatively nonreactive. Hamade et al. (2010) examined age-related effects of these exposure protocols and suggested that "… age considerably attenuates physiologic responses to O_3…. exposures."

Since O_3 has been shown to activate transient receptor potential (TRP)A1 cation channels on vagal C-fibers in the airways (Taylor-Clark and Undem, 2010), a role for O_3-induced autonomic nervous system synaptic reflexes in altering cardiac electrophysiology (as well as causing involuntary inhibition of full inspiration) has been generally accepted. Gackiere et al. (2011) provided evidence, in rats, that stimulation of airway receptors by O_3 exposure activates stress-responsive regions in the brainstem via the vagus nerve. More recent studies in rats support the existence of a neurohormonal systemic stress response to O_3 involving "activation of the sympathetic nervous system and the hypothalamic–pituitary–adrenal axis" (Kodavanti, 2016). In 14 healthy, younger volunteers exposed to 250 ppb O_3 or FA for 3 h with exercise, Tank et al. (2011) failed to find evidence for sympathetic activation at rest the morning after exposure. However, Miller et al. (2016) suggested that O_3-exposed volunteers may acutely develop increased circulating stress hormones and lipid metabolites. Even if acute O_3 exposure does stimulate a systemic stress response in humans, the cardiovascular consequences have not been defined.

21.6 STUDIES OF POPULATIONS EXPOSED TO OZONE IN AMBIENT AIR

Observational studies of the influence of O_3 on human health have often been difficult to interpret because people are also exposed to other pollutants in the ambient air that could affect the responses observed or to other environmental challenges that may produce comparable effects, such as environmental tobacco smoke, other pollutants in indoor air, and allergens found in indoor and outdoor air. For time-series studies of daily mortality and admissions to emergency departments, hospital admissions, and other health service providers, appropriate corrections need to be made for ambient temperature, which can covary with both O_3 concentrations and health effect indices.

21.6.1 Mortality

Kinney and Ozkaynak (1991) examined a 10-year record of daily mortality data from Los Angeles County. They demonstrated associations between short-term variations in total mortality (excluding accidents and suicides) and O_3, controlling for temperature. Similar results were detected for cardiovascular mortality. A parsimonious three-variable model (NO_2, 1-day lagged O_3, and temperature) explained 4% of the short-term variation in total mortality. Temperature and O_3 had the strongest association with mortality. However, in a follow-up study that included PM_{10} as an additional variable in a multiple regression analysis, Thurston and Kinney (1995) reported that the association between O_3 and daily mortality disappeared when PM_{10} was added to the model. These results suggest that the relationship, if any, between O_3 and daily mortality is weaker than that involving PM_{10} and that the univariate O_3 effect may have been due to O_3 acting as a PM_{10} surrogate. Furthermore, the results indicated that CO had an independent association with mortality that was of similar strength to that of PM_{10}. Similar findings were reported for Mexico City by Borja-Aburto et al. (1997). O_3 was significantly associated with daily mortality when considered alone. However, when a multiple regression analysis with total suspended particulate (TSP), SO_2, and O_3 was performed, only TSP remained significant.

Subsequent studies reported independent mortality effects of O_3 in multiple regression analyses. Sartor et al. (1995, 1997) found that O_3 affected mortality in Belgium during the summer of 1994 for both all ages and that for the elderly. Temperature potentiated the

response to O_3. Verhoeff et al. (1996) examined daily mortality in Amsterdam, The Netherlands, for 1986–1992 and reported that O_3 lagged 2 days was positively associated with mortality, along with current-day black smoke (BS) and PM_{10}. There was no association with SO_2 or CO. Anderson et al. (1996) studied air pollution and daily mortality in London, England, during 1987–1992. They reported that both same-day O_3 and BS were independently associated with all-cause mortality, which was greater on warm days, and independent of the effects of other pollutants. O_3 was also significantly associated with cardiovascular and respiratory disease mortality.

Touloumi et al. (1997) performed a combined analysis of daily mortality for six Western and Central European cities participating in the Air Pollution and Health: A European Approach (APHEA) project. They reported that a 50 $\mu g/m^3$ (25 ppb) increase in daily 1- h maximum O_3 concentration was associated with a 2.9% increase in the number of deaths and the effect was independent of the BS concentration change and consistent across the cities.

Thurston and Ito (2001) reexamined the data from a number of earlier time-series mortality studies that had not adequately corrected for ambient temperature. For all of the total mortality–air pollution time-series studies considered, the combined analysis yielded a relative risk (RR) = 1.036 per 100-ppb increase in daily 1-h maximum O_3 (95% CI: 1.023–1.050). However, the subset of studies that specified the nonlinear nature of the temperature–mortality association yielded a combined estimate of RR = 1.056 per 100 ppb (95% CI: 1.032–1.081). This indicated that prior time-series studies using linear temperature–mortality specifications had under-predicted the premature mortality effects of O_3. For Detroit, MI, an illustrative analysis of daily total mortality during 1986–1990 also indicated that the model weather specification choice can influence the O_3 health effect estimate. Results were intercompared for alternative weather specifications. Nonlinear specifications of temperature and relative humidity (RH) yielded lower intercorrelations with the O_3 coefficient, and larger O_3 RR estimates, than a base model employing a simple linear spline of hot and cold temperature. They concluded that, unlike for PM mass, the mortality effect estimates derived by time-series analyses for O_3 can be sensitive to the way that weather is addressed in the model. Generally, they found that the O_3–mortality effect estimate increased in size and statistical significance when the nonlinearity and the humidity interaction of the temperature–health effect association when incorporated into the model weather specification.

Many studies focused on the associations between short-term O_3 exposures and daily mortality rates in urban centers. The National Morbidity, Mortality, and Air Pollution Study (NMMAPS) used EPA's Atmospheric Information Retrieval System (AIRS) data on ambient O_3 from 95 U.S. communities and publicly available daily mortality data in a preselected analytical model. As shown in Fig. 21.7, a positive association was found in all but two communities, and a statistically significant association was shown for 7 communities and for the 95 as a whole (U.S. EPA, 2006). As shown in Fig. 21.8, the 95-community effect was strongest on the same day and highly significant on 1- and 2-day lags, as well as being even stronger when the distributed lag over 6 days was considered. In a follow-up study of NMMAPS data, Bell et al. (2005) estimated the excess mortality that would remain if EPA's 8-h O_3 NAAQS of 80 ppb was met in each of the communities. There was still a 0.30% (CI: 0.15–0.45) increase in mortality per 10 ppb in the average of the same day and previous day's O_3 level.

EPA commissioned three independent meta-analyses of time-series studies of the associations between ambient O_3 and daily mortality (Ito et al., 2005; Levy et al., 2005; Bell et al., 2005). They all produced daily mortality estimates that did not differ statistically from

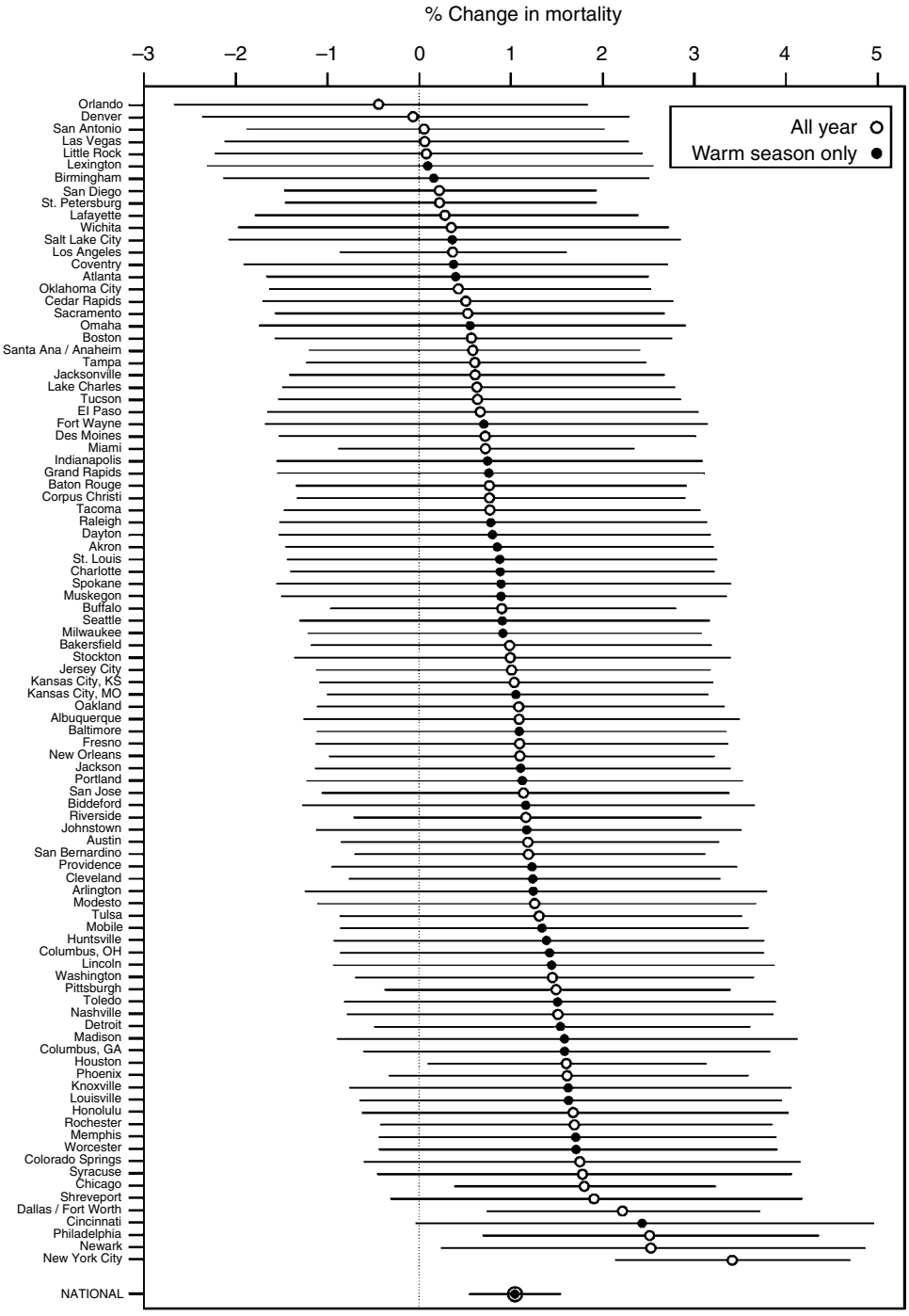

FIGURE 21.7 Bayesian city-specific and national average estimates for the percent change (95% CI) in daily mortality per 20- ppb increase in 24- h average. O_3 in the previous week using a constrained distributed lag model for 95 U.S. communities (NMMAPS), arranged by size of the effect estimate. *Source:* EPA O_3 PM. Data derived from Bell et al. (2004).

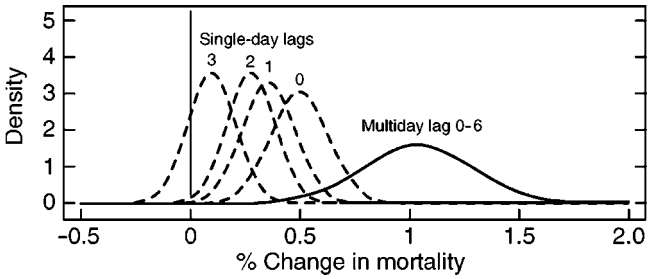

FIGURE 21.8 Comparison of single-day lags (0-, 1-, 2, and 3-day) to a cumulative multiday lag (0–6- day) for percent changes in all-cause mortality per 20- ppb increase in 24-year average O_3 in all ages. *Source*: EPA O_3CD (2005). Data derived from Bell et al. (2004).

each other. While O_3 did appear to have a significant impact on daily mortality rates, especially in the warmer months of the year, its average long-term concentration was not found to significantly influence annual mortality rate. In contrast, Pope et al. (2002) found that $PM_{2.5}$ was associated with significant excesses of cardiovascular and lung cancer mortality.

21.6.2 Morbidity

Associations between ambient air pollutants and respiratory morbidity were examined by Ostro and Rothschild (1989) using the Health Interview Survey (HIS), a large cross-sectional database collected by the National Center for Health Statistics. They examined the separate health consequences of O_3 and $PM_{2.5}$ using six separate HIS years. The results, using a fixed effects model that controls for intercity differences, indicated an association between $PM_{2.5}$ and both minor restrictions in activity and respiratory conditions severe enough to result in work loss and bed disability in adults. On the other hand, O_3 was associated only with more minor restrictions.

Bates and Sizto (1989) examined associations between ambient air pollutants and hospital admissions for respiratory disease in southern Ontario. They found a consistent association in summer between hospital admissions for respiratory disease and daily levels of O_3, SO_4^{2-}, and temperature, but no association for non-respiratory conditions. Multiple regression analyses have found that all environmental variables together accounted for 5.6% of the variability in respiratory admissions and that when temperature was included in the analysis first, it accounted for only 0.89% of the variability. Daily SO_4^{2-} data collected at one monitoring site in the center of the region were not correlated with respiratory admissions, whereas the SO_4^{2-} values collected every sixth day, on different days of the week, at 17 stations in the region had the highest correlation with respiratory admissions. They concluded that probably neither O_3 nor SO_4^{2-} alone is responsible for the observed associations with acute respiratory admissions but that either some unmeasured species or some pattern of sequential or cumulative exposure was responsible for the observed morbidity.

The Bates and Sizto (1989) study stimulated a series of additional studies. Burnett et al. (1994) also employed the Ontario acute care hospital database to analyze the effects of air pollution on hospital admissions, but their analysis considered all of Ontario and analyzed the data from each individual hospital, rather than aggregating the counts by region. Slow-moving temporal cycles, including seasonal and yearly effects, were removed, and day-of-week effects were controlled prior to the analysis. Poisson regression techniques were employed because of the low daily admission counts at individual hospitals. O_3

displayed a positive association with respiratory admissions in 91% of the 168 hospitals, and 5% of summertime (May through August) respiratory admissions (mean = 107/day) were attributed to O_3 (mean = 50 ppb). Positive associations were found in all age groups (0–1, 2–34, 35–64, and 65+). A parallel analysis of non-respiratory admissions showed no such associations. Lipfert and Hammerstrom (1992) reanalyzed the Bates and Sizto (1989) hospital admissions data set for 79 acute care hospitals in southern Ontario, incorporating more elaborate statistical methods and extending the data set through 1985. O_3, SO_4^{2-}, and SO_2 had significant associations with hospital admissions. By contrast, pollution associations with hospital admissions for accidental causes were nonsignificant. The pollutant mean effect accounted for 19–24% of all summer respiratory admissions.

Thurston et al. (1994) analyzed respiratory hospital admissions in the Toronto metropolitan area during the summers of 1986–1988, as well as strong particulate acidity (H^+) pollution on a daily basis at several sites in that city. Strong positive associations with asthma and respiratory admissions were found for both O_3 and H^+ and somewhat weaker significant associations with SO_4^{2-}, $PM_{2.5}$, PM_{10}, and TSP, as measured at a central site in downtown Toronto. Simultaneous regressions and sensitivity analyses indicated that O_3 was the summertime haze constituent of greatest importance to respiratory and asthma admissions, although elevated H^+ was suggested as a possible potentiator of this effect. During multi-pollutant simultaneous regressions on admissions, O_3 was consistently the most significant. Of the PM metrics, only H^+ remained statistically significant when entered into the admission regressions simultaneously with O_3. Sensitivity analyses also found that removing all days with 1-h O_3 above 120 ppb (2 of a total 117 days) did not significantly change the O_3 coefficients. The simultaneous O_3, H^+, and temperature model indicated that $21 \pm 8\%$ of all respiratory admissions during the three summers were associated with O_3 air pollution, on average, and that admissions rose an estimated $37 \pm 15\%$ above that otherwise expected on the highest O_3 day (159 ppb). Moreover, despite differing health-care systems, the Toronto regression results for the summer of 1988 were remarkably consistent with previously reported results for that same summer in Buffalo, NY (Thurston et al., 1992).

Delfino et al. (1994a) studied daily urgent hospital admissions for respiratory and other illnesses at 31 hospitals in Montreal, Canada, during the warm periods of the year between 1984 and 1988. Both 1- and 8-h maximum O_3 concentrations were considered in the analyses, as well as weather variables (temperature and RH) and PM measurements (Delfino et al., 1994b). For the months of July and August, all respiratory admissions were associated with 8-h daily and 1-h daily maximum O_3 4 days prior to admission, despite the low O_3 concentrations (90th percentile = 60 ppb O_3). No significant correlations were found between O_3 and non-respiratory control admissions.

Burnett et al. (1997) extended their study of the associations of O_3 with hospitalization for respiratory disease to 16 cities across Canada representing 12.6 million people from 1981 to 1991. There were 720,519 admissions for which the principal diagnosis was a respiratory disease. After controlling for SO_2, NO_2, CO, soiling index, and dew point, the daily high hour concentration of O_3 recorded 1 day previous to the date of admission was positively associated with respiratory admissions in the April to December period, but not in the winter months. The association between O_3 and respiratory hospitalizations varied among cities, with RR ranging from 1.000 to 1.088 after simultaneous covariate adjustment. PM and CO were also positively associated with respiratory hospitalizations.

Thurston et al. (1992) analyzed admissions to acute care hospitals in three New York State metropolitan areas during the summers of 1988 and 1989. Environmental variables considered included daily 1-h maximum O_3 and 24-h average SO_4^{2-} and H^+ concentrations,

as well as daily maximum temperature recorded at central sites in each community. The strongest O_3 respiratory admission associations were found during the period of high pollution in the summer of 1988 and in the most urbanized communities considered (i.e., Buffalo and New York City). After controlling for temperature effects via simultaneous regression, the summer haze pollutants (i.e., SO_4^{2-}, H^+, O_3) remained significantly related to total respiratory and asthma admissions, but high intercorrelation prevented the clear discrimination of a single pollutant as the causal agent. Depending on the index pollutant, the admission category, and the city considered, it was found that summer haze pollutants accounted for approximately 5–20% of June through August total respiratory and asthma admissions, on average, and that these admissions increased approximately 30% above average on the highest pollution days.

White et al. (1994) reported daily emergency room (ER) visit records from June through August 1990 at a large inner-city hospital in Atlanta, GA. Daily counts of visits for asthma or reactive airway disease by children (1–16 years) (mean = 6.6 visits/day) were related to daily levels of O_3, SO_2, PM_{10}, pollen, and temperature. The model yielded a 1.42 admission rate ratio ($p = 0.057$, 95% CI = 0.99–2.0) for the number of asthma visits following days with O_3 levels equal to or exceeding a 1-h maximum of 0.11 ppm, which is consistent with the RR values reported by Thurston et al. (1992, 1994).

In a study of Birmingham, AL, Schwartz (1994a) separately examined O_3 and PM_{10} influences on hospital admissions of the elderly for pneumonia (mean = 5.9 admissions/day) and COPD (mean = 2.2 admissions/day) from 1986 to 1989. Base model results (excluding winter months) yielded a 2-day lag RR estimate of 1.14 for pneumonia admissions from a 50- ppb increase in 24-h average O_3 (95% CI = 0.94–1.38). Excluding days exceeding 120 ppb yielded similar results (RR = 1.12, CI = 0.92–1.37). For COPD, the basic model yielded an RR = 1.17 (CI = 0.86–1.60), whereas excluding days above 120 ppb similarly gave nonsignificant RR = 1.18 (CI = 0.86–1.62).

Schwartz (1994b) analyzed O_3 and PM_{10} relationships with daily hospital admissions of persons ≥65 years in the Detroit, MI, metropolitan statistical area from 1986 to 1989. Daily counts for pneumonia (mean = 15.7 admissions/day), asthma (mean = 0.75 admissions/day), and all other COPDs (mean = 5.8 admissions/day) were regressed on the pollution variables. O_3 was analyzed with respect to both its daily 24-h average and 1-h maximum. Both O_3 and PM_{10} were significant in simultaneous pollutant models for pneumonia and COPD, but not for asthma (which was ascribed to the low daily counts for this category). Based on the regression coefficients and data presented, the mean effect for O_3 (11.6%) was double that for PM_{10} (5.7%) in the pneumonia model, but comparable for COPD (12.2% for O_3 vs. 10.2% for PM_{10}).

Schwartz (1994c) evaluated the associations of both PM_{10} and O_3 with respiratory hospital admissions by the elderly in Minneapolis–St. Paul, MN, from 1986 to 1989. Although no association was found for COPD in the elderly, O_3 did make a significant independent contribution to hospital admissions by the elderly for pneumonia (mean = 6.0/day), even after controlling for weather and PM_{10}.

Medina-Ramon et al. (2006) performed a case-crossover study in 36 U.S. cities of the influence of O_3 and PM_{10} on respiratory hospital admissions. Both pollutants were significantly associated with both COPD (at lag 1 day) and pneumonia admissions (at lag 0).

In summary, studies of the elderly suggest that a large portion of the O_3 effects on total respiratory hospital admissions are contributed by COPD and pneumonia cases in the elderly. Based on Thurston et al. (1992, 1994), the other major contributor is asthma admissions, which are usually more prevalent in younger age groups.

A variety of population studies also have analyzed associations between ambient O_3 and ER admissions. Cody et al. (1992) analyzed central New Jersey hospital ER visits to the high-O_3 season (May through August). For simultaneous regressions of respiratory visits on both temperature and O_3, there were significant associations for O_3 and a negative association for temperature. Day-of-week influences were considered, but found to be unimportant for these ER visit data.

Weisel et al. (1995) examined central New Jersey hospital ER visits for asthma (mean = 5.4/day) during the high-O_3 season (May through August) for 1986 through 1990. Using a stepwise regression analysis, a significant positive coefficient for O_3 and a negative coefficient for morning temperature were found. Other environmental factors were not correlated with asthma visits.

Stieb et al. (1996) examined the relationship of asthma ER visits to daily concentrations of O_3 and other air pollutants in Saint John, New Brunswick, Canada. Data on ER visits with a presenting complaint of asthma ($n = 1987$) were abstracted for the period 1984–1992 (May to September). Air pollution variables included O_3, SO_2, NO_2, SO_4^{2-}, and TSP; weather variables included temperature, dew point, and RH. The mean daily 1-h O_3 maximum during the study period was 41.6 ppb. A positive, statistically significant ($p < 0.05$) association was observed between O_3 and asthma ER visits 2 days later, and the strength of the association was greater in nonlinear models. The frequency of asthma ER visits was 33% higher (95% CI, 10–56%) when the daily 1-h O_3 maximum exceeded 75 ppb (the 95th percentile). The O_3 effect was not significantly influenced by the addition of weather or other pollutant variables into the model or by the exclusion of repeat ER visits.

Yang et al. (1997) examined the association between air pollution and the ER visits for asthma in Reno, Nevada, for the period 1992–1994. All 3 hospitals in the region were included, and there were a total of 1593 ER visits for asthma during this period. The air pollution variables were collected from seven monitoring stations, including PM_{10}, O_3, and CO. Levels of pollution were moderately elevated (the average concentrations of PM_{10}, CO, and O_3 were 38.0 $\mu g/m^3$, 4.55 ppm, and 51.0 ppb, respectively). Weighted least-squares (WLS) regression and autoregressive integrated moving average (ARIMA) time-series analyses were applied and compared. The daily 1-h maximum O_3 concentration was a significant predictor of asthma ER visits. Total asthma visits increased 33.7% (95% CI; range 6.0–61.5%) for each 100- ppb increase in the O_3 level. No association of the concentration of other measured pollutants with daily asthma ER visits was found.

Another index of respiratory morbidity that has been studied is clinic visits. Hernandez-Garduno et al. (1997) monitored patient visits for upper respiratory tract infections in Mexico City at five clinics and collected data on levels of O_3, NO_2, CO, SO_2, and climatological variables. Correlations of filtered data revealed that O_3 and NO_2 were associated with an increase in visits to clinics because of respiratory problems. Autoregressive analysis indicated that pollutant level/respiratory visit associations remained significant even after simultaneous inclusion of temperature, suggesting that air pollution was associated with 10–16% of the clinic visits. High levels of O_3 and NO_2 increased the total number of clinic visits to between 19 and 43% above average. The other pollutants and the control group did not demonstrate significant associations. Overall, these results are consistent with an O_3 effect on asthma morbidity.

In an analysis of respiratory hospital admissions in 14 Canadian cities, Burnett et al. (2001) founded that the effect was greatest at a 1- or 2-day lag, but greatest of all for a distributed lag over 4 days. Modifying factors such as ambient temperature, aeroallergens, and other co-pollutants (e.g., particles) also can contribute to this relationship. O_3 alone could account for a portion of summertime hospital admissions and ER visits for respiratory

causes. O_3 may therefore account for roughly one to three excess summertime respiratory hospital admissions per 100 ppb O_3. Yang et al. (2003) reported significant associations between O_3 respiratory hospital admissions for children less than 3 years and for the elderly in Vancouver, Canada, where the 24-h average O_3 concentration was only 13.4 ppb.

Silverman and Ito (2010) reported the O_3 concentration dependence on asthma hospital admissions in a two-pollutant mode with $PM_{2.5}$ (see Fig. 21.9).

In the Children's Health Study (CHS) in 12 Southern California communities, O_3 was associated with bronchitic symptoms in children with asthma, but the effects were more strongly associated with organic carbon (OC) and NO_2 than with O_3 (McConnell et al., 2003). An effect of O_3 in the CHS, which was not influenced by the other measured pollutants, was an excess in school absences due to respiratory illnesses (Gilliland et al., 2001). Similar effects were seen in a study of asthmatic children in seven U.S. communities, that is, a strong association of school absences associated most strongly with O_3 and respiratory symptoms more strongly associated with pollutants more closely associated with motor vehicle exhaust (O'Connor et al., 2008).

Another effect observed in the CHS that was more closely associated with O_3 than the other measured pollutants was incident cases of asthma, particularly in children who were engaged in three or more team sports (McConnell et al., 2002). Incident asthma in association with chronic O_3 exposure has also been reported in adult males in the AHSMOG cohort of Seventh-Day Adventists in California (McDonnell et al., 1999). The overall implications of the CHS studies on public health and cost–benefit considerations for air pollution control have been reviewed by Kunzli et al. (2003).

Another index of morbidity of respiratory morbidity in asthmatics is physician-authorized medication usage. In their study of children with moderate to severe asthma at a summer camp in the Connecticut River Valley, Thurston et al. (1997) found that the camp

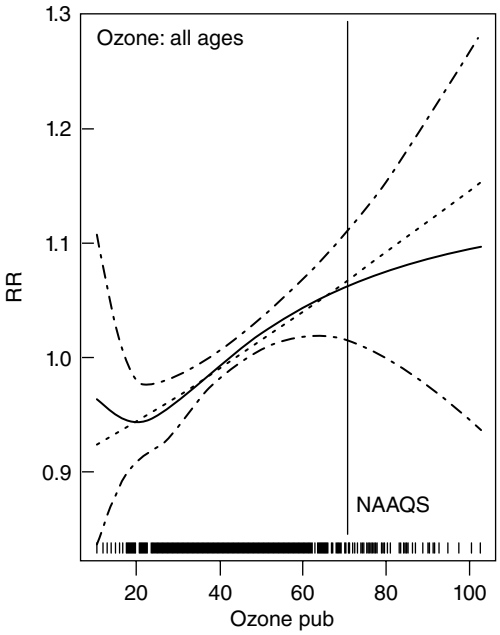

FIGURE 21.9 Estimated relative risks (RRs) of asthma hospital admissions for 8-h max O_3 at lag 0–1 allowing for possible nonlinear relationships using natural splines. *Source*: From U.S. EPA (2015).

physician authorized supplemental medication to children in the group at a rate proportional to the ambient O_3 concentration.

21.7 EFFECTS OBSERVED IN STUDIES IN LABORATORY ANIMALS

21.7.1 Effects on Physical Activity (Athletic Performance)

Animal models for decreased performance during O_3 exposure have been developed. Tepper et al. (1985) exposed rats or mice for 6 h to O_3 at 80, 120, 250, or 500 ppb while housed in running wheels. Running in either species decreased in a concentration-related manner during O_3 exposure, with the decrease being greater with increasing time of exposure. The decrease in running activity produced by O_3 persisted for several hours after exposure. At comparable concentrations, activity in rats decreased more than in mice.

21.7.2 Effects on Airway Reactivity

The basis for the effect of O_3 on airway reactivity was examined, by Gordon et al. (1981), in guinea pigs exposed for 1 h to either 100 or 800 ppb O_3. Both exposures significantly inhibited lung cholinesterase activity. Cholinesterase limits the response to acetylcholine at the neuromuscular junction, and thus inhibition of cholinesterase would prolong airway smooth contraction and lower the threshold dose of MCh.

The O_3-induced responsiveness may be centered in both the large and peripheral lung airways and be retained long after the O_3 exposure ceases. Beckett et al. (1988) exposed the peripheral lungs of anesthetized dogs to 1000 ppb O_3 for 2 h using a wedged bronchoscope technique. A contralateral sub-lobar segment was simultaneously exposed to air as a control. In the O_3-exposed segments, collateral resistance (R_{cs}) was increased within 15 min and remained elevated ~150% throughout the 2-h exposure period. At 15 h after the end of exposure, the R_{cs} remained increased in the O_3-exposed sub-lobar segments, and responsiveness to aerosolized acetylcholine (100 and 500 μg/mL) also increased. There were no differences in PMNs, mononuclear cells, or mast cells (numbers or degree of mast cell degranulation) between O_3- and air-exposed airways at 15 h. The airways of the lung thus are capable of remaining hyperresponsive hour after cessation of localized O_3 exposure, but this does not appear to be dependent on the persistent presence of inflammatory cells.

21.7.3 Effects on Airway Permeability

In laboratory tests in rats, Bhalla et al. (1987) exposed resting rats to 800 ppb O_3, increasing tracheal and bronchoalveolar permeability to DTPA at 1 h after the exposure. Bronchoalveolar permeability, but not tracheal permeability, remained elevated 24 h after the exposure. Exercise during O_3 exposure increased trachea and the bronchoalveolar permeability and prolonged the duration of tracheal (from 1 to 24 h) and bronchoalveolar (from 24 to 48 h) permeability. Exposure at rest to 600 ppb O_3 plus 2500 ppb NO_2 significantly increased bronchoalveolar permeability at 1 and 24 h after exposure, although exposure at rest to 600 ppb O_3 alone increased bronchoalveolar permeability only at 1 h after exposure. Exposure to O_3 and NO_2 during exercise led to significantly greater permeability than exercising exposure to O_3 alone. Nitric acid vapor can be formed in the O_3 + NO_2 atmosphere, suggesting that acidic components may have produced effects that were additive to the effect of O_3 by producing both an increase and prolongation of permeability.

Guth et al. (1986) examined changes in apparent lung permeability in rats by measurement of recovery of labeled bovine serum albumin in BALF after intravenous injection at the end of the O_3 exposure. Their permeability index increased in an exposure-concentration-dependent manner after 6 or 24 h ≥400 ppb O_3. It was also increased after 2 days of exposure to 200 ppb O_3. Abraham et al. (1984) measured changes in airway permeability of tritiated histamine in sheep after O_3 exposure. Permeability increased 24 h after a 2-h exposure to 500 ppb O_3. This persistently increased permeability is consistent with the observations of Bhalla et al. (1987) in rats.

21.7.4 Effects on Airway Inflammation

Arsalane et al. (1999) evaluated SCGB1A1 protein in the serum of rats after a single 3- h exposure to 300, 600, or 1000 ppb O_3. The urinary excretion of the protein was also studied in rats repeatedly exposed to 1000 ppb O_3, 3 h/day, for up to 10 days. The concentrations of SCGB1A1 in the lung or trachea homogenates, the lung SCGB1A1 mRNA levels, and classical markers of lung injury in BALF were also determined. O_3 produced a transient increase of SCGB1A1 concentration in serum that reached values that were, on average, 13 times above normal values 2 h after exposure to 1000 ppb O_3. The intravascular leakage of SCGB1A1 was dose dependent and correlated with the extent of lung injury as assessed by the levels of total protein, LDH, and inflammatory cells in BALF. This effect was most likely responsible for the concomitant marked reduction of SCGB1A1 concentrations in BALF and lung homogenate, because the lung SCGB1A1 mRNA levels were unchanged, and the absolute amounts of SCGB1A1 leaking into serum or lost from the respiratory tract were similar. These changes were paralleled by an elevation of the urinary excretion of SCGB1A1 resulting from an overloading of the tubular reabsorption process. These results demonstrated the utility of this assay to detect the increased lung epithelial permeability induced by O_3.

Broeckaert et al. (2000) applied this assay to humans, specifically to cyclists who exercised for 2 h during episodes of photochemical smog, and found that O_3 induces an early leakage of lung SCGB1A1 protein. The protein levels increased significantly into the serum from exposure levels as low as 60–84 ppb. These findings confirmed that there is almost no safety margin for the effects of ambient O_3 on airway permeability. The assay of SCGB1A1 in the serum represents a sensitive noninvasive test allowing the detection of early effects of ambient O_3 on the lung epithelial barrier.

Kahle et al. (2015) exposed 16 healthy adult volunteers for 2 h at 22 and 32.5°C to 0.3 ppm O_3, separated by 1 week or more from a control exposure to FA. At 22°C, the fibrinolysis pathway can activate, while at 32.5°C, there can be impairment, suggesting a basis for an interaction between temperature and mortality seen in some epidemiological studies. However, decrements in lung function were comparable at both temperatures, and no changes in systemic markers of lung inflammation were found.

21.7.5 Determinants of Responsiveness

There is a large genetic component to responsiveness. Kleeberger (1995) and Kleeberger et al. (1997) explored the contribution of genetic background to the pathogenesis of airway responses to O_3 using inbred mice strains, and a follow-up study with NO_2, another ambient air oxidant, examined the genetic basis for differences in response to the two agents. Kleeberger et al. (1997) determined significant genetic contributions in susceptibility to

lung injury and inflammation induced by single and repeated acute exposures to NO_2 and whether similar genetic factors control susceptibility to O_3. Nine strains of inbred mice (male, 5–6 weeks) were exposed for 3 h to FA or 15 ppm NO_2, and cellular inflammation, epithelial injury, and cytotoxicity were measured 2, 6, and 24 h thereafter. NO_2 exposure caused significant increases in cytotoxicity and lavageable macrophages, epithelial cells, PMNs, and protein in all strains. Inter-strain variation in each of these effects indicated that genetic background contributed a significant portion of the variance in responses to this oxidant. Two strains that were differentially susceptible to 3-h exposure to 15 ppm NO_2 [C57BL/6J (B6), C3H/HeJ (C3)] were also exposed for 6 h/day to 10 ppm NO_2 on 5 consecutive days. Each of the responses to NO_2 was completely adapted after 5 days in resistant C3 mice. Only the lavageable total protein response was adapted in susceptible B6 mice. To determine whether mechanisms of susceptibility to NO_2 and inflammation-prone (susceptible) C57BL/6J and inflammation-resistant C3H/HeJ inbred mice were exposed for 3 h to FA or 2 ppm O_3 and inflammation was assessed 6 and 24 h thereafter. Using recombinant inbred strains derived from these strains, a genetic locus on mouse chromosome 17 was identified. The region contained the gene encoding TNF. Subsequent studies also implicated genetic variants in the toll-like receptor 4 (*Tlr4*), and the transcription factor, nuclear factor, erythroid 2 like 2 (*Nfe2ls* aka *Nrf2*). Strain distribution patterns for responses to O_3 differed from those of NO_2, indicating that susceptibility mechanisms were different.

Studies in laboratory animals have examined the roles of O_3 concentration and exposure time on biochemical and cellular responses. Rombout et al. (1989) exposed mice and rats to 380, 750, 1250, and 2000 ppb O_3 for 1, 2, 4, and 8 h and measured BALF protein with both daytime and nighttime exposures. Observation times extended from 1 to 54 h. The responses varied with O_3 concentration, duration of exposure, time after the start of the exposure, and minute volume, with time of exposure having a greater than proportional influence. For 4- and 8-h exposures, the protein content of BALF peaked at 24 h and remained at elevated levels even at 54 h. As indicated previously, Devlin et al. (1991) found increased BALF protein in humans 18 h after 100 ppb O_3 exposure for 6.6 h.

Bhalla and Young (1992) studied the sequence of changes in lung epithelial permeability, free cells in the airways, PGE_2 l, PMN flux, and alveolar lesions in rats exposed to 800 ppb O_3 for 3 h and then studied at 4-h intervals up to 24- h post-exposure BALF protein increased immediately after O_3 exposure and returned to control levels by 16- h post-exposure. Albumin concentration in the BALF increased more gradually, and the albumin concentrations at 20- and 24- h post-exposure were still higher than the control levels. While the total protein in the BALF could be attributed to tissue injury and increased transmucosal transport, the albumin primarily reflected elevated transport from the serum. Total cells in the BALF decreased immediately after exposure, but returned to near-normal levels by 4 h. PGE_2 did not change significantly after O_3 exposure. PMNs in the lung sections increased with time, peaked at 8 h, and returned to normal levels by 16 h following exposure. The data suggested that the permeability changes may be produced by the direct toxic effects of O_3 on the airway epithelium, but the PMNs contribute to the injury process, especially at the later stages. Lung lesions of alveolar septal thickening and increased cellularity were present at 12- h post-exposure and increased with time, thus coinciding with declining permeability at the later stages. The morphological changes lag behind the functional perturbations and appear to represent a phase of functional recovery.

The weight of the evidence of functional and biochemical responses in humans and laboratory animals that accumulate over multiple hours and persist for many hours or days after exposure ceases is clear and compelling. Both functional changes and inflammatory

processes occur in humans following exposures to 100 ppb O_3 for 6.6 h, whereas higher concentrations were required to elicit comparable responses in rats. Thus, the rat data, which provide evidence of other effects as well, appear to provide conservative indications of effects in humans.

21.7.6 Effect of Single and Multiday Exposures on Particle Clearance

The effects of O_3 on mucociliary particle clearance were studied in rats and rabbits. Rats exposed for 4 h to 400–1200 ppb O_3 exhibited a slowing of particle clearance at ≥800 ppb but not at 400 ppb (Frager et al., 1979; Kenoyer et al., 1981). Rabbits exposed for 2 h to 100, 250, and 600 ppb O_3 exhibited a concentration-dependent trend of reduced clearance rate with increasing concentrations, with the change at 600 ppb being ~50% and significantly different from control (Schlesinger and Driscoll, 1987). It is not known why the animal tests find only retarded mucociliary clearance in response to O_3 exposure whereas the human tests note accelerated clearance. In corresponding tests with other irritants, that is, H_2SO_4 aerosol and cigarette smoke, both humans and animals have exhibited accelerated clearance at lower exposures and retarded clearance at higher exposures (Lippmann et al., 1987).

Phipps et al. (1986) examined the effects of acute O_3 exposure on some of the factors that affect mucociliary transport rates in studies in which sheep were exposed to 500 ppb O_3 for 2 h on 2 consecutive days. The exposures produced increased basal secretion of sulfated glycoproteins, but had no effect on ion fluxes. Histological examination indicated a moderate hypertrophy of submucosal glands in the lower trachea, and they concluded that the exposure caused airway mucus hypersecretion.

Studies of the effects of O_3 on alveolar macrophage-mediated particle clearance during the first few weeks have also been performed in rats and rabbits. Rats exposed for 4 h to 800 ppb O_3 had accelerated particle clearance (Frager et al., 1979). Rabbits exposed for 2 h to 100, 600, or 1200 ppb O_3 had accelerated clearance at 100 ppb and retarded clearance at 1200 ppb. Rabbits exposed for 2 h/day for 13 consecutive days to 100 or 600 ppb O_3 had accelerated clearance for the first 10 days, with a greater effect at 600 ppb (Driscoll et al., 1986).

The responses of the alveolar macrophages in rabbits were examined by Driscoll et al. (1987). A single 2-h exposure to 100 ppb O_3 resulted in increased BALF macrophages at 7 days, and repeated exposures resulted in an increase in BALF macrophages and PMNs on days 7 and 14. Macrophage phagocytosis was depressed immediately and 24 h after a single 2-h exposure to 100 ppb and immediately, 24 h and 7 days after a single 2-h exposure to 1200 ppb. Repeated exposures to 100 ppb (2 h/day × 13 days) decreased phagocytically active macrophages on days 3 and 7, with a return to control levels by day 14. Substrate attachment by macrophages was impaired immediately after exposure to 1200 ppb. The results of these studies demonstrated significant alterations in the numbers and functional properties of alveolar macrophages as a result of single or repeated exposure to 100 ppb O_3, a level frequently encountered in areas of high photochemical air pollution.

21.7.7 Time Scale for Biological Integration of Effects of Single O_3 Exposures

The time scale for the biological integration of the effects of a single O_3 exposure has also been examined in studies on laboratory animals. Costa et al. (1989) exposed Fischer 344 rats for 2, 4, or 8 h to 100, 200, 400, or 800 ppb O_3. Lung function was measured immediately after exposure, and BALF was performed immediately and 24 h later. Functional decrements increased with the product ppb-hour, leveling off at >6000, whereas BALF

proteins increased rapidly for ppb-hour >4000. In another test series involving 6.6 h of exposure with 8% CO_2 to stimulate respiration, rats exposed to 500 ppb O_3 had functional decrements closely matching those seen in humans at 120 ppb. Thus, rats can provide a good test model for the observed human responses to O_3, even though they are a less sensitive species than humans. The lesser responses to a given O_3 concentration reported here are consistent with the lesser retention of O_3 by rats, as discussed previously.

Highfill et al. (1992) examined relationships between concentration (C) and exposure time (T) and the impact of changes in the $C \times T$ product on toxic responses. Using BALF protein as an index of O_3-induced lung damage, models were developed from a matrix of C (0, 100, 200, 400, and 800 ppb) and T (2, 4, and 8 h) values in rat and guinea pig. Equal $C \times T$ products with different levels of C and T were incorporated into the protocol. Polynomial and exponential least-squares models were developed, and the lognormal linear model (Larsen et al., 1991) was evaluated for the rat and guinea pig data. For equal $C \times T$ products, the results showed similar BALF responses at low $C \times T$ products. Calculations from the data and the models showed that (1) the models were consistent with reported experiments (Hatch et al., 1986), (2) exercising humans were more responsive to O_3 exposure (without adjustments for ventilation rates) than either rats or guinea pigs as measured by changes in BALF (Koren et al., 1989), and (3) the exponential model provided more generality than Haber's law by providing estimates of BALF levels for various $C \times T$.

Ozone-induced bronchial hyperresponsiveness in dogs is inhibited by PMN depletion (O'Byrne et al., 1984a) and indomethacin pretreatment (O'Byrne et al., 1984b), suggesting that PMNs that infiltrate the airway after acute O_3 exposure (Holtzman et al., 1979; O'Byrne et al., 1984b) are the cells that release the cyclooxygenase products responsible for O_3-induced bronchial hyperreactivity. However, PMN infiltration is a relatively late effect (i.e., occurring >6- h post-exposure) and is not likely to account for the immediate responses. In a follow-up study (Jones et al., 1990), thromboxane antagonists were given to the dogs to further determine the role of thromboxane in O_3-induced airway hyperresponsiveness. The antagonists did not inhibit the response, indicating that thromboxane may not have an important role in causing O_3-induced airway hyperresponsiveness.

Leikauf et al. (1988) investigated the hypothesis that oxidant damage to the tracheal epithelium may result in elaboration of various eicosanoids. After exposure to O_3, epithelial cells derived from bovine trachea were isolated and grown to confluency. Monolayers were alternately exposed to O_3 and culture medium for 2 h in a specially designed *in vitro* chamber using a rotating inclined platform (Valentine, 1985). O_3 induced increases in cyclooxygenase and lipoxygenase product formation with significant increases in PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and leukotriene B_4. Release rates of immunoreactive products were dose dependent, and ozone concentrations as low as 100 ppb produced an increase in prostaglandin $F_{2\alpha}$. Thus, O_3 can augment eicosanoid metabolism in airway epithelial cells.

21.7.8 Effects of Single and Multiday Exposures on Lung Infectivity

Both *in vivo* and *in vitro* studies have demonstrated that O_3 can affect the ability of the immune system to defend against infection. Increased susceptibility to bacterial infection has been reported in mice at 80–100 ppb O_3 for a single 3-h exposure (Coffin et al., 1967; Ehrlich et al., 1977; Miller et al., 1978a). Related alterations of the pulmonary defenses caused by short-term exposures to O_3 include impaired ability to inactivate bacteria in rabbits and mice (Coffin et al., 1968; Coffin and Gardner, 1972; Goldstein et al., 1977; Ehrlich et al., 1979) and impaired macrophage phagocytic activity, mobility, fragility and

membrane alterations, and reduced lysosomal enzymatic activity (Dowell et al., 1970; Hurst et al., 1970; Goldstein et al., 1971a, 1971b; Hurst and Coffin, 1971; Amoruso et al., 1981; McAllen et al., 1981; Witz et al., 1983). Some of these effects occur in a variety of species including mice, rats, rabbits, guinea pigs, dogs, sheep, and monkeys.

Other studies indicate similar effects for short-term and subchronic exposures of mice to O_3 combined with pollutants such as SO_2, NO_2, H_2SO_4, and PM (Gardner et al., 1977; Ehrlich, 1980; Grose et al., 1980a, 1980b; Phalen et al., 1980; Aranyi et al., 1983). Similar to the human pulmonary function response to O_3, activity levels of mice exposed to O_3 play a role in determining the lowest effective concentration that alters the immune defenses (Illing et al., 1980). In addition, the duration of exposure must be considered. In groups of mice exposed to 200 ppb O_3 for 1, 3, or 6 h, superoxide anion radical production decreased 8, 18, and 35%, respectively, indicating a progressive decrease in bacteriocidal capacity with increasing duration of exposure (Amoruso and Goldstein, 1988).

The major limitation of this large body of data on the influence of inhaled O_3 on lung infectivity is that it requires uncertain interspecies extrapolating in order to estimate the possible effects of O_3 on infectivity in humans.

Gilmour and Selgrade (1993) compared the pulmonary defenses of rats and mice against streptococcal infection following O_3 exposures. In mice, 3- h exposures to 400 ppb O_3 resulted in bacterial proliferation and PMN influx in the lungs and excess mortality. By contrast, rats had only a transient impairment of microbial inactivation. These results indicate that caution is needed in translating the results from either species to predictions of human responses.

21.7.9 Genotoxic Effects

The 1996 EPA criteria document (U.S. EPA, 1996a) reviewed the genotoxic effects of ozone in detail. It summarized the available data. It reported that (1) some weakly positive data and some negative data exist on the genotoxicity of O_3 and (2) the *in vitro* studies are mechanistically interesting, but there were difficulties in the design of many of these studies. First, the concentrations used were orders of magnitude greater than those found in ambient air. Second, extrapolation of *in vitro* exposure concentrations to human exposure dose requires special methods that were not used in these studies. Third, direct exposure of isolated cells to O_3 is somewhat artifactual, because it bypasses host defenses and also results in chemical reactions between O_3 and culture media to generate chemical species that may not be produced *in vivo*. Therefore, for these reasons, the relevance and predictive value of *in vitro* genotoxicity studies to human health are questionable, and the more relevant data should therefore be obtained from *in vivo* studies.

21.8 EFFECTS OF OTHER POLLUTANTS ON RESPONSES TO OZONE

A study that addressed the issue of the potentiation of the characteristic functional response to inhaled O_3 by other environmental cofactors was performed in a rural area in New York (Tuxedo, NY) (Spektor et al., 1988b). It involved healthy adult nonsmokers engaged in a daily program of outdoor exercise with exposures to an ambient mixture containing low concentrations of acid aerosols and NO_2 as well as O_3. Each subject did the same exercise each day, but exercise intensity and duration varied widely between subjects, with minute ventilation ranging from 20 to 153 L/min, with an average of 79 L/min, and with duration of daily exercise ranging from 15 to 55 min, with an average of 29 min. Respiratory

function measurements were performed immediately before and after each exercise period. The O_3 concentrations during exercise ranged from 21 to 124 ppb. All functional indices measured had O_3-associated mean decrements. The functional decrements were similar, in proportion to lung volume, to those seen in children engaged in supervised recreational programs in summer camps. They were as large (FEV_1) as or much larger (FVC, FEF_{25-75}, PEFR) than those seen in controlled 1- and 2-h exposures in chambers. For the subgroup with the most comparable levels of physical activity, the responses in the field study were even greater. Since the ambient exposures of the adults exercising out of doors were for ~1/2 h, as compared with the 1- or 2-h exposures in the chamber studies, one can conclude that ambient cofactors potentiate the responses to O_3. Thus, the results of the exposures in chambers to O_3 in purified air may underestimate the O_3-associated responses that occur among populations engaged in normal outdoor recreational activity and exposed to O_3 in ambient air in the Northeastern United States.

The apparent potentiation of O_3-induced functional decrements seen by Spektor et al. (1988b) in rural New York was not seen by Avol et al. (1984) in a study in Southern California in which 42 healthy young men and 8 healthy young women were exposed for 1 h to ambient air containing an average of 153 ppb O_3 while exercising heavily in a chamber. The functional decrements were slightly but not significantly smaller than those produced in the same subjects on another day when they were exposed to 160 ppb O_3 in purified air. The ambient air in Southern California has much higher NO_2 concentrations and much lower acid aerosol concentrations than the ambient air in the Northeastern United States. Thus, this discrepancy suggests that acid aerosol is a possible causal factor for the potentiation seen by Spektor et al. (1988b) than NO_2.

However, the Spektor et al. (1988b) study on exercising adults and earlier studies on children at summer camps (Lioy et al., 1985; Spektor et al., 1988a) were not able to demonstrate the specific effect of any of the measured environmental variables, including heat stress and acid aerosol concentration, on the O_3-associated responses. The inability to find the individual effects of other environmental cofactors on the response to ambient O_3 may result from inadequate knowledge on the appropriate biological averaging time for these other factors. In the study of functional responses of children to ambient pollution in Mendham, NJ, a weeklong baseline shift in PEFR was associated with O_3 and H_2SO_4 of a preceding 4-day pollution episode (Lioy et al., 1985). A similar response to a brief episode with elevated O_3 and a much higher peak 4-h concentration of H_2SO_4 (46 $\mu g/m^3$) was seen among girls attending a summer camp in 1986 at Dunnville, Ontario, Canada, on the northeast shore of Lake Erie (Raizenne et al., 1989).

Several controlled human exposure studies in chambers have not demonstrated synergism in functional response between O_3 and NO_2 (Koenig et al., 1988) or between O_3 and H_2SO_4, although Stacy et al. (1983) did report that the mean responses to 400 ppb O_3 and 100 $\mu g/m^3$ H_2SO_4 after 2 h of exposure at rest were −9.0% for FVC and −11.5% for FEV_1, compared with corresponding values of −5.7 and −7.7% for O_3 alone, −1.4, and −1.2% for FA exposure, and +0.9 and +0.9% for H_2SO_4 alone. One possible reason why these mean differences, which appear to indicate an enhancement of the O_3 response by H_2SO_4, were not statistically significant was the very high variability of the sham exposure results. By contrast, Koenig et al. (1990) did demonstrate that prior 120 ppb O_3 exposure with intermittent exercise for 45 min potentiated the subsequent respiratory function response to a 15-min 100 ppb SO_2. Frampton et al. (1995) exposed 30 healthy and 30 asthmatic volunteers to either 100 $\mu g/m^3$ H_2SO_4 or NaCl aerosol for 3 h followed 24 h later by 3- h exposures to 80, 120, or 180 ppb O_3. For the healthy group, no convincing symptomatic or physiologic effects of exposure to either the aerosol or O_3 on lung function were found. For

the asthmatic group, pre-exposure to H_2SO_4 altered the pattern of response to O_3 in comparison with NaCl pre-exposure and appeared to enhance the small mean decrements in FVC that occurred in response to 180 ppb O_3. Individual responses among asthmatic subjects were quite variable, some demonstrating reductions in FEV_1 of more than 35% following O_3 exposure. Analysis of variance of changes in FVC or FEV_1 revealed evidence for interactions between aerosol and O_3 exposure both immediately and 4 h after exposure. When normal and asthmatic subjects were combined, four-way analysis of variance revealed an interaction between O_3 and aerosol for the entire group and a difference between normal and asthmatic subjects. There was no significant effect of exposures on symptoms for either normal or asthmatic subjects.

Pollutant interactions that potentiate the characteristic O_3 responses have also been reported in controlled exposure studies in animals. Osebold et al. (1980) exposed antigenically sensitized mice to 500 ppb O_3 for 3 days, with and without concurrent exposure to 1 mg/m^3 submicrometer H_2SO_4 aerosol. Atopic reactivity increased more following combined exposure than that for each pollutant alone. Lee et al. (1990) exposed 3-month-old male rats to either FA or 1200 ppb NO_2, 300 ppb O_3, or a combination of both oxidants continuously for 3 days. They measured lung weight and enzyme activities related to NADPH generation, sulfhydryl metabolism, and cellular detoxification. Relative to control, NO_2 exposure caused small (nonsignificant) changes in all the responses measured, O_3 caused significant increases in all the responses except for superoxide dismutase, and a combination of NO_2 and O_3 caused increases in all the parameters that were greater than those caused by NO_2 or O_3 alone. The effects of combined exposure were more than additive (synergistic) for 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase, glutathione reductase, and superoxide dismutase activities and additive for glutathione peroxidase and disulfide reductase activities, but not different from those of O_3 exposure for other enzyme activities.

Kleinman et al. (1989) reported that lesions in the gas exchange region of the lung were greater in size in rats exposed to mixtures containing O_3 with either H_2SO_4 or NO_2 than in rats exposed to O_3 alone. Graham et al. (1987) reported an interaction between O_3 and NO_2 in terms of mortality in mice challenged with streptococcal infection either immediately or 18 h after pollutant exposure. Last (1989) reported synergistic interaction in rats, in terms of a significant increase in BALF protein, following 9-day co-exposures at 200 ppb O_3 with 20 or 40 $\mu g/m^3$ H_2SO_4.

In summary, single O_3 exposures to healthy nonsmoking young adults at concentrations in the range of 80–200 ppb have produced a complex array of pulmonary responses including decreases in respiratory function and athletic performance and increases in symptoms, airway reactivity, BALF PMNs, and rate of mucociliary particle clearance. As shown in Table 21.3, the responses to O_3 in purified air in chambers occur at concentrations of 80 or 100 ppb when the exposures involve moderate exercise over 6 h or more and require concentrations of 180 or 200 ppb when the duration of exposure is 2 h or less. On the other hand, mean FEV_1 decrements >5% have been seen at 100 ppb of O_3 in ambient air for children at summer camps and for adults engaged in outdoor exercise for only 1/2 h. The apparently greater responses to O_3 in ambient air may result from the presence of, or prior exposures to, acidic aerosol, but further investigation of this tentative hypothesis is needed.

Further research is needed to establish the interrelationships between small transient functional decrements, such as FEV_1, PEFR, mucociliary clearance rates, and changes in symptoms, performance, reactivity, permeability, and PMN counts. The latter may be adverse, in themselves, or may be more closely related to the accumulation or progression of chronic lung damage. If transient changes in readily measured functions, such as

TABLE 21.3 Summary of Meta-Analysis of Time-Series Studies Published During the Period 1996–2001

Cause	Season	Lag	Timing (h)	No Studies		Random Effects Summary Coefficient[a]
				RR > 1	RR < 1	
Mortality	All	0	1	13 (8[b])	3 (0)	0.2 (0.1–0.3)
			8	9 (5[b])	3 (0)	0.4 (0.2–0.5)
			24	8 (5[b])	3 (1[b])	0.4 (0.1–0.6)
		Select[c]	1	17 (13[b])	3 (0)	0.3 (0.2–0.4)
			24	22 (8[b])	3 (0)	0.4 (0.3–0.6)
	Summer	Any	1	6 (5[b])	1 (0)	0.4 (0.1–0.6)
			8	6 (5[b])	1 (0)	0.6 (0.3–0.9)
			24	2 (2[b])	0	—
Hospital admission respiratory	All	Select	1	4 (2[b])	1 (0)	0.5 (0.1–1.0)
			8	10 (6[b])	1 (0)	0.7 (0.3–1.0)
			24	6 (2[b])	1 (0)	0.6 (0.2–1.0)
Asthma admissions in children	All	Select	1	3 (0)	1 (0)	0.1 (−0.4, 0.6)
			8	4 (2[b])	3 (2[b])	0.1 (−1.2, 1.3)

[a]Percentage change per 10 μg/m^3 increase and (95% confidence interval), preliminary results.
[b]N umber of single studies with a $p < 0.05$.
[c]"selected" lag = If results for more than one lag were presented the lag selected was chosen as lag focused on by the author, most statistically significant or largest estimate.

FEV_1 or PEFR, are closely correlated with other, more significant health effects than they could be established as useful surrogates in large-scale laboratory, field, and epidemiologic research as well as further retrospective analyses of published data on human exposure–response.

21.9 EFFECTS OF MULTIDAY AND AMBIENT EPISODE EXPOSURES

Because single exposures lasting for an hour or more at current peak ambient O_3 levels produce measurable biological responses in healthy humans and a single high-O_3 day can followed by several more high-O_3 days (Rao, 1988), it is important to know the extent to which the effects accumulate or progress over multiple days. The effects on PEFR can accumulate. This section also reviews the fairly substantial database on functional adaptation to repetitive exposures and the more limited database on biochemical and structural changes that such exposures produce. The data on functional adaptation is largely, but not exclusively, based on studies in human volunteers, whereas the database on biochemical and structural changes caused by O_3 is based entirely on studies in laboratory animals. Data on exposures lasting more than 2 weeks are discussed in Section 21.10.

It is well established that repetitive daily exposures, at a level that produces a functional response upon single exposure, result in an enhanced response on the second day, with diminishing responses on days 3 and 4 and virtually no response by day 5 (Hackney et al., 1977; Farrell et al., 1979; Folinsbee et al., 1980).

Brookes et al. (1989) found enhanced responses on the second day of successive exposures of exercising young adult males to 350 ppb O_3 for 1 h as well as an enhanced response to 250 ppb when the previous day's exposure was to 350 ppb. In older adults (60–89 years),

successive days of 2-h exposures to 450 ppb O_3 with light exercise led to small functional decrements on the first 2 days but no changes on successive days (Bedi et al., 1989).

For repeated 6.6 h/day exposures to 120 ppb O_3, the peak functional response occurs on the first day, with progressively lesser responses after the second, third, and fourth days of exposure. However, for these same subjects, their responsiveness to MCh challenge peaked on the second day and remained elevated throughout all 5 days of exposure (Folinsbee et al., 1994).

This kind of functional adaptation to exposure disappears about a week after exposure ceases (Horvath et al., 1981; Kulle et al., 1982). The adaptation phenomenon has led some people to conclude that transient functional decrements are not important health effects. On the other hand, recent research in animals has shown that persistent damage to lung cells accumulates even as functional adaptation takes place. Tepper et al. (1989) exposed rats to 350, 500, or 1000 ppb O_3 for 2.25 h on 5 consecutive days. Carbon dioxide (8%) was added to the exposure during alternate 15-min periods to stimulate breathing and therefore increase O_3 uptake and distribution. The consequences of exposure on pulmonary function, histology, macrophage phagocytosis, BALF protein, differential cell counts, and lung tissue antioxidants were assessed. Tidal volume, frequency of breathing, inspiratory time, expiratory time, and maximal tidal flows were all affected by O_3 during days 1 and 2 at all O_3 concentrations. By day 5, these O_3 responses were completely adapted at 350 ppb and greatly attenuated at 500 ppb, but showed no signs of adaptation in the group exposed to 1000 ppb. Unlike the pulmonary function data, light microscopy indicated a pattern of progressive epithelial damage and inflammatory changes associated with the terminal bronchiole region. Over the 5-day testing period, a sustained 37% increase in BALF protein and 60% decrease of macrophage phagocytic activity were observed with exposure to 500 ppb. Differential cell counts were unchanged. Lung glutathione initially increased but return to within the control range on days 4 and 5. Lung ascorbate was significantly elevated above control on days 3–5. These data suggest that attenuation of the pulmonary functional response occurs while aspects of the tissue response reveal progressive damage.

Van Bree et al. (1989) reported the influence of exposure time per day and number of exposure days on lung biochemical and cellular responses. Seven-day exposures to 800 ppb produced a loss of normal cilia, a secretoglobin cell hypertrophy, and an increase in cytochrome P450 enzyme activity, whereas 4-day exposures produced increases in protein and hexose-6-phosphate dehydrogenase/glucose 1-dehydrogenase (aka G6PDH). In rats exposed for 2, 4, 8, or 16 days to 400 ppb O_3 for 4, 8, or 24 h/day, the quantity of antioxidant in whole-lung tissue was influenced about twice as much by the exposure duration per day as by the number of exposure days. Finally, in rats exposed to 400 ppb O_3 for 12 h at either daytime or nighttime, the effects at night, when rats were active, were much greater, once again indicating physical activity increases O_3-induced responses.

Further indications that functional adaptation does not protect against the development of pathological changes as measured in the days and weeks following exposure were provided by Farman et al. (1997). Previously Last (1989) found that rats exposed to 800 ppb O_3 and 14.4 ppm NO_2 for 6 h/day daily developed progressive bronchiolitis and pulmonary fibrosis after about 8–10 weeks of exposure, with a high level of mortality. To understand what processes were occurring during the 2- to 2.5-month period of lesion development, Farman et al. studied the time course of evolution of fibrotic lesions in rats exposed to O_3 and NO_2. Rats were sampled weekly for 9 weeks from the onset of exposure, and biochemical and histopathological evaluations were performed. They also quantified airway epithelial proliferation by *in vivo* labeling with bromodeoxyuridine (BrdU) after 4

and 8 weeks of exposure. Histopathological evaluation indicated a triphasic response temporally: inflammatory and fibrotic changes increased mildly for the first 3 weeks, stabilized or apparently decreased during weeks 4–6, and increased markedly over weeks 7–9. Biochemical quantification of lung 4-hydroxyproline (a marker of collagen turnover) content was consistent with the histopathology with no differences from controls for the first 3 weeks, followed by increases after 4–5 weeks and a stabilization after 6 weeks of exposure. Cumulative BrdU labeling indices were normal (or slightly decreased) in the small airway and alveolar epithelium after 4 weeks, followed by with significantly diminished reparative capacity after 8 weeks. The diminished reparative capacity of the bronchiolar and alveolar epithelium may be causally linked with the rapid progressive fibrosis that occurred in this model after about 7–8 weeks of exposure to O_3 plus NO_2.

The effects of multiday O_3 exposures of laboratory animals on particle clearance from the lungs and on lung infectivity were reviewed previously. They also show that O_3-induced transient effects often become greater with repetitive exposures.

Effects in humans of a multiday episode-type exposure to O_3 in ambient air were described by Lioy et al. (1985). During a study focused on daily variations in lung function among 39 children attending a summer day camp in Mendham, NJ, a summer haze episode occurred in which the daily 1-h peak O_3 concentrations exceeded 120 ppb on 4 consecutive days, with the highest concentration being 185 ppb. During the week following the episode, lung function consistently deviated from the concentration versus peak flow regressions for the individual children, indicating a persistent loss of lung function during that time. In a subsequent reanalysis of this study, Lioy and Dyba (1989) suggested that the persistence of the reduced lung function during the week following the episode was more likely due to the cumulative daily exposure than by the daily peak concentrations. In any case, the exposure episode was apparently responsible for an approximately 1-week-long shift in the function baseline. They suggested that epithelial cell death and regeneration were involved and not just a reflex airway constriction.

In summary, successive days of exposure of adults in chambers to O_3 at current high ambient levels lead to a functional adaptation in that the responses are attenuated by the third day and are negligible by the fifth day. On the other hand, a comparable functional adaptation in rats does not prevent the progressive damage to the lung epithelium. Daily exposures of animals also increase other responses in comparison with single exposures, such as a loss of cilia, secretoglobin cell hypertrophy, alterations in macrophage function, and alterations in the rates of particle clearance from the lungs.

For children exposed to O_3 in ambient air, there was a weeklong baseline shift in peak flow following a summer haze exposure of 4 days' duration with daily peak O_3 concentrations ranging from 125 to 185 ppb. Since higher concentrations used in adult adaptation studies in chambers did not produce such effects, it is possible that baseline shifts require the presence of other pollutants in the ambient air. A baseline shift in peak flow in camp children was also reported by Raizenne et al. (1989) following a brief episode characterized by a peak O_3 concentration of 143 ppb and a peak acidic aerosol concentration of 559 nmoles/m^3.

21.10 CUMULATIVE EFFECTS OF AMBIENT OZONE EXPOSURES

The chronic effects database includes a limited amount of information on human effects and a more substantial data on effects seen in laboratory animals.

21.10.1 Controlled Laboratory Exposure Studies: Adaptation of Human Responses to Peak Exposures

A study by Linn et al. (1988) provided evidence for a seasonal acclimation of lung function in Southern California. In this study, a group of subjects selected for their relatively high functional responsiveness to O_3 had much greater functional decrements following 2-h exposure to 180 ppb O_3 with intermittent exercise in a chamber in the spring than they did in the following autumn or winter. Their responses in the following spring were equivalent to those in the preceding spring. These findings suggest that some of the variability in acute response coefficients reported for earlier controlled human exposures to O_3 in chambers could have been related to seasonal variations in responsiveness, which, in turn, may be related to an acclimation to chronic O_3 exposure.

21.10.2 Epidemiological Studies of Cumulative Effects of Chronic Exposures

Epidemiologic studies of populations living in Southern California suggest that chronic oxidant exposures do affect baseline respiratory function and emergency department visits.

21.10.2.1 Baseline Respiratory Function Cross-sectional studies suggest O_3-related decrements in respiratory function. Stern et al. (1994) examined differences in the respiratory health status of schoolchildren, 7–11 years, who resided in 10 rural Canadian communities of moderate and low exposure to regional SO_4^{2-} and O_3 pollution. Five communities were located in central Saskatchewan, a low exposure region, and five were located in southwestern Ontario, an area with moderately elevated exposures resulting from long-range atmospheric transport of polluted air masses. Summertime 1-h daily O_3 maxima means were 69 ppb in Ontario and 36 ppb in Saskatchewan. Concentrations of SO_4^{2-} were three times higher in Ontario than in Saskatchewan. Levels of SO_2 and NO_2 were low in both regions, and there were no significant differences in levels of PM_{10} or particulate nitrates. After controlling for the effects of age, sex, parental smoking, parental education, and gas cooking, no significant regional differences were observed in symptoms. Children living in Ontario had mean decrements of 1.7% FVC and 1.3% $FEV_{1.0}$ compared with Saskatchewan children, without changes in pulmonary flow parameters. The differences could have been due to exposures to either O_3 or SO_4^{2-}, or their combination.

A more definitive study by Kunzli et al. (1997) regressed mid- and end-expiratory flows ($FEF_{25-75\%}$, $FEF_{75\%}$) against effective O_3 exposure. A convenience sample of 130 UC Berkeley freshmen, ages 17–21, participated twice in the same tests (residential history, questionnaire, pulmonary function), 5–7 days apart. Groups were divided into students with lifelong residence in Northern or Southern California. Monthly ambient 8- h O_3 concentrations were assigned based on the lifetime residential history and nearby monitoring data for O_3. For a 20- ppb increase (interquartile range) in 8-h O_3, end-expiratory flow $FEF_{75\%}$ decreased 14% (95% Cl: 1.0–28.3%) and mid-expiratory flow $FEF_{25-75\%}$ decreased 7.2% as compared with the population mean values. Negative confounding factors were region, gender, and ethnicity. Lifetime 8-h average O_3 ranged from 16 to 74 ppb with little overlap between regions. There was no evidence for different O_3 effects across regions. Effects were independent of lifetime mean PM_{10}, NO_2, temperature, or humidity. Effects on FEV_1 tended to be negative, whereas those for FVC, although negative in some models, were inconsistent and small. The strong relationship of lifetime ambient O_3 on end- and

mid-expiratory flows of college freshmen and the lack of association with FEV_1 and FVC are consistent with biologic models of chronic effects of O_3 in the small airways.

Other evidences for chronic effects of O_3 were subsequently reported by Schwartz (1989) based upon an analysis of pulmonary function data in a national population study in 1976–1980, that is, the second National Health and Nutrition Examination Survey (NHANES II). Using ambient O_3 data from nearby monitoring sites, O_3-associated reduction in lung function was noted for people living in areas where the annual average O_3 concentrations exceeded 40 ppb. However, more recent studies of lung function growth in cohorts of children in 12 Southern California communities suggest that ambient air pollutants other than O_3 could be more responsible for the pulmonary function effects (Gauderman et al., 2000, 2002, 2005, Avol et al., 2001).

21.10.2.2 Emergency Department Visits Malig et al. (2016) performed a time-stratified case-crossover study of 3.7 million residents in California. These investigators reported that 2005–2008 O_3 concentrations were associated with increased emergency department visits for acute respiratory infections, asthma, pneumonia, COPD, and upper respiratory tract inflammation.

21.10.2.3 Lung Structure An autopsy study of 107 lungs from 14- to 25-year-old accident victims in Los Angeles County by Sherwin and Richters (1991) reported that 27% had what were judged to be severe degrees of structural abnormalities and bronchiolitis not expected for such young subjects, while another 48% had similar, but less severe, abnormalities. In the absence of corresponding analyses of lungs of comparable subjects from communities having much lower levels of air pollution, the possible association of the observed abnormalities with chronic O_3 exposure remains speculative. Some of the abnormalities observed could have been due to smoking and/or drug abuse, although the authors noted that published work on the association between smoking and small airway effects showed lesser degrees of abnormality (Cosio et al., 1980).

21.10.2.4 Development of Chronic Disease The effects of chronic exposure to O_3 and PM were followed for 10 years in a prospective cohort study by Abbey et al. (1995) in 6340 nonsmoking Seventh-Day Adventists living in California. Ambient air monitoring data were available for O_3, TSP, SO_4^{2-}, NO_2, and SO_2. O_3 was associated with increasing severity of asthma and with the development of asthma in males. Measured TSP and SO_4^{2-} and estimated $PM_{2.5}$ and PM_{10} were associated with the development of airway obstructive disease, chronic bronchitis, and asthma, and these were not confounded by the presence of the gaseous pollutants. No significant associations were found for NO_2 or SO_2.

21.10.2.5 Effect on Longevity The early evidence for an effect of O_3 on longevity was largely negative. Mendelsohn and Orcutt (1979) in a study utilizing the Public Use Sample containing data on two million individuals in the United States obtained both death certificate data and air pollution network data in eight U.S. regions. Consistent associations with mortality were found for SO_4^{2-}, and weaker and less consistent associations were seen for SO_2 and CO. No significant associations were found for O_3 or NO_2.

More recent multi-pollutant studies of the associations of annual mortality rates with long-term ambient air concentration of O_3 include the six cities study of Dockery et al. (1993), the American Cancer Society (ACS) studies of Pope et al. (2002) and Turner et al.

(2016), and the study of Crouse et al. (2015) of the Canadian Census Health and Environment Cohort. In the six cities prospective cohort study of 8111 adults over 14–16 years, consistent mortality effects were found for $PM_{2.5}$ and SO_4^{2-}, with smaller effects indicated for TSP, SO_2, and NO_2. The variations in O_3 were too small across the six cities. In the Pope et al. (2002) ACS study, associations of premature mortality were found with $PM_{2.5}$, SO_4^{2-}, and SO_2, but not with O_3.

In a later analysis of the ACS cohort by Turner et al. (2016) using updated mortality data and O_3 concentration estimates at the residences of cohort members, they observed positive associations between O_3, $PM_{2.5}$, and NO_2 concentrations and all-cause and cause-specific mortality. In two-pollutant models adjusted for $PM_{2.5}$, positive associations remained between O_3 and all-cause [hazard ratio (HR) per 10 ppb = 1.02; 95% CI: 1.01–1.04], circulatory (HR = 1.03; 95% CI: 1.01–1.05), and respiratory (HR = 1.12; 95% CI: 1.08–1.16) mortality that were unchanged with further adjustment for NO_2. Subsequently, Malley et al. (2017) extended the approach used by Turner et al. (2016) to data for respiratory mortality in adults >30 years of age using O_3 estimates using the GEOS-Chem model in various world regions and reported that the estimates were larger in other regions than in the United States.

Crouse et al. (2015) classified exposure estimates for $PM_{2.5}$, O_3, and NO_2 by postal code estimates for 2.5 million Canadians. For $PM_{2.5}$, O_3, and NO_2, the interquartile ranges IQRs were only 5.8, 9.9, and 10.5 $\mu g/m^3$. Despite the low concentration ranges, there were associations for these pollutants for cardiovascular disease, diabetes, and IHD mortality, with the highest for IHD.

Based on the increment in evidence of statistically significant associations between long-term mean O_3 concentrations and increases rates of annual mortality in very large cohort studies around the world, it now appears that chronic O_3 exposure is associated with reduce longevity, albeit to a considerably lesser extent than for $PM_{2.5}$.

21.11 CONTROLLED LABORATORY EXPOSURE STUDIES: ANIMAL RESPONSES

Most of the inhaled O_3 penetrates beyond the proximal sites in the airways that trigger the functional responses. In this distal region of the lung, at and just beyond the terminal bronchioles, the effects produced by O_3 include changes in biochemical indices, lung inflammation, and airway structure. Furthermore, the effects of O_3 exposure in this region appear to be cumulative and persistent, even in animals that have acclimated to the exposure in terms of respiratory mechanics (Frank et al., 2001)

In a series of inhalation studies, groups of rats were exposed to 120 or 250 ppb O_3 for 12 h/day for 6 and 12 weeks or to a daily cycle with a baseline of 60 ppb for 15 h with a broad peak for 8 h averaging 180 ppb for a period of 3–12 weeks. Hyperplasia of type I alveolar cells in the proximal alveoli was linearly related to the cumulative O_3 dose (Huang et al., 1988).

The highest O_3 dose is received at the pulmonary acinus, where the terminal bronchioles lead into alveolar ducts, and studies have found that the effects of inhaled O_3 on lung structure are also greatest in this region. Barry et al. (1985) reported that significant changes occurred in the alveoli just distal to the terminal bronchioles in rats exposed to 120 or 240 ppb O_3 for 12 h/day for 6 or 12 weeks. In both juvenile and adult rats, alveolar type I and type II epithelial cell number increased, and the interstitium and endothelium were altered.

Raub et al. (1983) reported increased vital capacity and end-expiratory volume that suggested alterations in distensibility of the lung tissue of rats with the equivalent exposure.

Barry et al. (1988) reported that 250 ppb O_3 exposure for 6 weeks produced alterations in the surface characteristics of ciliated and nonciliated (secretoglobin) cells in both groups. The surface area contributed by ciliated cells decreased (20–30%), the luminal surface of secretoglobin cells decreased (16–25%), and the number of brush cells per square millimeter of terminal bronchiolar basement membrane decreased. Thus, the normal structure of terminal bronchiolar epithelial cells was significantly altered. No interactions between the effects of O_3 and animal age at the beginning of the exposure were found, w ith a daily cycle with a baseline of 60 ppb for 13 h with a 5 day/week broad peak for 9 h averaging 180 ppb and containing a 1-h maximum of 250 ppb for a period of 3 or 12 weeks. Combining the results of these tests with the 6-week studies, Huang et al. (1988) and Chang et al. (1991) reported that hyperplasia of type I alveolar cells in the proximal alveoli was linearly related to the cumulative O_3 exposure in the four groups. Thus, there is, to date, no evidence for a threshold for cumulative lung damage.

Rats exposed for 6 weeks to clean air or to O_3 using the daily cyclic exposure regimen used by Huang et al. (1988) were exposed once for 5 h to an asbestos aerosol by Pinkerton et al. (1989). When sacrificed 30 days later, the fiber count in the lungs of the O_3-exposed animals was three times greater than in the sham-exposed animals. Thus, subchronic O_3 exposure can increase the effective dose of insoluble particles, which may have toxic and/or carcinogenic effects.

In rats exposed for 12 months by Grose et al. (1989) to the daily cycle used by Huang et al. (1988), the rate of lung nitrogen washout increased. RV and total lung capacity decreased. Glutathione peroxidase and reductase activities increased, but pulmonary superoxide dismutase was unchanged. Following exposure, BALF α-tocopherol decreased, ascorbic acid and protein increased, and cell number and differential were unchanged as compared with control. Immunological changes were not observed. Thus, 12 months of O_3 exposure caused (1) functional lung changes indicative of a "stiffer" lung, (2) biochemical changes suggestive of increased antioxidant metabolism, and (3) no observable immunological changes.

In a follow-up study in which the same exposure cycle was extended for up to 78 weeks, Tepper et al. (1991) found small, but statistically significant, changes in breathing patterns and mechanics in unanesthetized, restrained rats at weeks 1, 3, 13, 52, and 78 during post-exposure challenge with 0, 4, and 8% carbon dioxide (CO_2). The data indicate that O_3 exposure caused an overall increase in expiratory resistance (R_c), but particularly at 78 weeks. The spontaneous frequency of breathing and CO_2-induced hyperventilation decreased. The decreased frequency was dependent on a significant increase in the inspiratory time relative to control without a change in expiratory time. However, light microscopic evaluation of the lung did not reveal any lesions associated with O_3 exposure.

Chang et al. (1992) extended the analyses of animals exposed for 78 weeks to electron microscopic morphometry. Samples from proximal alveolar regions and terminal bronchioles were obtained by microdissection. Analysis of the proximal alveolar region revealed a biphasic response. Acute tissue reactions after 1 week of exposure included epithelial inflammation, interstitial edema, interstitial cell hypertrophy, and influx of macrophages. These responses subsided after 3 weeks of exposure. Progressive epithelial and interstitial tissue responses developed with prolonged exposure and included epithelial hyperplasia, fibroblast proliferation, and interstitial matrix accumulation. The epithelial responses involved both type I and type II epithelial cells. Alveolar type I cells increased in number,

became thicker, and covered a smaller average surface area. These changes persisted throughout the entire exposure and did not change during the recovery period, indicating the sensitivity of these cells to injury. The main response of type II epithelial cells was cell proliferation. The accumulation of interstitial matrix after chronic exposure consisted of deposition of both increased amounts of basement membrane and collagen fibers. Interstitial matrix accumulation underwent partial recovery during follow-up periods in air; however, the thickening of the basement membrane did not resolve. Analysis of terminal bronchioles found that short-term O_3 exposure caused a loss of ciliated cells and differentiation of preciliated and secretoglobin cells. The bronchiolar cell population stabilized on continued exposure; however, chronic exposure resulted in structural changes, suggesting injury to both ciliated and secretoglobin cells. Thus, chronic low-level O_3 exposure caused epithelial inflammation and interstitial fibrosis in the proximal alveolar region and bronchiolar epithelial cell injury in rats. The effects in distal lung observed in rodents may be considered relevant to human health, in view of our knowledge that humans receive even greater local doses of O_3 in the vicinity of the acinus than rats at the same O_3 exposure concentration.

Studies at relatively low O_3 concentrations have also been done in monkeys. Hyde et al. (1989) exposed rhesus monkeys to 150 or 300 ppb O_3 for 8 h/day for 6 or 90 days. In the nose, responses included ciliated cell necrosis, shortened cilia, and secretory cell hyperplasia with less stored glycoconjugates. Respiratory bronchiolitis was observed at 6 days and persisted to 90 days of exposure. Even at the lower concentration of 150 ppb O_3, nonciliated bronchiolar cells hypertrophy increased in respiratory bronchioles.

For some chronic effects, intermittent exposures can produce greater effects than those produced by a continuous exposure regime that results in higher cumulative exposures. For example, Tyler et al. (1988) exposed two groups of 7-month-old male rhesus monkeys to 250 ppb O_3 for 8 h/day either daily or, in the seasonal model, on days of alternate months during a total exposure period of 18 months. A control group breathed only FA. Monkeys from the seasonal exposure model, but not those exposed daily, had significantly increased total lung collagen content, chest wall compliance, and inspiratory capacity. All monkeys exposed to O_3 had respiratory bronchiolitis with significant increases in related morphometric measures. The only significant morphometric difference between seasonal and daily groups was in the volume fraction of macrophages. Even though the seasonally exposed monkeys were exposed to the same concentration of O_3 for only half as many days, they had larger biochemical and physiological alterations and equivalent morphometric changes as those exposed daily. Lung growth was not completely normal in either exposed group. Thus, long-term effects of intermittent (seasonal) oxidant air pollutants may be more dependent on the sequence of polluted and clean air than on the total number of days of pollutant exposure. Therefore, the estimation of the risks of human exposure to seasonal air pollutants from effects observed in animals exposed daily may underestimate long-term pulmonary damage. The effects observed with an intermittent protocol may be considered relevant to human health, in view of our knowledge that humans receive even greater local doses of O_3 in the vicinity of the acinus than rats.

A number of other interesting chronic exposure studies have been done in animals with O_3 concentrations in the range of 300–1000 ppb. Those that appear to provide useful insights into mechanisms of action will also be briefly reviewed.

Sherwin and Richters (1985) exposed newborn Swiss-Webster mice to intermittent 300 ppb O_3 for 7 h/day, 5 days/week for 6 weeks. O_3 exposure increased cell and wall measurements. In contrast to results previously reported for adult animals (Sherwin et al., 1983), there was a greater increase in mean type II cell area than in numbers of type II cells.

Effects on the type II cell population implicate damage to the type I alveolar lining cells. The increases in alveolar wall measurements that were found in both the adult and developing mouse lung imply an alteration of the lung scaffolding, raising the question of impaired regeneration of the epithelial lining.

The results of a chronic exposure study in rats by Gross and White (1987) again illustrate the importance of exposure pattern on the magnitude of the response. They exposed Fischer 344 rats to 500 ppb O_3 for 20 h/day, 7 days/week for 52 weeks. This protocol produced only mild functional changes (FRC, RV, and DL_{CO}), which returned to normal during 3 months of recovery. Grose et al. (1989) using a 23-h exposure ranging from 60 ppb to a peak 1-h maximum of 250 ppb for 5 days/week produced comparable functional changes at 1 year. Thus, as in the comparison of Tyler et al. (1988) in monkeys, intermittent exposures, modeled after realistic human exposure conditions, can produce much greater responses per unit dose than continuous exposure at high concentration. These results suggest that the damage results, at least in part, from the repeated attempts to adapt to the irritant challenge as well as to the direct effects of the irritant exposure.

To characterize the response of respiratory bronchioles to chronic high ambient levels of O_3, Moffatt et al. (1987) exposed bonnet monkeys 8 h/day for 90 days to 400 or 640 ppb O_3. Significant changes in respiratory bronchioles following exposure included (1) a thicker wall and a narrower lumen, (2) a thicker epithelial compartment and a much thicker interstitial compartment, (3) shifts in epithelial cell populations with many more nonciliated bronchiolar epithelial cells and fewer squamous type I epithelial cells, (4) larger nonciliated bronchiolar epithelial cells with a larger complement of cellular organelles associated with protein synthesis, (5) greater amounts of both interstitial fibers and amorphous ground substance, (6) greater numbers of interstitial smooth muscle cells per epithelial basal lamina surface area, and (7) greater volumes of interstitial smooth muscle, macrophages, mast cells, and PMNs per epithelial basal lamina surface area. These observations imply that chronic O_3 exposure causes a concentration-dependent reactive peribronchiolar inflammatory response and an adaptive response consisting of hypertrophy and hyperplasia of nonciliated bronchiolar epithelial cells.

Fujinaka et al. (1985) quantitated the response of respiratory bronchiolar epithelium and peribronchiolar connective tissue (PCT) to chronic exposure to high ambient levels of O_3. Adult male bonnet monkeys were exposed 8 h daily for 1 year to either 640 ppb or FA. Significant exposure-related changes were greater volume of RB in the lung, smaller diameter of RB lumen, thicker media and intima of peribronchiolar arterioles, thicker RB epithelium, and thicker PCT. Cellular numerical density increased in cuboidal bronchiolar cells and decreased in type I pneumocytes. Cell volume increases occurred in cuboidal bronchiolar, ciliated, and type II cells. PCT changes included more amorphous extracellular matrix, PMNs, and lymphocytes/plasma cells. It was concluded that centriacinar changes caused by exposure to long-term high ambient O_3 in bonnet monkeys result in narrowing of RBs primarily by peribronchiolar inflammation (inflammatory cells, fibers, amorphous matrix) and secondarily through hyperplasia of cuboidal bronchiolar cells.

Chronic O_3 exposure effects on lung collagen cross-linking were investigated by Reiser et al. (1987) in two groups of juvenile cynomolgus monkeys exposed to 610 ppb for 8 h/day for 1 year. Lung tissue was obtained immediately after the O_3 exposure or 6 months after the O_3 exposure, in which the monkeys were exposed to FA. Previous studies of these monkeys had revealed that lung collagen increased in both exposed groups (Last et al., 1984). In this study, the changes in the group killed at the termination of exposure were characteristic of those seen in lung tissue in the acute stage of experimental pulmonary fibrosis. Although the changes seen in the post-exposure group suggested, to the authors,

that the lung collagen being synthesized at the time the animals were killed was normal, "abnormal" collagen synthesized during the period of O_3 exposure was irreversibly deposited in the lungs. This study suggests that long-term exposure to relatively low levels of O_3 may cause irreversible changes in lung collagen structure.

Barr et al. (1988) exposed rats to either FA or 950 ppb O_3 8 h/day for 90 days and examined the centriacinar region of lungs morphologically and morphometrically. After O_3 exposure, terminal bronchiole luminal diameter decreased, but total terminal bronchiole volume was unchanged. The most notable change was a 3.4-fold increase in respiratory bronchiolar volume, which may have been related to changes in the centriacinar alveolar duct. Morphologic parameters supporting this conclusion included the presence of fused basement membrane beneath reactive respiratory bronchiolar epithelium, the presence of similar basal laminar changes in both the respiratory bronchioles and proximal alveolar duct septal tips, and the observation that most severe epithelial damage and inflammation occurred in the most proximal alveolar duct rather than in the terminal bronchiole. The severe injury within the acinus shifts distally as respiratory bronchiolar segments are formed. Hence, most of the damage occurs at the tips of alveolar septa at the respiratory bronchiolar–alveolar duct junction.

The issue of the effects of repetitive daily O_3 exposure during lung development was investigated by Tyler et al. (1987) in studies in 28-day-old rats exposed to FA or to 640 or 960 ppb O_3, 8 h/night, for 42 nights. A second control group was fed *ad libitum* and exposed to only FA. Half the rats were studied at the end of the 42-night exposures, the rest after a 42-day post-exposure period during which all rats breathed FA. Rats examined at the end of the exposure period had larger saline and fixed lung volumes. These larger lungs had greater volumes of parenchyma, alveoli, and respiratory bronchioles. Some of these changes persisted throughout a 42-day post-exposure period. Thus, O_3 inhalation by young rats alters lung growth and development in ways likely to be detrimental, and those changes persist after O_3 inhalation stops.

In summary, chronic exposures to ambient air appear to produce a functional adaptation that persists for at least a few months after the end of the O_3 season but dissipates by the spring. Several population-based studies of lung function indicate that there may be an accelerated aging of the lung associated with living in communities with persistently elevated ambient O_3, but the limited ability to accurately assign exposure classifications of the various populations in these studies makes a cautious assessment of these data prudent.

The plausibility of accelerated aging of the human lung from chronic O_3 exposure is greatly enhanced by the results of a series of chronic animal exposure studies in rats and monkeys, especially those in rats of Huang et al. (1988) and Grose et al. (1989) using a daily cycle with a 180 ppb average over 9 h superimposed on a 13-h base of 60 ppb and those in monkeys of Hyde et al. (1989) and Tyler et al. (1988) using 8 h/day of 150 and 250 ppb. The persistent cellular and morphometric changes produced by these exposures in the terminal bronchioles and proximal alveolar region and the functional changes consistent with a stiffening of the lung reported by Raub et al. (1983) and Tyler et al. (1988) are certainly consistent with the results of the epidemiological studies.

21.12 STANDARDS AND EXPOSURE GUIDELINES

The Occupational Safety and Health Administration's (OSHA) permissible exposure limit (PEL) for O_3 is 100 ppb, equivalent to 235 $\mu g/m^3$, as a time-weighted average for 8 h/day, along with a short-term exposure limit of 300 ppb for 15 min (U.S. DOL, 1989). The

American Conference of Governmental Industrial Hygienists (ACGIH, 2017) threshold limit value (TLV) for occupational exposure is 100 ppb as an 8-h time-weighted average for light work, 80 ppb for moderate work, and 50 ppb for heavy work. For a 2-h exposure at any workload, it is 200 ppb.

Health effects among the general community were first reported among high school athletes in California, in terms of lesser performance on high-exposure days (Wayne et al., 1967). The initial primary (health-based) NAAQS, established by the EPA in 1971, was 80 ppb of total oxidant as a 1-h maximum not to be exceeded more than once per year. The NAAQS was revised in 1979 to 120 ppb of O_3 as a 1-h maximum not to be exceeded more than four times in 3 years. This initial revision was based on the finding of DeLucia and Adams (1977) that exercising asthmatic adults exposed for 1 h to 150 ppb in a test chamber had increased cough, dyspnea, and wheezing, along with small but nonsignificant reductions in pulmonary function (U.S. EPA, 1986). A small margin of safety was applied to protect against adverse effects not yet uncovered by research and effects whose medical significance is a matter of disagreement.

EPA's review of the 1979 O_3 NAAQS began in 1983, and it decided, in March 1993, to maintain the existing standard, and to proceed, as rapidly as it deemed possible, with the next round of review. In July of 1997 the EPA Administrator promulgated a revised primary O_3 NAAQS of 80 ppb as an 8-h time-weighted average daily maximum, with no more than four annual exceedances, as averaged over 3 years (*Federal Register* 1997, 62:38762–38896). The change from one allowable annual exceedance to four was to minimize the designation of NAAQS non-attainment in a community that was triggered by rare meteorological conditions especially conducive to O_3 formation. The switch to an 8-h averaging time recognized that ambient O_3 in much of the United States has broad daily peaks and that human responses are more closely related to total daily exposure than to brief peaks of O_3 exposure.

In setting the 1997 NAAQS, the effects of concern with respect to acute response in the population at large were reductions in medically relevant lung function and increases in respiratory symptoms, airway reactivity, airway permeability, and airway inflammation. For persons with asthma, there are also increased rates of medication usage, as well as restriction in activities. Margin-of-safety considerations included (1) the influence of repetitive elicitation of such responses in the progression of chronic damage to the lung of the kinds seen in chronic exposure studies in rats and monkeys and (2) evidence from laboratory and field studies that ambient air co-pollutants potentiate the responses to O_3. The basis for these concerns is discussed later in this chapter.

In the process of considering a revision of the NAAQS for O_3 and related photochemical oxidants for 2007, the EPA Staff recommendations, contained in their July 2006 second draft of their Staff Paper, set forth two options with regard to revising the level and form of the standard: (1) retain the primary 8 h NAAQS of 0.08 ppm or (2) consider a reduction in level to 0.07 ppm. An external scientific advisory committee that was mandated by the Clean Air Act Amendments of 1977, the Clean Air Scientific Advisory Committee (CASAC), reviewed the Staff Paper for the NAAQS for ozone concluded in its October 20, 2006, letter: (1) there was no scientific justification for retaining the current primary 8-h NAAQS of 0.08 ppm, and (2) the primary 8-h NAAQS needs to be substantially reduced to protect human health, particularly in sensitive subpopulations. CASAC went on to recommend a range of 0.060–0.070 ppm for the primary NAAQS. It also endorsed the Staff Paper recommendation in support of the creation of an alternative secondary NAAQS to prevent damage to vegetation with a cumulative form that extends over an entire growing season.

In February 2013, the Integrated Science Assessment for Ozone and Related Photochemical Oxidants (EPA 600/R-10/07 6F) was released. On October 26, 2015, based on EPA's review of the air quality criteria for ozone (O_3) and related photochemical oxidants and for O_3, EPA announced a revision of the levels of both standards, with the primary and secondary ozone standard levels being reduced to 0.070 parts per million (ppm) and retaining their indicator (O_3) form (fourth highest daily maximum, averaged across 3 consecutive years) and averaging times (8 h).

The World Health Organization-Europe (WHO-EURO) issued air quality guidelines in 1987, 2000, and 2005, and the International WHO has adopted the 2000 and 2005 guidelines for the world as a whole as guidance to the member states that wish to establish national standards (WHO-EURO, 2017). The 2005 WHO guidelines, which were intended to be used worldwide, and not only in developed countries, were structured differently than those developed in 1987 and 2000 by WHO-EURO. Table 21.3 describes the guideline and interim targets for the daily maximum 8-h mean concentration. The higher interim targets were offered for those countries where concentrations currently greatly exceed the guidelines to demonstrate that important public health benefits could be achieved with phased progress in lowering exposures.

21.13 SUMMARY AND CONCLUSIONS

The apparently reversible respiratory effects that follow acute O_3 exposures lasting from 5 min to 6.6 h include changes in lung capacity, flow resistance, epithelial permeability, and reactivity to bronchoactive challenges. These effects may persist for many hours or days after the exposure ceases. Repetitive daily exposures over several days or weeks can exacerbate and prolong these effects.

Most of the data available on transient respiratory functional effects of O_3 were obtained from controlled human exposure studies and field studies of limited duration. Such studies can provide information on chronic pollutant effects only to the extent that prior exposures affect the transient response to single-exposure challenges. Furthermore, interpretation of the results of such tests is limited by our generally inadequate ability to characterize the nature and/or magnitude of the prior chronic exposures. Data on other effects of O_3 exposures on short-term responses is limited to epidemiological studies that implicate increased daily mortality due to cardiovascular and central nervous system responses, which are supported by long-term cumulative O_3 exposures implicating increased annual mortality. These important health impacts are summarized in Table 21.3 from the 2010 O_3 Integrated Science Assessment (U.S. EPA, 2013).

Epidemiological studies offer the prospect of establishing chronic health effects of long-term O_3 exposure in relevant populations and offer the possibility that the analyses can uncover the influence of other environmental factors on responses to O_3 exposure. On the other hand, the strengths of any association may be difficult to firmly establish because of the complications introduced by uncontrolled cofactors, which may confound or obscure the underlying causal factors.

One convenient and efficient way to study mechanisms and patterns of response to inhaled O_3 and of the influence of other pollutants and stresses on these responses is by controlled exposures of laboratory animals. One can study the transient functional responses to acute exposures and establish the interspecies differences in response among different animal species and between them and humans similarly exposed. One can also investigate

responses that require highly invasive procedures or serial sacrifice and gain insights that cannot be obtained from studies on human volunteers. Finally, one can use long-term exposure protocols to study cumulative responses and the pathogenesis of chronic disease in animals. Other advantages of studies on animals are the ability to examine the presence and basis for variations in response that are related to age, sex, species, strain, genetic factors, nutrition, the presence of other pollutants, and so on.

Another approach, which may prove to be more effective in the end, is to continue and expand fundamental mechanistic research along approaches summarized in Fig. 21.10.

Among the significant limitations to the use of exposure–response data from animal studies in human risk assessments is our quite limited ability to interpret the responses in relation to likely responses in humans who might be exposed to the same or lower levels. Controlled chronic exposure protocols can be very expensive, limiting the number of variables that can effectively be examined in any given study.

For studies focused on the biochemical mechanisms of epithelial cell responses to O_3, cells can be harvested from humans or animals and exposed to O_3 *in vitro*. Interspecies comparisons of cellular response can often be made, and relatively few animals can provide much study material. However, our ability to interpret the results of *in vitro* assays in relation to likely effects in humans *in vivo* is quite limited, even when the studies are done with human cells. The cellular response *in vitro* may differ from that of the same cells *in vivo* due to many factors including cell–cell interaction. The *in vivo* controls on cellular metabolism and function, which may play a significant role in the overall response, also are absent.

In terms of functional effects, we know that single O_3 exposures to healthy nonsmoking young adults at concentrations in the range of 80–200 ppb produce a complex array of pulmonary responses including decreases in respiratory function and athletic performance and increases in symptoms, airway reactivity, PMN content in BALF, and rate of mucociliary particle clearance. Responses to O_3 in purified air in chambers occur at concentrations

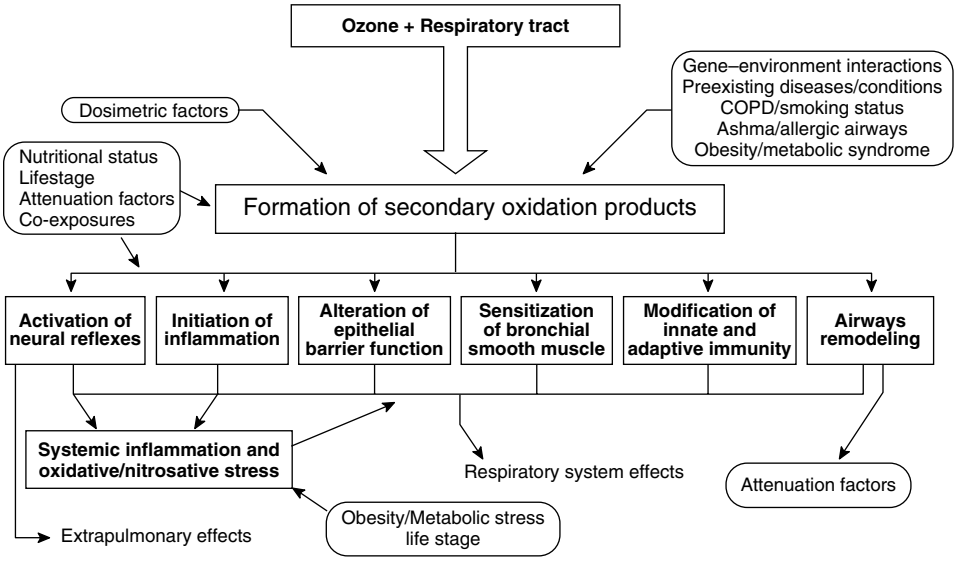

FIGURE 21.10 Some factors that likely contribute to the interindividual variability in responses to inhaled O_3. *Source*: From U.S. EPA (2015).

of 80 or 100 ppb when the exposures involve moderate exercise over 6 h or more and require concentrations of 180 or 200 ppb when the duration of exposure is 2 h or less. On the other hand, mean FEV_1 decrements >5% have been seen at 100 ppb of O_3 in ambient air for children at summer camps and for adults engaged in outdoor exercise for only 1/2 h. The apparently greater responses to O_3 in ambient air may be related to the presence of, or prior exposures to, acidic aerosol, but further investigation of this hypothesis is needed.

Further research is also needed to establish the interrelationships between small transient functional decrements, such as FEV_1, PEFR, and mucociliary clearance rates, which may not, in themselves, be adverse effects, and changes in symptoms, performance, reactivity, permeability, and PMN counts. The latter may be more closely associated with adversity or in the accumulation or progression of chronic lung damage.

Successive days of exposure of adults in chambers to O_3 at current high ambient levels lead to a functional acclimation in that the responses are attenuated by the third day and are negligible by the fifth day. On the other hand, a comparable functional acclimation in rats does not prevent the progressive damage to the lung epithelium. Daily exposures of animals also increase other responses in comparison with single exposures, such as a loss of cilia, secretoglobin cell hypertrophy, alterations in macrophage function, and alterations in the rates of particle clearance from the lungs.

Human exposures to ambient air appear to produce a functional acclimation that persists for at least a few months after the end of the O_3 season but dissipates by the spring. Several population-based studies of lung function indicate that living in communities with persistently elevated ambient O_3 may be associated with aging of the lung. However, the limited ability to accurately assign exposure classifications of the various populations in these studies makes a cautious assessment of these provocative data prudent.

The plausibility of accelerated aging of the human lung from chronic O_3 exposure is greatly enhanced by the results of a series of chronic animal exposure studies in rats and monkeys. There is little reason to expect humans to be less sensitive than rats or monkeys. On the contrary, humans have a greater dosage delivered to the respiratory acinus than rats for the same exposures. Another factor is that the rat and monkey exposures were to confined animals with little opportunity for heavy exercise. Thus, humans who are active outdoors during the warmer months may have greater effective O_3 exposures than the test animals. Finally, humans are exposed to O_3 in ambient mixtures. The potentiation of the characteristic O_3 responses by other ambient air constituents seen in the short-term exposure studies in humans and animals may also contribute toward the accumulation of chronic lung damage from long-term exposures to ambient air containing O_3.

Until recently, there was only limited evidence on chronic effects of ambient O_3 exposures on humans. In the recent analyses of the association of annual mortality with chronic exposure to O_3 in the ACS cohort by Turner et al. (2016), of 2.5 million Canadians by Crouse et al. (2015), and of Malley et al. (2017) on respiratory mortality using the GEOS-Chem model in various world regions, it now appears that chronic O_3 exposure does reduce longevity, albeit to a considerably lesser extent than $PM_{2.5}$.

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22

PESTICIDES

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Synthetic pesticides are a diverse group of chemical compounds, used to control insects, unwanted plants, fungi, rodents, and other pests. Hundreds of pesticide active ingredients are currently registered for use. These compounds are blended with "inert" ingredients to produce thousands of commercial pesticide formulations. In 2001, the most recent year for which data are available, the United States used over 1.1 billion pounds of pesticide active ingredients, and worldwide use was of 5.2 billion pounds (Grube et al., 2011). Pesticides occupy a unique position among chemicals in that they are deliberately added to the environment for the purpose of killing or injuring some form of life. Their injurious actions, however, are not always highly specific for undesirable targets, and many pesticides are often toxic to many nontarget species, including humans.

Pesticides are often classified on the basis of the target species they act on. The major classes (and their target pests) are the insecticides (insects), herbicides (weeds), fungicides (fungi, molds) and rodenticides (rodents). Within each class there are several subclasses, with substantially different chemical and toxicological characteristics (Table 22.1). For example, among insecticides there are organophosphates (OPs) and carbamates, organochlorines, pyrethroids, neonicotinoids, and many other chemicals. The agricultural sector is the primary consumer of pesticides, accounting for about 75% of use by volume. Industrial, commercial, and governmental users and home and garden users account for the remainder.

22.1 USES OF PESTICIDES

In many parts of the world, excessive loss of food crops to insects or other pests may contribute to possible starvation. In developed countries, pesticides allow production of abundant, inexpensive, and attractive fruits, vegetables, and grains. Along with insecticides, herbicides and fungicides play a major role in this endeavor, as pests have been estimated to cause 27–42% loss in production of major crops around the world, and this

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TABLE 22.1 Major Classes of Pesticides

Class	Subclass	Examples
Insecticides	Organophosphates	Chlorpyrifos, diazinon
	Carbamates	Propoxur, carbaryl
	Pyrethroids	Allethrin, deltamethrin
	Neonicotinoids	Imidacloprid, acetamiprid
	Organochlorines	DDT, lindane
	Phenylpyrazoles	Fipronil
	Bio-insecticides	*Bacillus thuringiensis*
	Rotenoids	Rotenone
Herbicides	Chlorophenoxy compounds	2,4-D
	Bipyridyl compounds	Paraquat
	Triazines	Atrazine
	Phosphonomethyl amino acids	Glyphosate
Fungicides	Dithiocarbamates	Zineb, thiram
	Benzimidazoles	Benomyl
	Benzonitriles	Chlorothalonil
	Chloroalkylthio compounds	Captan
Rodenticides	Anticoagulants	Warfarin, brodifacoum
	Thioureas	ANTU
	Inorganic compounds	Zinc phosphide

would rise to 48–83% without crop protection with pesticides (Oerke, 2006). Loss of harvested crops by post-harvest infestation by insects, fungi, and rodents is also a major problem that is dealt with by the use of fumigants and other pesticides. Herbicides find useful application in forestry, during reforestation, as well as the clearing of pests from roadways, train tracks, and utilities' rights of way. In the urban setting, pesticides find multiple uses in the home and garden area, to control insects, weeds, and other pests. Pesticides also play a major role in the control of vector-borne diseases, such as malaria, filariasis, yellow fever, viral encephalitis, typhus, and many others, which are a major threat to the health of large human populations (Novak and Lampman, 2001).

The use of pesticides has increased significantly from the 1950s to the 1980s, but in the past few decades it has actually reached a plateau in terms of quantity of active ingredients (Grube et al., 2011). This is due to the availability of more efficacious compounds that require less active ingredient (up to 100-fold less) to obtain the same degree of pest control (Lamberth et al., 2013). The introduction of integrated pest management approaches and the increased popularity of organic farming have also contributed to a decrease and/or stabilization of pesticide use in the developed countries. In the United States, almost half of the pesticides used are herbicides, while in other countries, particularly in Africa, Asia, and Central America, there is also a substantial use of insecticides.

22.2 HISTORY OF PESTICIDES

Pesticides have been used to a limited degree since ancient times, as reference to various preparations, particularly sulfur or arsenic, can be found in several publications dating back more than 25 centuries (Costa, 1987). *Veratrum album* and *V. nigrum*, two species of false

hellebore, were used by the Romans as rodenticides. Tobacco has been used as contact insecticide since the seventeenth century. Copper compounds were known since the early 1800s to have fungicidal value, and a hydrated lime and copper sulfate mixture was first used in France in the late 1800s. Until the 1930s, pesticides were of natural origins or inorganic compounds; arsenicals, sulfur, nicotine, rotenone, and mercury compounds were the primary chemicals used as pesticides. In 1939 the Swiss chemist Paul Mueller found that DDT (dichlorodiphenyltrichloroethane) had insecticidal properties. DDT was commercialized in 1942 and was used extensively and successfully for the control of typhus epidemics and particularly of malaria. Several other chlorinated hydrocarbon insecticides (e.g., lindane, chlordane, aldrin) were developed and commercialized in the following years. Though some organophosphorus compounds had been synthesized as early as 1820, Gerhard Schrader, a chemist at IG Farbenindustrie in Germany, is considered the "father" of modern organophosphorus insecticides. He synthesized several thousand molecules, and one (code name E605) was eventually introduced into the agricultural market under the trade name parathion, to become one of the most widely employed insecticides in this class. During those years, compounds of much greater toxicity than parathion were also synthesized as potential chemical warfare agents, among these being sarin, soman, and VX. The mechanism of action of OPs, that is, inhibition of acetylcholinesterase, was soon discovered, primarily by knowledge of the effects and mechanism of action of the carbamate physostigmine. Carbamate insecticides were introduced in the early 1950s. Pyrethrum flower and extracts had been used as insecticides for several centuries, and pyrethrins were characterized at the beginning of the twentieth century. Synthetic pyrethroids were developed in the early 1970s, while several other classes of insecticides (e.g., neonicotinoids, *N*-phenylpyrazoles) have been developed more recently (Casida, 2015; Casida and Durkin, 2017). Dozens of herbicides, fungicides, and rodenticides have also been developed in the past several decades (Casida and Durkin, 2017). Thioureas and anticoagulants such as warfarin were developed as rodenticides in the mid to late 1940s. A few years later, two important fumigants were introduced, 1,2-dichloropropene and methyl bromide. In the 1950s, chlorophenoxy compounds were developed as herbicides, together with the fungicides captan. Other important herbicides (e.g., triazines, paraquat), all widely used herbicides, were introduced in the 1960s, and so did the important class of dithiocarbamate fungicides, while the herbicide glyphosate (now the most sold pesticide worldwide) was introduced in the mid-1970s.

22.3 EXPOSURE TO PESTICIDES

Exposure to pesticides may occur through the skin, by inhalation, or by ingestion. Exposure may occur via the diet, in the workplace, in the yard or home, and in the community. In assessing exposure, it is important to understand that persons may simultaneously be exposed to multiple pesticides through several routes and that the effects of these multiple exposures may be additive or even synergistic (NRC, 1993). **Occupational exposure** to pesticides occurs among manufacturers and formulators; during transport and storage; among mixers, loaders, and applicators working in fields, green-houses, parks, and residential buildings; among vector control and structural applicators; and among farm workers entering fields or greenhouse workers handling foliage previously sprayed by pesticides (McConnell, 1994; Blondell, 1997). Crop duster aviation mechanics have also been reported to be at high risk for pesticide poisoning. Other groups occasionally exposed include emergency crews or sewer workers involved in cleanup. In developed countries, a

very large exposed group consists of building maintenance workers who apply insecticides in public and private housing, schools, hospitals, and commercial structures. **Environmental exposure** to pesticides can occur through ingestion of contaminated water and pesticide residues in food, inhalation of airborne spray drift, and dermal exposure to pesticides applied in the home, school, or community or from exposure to improperly disposed hazardous waste. The heaviest documented use of pesticides in the home occurred in inner-city neighborhoods for the control of roaches in apartments; for example, in New York State, the heaviest use of pesticides in all counties statewide occurred in Manhattan and Brooklyn (Landrigan et al., 1999). Seasonal contamination of drinking water by herbicides has been reported each spring in the American Midwest, a pattern that coincides with annual application of these compounds, atrazine in particular, prior to spring planting. Although nonoccupational exposure is usually at a low level, numerous episodes of acute illness have resulted from environmental exposure to pesticides (NRC, 1993).

Children are a group at particular risk of exposure to pesticides and major route of children's exposure is through their diet (NRC, 1993). They may also be exposed to pesticides applied in homes or schools, on lawns, and in gardens. Children employed in agriculture or living in migrant farm worker camps are particularly at high risk (McConnell, 1994). Children's tissues and organs are rapidly developing, and at various stages in early development, these growth processes create windows of great vulnerability to pesticides and other environmental toxicants. An analysis undertaken by the National Academy of Sciences (NAS) has established that the infants and children have an unique vulnerability to pesticides because of greater exposures and lower metabolic ability and the fact that their delicate developmental processes are easily disrupted and that they have more future years of life than most adults, that is, more time to develop chronic disease that may be initiated by early (NRC, 1993). In the past decade, since passage of the Food Quality Protection Act in 1996 (see below), levels of pesticide residues in domestically grown foods in the United States have steadily declined.

Several methodologies exist to assess occupational or even environmental exposures to pesticides. For example, pesticide levels can be quantified by passive dosimetry, using absorbent cloth or paper patches; biosensors or tracers followed by video imaging can also be utilized (Fenske et al., 1986; U.S. EPA, 1999; Chester, 2010). Biological monitoring is used to quantify the absorbed dose of pesticides. Analysis of body fluids and excreta, usually urine, for parent compound or metabolites, can provide both a quantitative and a qualitative measurement of absorbed dose. The advantage of such an approach over passive dosimetry is that it evaluates actual rather than potential absorption and integrates absorption from all routes of exposure (Sobus et al., 2010). In some cases, modifications of biochemical parameters or a consequence of exposure can be quantified as an indication of exposure and of a biological effect. This is the case, for example, of measurements of plasma or erythrocyte cholinesterases upon exposure to organophosphorus insecticides (Storm et al., 2000).

22.4 ACUTE POISONING WITH PESTICIDES

Pesticides are not always selective for their intended target species, and adverse health effects can occur in nontarget species, including humans (Calvert et al., 2010). From a global perspective, the major problem with pesticides remains that of acute human poisoning.

The World Health Organization (WHO) estimated that there are around three million hospital admissions for pesticide poisoning each year that result in around 220,000 deaths (WHO, 1990; Colosio et al., 2010). Most occur in developing countries, particularly in Southeast Asia, and a large percentage is due to intentional ingestion for suicide purposes (Gunnell and Eddleston, 2003). Self-poisoning with pesticides for suicidal purposes is very common in some countries; for example, in China, it accounts for about 50% of all suicides (Page et al., 2016). New estimates indicate that there are about >200,000 deaths due to pesticide self-poisoning each year, accounting for one-third of all suicides globally (Bertolote et al., 2006; Gunnell et al., 2007).

The best information on occupational pesticide poisoning in the United States comes from California, where in the mid-1990s, the average annual number of occupational pesticide poisoning cases reported in California was approximately 1500, of which 54% occurred in agriculture (Blondell, 1997). OPs were the class of compounds most frequently involved. By extrapolating California data to the nation, it has been estimated that there are between 10,000 and 20,000 cases of physician-diagnosed pesticide poisoning in the United States per year (U.S. EPA, 1992). Data on nonoccupational pesticide poisonings in the United States collected by the Consumer Product Safety Commission based on a statistical sample of emergency rooms in 6000 selected hospitals, indicated that in 1990–1992, there were an estimated 20,000 emergency room visits resulting from pesticide exposure. Incidence was disproportionately high in children, who accounted for 61% of cases. However, from 1995 to 2004, the overall number of pesticide poisonings in the United States declined, particularly for anticholinesterase insecticides (−70%) and for paraquat (−79%) (Blondell, 2007).

The WHO recommended a classification of pesticides by hazard, where acute oral or dermal toxicities in rats are considered (IPCS, 2010). As a class, insecticides are the most acutely toxic, and indeed, among the 86 active ingredients listed in class IA (extremely hazardous) and class IB (highly hazardous), 59% are insecticides (IPCS, 2010). Herbicides generally have moderate to low acute toxicity, one exception being paraquat. Fungicides vary in their acute toxicity, but they are usually low. In particular, reports of human poisonings worldwide underline the severe acute toxicity of certain anticholinesterase compounds and of paraquat. In recent years there has been an increasing use of the widely used herbicides glyphosate and glufosinate for suicidal attempts (Lee et al., 2015). Reducing the availability of highly acutely toxic pesticides can significantly reduce the number of fatal pesticide poisonings (Moebus and Bödeker, 2015).

22.5 TOXICITY OF PESTICIDES

Because the chemistry of pesticides is highly diverse, they are capable of causing a wide range of adverse health effects. Depending on the pesticide, or combination of pesticides, to which an individual or a population is exposed, these effects can involve virtually every organ system in the body. Pesticides can produce acutely toxic effects, delayed effects, and chronic effects. Also, some pesticides are developmental toxicants, while others are carcinogens or reproductive toxicants. The following sections summarize information on the toxic effects of the major classes of pesticides. Further information can be found in the following books or book chapters: Hayes (1982), Costa et al. (1987), Hayes and Laws (1991), Krieger (2001, 2010), Satoh and Gupta (2010), Costa (2006, 2013, 2015), and Vale and Lotti (2015).

22.5.1 Insecticides

All chemicals used as insecticides target the nervous systems of insects, which are not so different from those of mammals (Casida, 2009). Thus, insecticides are not very species specific with regard to toxicity, and humans are highly sensitive to their adverse effects. Species selectivity with older insecticides has usually been due to metabolic differences between insects and humans; with newer insecticides, differential interactions with the target usually explain enhanced selectivity.

22.5.1.1 Acetylcholinesterase (AChE) Inhibitors This class includes both the OPs and the carbamates. The toxicity of these two classes, which were developed in the 1940s to 1950s, is similar, with both inhibiting the enzyme AChE (Costa, 2006; Vale and Lotti, 2015). Inhibition of AChE results in the accumulation of acetylcholine, with resultant overstimulation of cholinergic receptors in the nervous system. The effects can be differentiated toxicologically into overstimulation of (1) the central nervous system (muscarinic and nicotinic receptors), (2) the nicotinic receptors (skeletal muscle and autonomic ganglia), and (3) the muscarinic receptors (secretory glands and postganglionic fibers in the parasympathetic nervous system). Together, these effects comprise what has been defined as a "cholinergic syndrome," which includes increasing sweating and salivation, profound bronchial secretion, bronchoconstriction, miosis, increased gastrointestinal motility, diarrhea, tremors, muscular twitching, and various central nervous system effects. The main difference between OPs and carbamates consists in the nature of inhibition of AChE. In the case of carbamates, carbamylated AChE undergoes relative rapid hydrolysis; thus inhibition of enzyme activity only lasts a few hours. In the case of OPs, the phosphorylated AChE is rather stable, and reactivation by hydrolysis may take hours to days. In addition, a chemical modification known as "aging" can occur, after which the enzyme can be considered irreversibly inhibited. The muscarinic receptor antagonist atropine represents the cornerstone for treating poisoning by AChE inhibitors. In the case of OPs, oximes such as 2-pralidoxime (2-PAM) are also useful, as they increase the rate of reactivation of phosphorylated AChE, but only before aging has occurred.

A few OPs (but not carbamates) may also cause another type of toxicity, known as organophosphate-induced delayed polyneuropathy (OPIDP), characterized by tingling of the hands and feet, followed by sensory loss, progressive muscle weakness and flaccidity of the distal skeletal muscles of the lower and upper extremities, and ataxia (Lotti, 1992; Lotti and Moretto, 2005). These may occur 2–3 weeks after a single exposure, when signs of the acute cholinergic syndrome have subsided. OPIDP is a distal sensorimotor axonopathy, presenting degenerative change in the distal parts of the axons and their terminals (Ehrich and Jortner, 2010). OPIDP is not related to AChE inhibition, but is believed to be related to inhibition of another esterase, named neuropathy target esterase (NTE). Phosphorylation and aging of at least 70% of NTE are necessary to initiate OPIDP. This two-step process occurs within hours of poisoning, and by the time the first clinical signs of OPIDP become evident some weeks later, NTE activity has recovered. Despite recent advances, the physiological role of NTE is still elusive, as is the exact chain of events occurring between phosphorylation and aging of NTE and axonal degeneration (Lotti and Moretto, 2005; Richardson et al., 2013). Several epidemics of OPIDP have occurred in the past (e.g., the Ginger Jake paralysis in the 1930s in the United States caused by tri-*ortho*-cresyl phosphate), but its occurrence in humans is now rare since, before commercialization, OPs must undergo specific neurotoxicity testing in the hen (one of the most sensitive species) to determine whether OPIDP is produced (Moretto, 1999).

22.5.1.2 Pyrethroids In the mid-1800s, pyrethrum extracts of chrysanthemum flowers (containing pyrethrin and other active ingredients) were found to be effective insecticides (Hayes and Laws, 1991). They are relatively less toxic than other commonly used insecticides but decompose rapidly in the light, hence having limited applications. Pyrethroid insecticides are closely related chemically to the naturally occurring pyrethrins but are all synthetic. They are more stable in the natural environment than the natural compounds and are used in agriculture, household pest control, soaked bed nets to prevent mosquito bites, and the topical treatment of head lice. Ingestion of large doses of pyrethroids causes salivation, nausea, vomiting, diarrhea, irritability, tremor, incoordination, seizures, and death. Toxicity is mediated through delay in the closing of sodium channels after discharge of an action potential, which results in repetitive neuronal discharge and a state of hyperexcitability of the nervous system (Narahashi, 1996). Depending on the chemical structure, pyrethroids can be divided into type I and type II; the latter, which contain a cyano (CN) group, may cause convulsions through inhibition of the gamma-aminobutyric acid A (GABA-A) receptor-associated chloride channel (Costa, 2015). In humans, dermal contact causes a reversible paresthesia with tickling, pricking, and burning of the skin. In contrast with AChE inhibitors, pyrethroids have low mammalian toxicity due to more effective detoxication systems in mammals and the higher sensitivity of insect sodium channels (Narahashi et al., 2007).

22.5.1.3 Neonicotinoids Nicotine, extracted from the leaves of tobacco plants, has been long used as an insecticide. It exerts its toxic effects in insects (as well as in mammals) by stimulating acetylcholine nicotinic receptors, which rapidly desensitize, resulting in paralysis. In the late 1970s, the neonicotinoids were developed by chemical modifications of the nicotine structure. As for nicotine, their insecticidal activity is due to activation of nicotinic receptors; however, they display very high selectivity and specificity toward insect nicotinic receptors (Sheets, 2010; Casida and Durkin, 2017). In recent years, neonicotinoids have been implicated in the decrease of the bee population, as it has been suggested that these insecticides may hamper the immune systems of bees, thus increasing their rate of infections (Sanchez-Bayo et al., 2016).

22.5.1.4 Organochlorines This class of insecticides includes **DDT**, **lindane**, and the cyclodienes **aldrin**, **dieldrin**, **endrin**, and **heptachlor**. Most uses of these chemically diverse insecticides were brought to market starting from the 1940s, and they enjoyed widespread use in agriculture, structure insect control, and malaria control programs. Subsequently, they were banned or restricted in the developed world because of their environmental persistence and bioaccumulation, two properties that have led to substantial damage to wildlife (Carson, 1962; Peakall, 1970). Levels of DDT and its metabolites in human adipose tissue have decreased markedly after DDT was banned in the United States in 1972; however, in many nations of the developing world, concentrations in human fat and milk from humans continue to be high. The use of DDT is still allowed in malaria-endemic nations, as it is effective against the malaria carrying mosquito (van Den Berg, 2009; Bouwman et al., 2011). DDT interferes with the sodium channel by a mechanism similar to that of pyrethroids and produces a period of increased neuronal excitability (Costa, 2015). As compared with DDT, dermal absorption of lindane, chlordane, or dieldrin is high. A prominent aspect of intoxication with these latter organochlorines is convulsions, ascribable to inhibition of GABA-A associated chloride channels. Many of these insecticides are also inducers of microsomial biotransformation enzymes and cause liver enlargements; several have also been shown to be carcinogenic in animals. Another

organochlorine insecticide is **chlordecone** (Kepone). One episode of poisoning involved 148 workers in a chlordecone producing factory in Hopewell, Virginia, between 1973 and 1975 (Taylor et al., 1978). The primary manifestation of chlordecone toxicity is the presence of tremors, which are observed in animals, as well as in humans, and whose precise mechanism has not been elucidated (Guzelian, 1982).

22.5.1.5 Other Insecticides A newer class of insecticides is that of phenylpyrazoles, of which **fipronil** was the first brought to market in 1990. Fipronil acts as a blocker of the GABA-A-gated chloride channel but binds to a site different from the picrotoxin binding site used by organochlorine insecticides (Casida and Durkin, 2017). It also has a much higher specificity for insect receptors over mammalian receptors (Narahashi et al., 2007; Wang et al., 2016). **Rotenone**, a natural compound, is derived from the roots of plants from South America and Asia. It is used as an agricultural insecticide/acaricide and is one of the few pesticides allowed in organic farming (Isman, 2006). Toxicity of rotenone in target and nontarget species is due to its ability to inhibit the mitochondrial respiratory chain at the level of complex I. In recent years, rotenone has received much attention for of its potential role in the etiology of Parkinson's disease. Indeed rotenone, when give repeatedly to rats, causes selective nigrostriatal degeneration, and other changes (e.g., Lewy bodies) as found in Parkinson's disease (Betarbet et al., 2002).

In recent years, increasing efforts have been devoted to the area of biopesticides, that is, pesticides derived from natural materials such as plants, bacteria, and fungi. Biopesticides, increasingly used when they are effective in integrated pest management programs, generally have a favorable environmental and toxicological profile (Glare et al., 2016). The most widely used microbial pesticides are subspecies and strains of ***Bacillus thuringiensis*** (Bt) that act as insecticides. Bt toxins, which are lethal to insects, have generally an unremarkable toxicological profile in mammals, though allergic reactions have been reported in humans (Rubio-Infante and Moreno-Fierros, 2016).

22.5.2 Insect Repellents

Diseases transmitted by insects are a major source of illness and death worldwide, and no part of the world is immune to their risks, as exemplified by the West Nile virus, Lyme disease, or, more recently, Zika virus. Insect repellents are widely used to provide protection against insect bites. The best known and most widely used insect repellent is DEET, while picaridin is demonstrating increasing success.

22.5.2.1 DEET DEET (*N*,*N*-diethyl-*m*-toluamide) has been available as an insect repellent since 1957. It is estimated that 30% of the U.S. population uses DEET every year. While effective at repelling insects, flies, fleas, and ticks, DEET does not appear to pose a significant health concerns to humans when used as directed. Because of case reports of neurotoxicity (seizures) in children associated with the use of DEET, it is recommended that toddlers and children should only be exposed to products with up to 10% DEET, while in all other individuals products with up to 30% DEET can be safely used (Sudakin and Trevathan, 2003; Antwi et al., 2008).

22.5.2.2 Picaridin Picaridin [1-piperidine carboxylic acid, 2-(hydroxyl-ethyl), 1-methyl propylester] was developed as an alternative to DEET. Insect repellent formulations containing 5–20% picaridin are highly effective against a variety of arthropod

pests, especially mosquitoes, ticks, and flies. The toxicological profile of picaridin is unremarkable (Antwi et al., 2008; Charlton et al., 2016).

22.5.3 Herbicides

Herbicides are the most important class of pesticides in terms of worldwide market share. Their use in agriculture and elsewhere is increasing steadily. This diverse class includes glyphosate, atrazine, 2,4-D, and paraquat. Most of these compounds, paraquat excepted, have low acute toxicity. Some herbicides, such as alachlor and atrazine, are important groundwater contaminants.

22.5.3.1 Glyphosate This compound is the most widely used herbicide (and pesticide) worldwide, particularly since transgenic crops that can tolerate glyphosate have been developed. Its target in plants is an enzyme not present in mammals, thus providing a high selectivity for this compound. The toxicity profile of glyphosate is mostly unremarkable, with low acute toxicity and adverse health effects often associated this other chemicals included in its formulations (Bradberry et al., 2004). Recently, however, the International Agency for Research on Cancer (IARC) has classified glyphosate as a "probable human carcinogen," based on animal, human, and mechanistic studies (Guyton et al., 2015; IARC, 2017). At the same time, the European Food Safety Authority (EFSA) has concluded that glyphosate is unlikely to pose any carcinogenicity risk for humans (EFSA, 2015). This situation has led to a wide debate among scientists supporting one or the other conclusion.

22.5.3.2 Atrazine Atrazine is a member of the family of triazines that exert their herbicidal action by inhibiting photosynthesis, a process unique to plants. As most herbicides, atrazine has low acute toxicity but is thought to have endocrine-disrupting effects (Hayes et al., 2011; van der Kraak et al., 2014). Contamination of drinking water with atrazine is an issue, and atrazine was banned in the European Union (EU) for this reason. Though animal studies suggested that atrazine may cause mammary tumors in rats, epidemiological studies of triazines and cancer in human have been inconclusive (Boffetta et al., 2013).

22.5.3.3 Chlorophenoxy Compounds Chlorophenoxy herbicides, of which 2,4 dichlorophenoxy acid (2,4-D) is the most common compound, have been used widely in the United States and elsewhere against broad-leaved plants. They are analogs of the plant growth hormone auxin and produce lethal growth in plants (Casida, 2009). During the Vietnam War, 2,4-D and 2,4,5-T (2,4,5-trichlorophenoxy acetic acid) were applied together for deforestation by American military forces, in a 50:50 mixture known as Agent Orange. 2,4,5-T is no longer marketed since it may become contaminated, during its manufacture, with dioxins, of which the most toxic and intensely studied is the tetrachlorinated 2,3,7,8-tetrachlorodibenzodioxin (TCDD) isomer. 2,4-D (which is not contaminated with dioxins) has moderate acute toxicity and a mostly benign toxicological profile (Kennepohl et al., 2010). However, some epidemiological studies have associated exposure to 2,4-D with certain cancers (non-Hodgkin's lymphoma, soft tissue sarcoma), and case reports describe possible neurotoxicity (Garabrandt and Philbert, 2002).

22.5.3.4 Paraquat High acute toxicity, lack of an effective antidote, and ready availability (because of low cost and herbicidal efficacy) have contributed to the notoriety of paraquat. Painful burns and bleeding of the gastrointestinal tract are common following

acute exposure to paraquat; approximately 20% of ingested paraquat is absorbed systemically, where it may cause renal toxicity. However, the most distinctive aspect of paraquat poisoning is delayed pulmonary toxicity. Paraquat is concentrated from the systemic circulation into the lungs, where pulmonary fibrosis may develop 2–4 days after ingestion. The mortality rate of pulmonary toxicity induced by paraquat is greater than 50% in most case series (Lock and Wilks, 2010). Paraquat is structurally similar to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), the contaminant of an illicit drug produced as a heroin substitute that caused an outbreak of acute-onset Parkinson's disease. Although it has been initially argued that paraquat does not cross the blood–brain barrier, it has been later shown that it can be transported into the brain by a neutral amino acid transporter (Shimizu et al., 2001). Animal studies and some epidemiological findings suggest an association between exposure to this herbicide and Parkinson's disease (McCormack et al., 2002; Moretto and Colosio, 2013).

22.5.4 Fungicides

Fungicides are applied to seeds, crops, and gardens to prevent fungal growth. This class of synthetic pesticides encompasses a wide variety of chemicals, including inorganic compounds (e.g., copper, sulfate), organometals (e.g., triphenyltin), dithiocarbamates (e.g., maneb, thiram), or other such as captan or chlorothalonil. Other fungicides such as methylmercury were used extensively as fungicides in the past for the prevention of seed-borne diseases in grains and cereals; however, given their high toxicity, particularly neurotoxicity, and large episodes of human poisoning (Bakir et al., 1973), their use has since been banned. With few exceptions, fungicides have low acute toxicity in mammals. However, some produce positive results in genotoxicity tests and may have carcinogenic potentials, while others are endocrine disruptors. The effects are often associated with the mechanisms by which these compounds act on their targets, the fungi.

22.5.4.1 Dithiocarbamates Dithiocarbamates and the related ethylenbisdithiocarbamate groups of fungicides have been widely used since the 1940s. The name of many of these compounds arises from the metal cations with which they are associated, that is, there are maneb (Mn), ziram and zineb (Zn), mancozeb (Mn and Zn), and ferbam (Fe). Thiram is a dithiocarbamate without a metal moiety. Some are metabolized to a common metabolite, ethylene thiourea (ETU), which is responsible for the effects of dithiocarbamates on the thyroid. Developmental toxicity and teratogenicity are observed with dithiocarbamates and ETU at maternally toxic doses, particularly in rats, and these effects are ascribed to an effect of ETU on the thyroid. There is also some evidence that dithiocarbamates may cause neurotoxicity by mechanisms not involving ETU, but rather to the release of the carbon disulfide moiety from a common metabolite, ethylene bisisothiocyanate sulfide (Hurt et al., 2010). Chronic exposure to maneb has been associated with Parkinsonism, which is likely ascribed to exposure to the manganese moiety, rather than the dithiocarbamate (Meco et al., 1994), particularly when there is co-exposure with paraquat (Costello et al., 2009). This fungicide has been withdrawn from the United States and the EU markets (Keigwin, 2010). The structure of dithiocarbamate fungicides resembles that of disulfiram, a compound used therapeutically to produce intolerance to alcohol, by virtue of its ability to inhibit aldehyde dehydrogenase. Interactions of dithiocarbamates with alcohol, leading to elevation in acetaldehyde levels, have been reported (Edwards et al., 1991).

22.5.4.2 Captan Captan is a broad spectrum protectant fungicide with low acute oral and dermal toxicity, but with irritant properties. Captan, as well as its metabolite thiophosgene, is mutagenic in *in vitro* tests, but not *in vivo*. Captan was classified by EPA as probable human carcinogens (category B2), but the classification has been changed to "not likely to be a human carcinogen when used according to label directions" (Gordon, 2007). Reentry intervals for farm workers are now based on the potential for eye irritation (Gordon, 2010). Because of its structural similarity to the potent teratogen thalidomide, captan has been extensively tested in reproductive/developmental studies in multiple species, but no evidence of teratogenicity has been found.

22.5.4.3 Other Fungicides **Chlorothalonil** is a halogenated benzonitrile widely used to treat vegetable, ornamental, and orchard diseases. Known adverse effects in humans are limited to its irritant effects on the eye and the skin, but reproductive toxicity and possible cancerogenicity have been observed in animals (Parsons, 2010). **Benomyl** inhibits fungal growth by inhibiting microtubule assembly in fungi. Allergic contact dermatitis caused by foliar benomyl residues has been reported. Teratogenic effects were observed following administration of high doses of benomyl, and anecdotal evidence suggests that maternal exposure to benomyl may result in anophthalmia in humans, but epidemiological studies did not demonstrate any convincing association (Spagnolo et al., 1994). **Azoxystrobin** is a broad spectrum fungicide obtained by chemical modification of natural strobilurins. Azoxystrobin is a quinone "outside" site (Qo) inhibitor fungicide, which inhibits mitochondrial respiration and energy production by blocking electron transfer at the Qo site, ultimately preventing generation of ATP (Casida and Durkin, 2017).

22.5.5 Rodenticides

There is a need to control rodent populations (vectors for several diseases), which can contaminate foodstuffs and consume large quantities of post-harvest stored foods. Rodenticides comprise a diverse range of chemical structures having a variety of mechanisms of action. The ultimate goal is to obtain a high species selectivity; however, with some common rodenticides the sites of action are common to most mammals. Toxicological problems can arise from acute accidental ingestions, particularly in children, or from suicidal/homicidal attempts.

22.5.5.1 Anticoagulants Warfarin, the more potent congener of dicoumarol, first isolated from spoiled sweet clover silage, was introduced as an extremely effective rodenticide in 1948. Warfarin antagonizes the action of vitamin K by inhibiting the enzyme vitamin K epoxide reductase, which regenerates reduced vitamin K necessary for sustained carboxylation and activation synthesis of clotting factors. In addition to their use as rodenticides, coumarin derivatives, including warfarin itself, are used as anticoagulant drugs and have become a mainstay for prevention of thromboembolic disease. Resistance to warfarin has led to the development of "superwarfarins," compounds that essentially act like warfarin but have prolonged half-lives and cause very long-lasting inhibition of coagulation. In 2014 almost 80% of all rodenticide poisonings were due to superwarfarins, and the use of these compounds is restricted to licensed pest control operators (King and Tran, 2015).

22.5.5.2 Bromethalin The toxicity of bromethalin in rodents is due to its metabolism to desmethylbromethalin, which targets the mitochondria, where it uncouples oxidative phosphorylation resulting in decreased ATP synthesis. Due to restrictions imposed on

superwarfarins, the use of bromethalin, which is readily available for over-the-counter purchase, has increased. A few hundred cases of human exposures to bromethalin have been reported to poison centers, with symptoms mostly of gastrointestinal and nervous system nature (Huntington et al., 2016).

22.5.5.3 Zinc Phosphide Zinc phosphide is widely used globally as single-dose rodenticide. Its toxicity is due to the phosphine gas formed during ingestion due to reactions with water or acids. Phosphine causes widespread cellular toxicity with injury to heart, liver, kidney, and the nervous system (Sciuto et al., 2016). The mechanism of toxicity involves generation of oxidative stress rather than inhibition of cytochrome c oxidase as initially suggested (Proudfoot, 2009; Sciuto et al., 2016). Exposure to phosphides by accidental or intentional ingestion is often lethal, and there are no known antidotes (Sciuto et al., 2016).

22.5.5.4 Norbormide This compound was introduced in 1964 as a selective rodenticide, lethal to rats, but not to other rodent species (e.g., mice) (Pelfrene, 2010). Such remarkable species difference in toxicity does not appear to be due to differences in toxicokinetics or biotransformation (Ravindran et al., 2009), but rather to differences in response of the peripheral blood vessels to norbormide-induced vasoconstriction. No cases of human intoxication with norbormide have been reported (Pelfrene, 2010).

22.5.6 Fumigants

Many chemicals, active toward insects, mites, nematodes, weed seeds, fungi, or rodents, have in common the property of being in the gaseous form at the time they exert their pesticidal action and are used for soil or structural fumigation or for fumigating post-harvest commodities. Compounds used as fumigants are usually nonselective, highly reactive, and cytotoxic. They provide a potential hazard, primarily for applicators, as fumigant residues in food commodities are usually extremely low. Several toxic fumigants used in the past have been phased out; examples are carbon tetrachloride (hepatotoxic), 1,2-dibromo-3-chloropropane (male reproductive toxicant), and ethylene dibromide (a carcinogen).

22.5.6.1 Methyl Bromide This compound is a broad spectrum pesticide and has been the most widely used fumigant for over 70 years. However, since the early 1990s, global use of methyl bromide has substantially decreased, because of environmental and toxicological concerns (Ruzo, 2006), and as of January 2005, it was officially phased out in the United States, though exemptions to the ban have been granted. Cases of systemic poisoning and fatalities have occurred over the years; acute exposure results in respiratory, gastrointestinal, and neurologic symptoms. As methyl bromide is an odorless and colorless gas, another fumigant, chloropicrin, which has a pungent odor and causes irritation of the eyes, was often used in conjunction with methyl bromide and other fumigant mixtures to warn against potentially harmful exposures. **Chloropicrin** has since emerged as an alternative to methyl bromide since its potency and short half-life make it beneficial as a fumigant (Ruzo, 2006). The main adverse effects of chloropicrin are due to its irritant properties.

22.5.6.2 1,3-Dichloropropene This soil fumigant is extensively utilized for controlling soil nematodes. It is an irritant and can cause redness and necrosis of the skin. Though it

has been phased out in the EU, 1,3-dichloropropene is one of the most widely used alternatives to methyl bromide for use as a soil nematocide in the United States (Sanchez-Moreno et al., 2009). However, it lacks herbicidal properties and is often formulated with chloropicrin, which is a better fungicide (Ajwa et al., 2010).

22.5.6.3 Metam-Sodium Metam-sodium, registered in the United States since 1954, is a widely used soil fumigant. Its toxic action toward soil nematodes, fungi, and weed seeds is due to its methyl isothiocyanate (MITC), a product of its hydrolysis. Main effects of acute exposure to MITC in the vapor state are irritated or burning eyes, nasal and throat irritation, nausea, coughing, shortness of breath, and contact dermatitis (Dourson et al., 2010; Deguigne et al., 2011).

22.5.6.4 Sulfur Elemental sulfur is considered the oldest of all pesticides, known to the ancient Greeks as early as 1000 BC. It is very effective for the control of many plant diseases, particularly fungal diseases, and is still widely used crop in the United States and worldwide. In agriculture, sulfur finds its major use in grapes and tomatoes, and it can be used in organic farming (Gammon et al., 2010). The primary health effect in humans associated with the agricultural use of elemental sulfur is dermatitis (Gammon et al., 2010).

22.6 PESTICIDES AS ENDOCRINE DISRUPTORS

Concern has arisen in recent years that certain pesticides may have adverse effects on the endocrine system. An endocrine disruptor is an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body, which are responsible for the maintenance of homeostasis, reproduction, development, and behavior (Stoker and Kavlock, 2010). For example, certain organochlorine compounds such as DDT can interfere with the effects of estrogen. Indeed, the *o*,*p*′-isomer of DDT, which comprise approximately 15% of the technical grade product, can act as an agonist at estrogen receptors, while a metabolite of DDT (*p*,*p*′-DDE) inhibits androgen binding to the androgen receptor (Kelce et al., 1995). It was the study of estrogenic effects of DDT in eagles and ospreys that led to Rachel Carson's original recognition of the ecotoxicology of the persistent chlorinated hydrocarbon compounds (Carson, 1962). Several other organochlorine insecticides have been found to be endocrine disruptors, including dieldrin, chlordane, toxaphene, endosulfan, and chlordecone (Guzelian, 1982; Soto et al., 1994; Kelce et al., 1995). Another pesticide that has been shown to have endocrine-disrupting activity is the herbicide atrazine (van der Kraak et al., 2014).

Because hormones play critical roles in the early development of the immune, nervous, and reproductive systems, even low-dose exposure to endocrine-disrupting pesticides during early windows of developmental vulnerability can have adverse effects on fetal life. The developmental effects of exposure to endocrine disrupters will vary depending on age at exposure and sex. It has been proposed that increased exposure to these agents may be involved in the incidence of undescended testes in male infants, which has been reported since 1960. Effects have also been documented in the sexual development of wildlife in areas where there are elevated levels of pesticides in the environment. For further discussion of endocrine disruptors, see Chapter 15.

22.7 PESTICIDES AND DEVELOPMENTAL NEUROTOXICITY

As discussed in the next section, a report from the NAS highlighted the potential higher exposure of children to pesticides (NRC, 1993), and the Food Quality Protection Act (FQPA) indicates that an additional safety factor should be included in the risk assessment process to ensure protection of children, who are presumed to be more sensitive to the effects of toxicants (FQPA, 1996). The concept that children are not simply "little adults" is now widely accepted; indeed, the developing organism, particularly the developing nervous system, can be differentially affected during development and in adulthood. The examples of ethanol or methylmercury, which cause profound neurotoxicity when exposure occurs *in utero*, and much less (and different) neurotoxicity in adults, are well known. Over the years innumerable *in vitro*, animal, and human epidemiological studies have investigated the potential developmental neurotoxicity of pesticides (Bjørlung-Poulsen et al., 2008; Abreu-Villaca and Levin, 2017). One of the most studied classes is that of the OPs. Young animals are more sensitive to the acute cholinergic toxicity of OPs, because of lower detoxication abilities (Costa, 2006). In contrast, the young appear to be more resistant to OPIDP (Lotti and Moretto, 2005). Animal and *in vitro* studies have shown that OPs can affect various cellular processes (e.g., DNA replication, neuronal survival, neurite outgrowth), and non-cholinergic pathways (e.g., serotoninergic synaptic functions, the adenylate cyclase system), and cause various behavioral abnormalities (Jett et al., 2001; Ricceri et al., 2003; Garcia et al., 2005). Such effects are at times seen at dose levels that produce no cholinergic signs of toxicity (Timofeeva and Levin, 2010). These findings, together with results of epidemiological studies (e.g., Eskenazi et al., 1999; Eaton et al., 2008; Muñoz-Quezada et al., 2013), have led to regulatory restrictions on the use of certain OPs.

Young animals have also been found to more sensitive to the acute toxicity of certain pyrethroids, such as deltamethrin and cypermethrin, most likely because of a lesser capacity for metabolic detoxification (Sheets, 2000). Other experimental studies have also suggested that certain pyrethroids may cause developmental neurotoxicity, but current evidence has been judged inadequate (Shafer et al., 2005). Pyrethroid exposure (presumably through residues in the diet) in children has been found to be very low. The possibility that some neonicotinoids may exert developmental neurotoxicity has also been raised, though the issue is still controversial (EFSA, 2013; Sheets et al., 2016).

22.8 LEGISLATIVE FRAMEWORK

The regulation of pesticides has been contingent on an assumed need to balance the economic benefits of these compounds against the risks associated with their use (NRC, 1993). That is the principle that underlays the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of 1947, the first federal statute controlling pesticide use in the United States. This law, which has undergone several revisions, regulated pesticides on the basis that they would not cause "unreasonable adverse effects." In the United States, the primary authority for pesticide regulation resides with the Environmental Protection Agency (EPA) under FIFRA and the Federal Food, Drug, and Cosmetic Act (FFDCA). Under FIFRA, EPA registers pesticides for use, while under FFDCA, EPA establishes maximum allowable levels of pesticide residues (tolerances) in foods and animal feeds, which are enforced by other federal agencies (Fenner-Crisp, 2001). Due to rising concern

about the health effects of pesticides in foods, particularly regarding the potential of some agents to cause cancer, Congress passed the Delaney clause in 1958, as an amendment to the FFDCA. This clause banned any "additive," including any pesticide that had been shown to cause cancer in humans or animals from processed foods. From the beginning, the Delaney clause was highly controversial. Representatives of the pesticide and food industries argued that the law was too inflexible as it prohibited processed foods from containing even the smallest amount of carcinogenic chemicals; environmentalists, in contrast, considered the Delaney clause a bulwark of public health and environmental protection. The Delaney clause posed a policy dilemma by establishing a standard for pesticides in processed foods much stricter than that established for pesticides in raw foods—this was termed the "Delaney paradox" (NRC, 1987). To cope with this paradox, the EPA for many years did not strictly enforce the clause and allowed very low, or "de minimis," levels of carcinogenic pesticides in processed foods. In the Agency's opinion, these levels posed no more than a "minimal risk" to health. In 1992, however, the U.S. Court of Appeals ruled that this approach contravened the intent of the Delaney clause and thus was not legal. This decision set the stage for passage in 1996 of the Food Quality Protection Act (FQPA), the major statute regulating pesticides in the United States today (FQPA, 1996). Under FQPA, pesticide residues are excluded from the definition of food additive, and the Delaney clause no longer applies to residues in food. Thus, tolerances can be set also for carcinogens.

A 1993 report by the NAS found that the then current laws and regulations did not adequately protect children from the risks of pesticides in foods (NRC, 1993). The report recommended that Congress enact a legislation that would specifically consider the effects of pesticide residues on children's health. Following that guidance, the FQPA requires that (1) children's sensitivities and unique exposure patterns to pesticides should be considered in setting pesticide standards, (2) tolerance levels are safe for children, and (3) an additional safety factor of up to 10-fold must be added to account for uncertainty in the database relative to children and to reflect children's greater exposure and greater susceptibility to pesticides, unless there is reliable evidence that a different factor should be used. FQPA also directs EPA to consider aggregate exposure in the risk assessment process, that is, exposure that occurs from all food uses for a pesticide, as well as from exposures that occur from nonoccupational sources (e.g., drinking water, indoor residential or school use, etc.). Additionally, EPA must consider whether certain pesticides, as well as other substances, may have the same mechanism of toxicity and, if so, carry out a cumulative risk assessment. Finally, provisions are included for EPA to determine whether certain substances may have endocrine-disrupting effects in humans.

To register a pesticide or a formulated product for sale in the United States, over 140 studies are required, which include information on product and residue chemistry, environmental fate, toxicology, biotransformation/degradation, occupational exposure and reentry protection, spray drift, environmental impact on nontarget species (e.g., birds, aquatic organisms, soil organisms), environmental persistence and bioaccumulation, and product performance and efficacy. In addition to all this, a whole set of toxicology data are also required (Costa, 2013). Older products, registered before 1984, must undergo a re-registration process. Agencies similar to the U.S. EPA exist in other countries (e.g., EU, Japan, Canada). In contrast, some developing nations have adopted the regulatory framework of one or another industrialized nation. The WHO provides guidance, particularly with the setting of acceptably daily intake (ADI) values for pesticides.

22.9 CONCLUSION

The past 70 years have seen the development of hundreds of pesticide active ingredients and thousands of formulations that have provided important chemical tools for the production of food and fiber for the increasing world population. Together with this positive impact, adverse environmental and human health issues have also been associated with increasing use of pesticides. Despite the increased favor encountered by organic farming, the use of pesticides is expected to continue, particularly in rapid developing countries with fast growing populations. The right strategy is to develop new pesticides that will exert their expected pest control and overcome increasing resistance, that will no harm the environment, and that will be highly selective, with no or minimal human toxicity. The research in this area is indeed going in this direction (Casida, 2017; Casida and Durkin, 2017).

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23

RADON AND LUNG CANCER

NAOMI H. HARLEY

23.1 INTRODUCTION

Everyone is exposed to natural radiation background. This includes both external radiation and internal radioactivity. The major components of natural background radioactivity exposure arise from the uranium-238 (^{238}U) and thorium-232 (^{232}Th) primordial series, plus potassium-40 (^{40}K), present in all Earth's native substances. Both the ^{238}U and ^{232}Th primordial series and potassium have internal and external components. External exposure occurs through gamma ray exposure from these ground/soil source nuclides present in all materials associated with soil. The internal exposure arises mainly through inhaled alpha and beta radioactivity and dietary/water intake of these same radionuclides. The primordial series ^{238}U and ^{232}Th are shown in Figs. 23.1 and 23.2.

The radionuclides radon, ^{222}Rn, and thoron, ^{220}Rn, are decay products in the ^{238}U and ^{232}Th series. These and ^{40}K were present during planet Earth's formation. The primordial series and ^{40}K are easily measured through their alpha, beta, or gamma ray radioactivity. All are present today in natural materials because of their long half-lives, 4.5×10^9, 14×10^9, and 1.3×10^9 years, respectively. The ^{238}U and ^{232}Th series support decay products that are both alpha and beta emitters. Potassium-40, present in every cell in the human body, is a beta and gamma ray emitter and a very small fraction, 0.0117%, of all potassium, ^{39}K, decaying to stable ^{40}Ar (argon), the source of the third most abundant gas in our atmosphere, about 1.0%.

Alpha particles are energetic helium nuclei, ^{4}He, released in alpha particle decay. When released by an emitter such as radon, ^{222}Rn, an alpha particle has significant kinetic energy. The alpha particle then slows by interaction with matter and becomes ^{4}He, a stable gas atom, the source of all Earth's helium, a valuable industrial product. Beta particles are energetic electrons emitted by the nucleus in beta decay, and gamma rays are photons emitted usually as secondary radiation along with alpha and beta radiation.

Environmental Toxicants: Human Exposures and Their Health Effects, Fourth Edition.
Edited by Morton Lippmann and George D. Leikauf.

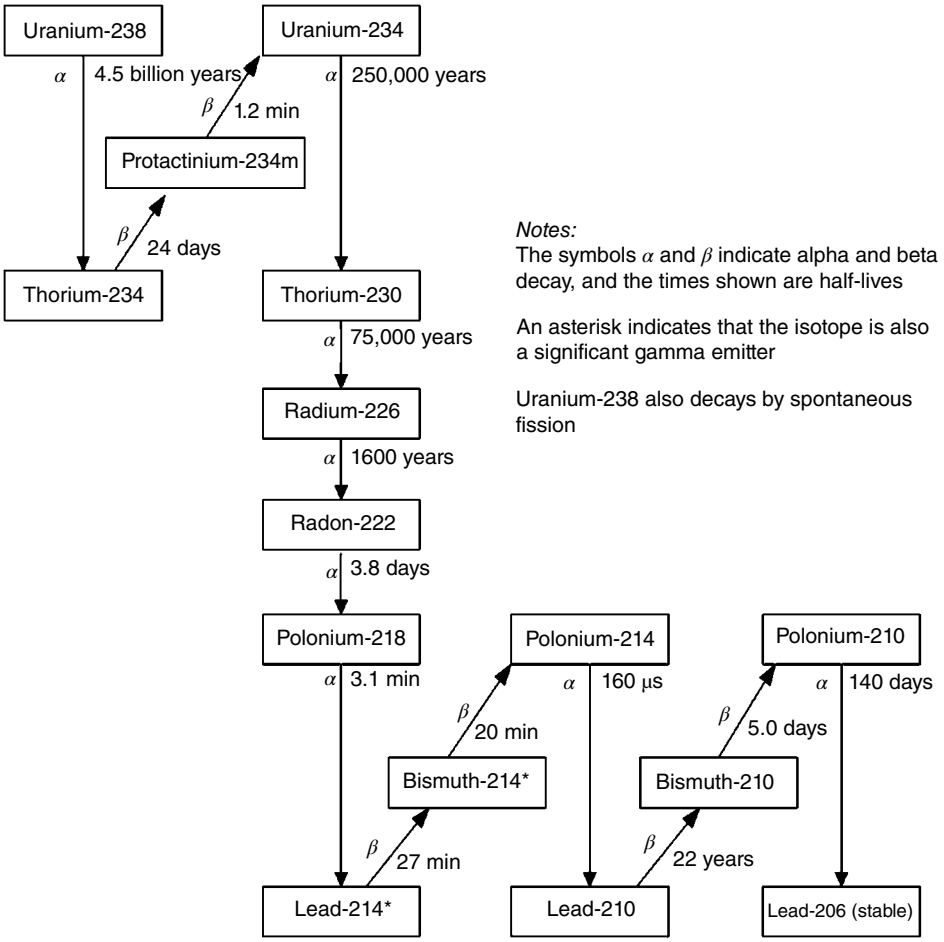

FIGURE 23.1 ^{238}U primordial decay series (ICRU, 2015).

Planetary substances may include the same natural radionuclides, for example, ^{232}Th has been measured in lunar samples (Potdar and Bhandari, 1979). In 1971, Apollo-15 passed 110 km (68 mi) above the Aristarchus plateau on the Moon and detected a significant rise in alpha particles thought to be caused by the decay of ^{222}Rn. The presence of ^{222}Rn was inferred later from data obtained from the Lunar Prospector alpha particle spectrometer (Lawson et al., 2005).

The major contributor to human internal radiation dose is the inhalation of short-lived ^{222}Rn decay products, ^{218}Po, ^{214}Pb, ^{214}Bi/Po, and to a lesser extent ^{220}Rn and its decay products. Radon-222 and decay products are members of the ^{238}U primordial series, while ^{220}Rn and decay products are members of the ^{232}Th series. It is well documented that ^{222}Rn decay products are the leading lung carcinogen following tobacco-related disease. The external gamma ray radiation exposure from the ^{238}U and ^{232}Th series affects the global population to varying levels, while ^{222}Rn inhalation exposes the entire global population.

Other ways of exposure to natural radiation radioactivity exist, such as dissolved ^{222}Rn in drinking water, dietary intake of ^{238}U, ^{232}Th, ^{226}Ra, ^{210}Pb, and ^{210}Po, and cosmic rays. Enhanced exposures to natural radioactivity exist, such as ^{226}Ra in certain industrial

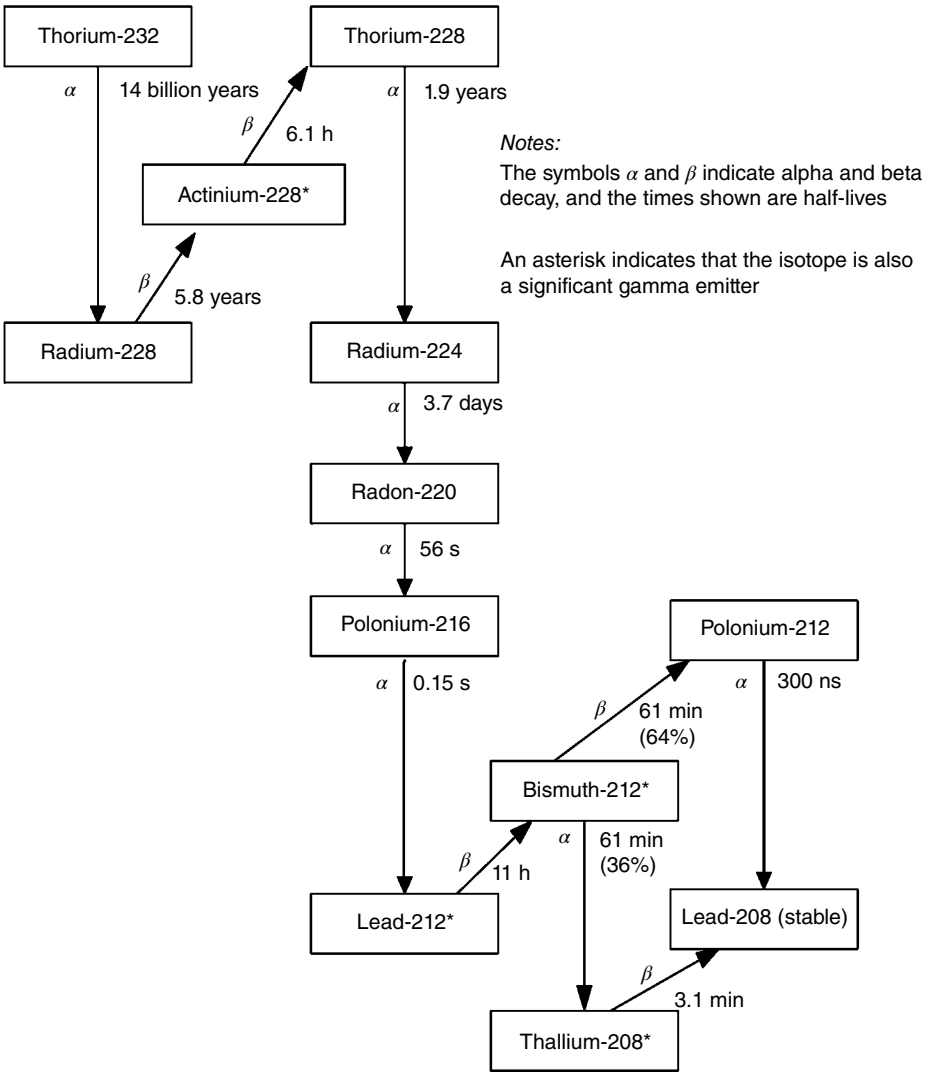

FIGURE 23.2 ^{232}Th primordial decay series (ICRU, 2015).

operations and drilling for oil, natural gas, areas of high levels of natural radionuclides, for example, ^{232}Th deposits in Brazil and India. Consumer products contain specific members of the primordial series such as ^{210}Pb/^{210}Po in native tobacco products and are inhaled in tobacco smoke, producing a significant bronchial lung dose.

The details of measurement, primarily ^{222}Rn, and levels of this radiation exposure, the internal dosimetry, and the health risk, namely, cancer, are included in the following sections. Because ^{222}Rn produces the highest numbers of lung cancers per year in the global population, second only to tobacco-related lung cancer, emphasis is given to ^{222}Rn measurement and its decay products. These include ^{222}Rn air concentrations, the bronchial dose resulting from inhalation, and the excess lung cancer risk that is firmly established for ^{222}Rn exposure through many epidemiologic studies in underground mines and residences.

23.2 HISTORY OF RADON AND DECAY PRODUCT MEASUREMENT

Measurements, made around 1900, reported an unknown radioactive gas when working with uranium or thorium salts. These measurements were first made using electrometers and later using ionization detection chambers after radiation detection instrumentation was developed. Owens and Rutherford observed electrometer readings and later measurements with an ionization chamber when working with thorium salts. Rutherford and Soddy were able to condense the gas ^{222}Rn, and Soddy was able to prove that ^{222}Rn was a member of the inert gas family (NCRP, 1988).

In the 1950s the scintillation cell was developed and provided an easy measurement technique for ^{222}Rn gas. The scintillation cells were made with radiation detectors of different volumes having a clear window on one side and a coating of zinc sulfide (ZnS) on the interior walls. An alpha particle striking the ZnS produced a light pulse and was detected with a photomultiplier tube and electronic scaler. A grab sample of air was captured for the sample; for a more integrated sample, a collapsible bag was slowly filled, and a fraction of the air in the bag transferred to the scintillation cell.

The original concentration unit for measurements of ^{222}Rn was pCi/L, the gas concentration in air. Now, the SI unit Bequerel (Bq)/m^3 is the generally accepted air concentration unit, although some U.S. agencies retain the original unit.

Early measurements of ^{222}Rn gas were made in laboratories or in occupational environments, such as underground uranium mines and laboratories. Essentially no residential measurements were made, and it was not recognized until the 1970s, when residential measurements were made, that ^{222}Rn concentrations in homes could reach levels reported in mines (Steinhausler, 1975; Evans et al., 1981; George, 1984). It became evident that home exposure to ^{222}Rn could be related to health effects, especially lung cancer (NCRP, 1984).

Measurements made in mines needed to be rapid and extensive, so they were usually made taking filtered air samples of the solid decay products ^{218}Po, ^{214}Pb, ^{214}Bi/Po, and alpha counting the filter with a handheld survey meter. The main method was the Kusnetz technique and was widely used in mines for 30 years (Kusnetz, 1956). Air samples, 2–20 lpm, taken for 5 min were counted after a delay of 40–90 min. This single count, divided by a factor, is directly related to the working level (WL), an exposure unit used mainly for mines. This method is relatively insensitive to the airborne decay product ratios (NCRP, 1987).

A method to measure the individual decay products was developed by Tsivoglou et al. (1953). This technique was alpha counting the filtered air sample over three counting intervals, a total of 30 min duration, starting 5 min after sampling. This allowed the equilibrium concentration, C_{eq}, of ^{222}Rn to be calculated.

In 1958, the Nuclear Standards Board of the American National Standards Association (later Institute [ANSI]) established a committee to develop a standard for uranium mines and mills. This standard was adopted in 1960 and denoted the WL that numerically equals the total alpha energy released from an equilibrium concentration of 100 pCi/L ^{222}Rn and its decay products ^{218}Po, ^{214}Pb, and ^{214}Bi in 1 L of air. The alpha particle energy released equals 1.3×10^5 MeV/L. The utility of the WL was because it was thought to be directly related to lung dose (Harley, 2018). Measurements in mines were then made in WL units.

Current ^{222}Rn decay product measurements in mines are made with real-time equipment for occupational exposure assessment of WL because of the equipment's ability to measure individual alpha emitting decay products and therefore WL. The real-time monitors measure the alpha particle energies in a filtered air sample to obtain the individual

decay products. In some countries alpha track detectors are used. One such type still in use is the one first described by Duport et al. (1980) for French uranium mines. This detector uses a pump to collect the radon decay products, which then pass through separate collimators having different filters for energy discrimination. The filter activity is subsequently recorded on a solid state polycarbonate nuclear track detector (ICRU, 2015).

The guidelines and regulations for measured ^{222}Rn in homes and mines are discussed in Section 23.11.

23.3 INDOOR MEASUREMENTS OF ^{222}RN

Early measurements of ^{222}Rn were made exclusively in underground mines or occupational settings. One of the first surveys of indoor ^{222}Rn measurements was made in Innsbruch, Austria (Steinhausler, 1975). Radon-222 decay product measurements were made with filtered air sampling, and ^{222}Rn gas samples were measured in an ionization chamber. It should be noted that a so-called ^{222}Rn measurement is usually a measurement of ^{222}Rn including its decay products or the decay products alone.

Radon-222 survey measurements in homes began in the 1980s, when lung cancer epidemiology in mines implicated lung cancer to ^{222}Rn exposure (NCRP, 1984). Measurements were made in the 1980s with solid state nuclear track detectors (SSNTD) and with activated charcoal samplers that were developed for short- or long-term residential monitoring. SSNTD can be deployed for over a year to best estimate average exposure. Alpha particles striking the SSNTD produce damage/tracks in the plastic material that are visible after chemical etching, followed by track counting with a microscope and software. Calibration is usually with SSNTD or activated charcoal detectors exposed in calibration chambers to obtain tracks mm^2 per unit ^{222}Rn concentration.

Radon-222 entry and removal in homes is a function of the ^{222}Rn concentration in the soil beneath the home, outdoor concentration, pressure-driven flow from soil gas due to temperature differences, home construction, floor level, and ventilation rate. Apartment buildings usually have low ^{222}Rn concentration due to the lowest-level floor thickness and ventilation in upper floors. Year-to-year home concentrations vary by about 25% in the lowest level (basement) (Steck, 2009; Harley et al., 2011).

Attempts have been made to model year-to-year ^{222}Rn concentrations in homes without success. This is most likely due to the ever-changing values of the home entry and removal parameters.

Rapid measurement, required by law for some home sales, is made within a small closed bag containing activated charcoal that efficiently absorbs ^{222}Rn. The bag is exposed to the sampling atmosphere for about a week, usually in the basement, where the highest ^{222}Rn concentrations exist, and the decay products that build up in the activated charcoal are gamma ray counted for ^{222}Rn concentration measurements. SSNTD and charcoal measurements are simple, inexpensive, and currently in use in residences during home transfers or sales to determine whether ^{222}Rn concentrations comply with local or state regulations.

Calibration of detectors for home sales is mandatory and carried out by EPA or by well-known commercial firms such as Bowser–Morner, Dayton, OH, who maintain chambers with verifiable, traceable, concentrations of ^{222}Rn gas.

Because of the known lung cancer risk discussed later, many countries have established programs for large-scale environmental surveys to determine country-wide average ^{222}Rn concentrations. Figure 23.3 shows results of ^{222}Rn survey measurements in 50 countries.

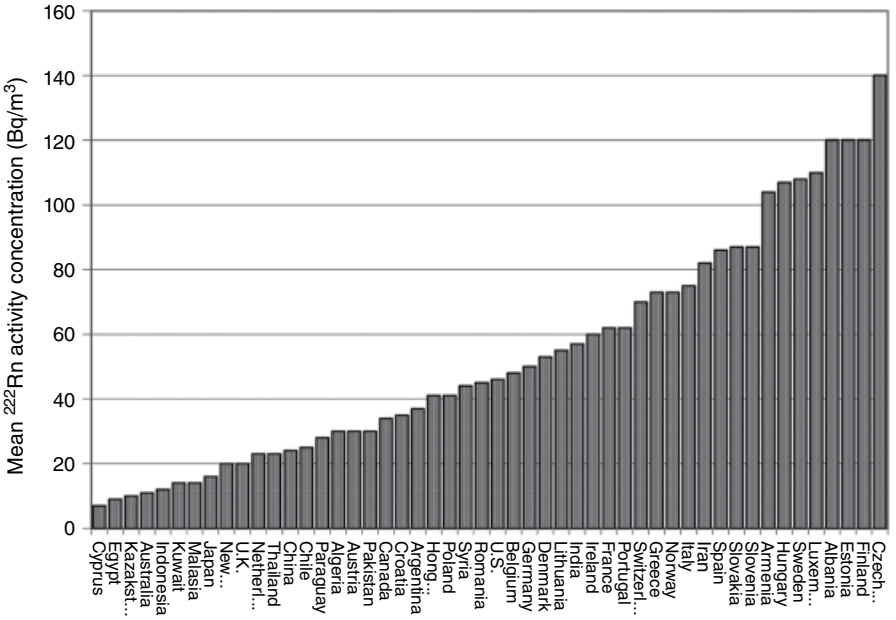

FIGURE 23.3 Average ^{222}Rn concentrations measured in surveys from 50 countries (UNSCEAR, 2008).

23.4 OUTDOOR MEASUREMENTS OF ^{222}RN

Land masses, continents, are the source of outdoor ^{222}Rn because of the ubiquitous uranium soil concentration. All ^{238}U decay products in native materials are typically in equilibrium with the parent ^{238}U. Oceans have very little uranium and are sinks rather than sources. There are fewer outdoor measurements than indoor because ^{222}Rn concentrations are low, typically 7–50% of indoor concentrations (Harley, 1990). There are areas with relatively high outdoor ^{222}Rn concentrations, depending upon the local soil characteristics. Outdoor ^{222}Rn concentrations are useful when exposure comparisons are made within various environments to suggest concentrations relative to a baseline values.

The first ^{222}Rn published measurements in ground-level outdoor air were made in Innsbruch, Austria, from 1921to 1937 (Steinhausler, 1975).

In the upper atmosphere, ^{222}Rn has been measured to heights of 20 km with concentrations of 1 mBq/m^3 at 20 km and 15,000 mBq/m^3 at ground level (Harley, 1990; Fisenne et al., 2005). Near equilibrium with its decay products, ^{218}Po, ^{214}Pb, ^{214}Bi/Po, exists at heights. At ground level, 60% equilibrium is used as an average (UNSCEAR, 2008).

Few measurements of ^{222}Rn in the troposphere or stratosphere have ever been made. Fisenne et al. (2005) collected stratospheric air samples in 1962 using WB-57 aircraft of the U.S. Weather Bureau (now NOAA) to explore the possibility of using radon profiles as an atmospheric tracer.

Samples were collected from tropospheric and stratospheric air by pressurizing low ^{226}Ra background steel spheres. Radium-226 in steel releases ^{222}Rn into the sphere, and sample background increases detection limits. The stratospheric sampling locations were

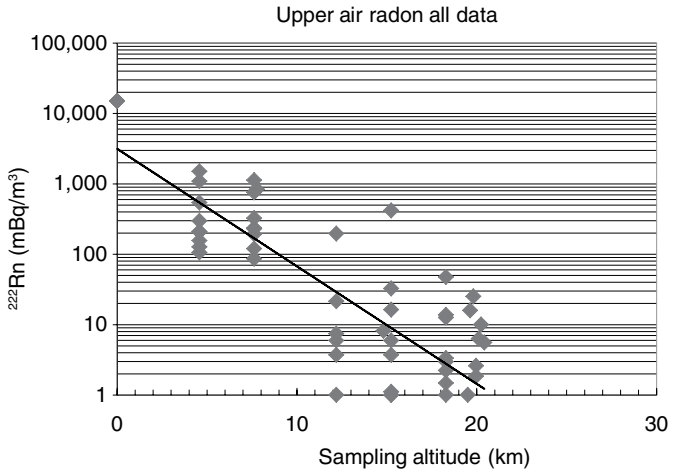

FIGURE 23.4 Measured upper atmosphere ^{222}Rn concentrations over Alaska, the Canal Zone, and Southwest United States. *Source*: From Fisenne et al. (2005).

Alaska, the Southwest United States, and the Panama Canal Zone. The samples were obtained from 3 to 6 different altitudes, ranging from 4 to 20 km both below and above the tropopause, at which height varied from 8 to 17 km. The ^{222}Rn concentrations ranged from 1 mBq/m^3 at 20 km to 1000 mBq/m^3 at 4 km, compared with average ground-level concentration of 15,000 mBq/m^3. The ^{222}Rn concentration profiles over the Canal Zone and the Southwest United States were similar with height, while the Alaskan profiles were lower by a factor of 5–10. The tropopause appears to be a more effective barrier to ^{222}Rn transport in the Canal Zone and over the Southwest United States than over Alaska. The reason for this is not known but may reflect the bearing of the jet stream on lower altitude turbulence. Figure 23.4 shows all ^{222}Rn data collected over the Canal Zone, Alaska, and Southwest United States (Fisenne et al., 2005).

Machta and Lucas (1962) initiated ^{222}Rn measurements in the troposphere and stratosphere to identify the top of the troposphere bearing on fission product transfer from nuclear weapons testing. On average, the troposphere is well mixed, and the 3.8-day ^{222}Rn half-life permits it to be carried upward without significant decay loss while participating in normal atmospheric transport processes. Fission products were introduced to the stratosphere from weapons testing up to 1964, and mixing processes were important to understand. Attempts were made with WB-57 aircraft using steel sampling bottles to measure ^{222}Rn below and above the tropopause and identify the mixing process. They confirmed that turbulent mixing of ^{222}Rn occurs in the troposphere, but a rising current rather than turbulent mixing exists in the stratosphere. There were problems with contamination in the steel of the sampling bottles, and further work was given to the Health and Safety Laboratory (HASL) U.S. Atomic Energy Commission; see Fisenne et al. (2005). The concentrations measured by Machta and Lucas (1962), were in general agreement with those of Fisenne et al. (2005).

EPA has conducted measurements of outdoor radon at ground level in all 50 states. This survey and other state measurements were summarized in NAS/NRC (1999a). A mean for the United States from 437 outdoor measurements is 14.8 ± 5.3 Bq/m^3 (ICRU, 2015). The seasonal ambient ^{222}Rn measurements are shown in Fig. 23.5.

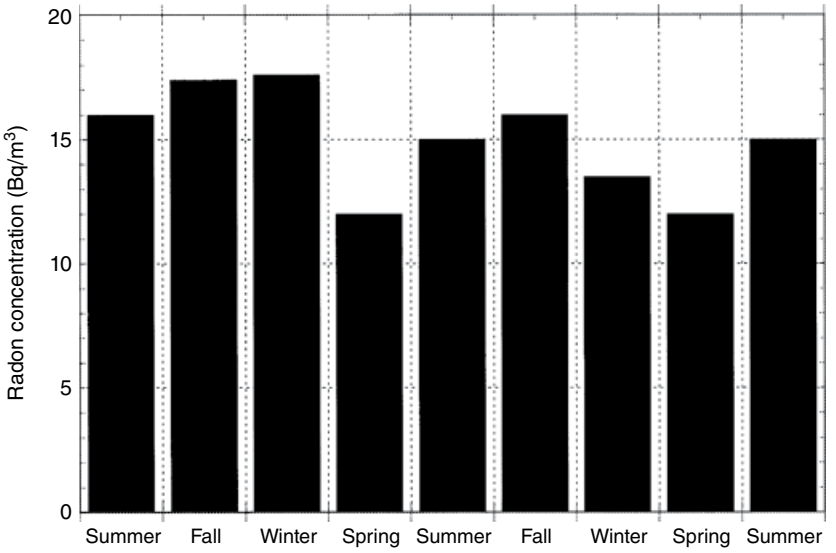

FIGURE 23.5 National EPA 50 site ambient (outdoor) ^{222}Rn survey. *Source*: From Hopper et al. (1991).

23.5 MEASUREMENT OF ^{222}RN DECAY PRODUCTS

Radon-222 and decay products (progeny) are part of the ^{238}U primordial decay series (Fig. 23.1). Radon-222 is present in all atmospheres and must be present to support its short-lived chain ^{218}Po, ^{214}Pb, and ^{214}Bi, having a combined half-life of 33 min (Harley, 1980 [1952]).

In 1955, the U.S. Public Health Service (PHS) developed the concept of expressing a tolerance level in terms of the potential alpha energy of radon decay products in air. This was called the working level, numerically equal to the total alpha energy release from decay of the ^{218}Po, ^{214}Pb, and ^{214}Bi/Po in 1 L of air (Harley, 2018) (see Section 23.1).

Measurement of the airborne ^{222}Rn decay products in mines was made in units of the WL. This was developed for measurements in underground uranium mines, where very high concentrations of ^{222}Rn had been measured. The first ^{222}Rn decay product measurements were in the Utah and Colorado mines. Measurements in about 40 mines in Utah and Colorado confirmed high concentrations averaging over 92,000 Bq/m^3 (2500 pCi/L) (Harley, 2018).

However, ^{222}Rn is rarely in near equilibrium with its decay products. The calculation of WL requires a measurement or estimate of the equilibrium equivalent concentration (EEC). This estimate, C_{eq}, is a concentration of ^{222}Rn in air, in equilibrium with its short-lived decay products that has the same potential alpha energy concentration as the existing nonequilibrium ^{222}Rn concentration. The estimated average EEC frequently used is described later in this section.

The EEC in general use was obtained from many measured ^{222}Rn and decay product concentrations and was averaged to obtain an equilibrium factor (F_{eq}). The estimated F_{eq} is the average fraction of the ^{222}Rn concentration, in equilibrium with its short-lived decay products measured in many conditions.

The equilibrium factor, F_{eq}, is defined as the ratio of C_{eq}, the EEC, and the measured ^{222}Rn gas concentration. As indicated above it can be a specific value from measurements or an adopted average:

$$C_{eq} = 0.104\left(^{218}\text{Po}\right) + 0.516\left(^{214}\text{Pb}\right) + 0.380\left(^{214}\text{Bi}\right)$$

$$F_{eq} = \frac{C_{eq}\ \text{Bq/m}^3}{^{222}\text{Rn}\ \text{Bq/m}^3} \quad \text{or} \quad F_{eq} = \frac{C_{eq}\ \text{pCi/L}}{^{222}\text{Rn}\ \text{pCi/L}}$$

$$\text{WL} = F_{eq} \times \left(\frac{^{222}\text{Rn}}{3700}\right) ^{222}\text{Rn in Bq/m}^3$$

Working Level Months (WLM). The cumulative exposure usually annual exposure in units of work month = hours worked/170 h per work month

$$\text{WLM} = F_{eq} \times \left(\frac{^{222}\text{Rn}}{3700}\right) \times \left(\frac{\text{hours worked per year}}{170}\right) ^{222}\text{Rn in Bq/m}^3$$

Because individual decay products are rarely measured, especially in residences, ^{222}Rn gas measurements are multiplied by adopted values of F_{eq} for indoor, outdoor, or mine environments to obtain a calculated value of C_{eq}.

The values of F_{eq} often used for indoor and outdoor ^{222}Rn environments are 0.4 and 0.6, respectively (UNSCEAR, 2008). Chen and Harley (2018a, 2019) reviewed published measurements and found that the UNSCEAR selected typical values of $F_{eq} = 0.4$ for indoor and $F_{eq} = 0.6$ outdoors and 0.38 for mines are adequate when the local environment-specific F_{eq} is unknown or not well known. However, this is with the understanding that the variability in these F_{eq} values can be more than ±50%.

The average concentrations throughout U.S. mines in the 1940s and 1950s were high (7–15 WL), and mean exposures accumulated over the 1950s and 1960s were about 800 WLM (Evans et al., 1981; NAS/NRC, 1988, 1999b).

The WL is still in use, mainly in underground mines, and can be measured with direct-reading instrumentation.

23.6 GROUNDWATER AS A SOURCE OF INDOOR ^{222}RN

Radon-222, similar to other noble gases, is slightly soluble in water. Groundwater has dissolved ^{222}Rn that emanates from ^{226}Ra in soil. Radon released indoors from water use increases air concentration. The transfer of ^{222}Rn to indoor air depends upon six factors: groundwater concentrations, seasonal temperature changes in ^{222}Rn solubility in soil, water use in the residence, the type of water use per occupant, the volume of the residence, and the air exchange rate.

Two major national databases of radioactivity in public water supplies in 48 U.S. states, one by USEPA and one by Hess et al., were summarized by NAS/NRC (1999a). The summary of published data for radon in public water systems showed averages ranging from 4 to 370 Bq/L. Hess et al. (1985) measured ^{222}Rn in all 50 U.S. states, with geometric mean (GM) values for private wells, public water supplies, public groundwater supplies, and public surface water as 34, 2.5, 4.8, and 0.04 Bq/L, respectively. Radon-222 from above groundwater supplies, usually reservoirs, has a low ^{222}Rn concentration. Private

underground wells or those suppling public groundwater distribution can achieve high ^{222}Rn concentrations, depending upon the ^{226}Ra content of the local rock or soil. Nazaroff et al. (1987) summarized EPA data for radon concentrations in potable water in 41 U.S. states. They derived a GM water concentration of 5.2 and 36 Bq/L for public and private groundwater supplies, respectively. Surface water had a GM of 0.3 Bq/L. In the United States, New England has the highest groundwater concentrations of ^{222}Rn, with reported concentrations in Maine of 37,000 Bq/L (Hess et al., 1985). See Section 23.11 for ^{222}Rn in drinking water regulations.

The amount of ^{222}Rn released to air differs among residences, depending on water use. Bathrooms and kitchens usually have the highest ^{222}Rn releases because of greater water use. The total amount of water use in different sections of the United States varies widely, but individual use is less variable with estimates of $0.28 \pm 0.20\,m^3$ with geometric mean 0.23 GSD $1.8\,m^3$ per person per day (NAS/NRC, 1999a).

The transfer coefficient is usually defined as

$$\text{Transfer coefficient} = \frac{\Delta C_a}{C_w}$$

where ΔC_a is the average incremental ^{222}Rn concentration throughout the dwelling and C_w is the average ^{222}Rn water concentration (NAS/NRC, 1999a).

There are relatively few measurements of the water to air transfer coefficient. The published measurements are shown in Fig. 23.6 (NAS/NRC, 1999a).

The NAS/NRC committee on the risk assessment of radon in drinking water recommended that the best values are between 0.8 and 1.2×10^{-4}; however, the committee recommended that EPA continues to use 1.0×10^{-4} as the best central estimate of the transfer coefficient, based on the available data. That is, on average 10^4 Bq/m^3 in water contributes to 1 Bq/m^3 ^{222}Rn to indoor air.

One study of the transfer coefficient in a bathroom in an energy efficient home with a private well with a ^{222}Rn concentration of 69 ± 2 Bq/L measured a 92% release rate in the shower (Harley et al., 2014). The transfer coefficient for the bathroom increased and was 1/2300 due to the small room volume.

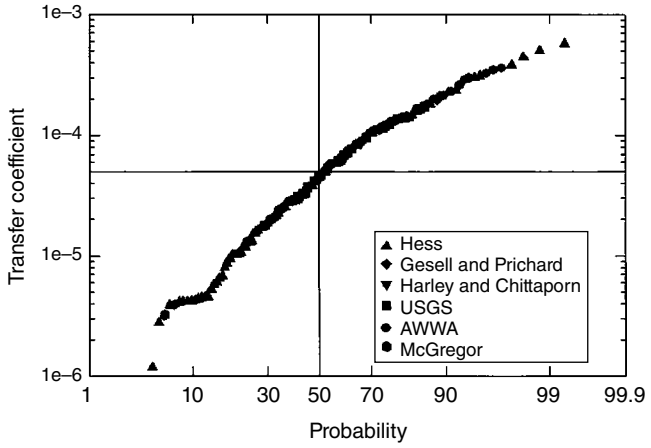

FIGURE 23.6 Cumulative probability distribution of measurements of water to air transfer coefficient (NAS/NRC, 1999a).

The organ dose from ingested water is low. At the request of the EPA Safe Drinking Water Act, the U.S. National Research Council (NRC) appointed a committee to conduct a study and report on the health risks associated with exposure to ^{222}Rn in drinking water (NAS/NRC, 1999a). Radon-222 can diffuse through the stomach wall, but this diffusion is very low. The calculated effective dose was about 4×10^{-9} Sv/Bq ingested. ICRP published a dose coefficient for ingested water as 6.9×10^{-10} Sv/Bq (ICRP, 2017).

Of special concern is the fetal dose from maternal ingested water. This requires a fetal dose model. The model developed estimated that if an average of 0.6 L of raw tap water at a ^{222}Rn concentration of 100 Bq/L were consumed per day, the calculated total dose to the fetus over the term of the pregnancy would be about 250 μSv or 25% of the normal background radiation dose over the fetal lifetime (Robbins et al., 1990; Robbins and Harley, 2005).

23.7 ^{220}RN (THORON) THE OTHER RADON

23.7.1 Indoor Measurement

Radon-220, thoron, is the noble gas produced as a decay product of ^{224}Ra in the primordial ^{232}Th decay series (Fig. 23.2). Thoron has a very short half-life, 55.6 s, compared with ^{222}Rn, 3.82 days. For this reason, many assume that its emanation from floor and wall surfaces indoors poses no problem because it decays before mixing in room air. However, this is not supported by measurements, and the mean life of a ^{220}Rn atom $T_{1/2}/\ln 2 = 80$ s is sufficient time for complete mixing of gas atoms with the room atmosphere. One publication measured ^{220}Rn in a laboratory that had the floor contaminated with ^{232}Th and an epoxy seal. The measurements were made using alpha track detectors placed on a string attached from ceiling to floor. The ^{220}Rn measurements at 0.5 m intervals above the floor source showed that the room concentration of ^{220}Rn was uniformly mixed. In spite of its short (55.6 s) half-life, the mean life of a ^{220}Rn atom, 80 s, is long enough for the gas atoms to mix uniformly into the room space where normal human breathing exists (Harley et al., 2010).

Thoron decays to ^{212}Po ($T_{1/2}$ 0.3 μs), ^{212}Pb ($T_{1/2}$ 10.6 h), and ^{212}Bi ($T_{1/2}$ = 60.5 min). Lead-212 is a beta emitter and produces little bronchial dose when inhaled; ^{212}Bi is an alpha emitter and produces the bronchial dose from ^{220}Rn inhaled in any atmosphere. Similar to ^{222}Rn, the decay products rapidly attach to local aerosol particles and, when inhaled, are deposited on the bronchial airways, producing the relevant dose. The buildup of ^{212}Pb and ^{212}Bi in air through 10.6 h ^{212}Pb is therefore small and means that airborne concentrations of the alpha emitters relevant to lung dose are low. Airborne concentrations are reduced by typical removal rates of aerosol particles onto surfaces and by ventilation of 0.2 h^{-1}.

The awareness of the importance of ^{220}Rn, in terms of its radiological impact, is increasing not only because of its bronchial dose, but mainly because of awareness of measurement difficulties. Part of the measured alpha activity for ^{222}Rn and decay products can include the thoron decay product ^{212}Bi. Guidelines for ^{222}Rn dosimetry are presently under the discussion section, and it is anticipated that ^{220}Rn will also be involved. Compared with ^{222}Rn, there are few published survey data on environmental ^{220}Rn presently available. More effort should be put into ^{220}Rn-related studies to better understand accurate measurements of residential ^{222}Rn exposure and lung dose (Fisenne et al., 1990; Tokonami et al., 2010).

The bronchial dose due to inhalation of ^{220}Rn decay products is similar to ^{222}Rn. The bronchial dosimetry is discussed in Section 23.11.

23.8 RADON EPIDEMIOLOGY IN UNDERGROUND MINES AND LUNG CANCER RISK

Mining started at the beginning of the sixteenth century in Schneeberg (now Germany) and at the beginning of the fifteenth century in Joachimsthal (now Czech Republic). Both are situated in the Erzgebirge, or Ore Mountains, which form the border between Saxony and Bohemia. The mountains are famous for their mineral wealth. Besides copper and iron, silver was mined first, and later cobalt, arsenic, bismuth, and nickel. In the latter part of the nineteenth century pitchblende was mined, especially in Joachimsthal, and was used in the manufacture of uranium dye. In 1909 the manufacture of radium was started in Joachimsthal, and from 1909 to 1925, 26 g of radium was produced (Lorenz, 1944).

Deaths from lung cancer were observed, especially in the uranium mines in the Erzgebirge of Saxony in the former German Democratic Republic. The history of disease in these miners extended over five centuries with the first observations of health hazard starting in the Middle Ages (Lorenz, 1944).

Many of the miners of these districts were known to die in middle age of a pulmonary disease, which the miners call "Bergkrankheit" or mountain disease. As early as the beginning of the sixteenth century, descriptions of the disease could be found in old chronicles (Lorenz, 1944). The "Bergkrankheit" was first diagnosed as cancer of the lung by Harting and Hesse (1879). They determined that a miner inhaled approximately 6 g of dust in the mines in 7 h. The first detailed physical investigations in Schneeberg were carried out by Ludewig and Lorenser (1924), who found that the ^{222}Rn content of the air in the mines varied from 3.6×10^{-10} to 1.8×10^{-8} curies (Ci)/L and most values were below 3.6×10^{-9} Ci/L (0.013, 0.67, 0.13 kBq/m^3, respectively).

The early Schneeburg and Joachimsthal experience in silver, bismuth, nickel, radium, and arsenic miners reported that lung cancer was the cause of between 30 and 70% of deaths (NCRP, 1984). The first underground uranium mining cohort was established for Colorado miners from 1950 to 1963 (Lundin et al., 1971). It included 3362 white miners and 780 mostly Navajo Indian miners. Later cohorts were established for Czech, Swedish, Canadian, and Newfoundland miners. NCRP (1984) used these four lung cancer cohorts to develop the first risk model for residential ^{222}Rn decay product related to lung cancer (Harley and Pasternack, 1981).

The NCRP (1984) risk model used low ^{222}Rn exposure mine estimates to apply the risk to residences and developed the first model to establish that risk decreases after the last exposure to ^{222}Rn and decay products, that is, a time since exposure effect. Lung cancer risk was estimated to decrease with a half-life of 20 years following exposure. The first anatomical model was developed to calculate the ^{222}Rn dose factor in miners and the environmental dose factor for men, women, and children. This average environmental dose factor for adults was 6 rem/WLM or 9.6 mSv/WLM (NCRP, 1984).

Various organizations obtained the occupational dose and employment records, and three large studies evaluated the epidemiologic data (NAS/NRC, 1988, 1999b; Lubin et al., 1995). Table 23.1 shows the characteristics of the BEIR VI study for 11 cohorts and includes the ^{222}Rn exposure in WLM, number of miners, the type of mine, follow-up period, and excess relative risk, ERR/100 WLM. The weakness in the early data was that relatively scant exposure measurements existed and that no information was available on smoking. The exception for smoking was the Colorado occupational records that had smoking information.

The studies have also been used to identify health issues other than lung cancers, but so far lung cancer is the only identified risk, with some suggestion of stomach cancer due

TABLE 23.1 Characteristics of the Cohorts Considered in the BEIR VI Report (NAS/NRC, 1999b)

Place	Country	Type of Mine	Follow-Up Period	No. of Miners	Cumulative Exposure (WLM)	Person-Years[a]	ERR per 100 (WLM)	
Yunnan	China	Tin	1976–1987	13,649	286.0	134,842	0.17	
W-Bohemia	Czech Republic	Uranium	1952–1990	4,320	196.8	102,650	0.67	
Colorado	USA	Uranium	1950–1990	3,347	578.6	79,556	0.44	
Ontario	Canada	Uranium	1955–1986	21,346	31.0	300,608	0.82	
Newfoundland	Canada	Fluorspar	1950–1984	1,751	388.4	33,795	0.82	
Malmberget	Sweden	Iron	1951–1991	1,294	80.6	32,452	1.04	
New Mexico	USA	Uranium	1943–1985	3,457	110.9	46,800	1.58	
Beaverlodge	Canada	Uranium	1950–1999	6,895	21.2	67,080	0.55	
Port Radium	Canada	Uranium	1950–1980	1,420	243.0	31,454	0.24	
Radium Hill	Australia	Uranium	1948–1987	1,457	7.6	24,138	2.75	
CEA-COGEMA	France	Uranium	1948–1986	1,769	59.4	39,172	0.51	
Total				**60,606**	**164.4**	**888,906**	**0.59**	**1.32**

[a]Among exposed.
ERR, excess relative risk; WLM, Working Level Month.

to decay product clearance from the lung to the stomach (Darby et al., 1995, 2005). The results from 11 collaborative studies provide considerable evidence that neither inhaled ^{222}Rn nor ^{222}Rn progeny deposited on the skin causes a material risk of mortality from cancers other than lung cancer. It therefore seems appropriate that protection standards against atmospheric ^{222}Rn should continue to be based on consideration of the lung cancer risk alone (Darby et al., 1995, 2005).

One of the most recent analyses of lung cancer risk and ^{222}Rn exposure was carried out using restrictions to a nested case–control study on uranium miners in the Czech Republic, France, and Germany (Hunter et al., 2013). With the data restricted to cumulative exposures below 300 WLM and adjustment for smoking status, the ERR/WLM was 0.0174 (95% CI: 0.009–0.035) compared with the estimate of 0.008 (95% CI: 0.004–0.014) using the unrestricted data.

The study of Hunter et al. (2013) can be shown to be in good agreement with the lifetime ^{222}Rn risk from the residential study of 13 pooled European studies (Darby et al., 2005). The pooled European study estimated a lifetime ERR of 0.16 for a ^{222}Rn exposure of 100 Bq/m^3. Hunter et al. (2013) can be shown to yield a lifetime ERR of 0.19 or 0.09 for the restricted and unrestricted data, respectively. The details of this calculation are given in Section 23.9.

Analysis of both the restricted and unrestricted data in Hunter et al. (2013) showed that time since exposure windows had a major effect; the ERR/WLM was six times higher for more recent exposures (5–24 years) than for more distant exposures (25 years or more). There was no statistically significant difference in the ERR/WLM by smoking status, that is, never smoked or smoker.

The same ERR/WLM for smokers and never smokers means that the higher risk for smokers, often noted, results from the higher baseline (background) lung cancer risk for smokers, not from synergy as sometimes stated; see Table 23.5 in Section 23.12 for lung cancer risk in smokers and never smokers.

The pooled BEIR VI analysis for the 11 cohorts was ERR/100 WLM which was 0.48 (CI: 0.18–1.27) among ever-smokers. Among these same miners, the overall ERR/100 WLM, ignoring smoking status, was 0.53 (CI: 0.20–1.38).

The most recent analysis of a very large miner population is the East German Wismut study. Extensive uranium extraction took place from 1946 until 1990 at the former Wismut mining company in East Germany. A total of 58,987 male former employees of this company form the largest single uranium miner cohort that has been followed up for causes of mortality occurring from the beginning of 1946 to the end of 2008. The simple cohort excess relative risk (ERR/WLM) for lung cancer was estimated as 0.0019 (95% CI: 0.0016–0.0022) (Walsh et al., 2015) and ERR/WLM = 0.013; 95% CI: 0.007–0.021 (Kreuzer et al., 2015). The difference is probably related to the models used. The BEIR VI model applied a 51% decrease in risk with each decade following time since exposure that may overestimate risk.

23.9 RESIDENTIAL RADON EPIDEMIOLOGY LUNG CANCER MODELS AND LUNG CANCER RISK

The first calculations of lifetime lung cancer risk from residential ^{222}Rn exposure were published in NCRP Report 78 (NCRP, 1984). The lifetime risk calculations were based on the five existing epidemiologic studies at the time, United States, Sweden, Czechoslovakia, Canada, and Newfoundland.

The risk model was applied to residential exposure but was extrapolated from the uranium underground mining epidemiology. The NCRP (1984) model was the first to establish a reduction in risk with time following exposure, using a 20-year half-life reducing the risk. The published residential epidemiological studies are summarized in Fig. 23.7, giving both the individual studies and the pooled studies. There are 22 residential case–control studies and seven pooled studies shown in Fig. 23.7.

The excess ERR most used for residences is 0.16 $(100\,\text{Bq/m}^3)^{-1}$ or a 16% increase from background (baseline) lung cancer, regardless of smoking status. This was derived from a pooled study of 13 individual European epidemiologic studies (Darby et al., 2005). The Darby et al. (2005) ERR was first calculated as 8% but then corrected to 16% by

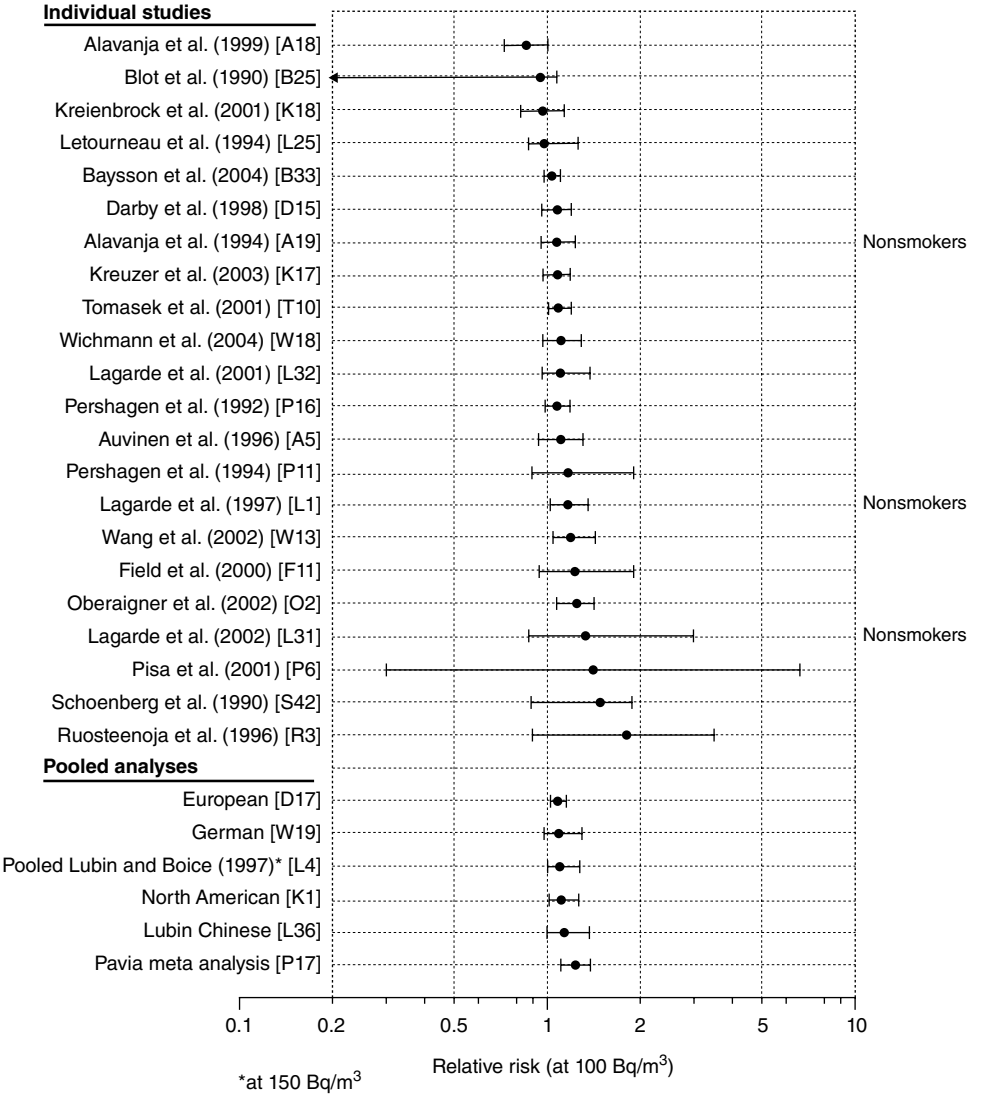

FIGURE 23.7 The summary relative risks for individual and pooled residential studies. Exposure at $100\,\text{Bq/m}^3$ [$150\,\text{Bq/m}^3$ for (L4, P17)] and the corresponding 95% confidence intervals (UNSCEAR, 2008).

including the possible effect of year-to-year variations in measurements of annual exposure. At usual ^{222}Rn levels of 0, 100, 400, and 800 Bq/m^3, respectively, cumulative absolute risks of lung cancer by age 75 years would be 0.41, 0.47, 0.67, and 0.93% in lifelong nonsmokers and 10.1, 11.6, 16.0, and 21.6% in cigarette smokers. The risk depends only on the baseline lung cancer rate because ERR is the same for smokers and nonsmokers (Darby et al., 2005).

The Darby et al. (2005) pooled analysis of 13 individual studies have the best precision so far, also of evidence of statistical significance. It should be noted that better ^{222}Rn measurements improves the precision in the epidemiologic studies.

In agreement with mining studies, analyses of lung cancer risk were carried out using restrictions to nested case–control data on uranium miners in the Czech Republic, France, and Germany (Hunter et al., 2013). With the data restricted to cumulative exposures <300 WLM and adjustment for smoking status, the ERR/WLM was 0.0174 (95% CI: 0.009–0.035), compared with the estimate of 0.008 (95% CI: 0.004–0.014) using the unrestricted data. These values were compared to the Table 23.1 average of 0.59 ERR/100 WLM. A ^{222}Rn exposure of 100 Bq/m^3 can be calculated in units of WLM to compare the mining and residential risks. The residential exposure to ^{222}Rn gas of 100 Bq/m^3 is equal to 0.54 WLM/year, and the 20-year exposure assumed for residential exposure (Darby et al.) is a lifetime exposure of 20×0.54 or 11 WLM. The ERR values of 0.008 and 0.0174 WLM^{-1} derived from Hunter et al. equal an ERR of 9 and 19% per 100 Bq/m^3 for the unrestricted and restricted analyses, respectively. The Hunter et al. (2013) studies of three uranium miners is in good agreement with the residential study, ERR = 16%, of Darby et al. (2005).

The ERR for bronchogenic lung cancer in underground mines and residences show no striking differences; thus the dose pathways for production of lung cancer appear to be similar. The bronchial dose from exposure in mines and residences is described in detail in Section 23.10.

23.10 LUNG DOSIMETRY

23.10.1 ^{222}Rn (Radon) Bronchial Dosimetry

23.10.1.1 Dosimetry Calculations In 1958, the Nuclear Standards Board of the American National Standards Association [later Institute (ANSI)] established a committee to develop a standard for uranium mines and mills. This standard, adopted in 1960, was denoted the WL, numerically equal to the total alpha energy release from decay of the ^{218}Po, ^{214}Pb, and ^{214}Bi in 1 L of air equivalent to 1.3×10^5 MeV/L. The annual exposure was in WLM equal to WL × (hours exposed/month)/(170 hours per work month).

In 1960, 1 WL and 12 WLM/year were adopted as a standard and were responsible for a significant decrease in miner exposures in the United States. Companies began implementing control, and in 1971, the standard was reduced from 12 to 4 WLM/year and adopted by the U.S. Bureau of Mines (USBM) because of the emerging epidemiologic studies of ^{222}Rn and lung cancer (Lundin et al., 1971). This standard is still in effect in mines in the United States, at the USBM and NRC (Harley, 2018).

23.10.1.2 ^{222}Rn Dosimetry Models Guidelines for ^{222}Rn exposure are based on gas concentrations for residences or WLM for mines and most occupational settings. Some countries and ICRP are considering application of these values to a dose limitation system or for comparison with other radiation dose exposures, even though such a comparison is rare.

The relevant dose from ^{222}Rn is the alpha radiation dose to basal and secretory cells in the airway bronchial epithelium. Radon-222 does deliver a small dose to other organs; however, the specific carcinogenic dose is to the lung bronchial epithelium. This requires a dose model to estimate alpha particle dose resulting from the decay products deposited on the bronchial airways part of the tracheobronchial tree. The dose from ^{222}Rn gas itself is about a factor of 5 less than the decay product dose because of the large difference in distance to target cells within the epithelium from gas atoms in the airway volume versus decay product atoms deposited on the epithelial surface (Harley, 2018).

A bronchial dose factor model has been used by several organizations and originally published by individuals. The models considered 5 factors to deposit inhaled decay products on the epithelium. These are breathing rate, inhaled activity particle size, lung deposition and mucociliary clearance of particles on the airways, target cells, and depth within the bronchial epithelium. Published models selected different characteristics, resulting in a variety of dose factors values (Harley, 2018). The basic alpha absorbed dose factor mGy/WLM is expressed as equivalent dose using the same unit mGy/WLM by multiplying absorbed dose by 20 (the radiation weighting factor). The dose in effective dose units is mSv/WLM multiplying by 0.08, the tissue weighting factor for bronchial epithelium. The traditional dose unit is the WL and total or annual dose WLM is the exposure guideline used in mines.

One factor that has been widely used for occupational dose records is the ICRP (1993) dose conversion convention (DCC) based on the ratio of ^{222}Rn lifetime excess absolute risk (LEAR) to A-bomb effective dose risk. Because of the results of ongoing epidemiology, the calculated dose conversion convention increased by a factor of 2 because both ^{222}Rn and A-bomb risk values changed. The conversion convention risk ratio is replaced by a biokinetic/dosimetric model (ICRP, 2014, 2017). The values for the former and recent dose conversion convention are listed in Table 23.2.

The dose conversion convention is a mixed unit dose factor because the ^{222}Rn lifetime risk is an alpha dose specific to lung, while the effective dose is a whole-body dose based on external gamma ray radiation. The DCC is based on risk.

For many years, publications concerning ^{222}Rn exposure in different countries and under different exposure conditions used the UNSCEAR bronchial dose factor of 9 nSv per Bq h/m^3 (EEC). This is equal to 6 mSv/WLM, and because it is within the range of published dose factors, it continues to be used (UNSCEAR, 1988, 1993, 2000, 2008, 2019).

The bronchial dose from ^{222}Rn is due to the inhalation of the solid, alpha emitting, short-lived decay products. The decay products attach rapidly to 100–400 nm aerosol particles and are never in equilibrium with the parent ^{222}Rn. This is because they are removed by surface deposition, by ventilation in indoor environments, and by diffusion. The EEC of the decay products is needed for the dose calculation because the factor WL requires equilibrium. The equilibrium concentration is determined by measurement of the decay product concentrations and is measured originally using alpha counting over three intervals after a filtered

TABLE 23.2 Estimated Dose Factors, mSv/WLM, Using the Dose Conversion Convention Risk Factors (ICRP, 1993, 2010)

Reference	LEAR ^{222}Rn Detriment/WLM	Workers Detriment/ Effective Dose (mSv)	Dose Conversion Convention (mSv/WLM)
ICRP (1993)	2.8×10^{-4}	5.6×10^{-5}	5.0
ICRP (2010)	5.0×10^{-4}	4.2×10^{-5}	11.9

air sample is collected. The EEC is currently measured using portable alpha spectrometers analyzing over two or three alpha particle energy regions to measure WL directly.

The EEC ^{222}Rn concentration C_{eq} is

$$C_{eq} = 0.104\left(^{218}\text{Po}\right) + 0.516\left(^{214}\text{Pb}\right) + 0.380\left(^{214}\text{Bi}\right)$$

where ^{218}Po, ^{214}Pb, and ^{214}Bi are the measured concentrations of ^{218}Po (Bq/m^3).

When C_{eq} is measured, an equilibrium factor, F_{eq}, is used to calculate the EEC:

$$F_{eq} = \frac{C_{eq}}{^{222}\text{Rn}}\,\text{Bq/m}^3$$

The WL is calculated from the definition of 100 pCi/L ^{222}Rn in equilibrium with its decay products (equal to a total energy release in air 1.3×10^5 MeV/L):

$$\text{WL} = F_{eq} \times \frac{^{222}\text{Rn Bq/m}^3}{3700}$$

The annual WLM or any exposure interval in months is

$$\text{WLM} = \left[F_{eq} \times \frac{^{222}\text{Rn Bq/m}^3}{3700}\right] \times \left[\frac{\text{hours exposed}}{\text{170 hours per work month}}\right]$$

A summary of published bronchial dose factors is shown in Table 23.3. In Table 23.3, the average effective bronchial dose conversion factor (DCF) for seven measurements in homes is 9.2 ± 1.4 mSv/WLM, and for six measurements of workplaces and mines is 10.4 ± 0.7 mSv/WLM. UNSCEAR summarized 13 published bronchial effective dose models and found 13 ± 24 mSv/WLM.

The ICRP (2017) recommendation for the ^{222}Rn effective dose factor for indoors and workplaces is 20 and 12 mSv/WLM for mines. The activity median particle diameter selected was 500 nm, F_{eq} 0.4 for indoor workplaces, and 250 nm, F_{eq} 0.2 for mines.

The ICRP (2017) indoor and workplace particle size distribution contains a 30% inclusion of an unusual mode, the nucleation mode with 60 nm diameter. The nucleation mode is a transient home or workplace aerosol particle injection due to cooking or a specific process. The nucleation activity size distribution is usually assumed to be from about 10 to 100 nm. Whenever nucleation is included in a dose model, it increases the bronchial dose factor by about 30%. A nucleation mode should not be considered as a typical, ever present aerosol particle size distribution unless calculation is for a specific atmosphere. The ICRP (2017) parameters selected for their model are regulatory and conservative, not necessarily typical for indoor or mine environments.

One of the major parameters affecting dose in the dose models is the inhaled aerosol particle size. Radon-222 decays to ^{218}Po that exists for up to 30 or 60 s as a cluster with an activity median diameter 1–4 nm. This is called the unattached decay product mode. The cluster rapidly attaches to the local aerosol particles with an activity median diameter of about 100–300 nm diameter, called the attached mode. A typical activity particle size spectrum for residences is shown in Fig. 23.8.

An illustration to show the variability of the bronchial dose factor's dependence upon inhaled particle diameter and breathing rate for unattached mode fpot of 0.05 is shown in Fig. 23.9.

TABLE 23.3 Published Values of Effective Dose to an Adult Male from the Inhalation of Radon and Its Progeny Calculated Using Dosimetric Models

Publication	Model Type	Exposure Scenario	Effective Dose (mSv/WLM)	Effective Dose [mSv/(mJh/m^3)]
ICRP (1987)	NEA (1983)	Indoors	6.4	1.8
		Outdoors	8.9	2.5
UNSCEAR (2008)	NEA (1983)	Indoors and outdoors	5.7	1.6
Harley et al. (1996)	Anatomical lung	Indoors and mines	9.6[a]	2.7
Porstendorfer (2001)	Zock et al. (1996)	Home[b]	8	2.3
		Workplace	11.5	3.2
		Outdoor	10.6	3.0
Winkler-Heil and Hofmann (2002)	Deterministic airway generation model	Home	7.6	2.1
Winkler-Heil et al. (2007)	Deterministic airway generation model	Mine	8.3	2.3
	Stochastic airway generation model	Mine	8.9	2.5
	HRTM (ICRP, 1993)	Mine	11.8	3.3
Marsh and Birchall (2000)	HRTM (ICRP, 1993)	Home	15	4.2
James et al. (2004)	HRTM (ICRP, 1993)	Mine[c]	16.8	5.9
		Home[b]	20.9	6.0
Marsh et al. (2005)	HRTM (ICRP, 1993)	Mine	12.5	3.5
		Home[b]	12.9	3.6

[a]An absorbed dose of 6 mGy/WLM [1.7 mGy/(mJh/m^3)] was calculated for the bronchial region. The effective dose per unit exposure calculated with a radiation weighting factor for alpha particles of 20 and a tissue weighting factor of 0.08 (=2/3 × 0.12) for the bronchial and bronchiolar regions of the lung (NCRP, 1984).
[b]Home without cigarette smoke.
[c]No hygroscopic growth was assumed.
WLM, Working Level Month; HRTM, Human Respiratory Tract Model.

One difficulty in calculating bronchial dose from inhaled ^{222}Rn decay products is that the input to the model, WLM, requires the value of F_{eq}. The equilibrium factor, F_{eq}, is quite variable depending upon the environment chosen. In published results from China, India, and 13 other countries, F_{eq} was 0.41 ± 0.11 in dwellings, 0.44 ± 0.16 at indoor workplaces, and 0.54 ± 0.13 outdoors with a range from 0.1 to 0.8 (Chen and Harley, 2018a).

UNSCEAR (2008) adopted F_{eq} values for indoors = 0.4 and outdoors = 0.6 to use for dose calculation. These adopted values can have an error of ±50% and are used if measurements are not available for specific environments (Chen and Harley, 2018a).

In mines, a value of 0.3 for F_{eq} is commonly used based on sparse measurements and noting the higher ventilation rates in mines than for other occupations. Chen and Harley (2019) in a review of 173 mines found the average F_{eq} to be 0.38. The F_{eq} value can be highly variable depending on the location involved. More measurements are required if dose units are to be calculated in mining atmospheres for exposure records.

A summary of models developed for application of bronchial dose factors to ^{222}Rn measurements is shown in Table 23.3 from ICRP Publication 115 (ICRP, 2010).

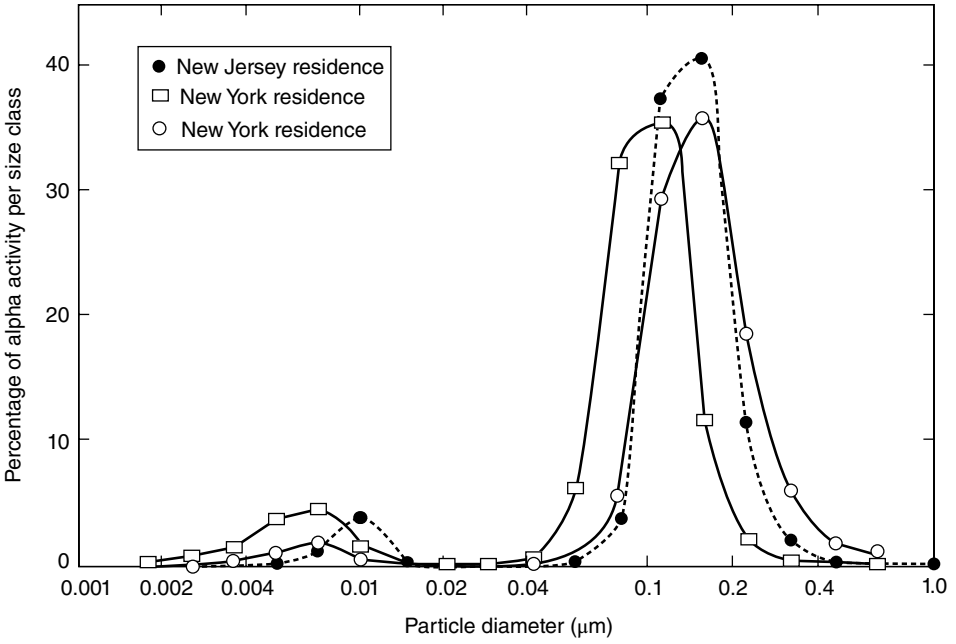

FIGURE 23.8 Indoor ^{222}Rn decay product size distributions (George and Breslin, 1980; NCRP, 1984).

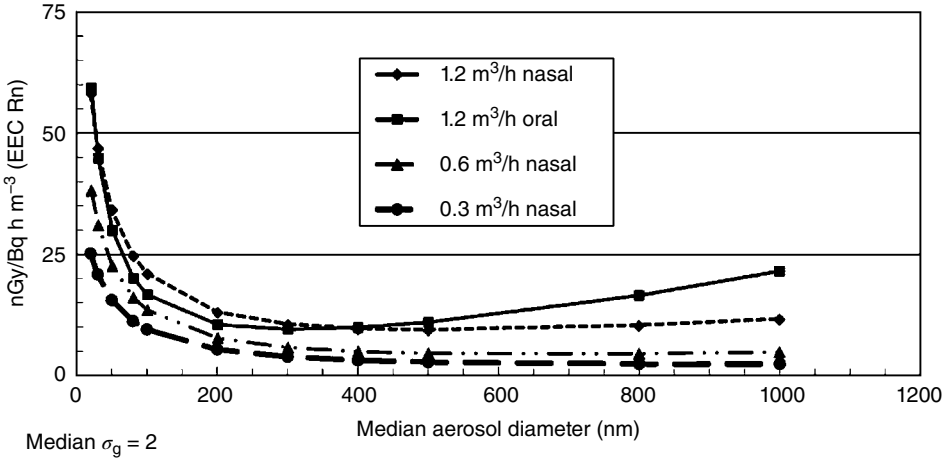

FIGURE 23.9 Dose factors for selected breathing rates, fpot 0.05 for mSv/WLM (UNSCEAR, 2008). Multiply by 0.99 for mSv/WLM.

23.10.2 ^{220}Rn (Radon) Dosimetry

Radon-220 (Thoron) is present in most atmospheres because the parent chain headed by ^{232}Th, half-life 14×10^9 years, is present in all Earth's elements. The very short half-life of ^{220}Rn, 55.6 s, decaying to ^{212}Pb, 10.6 h, ensures a low concentration in outdoor and indoor air. There are few surveys of ^{220}Rn, and some show a concentration about equal to the ^{222}Rn concentration.

However, because of the gas' short half-life and long decay product half-life, the EEC of ^{220}Rn is low. The measurement of the average equilibrium factor, average F_{eq} for ^{220}Rn, reported in China, India, and 13 other countries is 0.042 ± 0.031 with a range from 0.0.003 to 0.14. In this review, ^{220}Rn concentrations ranged from 11 to 1247 Bq/m^3 (Chen and Harley, 2018b).

UNSCEAR adopted average values of F_{eq} = 0.02 for indoors and F_{eq} = 0.003 for outdoors, based on very limited data at the time.

UNSCEAR (2008) reported 10 ^{220}Rn bronchial dose models with an average effective dose of 3.5 ± 0.3 mSv/WLM. ICRP (2017) recommends 5.6 and 4.8 mSv/WLM with atmospheric characteristics similar to ^{222}Rn, including a nucleation mode.

The measurement of ^{222}Rn is often accompanied by an unknown ^{220}Rn component. This measurement difficulty is especially evident in alpha track detectors without a ^{220}Rn barrier to prevent inclusion of the ^{220}Rn signal. This increases the calculated ^{222}Rn concentration producing a compromised value. Radon-222 gas measurements should universally include a ^{220}Rn barrier for accurate exposure assessment (Tokonami et al., 2010).

23.11 REGULATIONS AND GUIDELINES FOR ^{222}RN EXPOSURE

The first regulations were for underground uranium mine exposures following the reports of lung cancer in mining population (see Section 23.10).

The first guideline for home exposure to ^{222}Rn was set by NCRP at 2 WLM/year or one-half the 4 WLM/year limit for underground mines and was based on lung cancer risk estimates (NCRP, 1984). The NCRP guideline, however, was for annual personal exposure, not for a home. The epidemiologic studies of ^{222}Rn in homes and associated with lung cancer are convincing in that evidence provided by measurements of ^{222}Rn supports an exposure risk relationship.

The exposure guideline for ^{222}Rn was given in units of WL and cumulative exposure in WLM. This is the exposure times the hours worked in work months and usually calculated for annual exposure. The WL multiplied by the time in work hours per year, divided by 170 h per work month, equals the annual WLM cumulative exposure (see Section 23.10 for details).

Guidelines for ^{222}Rn exposure in residences are currently based on air concentrations of ^{222}Rn gas (Bq/m^3) or ^{222}Rn decay products and WLM for occupational exposure.

Guidelines for ^{222}Rn exposure emerged as the epidemiology of ^{222}Rn exposure and lung cancer in residences was studied, and it was discovered that homes could attain ^{222}Rn concentrations equal to that in underground uranium mines. The first case referent epidemiologic study of ^{222}Rn in residences was Axelson et al. (1979). This was followed by Edling et al. (1984), Svensson et al. (1987, 1989), Axelson et al. (1988), and Schoenberg et al. (1989).

The difficulty in converting air concentration to a bronchial dose guideline is that individual decay products are rarely measured, especially in residences, and dose factors must be used. Gas measurements must be multiplied by a dose factor and an adopted value of F_{eq} for indoor or outdoor environments. To obtain a value of C_{eq}, the ^{222}Rn EEC, values of measurements of F_{eq} for ^{222}Rn or ^{220}Rn (Chen and Harley, 2018a, 2018b) (see Section 23.10) must be used and dose factors from Table 23.3. Regulatory and residential guidelines based on these variables are shown in Table 23.4.

TABLE 23.4 Occupational and Residential Guidelines for ^{222}Rn Exposure

Organization	Residential	Occupational
ICRP 2012	100–300 Bq/m^3	1000 Bq/m^3
NCRP 1984	2 WLM/year personal exposure	4 WLM/year
U.S. EPA 2016	148 Bq/m^3	
WHO 2009	100–300 Bq/m^3	
U.S. Bureau of Mines (USBM)		4 WLM/year
NRC		4 WLM/year
Health Canada 2011, 2017	200 Bq/m^3	200 Bq/m^3
European Union (EU)[a]	200 Bq/m^3	

[a]For EU value see Canada residential guidelines.

Guidelines follow the epidemiologic studies becoming regulations that were adopted from prudent estimates of what risk envisioned, not actually risk based.

Because drinking water use can increase ^{222}Rn concentrations in a home, the Safe Drinking Water Act (SDWA) directs the EPA to promulgate a maximum contaminant level (MCL) for ^{222}Rn in drinking water. Radon in drinking water is based on a multimedia approach designed to achieve greater risk reduction by addressing radon risks in indoor air, with public water systems providing protection from the highest levels of ^{222}Rn in their groundwater supplies (U.S. EPA, 1999).

Jurisdictions without an approved multimedia mitigation MMM program plan, serving >10,000 people, must comply with an MCL of 300 pCi/L. If they comply with the MMM an alternative, AMCL of 4000 pCi/L applies. Small community water systems serving 10,000 or fewer people must comply with 4000 pCi/L and implement a state-approved multimedia mitigation plan (see Section 23.5 on groundwater).

23.12 RADON AND SMOKING

Cigarette tobacco contains ^{210}Pb and some ^{226}Ra taken up during plant growth mainly through root absorption from the soil (Tso and Fisenne, 1968). A small fraction of the ^{210}Pb in tobacco is deposited on the tobacco leaf from atmospheric ^{222}Rn decay products. It is about 3 years before cigarettes are manufactured from cured tobacco, and the ^{210}Po decay product of ^{210}Pb builds up in the tobacco to equal the concentration of the ^{210}Pb. At temperature of a burning cigarette, the ^{210}Po is more volatile than ^{210}Pb, and alpha emitting ^{210}Po is the relevant radiation carcinogen. Nicotine is extracted from plants in the tobacco family for e-cigarette cartridges but so far ^{210}Po has not been detected in e-cigarette vapor. Tobacco also contains measured ^{226}Ra, but ^{226}Ra is not volatile at burn temperature. Schayer et al. (2009) calculated the average concentration of ^{210}Po measured in cigarette tobacco from 17 countries as 15 ± 4 mBq/cig (15 ± 4 mBq/g) and measured ^{210}Po in 12 brands of Chinese cigarettes that showed a 50% greater concentration, 23 ± 4 mBq/cig.

The amount of ^{210}Po and ^{210}Pb transferred to mainstream smoke during the cigarette burn determines the bronchial dose. Iwaoka and Yonahara (2012) calculated the average of 11 studies that measured ^{210}Po transfer to mainstream smoke as 16 ± 4%, the transfer of ^{210}Pb to mainstream smoke is about half this value, and, because it is a beta particle emitter, the dose is very small compared with the ^{210}Po bronchial dose. One lung dose estimate for inhaled ^{210}Po is 1.4×10^{-5} Sv/Bq (Iwaoka and Yonahara, 2012). The typical smoking duration is 40 years. Smoking one pack of cigarettes (20) per day for 40 years yields an estimate

TABLE 23.5 The Lifetime Risk of Lung Cancer in Residences for Smokers and Never Smokers

Smoking Status	% Lifetime Baseline Lung CA[a]	^{222}Rn Exposure (Bq/m^3)	% Lifetime Lung CA with Radon Exposure[b]
Men nonsmoker	0.91	100	$0.91 \times 1.16 = 1.06$
Men smoker	11.6	100	$11.6 \times 1.16 = 13.5$
Women nonsmoker	0.59	100	$0.59 \times 1.16 = 0.68$
Women smoker	6.8	100	$6.8 \times 1.16 = 7.89$

[a]Source NAS/NRC (1999a).
[b]Source ERR for residential studies 0.16 (Darby et al., 2005).

of the lung dose as 0.25 mSv/year and 10 mSv for 40 years. The average annual natural background dose from all sources is 3.1 mSv/year so typical cigarette smoking corresponds to 0.25/3.1 or 8% of all-natural radiation background (NCRP, 2009).

There are two large epidemiologic studies of ^{222}Rn and lung cancer that deal with smoking. One is for uranium miners (Hunter et al., 2013), and the other is the pooled residential study of Darby et al. (2005). Hunter et al. (2013) carried out an analysis of lung cancer risk using restrictions to data on uranium miners in the Czech Republic, France, and Germany. The data were restricted to cumulative exposures below 300 WLM and adjustment for smoking status; the ERR/WLM was 0.0174 (95% CI: 0.009–0.035), compared with the estimate of 0.008 (95% CI: 0.004–0.014) using the unrestricted data. There was no statistically significant variation in the ERR/WLM by smoking status. The calculated pooled residential study had an ERR = 0.16. An annual ^{222}Rn exposure of 100 Bq/m^3 is equal to 0.54 WLM/year and thus a 20-year lifetime exposure of 11 WLM.

An ERR of 0.0174 WLM^{-1} for the entire cohort, adjusted for smoking, is numerically equal to a lifetime risk of 0.0174×11, or 0.19. This is very good agreement with the residential study lifetime risk result of 0.16. The calculated mining risk changes depending upon the ERR value used; however, there is general agreement of the risk results for both mining and residential exposure (Hunter et al., 2013). It is important to obtain smoking information and best ^{222}Rn measurements in any ^{222}Rn and lung cancer study because this improves the precision of the risk estimates. See Section 23.9 for details of residential versus mining smoking risk.

As an example, the lifetime risk of lung cancer in residences for smokers and never smokers is shown in Table 23.5.

The increased risk from ^{222}Rn and smoking is often said to be synergistic. However, the ERR is the same value, 0.16, for both smokers and nonsmokers (Darby et al., 2005). The larger ^{222}Rn risk for smokers versus nonsmokers is a function of the larger baseline lifetime lung cancer risk.

23.13 CHILDHOOD ^{222}RN EXPOSURE

Childhood cancer is not unknown but relatively rare in normal situations. Some attempts are made to associate it with ^{222}Rn exposure because of a higher dose due to smaller lung size. There are six large epidemiological studies of residential ^{222}Rn and childhood leukemia, a dose assessment of fetal dose from maternal ^{222}Rn in drinking water and one study of childhood lung cancer in tin mines in China.

For a given high radiation dose, that is, therapy, children are generally at more risk of tumor induction than are adults. At present, projections of lifetime risk for specific cancer types following high exposure at young ages are statistically insufficient (UNSCEAR, 2013).

Robbins and Harley (2005) calculated the dose to the fetus from ^{222}Rn in maternal drinking water. They assumed that an average of 0.6 L of raw tap water at a concentration of 100 Bq/L is consumed per day and the calculated total dose to the fetus over the term of the pregnancy is about 0.25 mSv or 7% of the normal background radiation dose.

A possible association between residential ^{222}Rn and pediatric cancers in Devon and Cornwall compared the incidence of childhood cancers in 238 postal sectors between low ^{222}Rn sectors (<100 Bq/m^3), with an average of 57 Bq/m^3, and high radon sectors (>100 Bq/m^3), with an average radon level of 183 Bq/m^3. The authors found no significant difference in cancer incidence rate between high and low exposure sectors (Thorne et al., 1996).

In an investigation of residential ^{222}Rn exposure and risk of childhood acute myeloid leukemia (AML) alpha track detectors were placed in the houses of 173 cases and 254 controls for a year. Overall, there was no association between residential radon and the risk of AML (Steinbuch et al., 1999).

Evrard et al. (2005) evaluated the ecological association between indoor ^{222}Rn concentration and acute leukemia (AL) incidence among children <15 years of age in France. The study considered the whole country, divided in 348 geographical units. Incidence data included 4015 cases of AL registered by the French National Registry of Childhood Leukemia and Lymphoma between 1990 and 1998. Exposure was based on a national campaign of 13,240 indoor ^{222}Rn measurements. A positive ecological association was observed between indoor ^{222}Rn concentration and childhood leukemia incidence, on the borderline of statistical significance ($p = 0.053$). A significant association was observed for AML ($p = 0.004$) but not for acute lymphoid leukemia ($p = 0.49$).

Raaschou-Nielsen et al. (2008) performed a study of childhood leukemia in Denmark (2400 cases; 6697 controls) from 1968 to 1994. This study suggested a weak, but statistically significant, association of residential radon exposure and childhood ALL. The Danish study estimated a relative risk (RR) of 1.56 (95% CI, 1.05–2.30) for a cumulative exposure of 1000 Bq/m^3 year. For an exposure duration of 10 years, their RR corresponds to a radon concentration of 100 Bq/m^3.

Commenting on the Danish study, Harley and Robbins (2009) compared about 1 mSv/year from natural external background to ^{222}Rn and decay products that contribute an additional 10–60% to the bone marrow lymphocyte equivalent dose. However, another pathway for exposure of T (or B) lymphocytes is within the tracheobronchial epithelium (TBE). Inhaled ^{222}Rn decay products deposit on the relatively small area of airway surfaces and deliver a significant dose to the nearby basal or mucous cells implicated in human lung cancer. Lymphocytes are co-located with basal cells and are half as abundant. Using a 10-year exposure to 100 Bq/m^3, our dose estimates suggest that the equivalent dose to these lymphocytes could approach 1 Sv. The relatively high dose estimate to lymphocytes circulating through the BE, potential precursor cells for ALL, provides a dose pathway for an association, but no association has been found.

Demoury et al. (2017) studied childhood AL and natural background in France. The risk of AL following exposure to low doses due to natural background radiation (NBR) is not conclusively determined. AL cases diagnosed over 1990–2009 (9056 cases) were identified, and their municipality of residence at diagnosis collected by the National Registry of Childhood Cancers. The Geocap study, which included the 2763 cases in

2002–2007 and 30,000 population controls, was used for complementary analyses. AL risk, irrespective of subtype and age group, was not associated with the exposure of municipalities to radon or gamma radiation in terms of yearly exposure at age reached, cumulative exposure, or RBM dose. The findings do not support the hypothesis that residential exposure to NBR increases the risk of AL, despite the large size of the study, fine-scale exposure estimates, and a wide range of exposures over France.

Kollerud et al. (2014) studied the risk of leukemia or cancer in the central nervous system among children living in an area with high indoor ^{222}Rn concentrations. The results of this study in Norway used a cohort approach of 0–15-year-old children to examine whether residential ^{222}Rn exposure was associated with childhood leukemia and cancer in the central nervous system in the Oslo region. The study was based on Norwegian population registers and identified cancer cases from the Cancer Registry of Norway. The residence of every child was geo-coded and assigned a ^{222}Rn exposure. In all, 712,674 children were followed from 1967 to 2009 from birth to date of cancer diagnosis, death, emigration, or 15 years of age. A total of 864 cancer cases were identified: 437 children had leukemia and 427 had cancer in the central nervous system. The on-site indoor ^{222}Rn measurements accounted for only 6% of the residences in the study region. A buffer model with different radius size was used to estimate ^{222}Rn exposure to the rest of the cohort. Exposure was grouped as <50, 50–100, and >100 Bq/m^3. No association was found for ^{222}Rn and childhood leukemia.

Lubin et al. (1998) investigated the association between the incidence of ALL in children under age 15 years and indoor ^{222}Rn exposure. Detectors were placed in current and previous homes of subjects where they resided for 6 months or longer. Radon levels could be estimated for 97% of the exposure period for the eligible 505 case subjects and 443 control subjects. Mean ^{222}Rn concentration was lower for case subjects (65.4 Bq/m^3) than for control subjects (79.1 Bq/m^3). For categories less than 37, 37–73, 74–147, and 148 or more Bq/m^3 of ^{222}Rn exposure, RRs based on matched case–control pairs were 1.00, 1.22, 0.82, and 1.02, respectively. In contrast to prior ecologic studies, the results from this analytic study provide no evidence for an association between indoor ^{222}Rn exposure and childhood ALL.

Lubin et al. (1990) performed a case–control study of childhood acute lymphoblastic leukemia and residential ^{222}Rn exposure in tin mining carried out by the Yunnan Tin Corporation (YTC), China. YTC exposed adult miners and children under the age of 14. UNSCEAR (2008) summarized the YTC epidemiology regarding children. About 90% of lung cancer cases at YTC had history of working underground prior to 1950, the principal work involved boys and men carrying ore on their backs in small tunnels. That working style was gradually abolished after 1953 as mechanical exploitation started to be introduced at YTC during the early 1950s. YTC miners represented a stable population without significant loss of follow-up. Miners generally started mining before 1950, some as early as the 1920s. Most miners with lung cancer began mining as children under the age of 14, but this group's age of death and risk of lung cancer showed no prominent differences with miners who started mining after the age of 15 or 20.

The studies of residential concentration of ^{222}Rn do not support any association of childhood leukemia. The Raaschou-Nielsen et al. (2008) study had a weak ALL response, but the ^{222}Rn exposure data was estimated from other studies. The study of high ^{222}Rn exposure in underground mines where children were used showed no difference from adult miners.

23.14 OTHER NATURAL BACKGROUND EXPOSURE

Other natural radionuclides, ^{238}U, ^{232}Th, ^{226}Ra, ^{210}Pb, ^{210}Po, and ^{40}K that deliver an internal radiation dose to the global population, are generally found in the diet or drinking water. This internal body radioactivity derives from the ^{238}U, ^{226}Ra, ^{232}Th, and ^{210}Pb in the food chain due to plant uptake of these elements present in soil. Ever present external gamma ray radiation is present from the ^{238}U, ^{232}Th, ^{226}Ra, and ^{40}K in soil plus cosmic ray radiation.

The major internal dose from a natural nuclide is from ^{40}K, a fraction of the necessary component of the body's element stable ^{39}K. Potassium-40 is present as a fraction (0.0117%) of all stable ^{39}K. Every cell in the body requires ^{39}K ions for normal function. The average body content of ^{39}K is about 100 g.

A summary of all major radioactivity sources and annual exposure is shown in Table 23.6 and Fig. 23.10.

TABLE 23.6 Natural Background Exposure and Sources in the United States (NCRP, 2009)

Background Component	Percent Contribution	mSv/year	Exposure Source
^{222}Rn	68	2.1	Inhalation
Space	11	0.34	Cosmic rays
Terrestrial	7	0.22	Soil content U, Th, 40K
^{220}Rn	5	0.16	Inhalation
Potassium-40	5	0.16	Internal body content
Uranium and thorium series	4	0.12	Internal body content
Other	<1	—	—
Total	**100**	**3.1**	

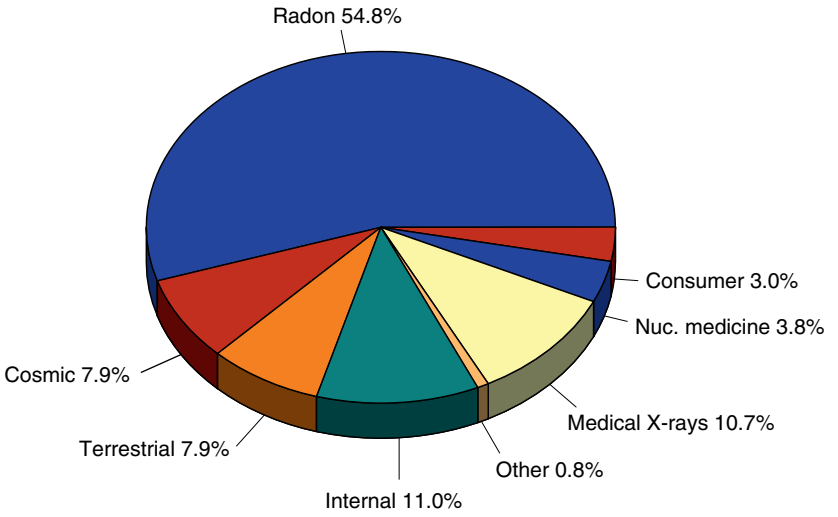

FIGURE 23.10 Annual average effective dose 3.6 mSv (360 millirem) from NCRP (2009).

23.15 SUMMARY

This chapter explains the details of exposure of the global population to natural background radionuclides. The major radiation dose from background exposure is due to an element in the primordial ^{238}U series, that is, airborne ^{222}Rn (radon), half-life 3.82 days, and its decay products, ^{218}Po, ^{214}Pb, ^{214}Bi(Po). Radiation exposure exists through many pathways either occupational or at home. The ^{222}Rn concentrations encountered in underground mines, in homes, outdoors, and in ground water are detailed. The reason ^{222}Rn, such a short-lived gas, is present globally in all atmospheres is because of the release and diffusion of the gas from ^{226}Ra, half-life1600 years, present in all of Earth's natural substances. Two chains of primordial radioactive nuclides ^{238}U and ^{232}Th—both include radon isotopes—are present in any of Earth's natural substance and parents of all radionuclides present on planet Earth. Various elements in these chains yield everyone's radiation exposure. The exception is ^{40}K, a small fraction of all stable potassium, ^{39}K, present in Earth's native substances.

One of the primordial chains starts with ^{238}U, half-life 4.5×10^9 years, and supports 14 radioactive nuclides (Fig. 23.1). The ^{238}U chain includes ^{226}Ra, ^{222}Rn, and ^{210}Pb, the important nuclides for personal exposure. The other chain, headed by ^{232}Th, half-life 1.4×10^{10} years, supports 12 radioactive nuclides, including ^{220}Rn (thoron), a noble gas like ^{222}Rn, but with half-life, 55.6 s (Fig. 23.2). The base native elements, uranium and thorium, are used industrially in many applications. The alpha particle (^{4}He) resulting from decay of many nuclides in these two chains is the source of all helium on earth. Helium has many important uses in medical and scientific technology.

The ^{222}Rn concentrations in both mines and residences have been measured over many years for the purpose of estimating lung cancer risk, a fundamental of occupational and environmental toxicology. Guidelines are in place, intended to limit radiation risk, and this chapter explains the difficulties in the process.

GLOSSARY

Absorbed dose The decay energy from ionizing radiation absorbed per unit mass. The special unit of absorbed dose is the Gray numerically equal to 1 Joule/kg (J/kg). The older unit of absorbed dose is the rad, 100 erg/g.

Activity size distribution As used here, the diameter of particles associated with ^{222}Rn decay product activity in several size modes, unattached mode, 1–4 nm, accumulation mode, 100–400 nm, coarse mode, >1000 nm.

Alpha particle The positively charged helium atom nucleus ejected from some radionuclides during radioactive decay.

Basal cell The target cell at risk in lung cancer production whose depth is 25 μm below the surface of bronchial epithelium. The basal cells are dividing stem cells producing replacement cells for those lost normally from the bronchial epithelium.

Becquerel (Bq) The activity unit for radioactive decay equal to 1 nuclear disintegration (transformation) per second.

Bronchial airway generations The branches of lung airways from the trachea to the gas exchange region. The branches are numbered sequentially from 0, the trachea, 1 main bronchi, 2–8 bronchi, 9–14 bronchioles, 15 terminal bronchioles, and 16–18 respiratory bronchioles.

Bronchial epithelium The surface layer of cells lining the conducting airways. The airway thickness decreases with bronchial airway generations from 1 to 18 from about 50 μm in the trachea to 10 μm in the smallest airways.

Cilia The hairlike structures on some bronchial surface cells, about 6 μm length, which propel the mucus and deposited material upward to the pharynx, mucociliary clearance.

Curie (Ci) The older special unit of radioactivity. One curie equals 3.7×10^{10} nuclear disintegrations (transformations) per second. The curie was replaced with the Bq by some organizations.

Dose conversion factor (DCF) The absorbed dose in Gray (Gy) per working Level Month (WLM). The radon DCF converts exposure in WLM to absorbed bronchial dose.

Dosimetry The measurement or calculation of the energy absorbed per unit matter of tissue, air, or other substances.

Epidemiology The study of health and illness in human populations. Now epidemiologic studies are carried out in underground mines and residences to determine ^{222}Rn lung cancer risk in people.

Epithelium The surface cells that line the bronchial airways. See bronchial epithelium.

Equilibrium When the activity of all the short-lived radon decay products is equal to the parent radon activity. This is rarely achieved, and the decay product activities are usually less than the radon activity.

Gray The unit of absorbed dose equal to 1 J/kg.

Lung deposition The inhaled and exhaled particles deposited on the bronchial airways and in the lung alveoli.

MeV A unit of energy. The energy acquired by an electron accelerated through a potential difference of 1 million volts. Currently the unit of decay energy for radionuclides.

Mucous cell (goblet cell) The mucous producing cell in bronchial epithelium.

Rad The older special unit of radiation absorbed dose. One rad is the absorption of 100 ergs/gram of absorbing material. The unit currently used to replace the rad is the Gray, Joule/kg (J/kg).

Radiation detectors Electronic devices used to measure the alpha, beta, or gamma rays emitted by radioactive substances.

Radon ^{222}Rn The radioactive noble gas in the ^{238}U primordial series.

Radon decay products The short-lived radionuclides formed as a result of ^{222}Rn decay. The decay products include ^{2l8}Po, ^{214}Pb, ^{214}Bi, and ^{214}Po. Their effective combined half-life is about 33 min.

Risk The lung cancer mortality per unit exposure following a suitable latent interval. Expressed as excess relative risk (ERR) per 100 Bq/m^3 ^{222}Rn or as lifetime lung cancer deaths per WLM.

Sievert (Sv, mSv) The unit for effective dose, ED, that calculates an effective whole-body dose from an absorbed dose D. ED = $D \times W_r \times W_t$. Radiation and tissue weighting factors are W_r and W_t, respectively.

Thoron ^{220}Rn The radioactive noble gas in the ^{232}Th chain.

Tracheobronchial tree The portion of the respiratory system which conducts air to the alveoli, the gas exchange region.

Unattached decay products The fraction of the equilibrium amount of ^{218}Po and a lesser amount of ^{214}Pb, usually equal to 1/10 ^{218}Po, that is not attached to the ambient aerosol.

Working level (WL) Based on 100 pCi ^{222}Rn per 1 L of air in equilibrium with the decay products. Any combination of short-lived radon decay products in 1 L of air that will result in the emission of 1.3×10^5 MeV of potential alpha energy.

Working Level Month (WLM) The cumulative ^{222}Rn decay product exposure from breathing an air concentration of one working level (WL), for 170 h, a working month. For an annual exposure, WLM = WL × (hours exposed)/170.

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24

SECONDHAND TOBACCO SMOKE

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24.1 INTRODUCTION

Extensive toxicological, experimental, and epidemiological data, largely collected since the 1950s, have established that active cigarette smoking is the major preventable cause of morbidity and mortality in the United States (USDHHS, 2014). Since the 1970s, involuntary exposure to tobacco smoke has been investigated as a risk factor for disease and also found to be a cause of preventable morbidity and mortality in nonsmokers. The 1986 report of the U.S. Surgeon General (USSG) on smoking and health and a report by the National Research Council (NRC), also published in 1986, comprehensively reviewed the data on involuntary (secondhand) exposure to environmental tobacco smoke (ETS) and reached comparable conclusions with significant public health implications; both reports concluded that involuntary smoking causes disease in nonsmokers (National Research Council and Committee on Passive Smoking, 1986; USDHHS, 1986). The U.S. Environmental Protection Agency (EPA) reached a similar conclusion in its 1992 risk assessment, which classified ETS as a class A carcinogen (U.S. Environmental Protection Agency, 1992). These conclusions have had lasting and significant impact on public policy and public health in the United States and elsewhere.

Subsequently, a now massive body of evidence has continued to identify new diseases, as well as other adverse effects of secondhand smoke (SHS) as reviewed in numerous authoritative reports (CalEPA, 1997; Scientific Committee on Tobacco and Health, 1998; World Health Assembly, 1999; IARC, 2004, 2009; CalEPA and Air Resources Board, 2005). The 2006 and 2010 USSG reports leave no doubt that any exposure to tobacco smoke is harmful to human health (USDHHS, 2004, 2010). The most recent report updating conclusions on passive smoking is the 2014 50th Anniversary USSG report (USDHHS, 2014). The findings on SHS and disease have been the foundation of the drive for smoke-free indoor environments and for educating parents concerning the effects of their smoking on their children's health.

Environmental Toxicants: Human Exposures and Their Health Effects, Fourth Edition.
Edited by Morton Lippmann and George D. Leikauf.

This chapter provides an overview of the evidence on SHS and its impact on the health of children and adults. It covers the conclusions of the major recent reports that have systematically evaluated the evidence. The chapter describes the findings of a few key representative studies, but is not systematic in approach given the current scope of the voluminous literature. We also note that there has been a long-standing campaign by the tobacco industry to discredit the evidence on SHS and health in order to maintain an apparent controversy as a basis for slowing tobacco control. These tactics are well described through research based on the tobacco industry's own documents, obtained as a result of litigation (Proctor, 2012). The influence of this campaign has extended to the peer-reviewed literature, including reports on methodologic issues, exposures, epidemiological studies, risk estimates, and control measures.

24.2 EXPOSURE TO SECONDHAND SMOKE (SHS)

24.2.1 Characteristics of SHS

Nonsmokers inhale the combination of the sidestream smoke that is released from the cigarette's burning end and the mainstream smoke exhaled by the active smoker (USDHHS, 2006). This mixture has also been referred to as environmental tobacco smoke (ETS, or as SHS, the preferred scientific term as ETS originated with the industry). The inhalation of SHS is also referred to as passive smoking or involuntary smoking. The exposures of involuntary and active smoking differ quantitatively and, to some extent, qualitatively (IARC, 2004). Because of the lower temperature in the burning cone of the smoldering cigarette, most partial pyrolysis products are enriched in sidestream as compared with mainstream smoke. Consequently, sidestream smoke has higher concentrations of some toxic and carcinogenic substances than mainstream smoke; however, dilution by room air markedly reduces the concentrations inhaled by the involuntary smoker in comparison with those inhaled by the active smoker. Nevertheless, involuntary smoking is accompanied by exposure to toxic agents generated by tobacco combustion (USDHHS, 1986; IARC, 2004).

24.2.2 Secondhand Smoke Concentrations

Tobacco smoke is a complex mixture of gases and particulate matter (PM) that contains myriad chemical species (U.S. Department of Health and Human Services, 1984; Guerin et al., 1992; IARC, 2004). Not surprisingly, tobacco smoking in indoor environments increases levels of respirable particles, nicotine, polycyclic aromatic hydrocarbons (PAHs), carbon monoxide (CO), acrolein, nitrogen dioxide (NO_2), and many other substances. The extent of the increase in concentrations of tobacco smoke components varies with the number of smokers, the intensity of smoking, the rate of exchange between the indoor air space and with the outdoor air, and the use of air-cleaning devices. Ott has used mass balance models to characterize factors influencing concentrations of tobacco smoke indoors (Ott, 1999). Using information on the source strength (i.e., the generation of emissions by cigarettes) and on the air exchange rate, researchers can apply mass balance models to predict tobacco smoke concentrations. Such models can be used to estimate exposures and to project the consequences of control measures. Repace and colleagues proposed a biologically based model for SHS exposure that models risk based on pharmacokinetic principles (Repace, 2007).

Diverse components of cigarette smoke have been measured in indoor environments as markers of the contribution of tobacco combustion to indoor air pollution. PM mass concentrations have been measured most often because both sidestream and mainstream smoke contain high concentrations of PM in the respirable size range (USDHHS, 1986; IARC, 2004). PM is a nonspecific marker of tobacco smoke contamination, however, because numerous sources other than tobacco combustion add PM to indoor air. Other more specific markers have also been measured, including nicotine, solanesol, and ultraviolet (UV) light absorption of PM (Guerin et al., 1992). Nicotine, which is present in the gas phase in SHS, is usually measured with passive diffusion badges (Leaderer and Hammond, 1991; Guerin et al., 1992; USDHHS, 2006). Nicotine has become the principal marker because of its specificity to cigarette smoke and the ease of measurement with the passive monitors. In past decades, many studies of levels of SHS components have been conducted in public buildings; fewer studies were conducted in homes and offices (USDHHS, 1986, 2006). These older studies provide insights into exposures under circumstances when smoking was still widespread.

The contribution of various environments to personal exposure to tobacco smoke varies with the time–activity pattern, namely, the distribution of time spent in different locations. Time–activity patterns may heavily influence lung airway exposures in particular environments for certain groups of individuals. Most of the studies that follow were carried out in an era when smoking was still widespread in indoor environments and likely have more limited applicability at present in the United States where smoking indoors has declined greatly over the last three decades; nonetheless, the findings were a critical component of the impetus for restricting smoking indoors. For example, exposure in the home predominated for infants who did not attend day care (Harlos et al., 1987). For adults residing with nonsmokers, the workplace was typically the principal location where exposure took place. A nationwide study, supported by the tobacco industry, assessed exposures of nonsmokers in 16 metropolitan areas of the United States (Jenkins et al., 1996). This study, involving 100 persons in each location, was directed at workplace exposure and included measurements of respirable PM and other markers. The results showed that in 1993 and 1994, exposures to SHS in the home were generally much greater than those in the workplace.

The contribution of smoking in the home to indoor air pollution has been demonstrated by studies using personal monitoring and monitoring of homes for respirable PM. In one of the earliest studies, Spengler et al. monitored homes in six U.S. cities for respirable PM concentrations over several years and found that a smoker of one pack of cigarettes daily contributed about $20\,\mu g/m^3$ to 24-h indoor PM concentrations (Spengler and Tosteson, 1981). In homes with two or more heavy smokers, this study showed that the pre-1987 24-h National Ambient Air Quality Standard (NAAQS) of $260\,\mu g/m^3$ for total suspended particles (TSP) could be exceeded as would the current NAAQS. Because cigarettes are not smoked uniformly over the day, higher peak concentrations must occur when cigarettes are actually smoked. Spengler et al. measured the personal exposures to respirable PM sustained by nonsmoking adults in two rural Tennessee communities (Spengler et al., 1985). The mean 24-h exposures were substantially higher for those exposed to smoke at home: $64\,\mu g/m^3$ for those exposed versus $36\,\mu g/m^3$ for those not exposed.

Nicotine levels were measured in multiple homes in the United States (Hammond et al., 1989; Henderson et al., 1989; Leaderer and Hammond, 1991; Marbury et al., 1993; Jenkins et al., 1996; Emmons et al., 2001; USDHHS, 2006). While the cited studies extend back several decades, the measurements remain reflective of concentrations during ad lib

smoking in homes. In homes with smokers, mean values ranged from about 2 to 5 $\mu g/m^3$ in the various studies, but maximum values in some homes were much higher. Additionally these measures reflected the average concentration across the time of measurement, but not the values when nonsmokers were actually being exposed.

Cigarette combustion is a strong source of many volatile organic compounds. Indoor monitoring has shown increased concentrations of benzene, xylenes, ethylbenzene, and styrene in homes with smokers compared with homes without smokers.

Monitoring in locations where smoking may be intense, such as bars and restaurants, generally showed substantial elevations of PM and other markers of smoke pollution while smoking was taking place. For example, Repace and Lowrey, in a pioneering study, used a portable piezobalance (an instrument for measuring PM) to sample aerosols in restaurants, bars, and other locations (Repace and Lowrey, 1980). In the places sampled, respirable PM levels ranged up to 700 $\mu g/m^3$, and the levels varied with the intensity of smoking (National Research Council and Committee on Passive Smoking, 1986; Guerin et al., 1992; CalEPA, 1997). Studies summarized in the 2006 USSG report showed the widespread presence of nicotine in workplaces and other locations with smoking allowed and the potential for maximum concentrations to be extremely high (USDHHS, 2006). Monitoring studies document the effectiveness of workplace smoking policies for sharply reducing nicotine concentrations. After bans were implemented, studies showed low concentrations in many workplace settings, reflecting declining the smoking prevalence in recent years and changing practices of smoking in the workplace. Using passive nicotine samplers, Hammond showed that worksite smoking policies can sharply reduce SHS exposure (Hammond, 1999).

In the United States and worldwide, transportation environments were long polluted by cigarette smoking. Contamination of air in trains, buses, automobiles, airplanes, and even submarines was widely documented (National Research Council and Committee on Passive Smoking, 1986; USDHHS, 1986). An NRC report on air quality in airliners summarized studies for tobacco smoke pollutants in commercial aircraft [National Research Council (US) Committee on Airliner Cabin Air Quality, 1986]. In one study, during a single flight, the NO_2 concentration varied with the number of passengers with a lighted cigarette. In another study, respirable PM concentrations in the smoking section were measured at five or more times higher than in the nonsmoking section, with peaks as high as 1000 $\mu g/m^3$ in the smoking section. Mattson et al. used personal exposure monitors to assess nicotine exposures of passengers and flight attendants (Mattson et al., 1989). All persons were exposed to nicotine, even if seated in the nonsmoking portion of the cabin. These studies are now of historical interest only, as almost all commercial flights worldwide are smoke-free, largely because of the emergence of evidence on the risks of SHS. Automobiles, however, are potential sites of high levels of exposure: preliminary data from a study in Greece show that levels of concentrations can reach excessive heights when a smoker in a car exposes others to SHS (Vardavas et al., 2006). Looking back, these studies documented the ubiquity of SHS and the need to maintain smoking bans in offices and public places. They were key drivers in implementing smoke-free policies.

24.2.3 Thirdhand Smoke Exposure

Thirdhand smoke (THS) exposure refers to exposure to smoke components and their metabolic by-products from contact with surfaces that have adsorbed smoke. Specifically, THS are tobacco chemicals that "remain, react, re-emit, and/or are resuspended long after active

smoking ends"(Jacob et al., 2017). The smoke leaves a residue of nicotine and other toxic substances in household dust and on surfaces. Both smoke residues and byproducts—generated from smoke constituents reacting with atmospheric oxidants—may be re-emitted into the air and airborne PM settled on surfaces that may be resuspended (Jacob et al., 2017). Although not yet well studied, there is concern that contact with THS will result in absorption of toxins through the skin or by ingestion from contamination of the hands. Inhalation of resuspended dust is another potential route for entry into the body (Benowitz et al., 2009; Winickoff et al., 2009; Sleiman et al., 2010). Although direct health effects from THS have not been established, many of the toxins that are deposited on surfaces are group 1 carcinogens, raising concerns about chronic exposure, even at low levels. The persistence of these substances in the home environment for months, or even years, after smoking behaviors cease represents an unappreciated health hazard through dermal exposure, dust inhalation, and ingestion (California Consortium for Thirdhand Smoke, 2018). Mitigation of THS in the home through repainting or replacement of drywall, carpeting, and other tobacco smoke-affected materials may be necessary as well as a thorough, professional cleaning (California Consortium for Thirdhand Smoke, 2018). However, the efficacy of these mitigation methods is still unknown, particularly if there has been long-term smoking in the home (California Consortium for Thirdhand Smoke, 2018).

24.2.4 Biological Markers of Exposure

Biological markers have been used to describe the prevalence of exposure to SHS, and its changing prevalence over time, in order to investigate the dosimetry of involuntary smoking and to validate questionnaire-based measures of exposure. In both active and involuntary smokers, the detection of tobacco smoke components or their metabolites in body fluids or alveolar air provides evidence of exposure to tobacco smoke, and levels of these markers can be used to gauge the intensity of exposure. The risk of involuntary smoking has also been estimated by comparing levels of biological markers in active and involuntary smokers.

At present, the most sensitive and specific markers for tobacco smoke exposure are nicotine and its metabolite, cotinine (Jarvis and Russell, 1984; USDHHS, 1988, 2006; IARC, 2004). Neither nicotine nor cotinine is present in body fluids in the absence of exposure to tobacco smoke, although unusually large intakes of some foods could produce measurable levels of nicotine and cotinine (Idle, 1990). Cotinine, formed by oxidation of nicotine by cytochrome P450, is one of several primary metabolites of nicotine (USDHHS, 1988). Cotinine itself is extensively metabolized, and only about 17% of cotinine is excreted unchanged in the urine (IARC, 2004).

Because the circulating half-life of nicotine is generally shorter than 2 h, nicotine concentrations in body fluids reflect more recent exposures (Rosenberg et al., 1980). Nicotine can be measured in hair, as it is incorporated into the growing hair. By using several centimeters of hair, the level of nicotine reflects exposure over several weeks (Jaakkola and Jaakkola, 1997). In contrast to the short half-life of nicotine in the blood, cotinine has a half-life in the blood or plasma of active smokers of about 10 h and in nonsmokers of about 20 h (USDHHS, 2006); hence, cotinine levels in blood, urine, or saliva provide information about exposure to tobacco smoke of involuntary smokers over periods of several days (Turner et al., 1987; Wall et al., 1988). Concerns about nonspecificity of cotinine, arising

from eating nicotine-containing foods, have been set aside (Benowitz, 1996). Thiocyanate concentration in body fluids, concentration of CO in expired air, and carboxyhemoglobin (COhb) level distinguish active smokers from nonsmokers, but are not as sensitive and specific as cotinine for assessing involuntary exposure to tobacco smoke (Jarvis and Russell, 1984; USDHHS, 2006).

Cotinine levels have been measured in adult nonsmokers and in children (USDHHS, 2006). In studies of adult nonsmokers, exposures at home, in the workplace, and in other settings determined cotinine concentrations in urine and saliva. The cotinine levels in involuntary smokers ranged from less than 1% to about 8% of cotinine levels measured in active smokers. Smoking by parents is the predominant determinant of the cotinine levels in their children. In 1988–1991, 88% of nonsmokers had a detectable level of serum cotinine using liquid chromatography–mass spectrometry as the assay method (Pirkle et al., 1996, 2006; USDHHS, 2006). Cotinine levels in this national sample increased with the number of smokers in the household and the hours exposed in the workplace. In subsequent waves of the National Health and Nutrition Examination Survey (NHANES), the proportion of participants with a detectable level of cotinine and the mean level have dropped substantially with the most recent findings indicating the prevalence of SHS exposure in nonsmokers declined by half during the period of 1999/2000 and 2011/2012 (Homa et al., 2015).

Viewed from a historical perspective, the SHS biomarkers were key for supporting research, supporting the plausibility of SHS as a cause of disease, and motivating smoke-free policies. The results of studies on biological markers had important implications for research on involuntary smoking and added to the biological plausibility of associations between involuntary smoking and diseases documented in epidemiological studies (Benowitz, 1996). The data on marker levels provided ample evidence that involuntary exposure leads to absorption, circulation, and excretion of tobacco smoke components. The studies of biological markers also confirmed the once high prevalence of involuntary smoking, as ascertained by questionnaire and the decline over time (Benowitz, 1996; Pirkle et al., 1996; Coultas et al., 1987). The observed correlations between reported exposures and levels of markers suggested that questionnaire methods for assessing recent exposure have some validity.

24.2.5 Exposure Assessment

The information on the health effects of involuntary smoking has been largely derived from observational epidemiological studies. In these studies, exposure to SHS has been estimated primarily by responses to questionnaires concerning the smoking habits of household members or of fellow employees; attempts have been made to quantitate exposures by determining the number of cigarettes smoked by family members and the duration of exposure. Biomarkers have also been used in some studies. Limitations of the questionnaire approach were discussed extensively in the 1986 report of the USGS and again in the 2006 report (USDHHS, 1986, 2006). The potential for information bias to have introduced positive associations of SHS exposure with disease risk was a focus of debate, in part driven by tobacco industry consultants and surrogates (Muggli et al., 2004; Tong et al., 2005).

A number of older studies have addressed characteristics of questionnaires and biological markers for assessing exposure to SHS. As addressed in the 2006 report of the USGS, questionnaires on SHS exposure have sufficient reliability and validity for

research purposes (USDHHS, 2006). Measurement of nicotine concentration and biomarkers provide complementary data.

24.3 HEALTH EFFECTS OF INVOLUNTARY SMOKING

This section highlights the extensively researched health effects of SHS exposure that have been found to be causal in major authoritative reports (Table 24.1). Initially, lung cancer in adults was considered the most important health effect from SHS exposure from the regulatory and policy perspective. However, major reports also found substantial evidence for causal effects of SHS on multiple diseases and other health outcomes in both children and adults. The findings on children motivated educational efforts and in-home smoking policies at the household level. Given the now voluminous literature on SHS and health, we do not provide comprehensive reviews of studies. We provide examples of several historically important studies.

24.3.1 Health Effects in Children

The adverse consequences of SHS begin with the fetus, subject to exposure to tobacco smoke components from maternal smoking and from the smoking of others. Infants and children are affected from birth on, as they are exposed to SHS at home and elsewhere (Table 24.2). Researchers have demonstrated that active smoking by mothers results in a variety of adverse health effects in children (USDHHS, 2014).

Some developmental health effects result predominantly from transplacental exposure of the fetus to tobacco smoke components. For example, paternal smoking in the presence of a pregnant mother may lead to perinatal health effects manifested upon birth of the baby, and either maternal or paternal smoking in the presence of a newborn child may lead to postnatal health effects in the developing child. Health effects on the fetus resulting from SHS include fetal growth effects (decreased birth weight, growth retardation, or prematurity), fetal loss (spontaneous abortion and perinatal mortality), and congenital malformations.

Studies have also investigated and demonstrated associations between adverse health effects in children and exposure to SHS. Health effects on the child postnatally, resulting from either SHS exposure to the fetus or to the newborn child, include sudden infant death syndrome (SIDS) and adverse effects on neuropsychological development and physical growth. Possible longer-term health effects of fetal SHS exposure include childhood cancers of the brain, leukemia, and lymphomas, among others.

In both pre- and postnatal stages, the respiratory tract is adversely affected by SHS exposure by multiple mechanisms. Extensive research has shown a causal relationship between SHS and lower respiratory illnesses, respiratory symptoms such as such as cough and wheezing, and the onset and exacerbation of asthma in children (USDHHS, 2006). A large body of research on the effects of SHS on pre- and postnatal development has established a causal relationship between SHS and lower levels of lung growth and function (USDHHS, 2006). Prenatally, maternal smoking leads to a reduction in lung growth, the adverse effects of which are persistent through childhood as lung growth continues (USDHHS, 2006). In infancy and childhood, SHS exposure leads to a lower level of lung function (USDHHS, 2006). The effects of SHS persist through infancy and childhood into adulthood as the maximum achieved level of lung growth and function is reduced by exposure to SHS, increasing the risk of future chronic lung disease as adults (USDHHS, 2006).

TABLE 24.1 Adverse Effects from Exposure to Tobacco Smoke

Health Effect	SGR 1984	SGR 1986	EPA 1992	CalEPA 1997	UK 1998	WHO 1999	IARC 2004	CalEPA[a] 2005	SGR 2006	SGR 2014
Increased prevalence of chronic respiratory symptoms	Yes/a	Yes/a	Yes/c	Yes/c	Yes/c	Yes/c		Yes/c	Yes/c	
Decrement in pulmonary function	Yes/a	Yes/a	Yes/a	Yes/a		Yes/c		Yes/a	Yes/c	
Increased occurrence of acute respiratory illnesses	Yes/a	Yes/a	Yes/a	Yes/c		Yes/c		Yes/c	Yes/c	
Increased occurrence of middle ear disease		Yes/a	Yes/c	Yes/c	Yes/c	Yes/c		Yes/c	Yes/c	
Increased severity of asthma episodes and symptoms			Yes/c	Yes/c		Yes/c		Yes/c	Yes/c	
New asthma			Yes/a	Yes/c				Yes/c	Yes/c	
SIDS				Yes/c	Yes/a	Yes/c		Yes/c	Yes/c	
Lung cancer in adults		Yes/c	Yes/c	Yes/c	Yes/c		Yes/c	Yes/c	Yes/c	
Breast cancer for younger, primarily postmenopausal women								Yes/c		Yes/a
Nasal sinus cancer								Yes/c		
Heart disease in adults				Yes/c	Yes/c			Yes/c	Yes/c	
Stroke									Yes/a	Yes/c

Yes/a = association; Yes/c = causal. Table adapted from U.S. Department of Health and Human Services (2006); SGR 1984: U.S. Department of Health and Human Services (1984); SGR 1986: U.S. Department of Health and Human Services (1986); EPA 1992: U.S. Environmental Protection Agency (1992); Cal/EPA 1997: California Environmental Protection Agency and Office of Environmental Health Hazard Assessment (1997); UK 1998: Scientific Committee on Tobacco and Health and HSMO (1998); WHO 1999: World Health Organization (1999); IARC 2004: International Agency for Research on Cancer (2004); Cal/EPA 2005: California Environmental Protection Agency and Air Resources Board (2005); SGR 2006: U.S. Department of Health and Human Services (2006); SGR 2014: U.S. Department of Health and Human Services (2014).
[a]Only effects causally associated with SHS exposure are included.

TABLE 24.2 Health Effects in Children

Health Effect	Specific Outcomes
Fetal	
Fetal loss	Spontaneous abortion[A]
	Perinatal mortality[A]
Fetal growth effects	Decreased birth weight[C]
	Growth retardation[C]
	Preterm delivery[A]
Congenital malformations[A]	
Postnatal/childhood	
Infant mortality	
Low birth weight[C]	
Sudden infant death syndrome (SIDS)[C]	
Cognitive development	
Behavioral development	
Growth deficiencies	Height
	Lung[C]
Childhood cancers[A]	Brain cancer
	Leukemia
	Lymphomas
Lower respiratory illnesses[C]	
Respiratory symptoms and illness[C]	Asthma onset[A]
	Exacerbation of asthma symptoms[C]
Middle ear disease[C]	
Decreased lung function[C]	

Source: USDHHS (2006).
A = association; C = causal.

24.3.2 Health Effects in Adults

Extensive evidence has shown that active smoking causes respiratory symptoms and illness, multiple types of cancer, and coronary heart disease and stroke (USDHHS, 2004, 2014). Studies have also investigated and demonstrated associations between adverse health effects in adults and exposure to SHS (Table 24.3). Adverse health effects on adults resulting from exposure to SHS that have been investigated include chronic and acute respiratory symptoms and illnesses (adult-onset asthma, exacerbation of asthma symptoms, and impaired or reduced lung function), cancers (lung, breast, and nasal sinus), and cardiovascular disease (including coronary heart disease and stroke).

SHS has adverse effects on the adult respiratory system through onset and exacerbation of acute and chronic respiratory symptoms, a decline in lung function, onset and exacerbation of adult-onset asthma, and chronic obstructive pulmonary disease (USDHHS, 2006). Both short- and long-term SHS exposures have been found to adversely affect lung function in adults (USDHHS, 2006). SHS has been found to not only contribute to adult-onset asthma but also to the worsening of asthma symptoms, effects that are consistent with those of active smoking (USDHHS, 2004, 2006, 2014).

Historically, the findings on SHS and lung cancer risks were pivotal in driving policies to control SHS, given the contamination of workplaces and public places by a proven carcinogen. In 1981, published reports from Japan and Greece indicated increased lung

TABLE 24.3 Health Effects in Adults

Health Effect	Specific Outcome
Cancer	Lung cancer[C]
	Breast cancer[A]
	Nasal sinus cancer[C]
	Nasopharyngeal cancer
	Cervical cancer
	Additional cancers[A]
Cardiovascular disease	Coronary heart disease[C]
	Stroke[C]
Respiratory symptoms and illnesses[A]	Adult-onset asthma[A]
	Exacerbation of asthma symptoms[A]
Decline in lung function[C]	
Chronic obstructive pulmonary disease[A]	

Source: USDHHS (2006).
A = association; C = causal.

cancer risk in nonsmoking women married to cigarette smokers. Subsequently this association was examined in numerous investigations conducted in the United States and other countries (Hirayama, 1981; Trichopoulos et al., 1981). The first major studies on SHS and lung cancer were reported in 1981. Hirayama's early report was based on a prospective cohort study of 91,540 nonsmoking women in Japan (Hirayama, 1981). Standardized mortality ratios (SMRs) for lung cancer increased significantly with the amount of smoking by the husbands. The findings could not be explained by confounding factors and were unchanged when follow-up of the study group was extended (Hirayama, 1984). However, the tobacco industry launched a massive and long-lived attack on the findings. In 1981, Trichopoulos et al. also reported increased lung cancer risk in nonsmoking women married to cigarette smokers based on a case–control study in Athens, Greece (Trichopoulos et al., 1981).

By 1986, the evidence had mounted, and three reports published in that year concluded that SHS was a cause of lung cancer. The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) concluded that "passive smoking gives rise to some risk of cancer" (IARC, 1986). In its Monograph 38 on tobacco smoking, the agency supported this conclusion on the basis of the characteristics of sidestream and mainstream smoke, the absorption of tobacco smoke materials during involuntary smoking, and the nature of dose–response relationships for carcinogenesis. In the same year, the U.S. NRC and the USSG also concluded that involuntary smoking increases the incidence of lung cancer in nonsmokers (National Research Council and Committee on Passive Smoking, 1986; USDHHS, 1986). In reaching this conclusion, the NRC cited the biological plausibility of the association between exposure to SHS and lung cancer and the supporting epidemiological evidence (National Research Council and Committee on Passive Smoking, 1986). Based on a pooled analysis of the epidemiological data adjusted for bias, the report concluded that the best estimate for the excess risk of lung cancer in nonsmokers married to smokers characterized involuntary smoking as a cause of lung cancer in nonsmokers. Numerous subsequent reports have reached the same conclusion (Table 24.1), supporting the call for smoking bans (USDHHS, 1986).

The extent of the lung cancer hazard associated with involuntary smoking in the United States and in other countries remains subject to some uncertainty; however, estimates have been made that are useful indications of the magnitude of the disease risk (USDHHS, 1986, 2006, 2014; Weiss, 1986). The 2014 USSG report estimated that 7330 (4.63%) U.S. lung cancer deaths and 33,950 (8.23%) U.S. deaths from coronary heart disease were attributable to secondhand smoking from 2005 to 2009 (USDHHS, 2014).

24.4 CONTROL OF EXPOSURE TO SECONDHAND SMOKE

Since the 1980s, there has been growing momentum for making public places and workplaces smoke-free. The public health basis for this movement lies in the strong causal findings on the health risks of SHS (Table 24.1) and on the need to eliminate smoking indoors to fully protect nonsmokers from inhaling SHS. Cigarettes are strong sources of gaseous and PM emissions, and use of mass balance models implies that concentrations of SHS components indoors could not be controlled by either ventilation or air cleaning (USDHHS, 2006). Such consideration led the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) to conclude that ventilation was not a sufficient control measure for SHS (Samet, 2005). The 2006 USSG report reached a similar conclusion (USDHHS, 2006).

There are regulatory and non-regulatory approaches to eliminating smoking indoors. While there is no national ban on smoking indoors in the United States, local and state ordinances now cover public places and workplaces in the majority of locales, and most large companies have policies in place that prohibit smoking indoors. Major hotel chains are now smoke-free, as are rental cars from national agencies. The majority of employed people report working under a smoke-free policy, although there is some variation by type of workplace and region of the country (USDHHS, 2006). Blue-collar and farm workers are least likely to be covered. To date, the United States lacks a federal regulation on SHS exposure in the workplace, although 35 states have enacted statewide bans on smoking in public and/or private workplaces (ANRF, 2018b). Despite this progress, an estimated 41% of current U.S. workers are still exposed to SHS on the job (ANRF, 2018a).

The home is not subject to regulation, but increasing numbers of households in the United States have voluntary policies in place (USDHHS, 2006). The majority of households are now smoke-free, and increasing numbers of households with smokers have smoke-free policies in place. In 2017, the U.S. Department of Housing and Urban Development (HUD) released a policy prohibiting the use of certain combustible tobacco products, including cigarettes, cigars, pipes, and hookahs, in public housing properties (HUD, 2017). The effectiveness of these policies is still inadequate, and research is in progress to develop more efficacious approaches to reduce SHS exposure in homes, particularly for people, for example, children with asthma, who are especially susceptible to the adverse health effects of SHS.

Globally, exposure to SHS is a major concern that prompted the first international treaty negotiated under the auspices of WHO. The WHO Framework Convention on Tobacco Control (WHO FCTC) was developed in response to the globalization of the tobacco epidemic to address the causes of the epidemic and actively progress tobacco control worldwide. WHO FCTC introduced the MPOWER measures to help countries

implement effective interventions to reduce tobacco use and demand. The MPOWER measures include monitoring tobacco use and prevention policies; protecting people from tobacco smoke; offering help to quit tobacco use; warning about the dangers of tobacco; enforcing bans on tobacco advertising, promotion, and sponsorship; and raising taxes on tobacco. Since its introduction, 121 countries, comprising 63% of the world's population, have introduced at least one MPOWER measure, and about 4.7 billion people are now covered by at least one best-practice policy intervention at the national level (WHO, 2017). While progress has been made in the implementation of the WHO MPOWER measures, protecting the world's population from SHS has not been as successful. As of 2017, 55 countries have adopted comprehensive smoke-free legislation, covering almost 1.5 billion people (WHO, 2017). However, only 22 of these countries report a high compliance rate, leaving millions of people vulnerable to SHS despite smoke-free laws in place to protect them (WHO, 2017).

24.5 SUMMARY

The effects of active smoking and the toxicology of cigarette smoking have been comprehensively examined, providing a solid foundation of research for policy formation on involuntary smoking. The health risks of involuntary smoking have been identified, and causal conclusions reached, beginning in the mid-1980s. The 1986 report of the USSG concluded that involuntary exposure to tobacco smoke causes respiratory infections in children, increases the prevalence of respiratory symptoms in children, reduces the rate of functional growth as the lung matures, and causes lung cancer in nonsmokers (National Research Council and Committee on Passive Smoking, 1986; USDHHS, 1986). These conclusions have been reaffirmed in numerous subsequent reports and new conclusions were added (U.S. Environmental Protection Agency, 1992; CalEPA, 1997; USDHHS, 2006, 2014). Involuntary smoking is now considered as a cause of asthma and a factor exacerbating asthma (U.S. Environmental Protection Agency, 1992; CalEPA, 1997; USDHHS, 2006) and as a cause of heart disease (CalEPA, 1997; USDHHS, 2006). The 2006 USSG report leaves no doubt: SHS causes premature death and disease in children and adults who do not smoke (USDHHS, 2006, p. 11).

The adverse effects of involuntary exposure to tobacco smoke have provided a strong rationale for policies directed at reducing and eliminating exposure of nonsmokers to SHS (USDHHS, 1986). Complete protection of nonsmokers in public locations and the workplace requires the banning of smoking indoors. To date, there is no national-level smoke-free legislation in the United States; instead protection is left to the state and local governments. Households are points of exposure that are uniquely outside the reach of legislation with smoke-free policies being at the will of the resident. However, great progress has been made with the majority of U.S. households reporting having a smoke-free policy in place (USDHHS, 2006). Motor vehicles are another point of exposure where no national-level policies exist to protect passengers (ANRF, 2018a). To date, only eight U.S. states and Puerto Rico have laws prohibiting smoking in vehicles (ANRF, 2018a). However, these laws only pertain to vehicles transporting children and the age range varies by state. The 1986 USSG report concluded that "the simple separation of smokers and nonsmokers within the same air space may reduce, but does not eliminate, the exposure of nonsmokers to environmental tobacco smoke" (USDHHS, 1986). The 2006 USSG report went even further: "the scientific evidence indicates that there is no risk-free level of

exposure to secondhand smoke" and "separating smokers from nonsmokers, cleaning the air, and ventilating buildings cannot eliminate exposures of nonsmokers to secondhand smoke" (USDHHS, 2006, p. 11). Together, these conclusions are a sufficient basis for banning smoking in indoor environments.

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SULFUR OXIDES (SO_X): SO_2, H_2SO_4, NH_4HSO_4, AND $(NH_4)_2SO_4$

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25.1 INTRODUCTION

A variety of gaseous and particulate matter (PM) chemicals in the ambient community air are acidic, and some of them have been associated with adverse health effects in observational epidemiological studies of human populations. Some of these effects have also been documented in controlled laboratory-based exposures of human volunteers to specific sulfur oxides (SO_x) compounds. The concentrations of some of these compounds occurring in ambient air have been associated with both acute bronchoconstriction and elevated short-term morbidity and mortality rates in human populations. These SO_x compounds include sulfur dioxide vapor (SO_2); strong acids in PM, that is, highly irritating sulfuric acids (H_2SO_4) and ammonium bisulfate (NH_4HSO_4); and ammonium sulfate $(NH_4)_2SO_4$, a weak acid. Inhaled SO_2, when deposited onto airway surfaces, hydrolyzes as sulfurous acid (NH_4SO_3), a weak acid. Since the National Ambient Air Quality Standard (NAAQS) for SO_x in the United States relies on SO_2 as its indicator, routine air concentration monitoring for SO_x has been limited to SO_2. The observed human health effects that have been associated with elevated SO_2 concentrations in ambient air may have been due, at least in part, caused to its oxidation products resulting from atmospheric transformation of SO_2 to other SO_x forms, as well as to other PM pollutants that often coexist with SO_2. The U.S. Clean Air Act (CAA) requires the Environmental Protection Agency (EPA) to review its NAAQS at 5-year intervals. Since these periodic NAAQS review cycles usually take more than 5 years. When the revisions are delayed, and do not reflect a need to lower the limits based on new peer-reviewed science, they may no longer protective of human health and/or welfare. The last NAAQS revision of the NAAQS for SO_x was in 2010. As of this writing (2019), EPA was still in the process of completing its next review of the relevant peer-reviewed literature, now known as an Integrated Science Assessment (ISA).

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The NAAQS-related focus in recent years was primarily focused on PM and O_3, and the SO_x ISA will have to report that there has been no new literature from controlled SO_x human exposure studies and that there has been very little new literature showing significant associations with ambient air concentrations of SO_x. As a result, the next very thorough ISA for SO_x will, once again, be focused, almost exclusively, on the effects attributable to SO_2. Thus, there is still a dearth of knowledge on the health effects of other SO_x chemical components of the air pollution mixture that account for a significant mass fraction of the ambient air PM.

This chapter summarizes and discusses the very extensive, but mostly quite dated, peer-reviewed literature describing ambient air concentrations of SO_2 in the United States and the health effects that have been associated with ambient air SO_2 exposures at concentrations that have been routinely monitored or measured in community atmospheres. It also describes the much sparser literature on ambient air concentrations and inhalation exposures to acidic SO_x aerosols and their health effects, which may, at times, be greater than those of SO_2. A summary of these effects of SO_x aerosols is also discussed in Chapter 9 on the complex chemical mixture known as ambient air PM.

This chapter does not provide in-depth discussions of health effects associated with occupational exposures to SO_x pollutants at much higher concentrations because of their lack of relevance to the subject at issue, that is, the health effects in the population as a whole.

25.2 SOURCES AND EXPOSURES

25.2.1 Sources

Most of the sulfur (S) in fossil fuel is converted into SO_2 in the combustion zones of stationary and motor vehicle sources, with less than 10% of the SO_x emerging from the stack or tailpipe as H_2SO_4. Some of the H_2SO_4 forms a surface film on ultrafine-sized mineral ash particles in the exhaust stream, where it can convert transition metals in or on the ash particles into soluble SO_4^{2-} salts. When the discharge point is a tall stack, most of the SO_2 escapes local deposition on terrestrial surfaces and is gradually (1–10%/h) converted into sulfur trioxide (SO_3), a highly hygroscopic vapor that rapidly combines with atmospheric water vapor to produce ultrafine nonvolatile droplet aerosols of H_2SO_4. The H_2SO_4 in the aqueous droplets is then gradually neutralized by atmospheric ammonia (NH_3), first to the strong acid ammonium bisulfate (NH_4HSO_4) and then to ammonium sulfate $(NH_4)_2SO_4$, a nearly neutral salt. Rates of NH_3 neutralization vary widely, depending on NH_3 emission rates from ground-based sources. Neutralization rates are higher over cities and agricultural areas because of NH_3 generated by biological decay of waste products, are lower in concentration over forests, and are virtually nil over deepwater bodies.

The ratios between SO_2, H_2SO_4, NH_4HSO_4, and total particulate sulfate ion (SO_4^{2-}) in the atmosphere are highly variable in space and time. While ambient concentration data are relatively plentiful for SO_2 and SO_4^{2-}, they are, unfortunately, very sparse for the hydrogen ion (H^+) in the H_2SO_4 and NH_4HSO_4. The H^+ component may account for much of the excess annual mortality and morbidity historically associated with mixtures of SO_2 and PM. SO_2 is a very poor surrogate index for ambient concentrations of H^+ in the PM, but SO_4^{2-} can often serve as a useful surrogate index of overall SO_x concentration for observational epidemiology in some parts of the United States, Europe, and Asia.

One of the few significant indoor sources of SO_2 and H_2SO_4 was the unvented kerosene space heater, whose usage has fortunately declined in recent years due to the decline

in S content in kerosene. Leaderer et al. (1990) studied pollutant emissions from four portable kerosene space heaters using kerosene containing 0.039% S. The heaters were operated in a 34-m^3 room at 1.4 air changes per hour. Background chamber pollution levels were low. On a mass balance basis, SO_4^{2-} accounted for 2–26% of the S in the fuel, with the balance emitted as SO_2. SO_4^{2-} concentrations ranged from 33 to 693 $\mu g/m^3$, and acidic particulates, as H_2SO_4, ranged from 1.3 to 75 $\mu g/m^3$. Since the S content of kerosene has been substantially reduced since 1990, these concentrations represent upper limits.

25.2.2 Exposures

The current U.S. ambient air levels of SO_2 are generally well within the primary NAAQS established in 2010 that specifies that the 99th percentile of 1-h maximum concentration averages, averaged over 3 years, of 75 ppb (195 $\mu g/m^3$), shall not to be exceeded. There is an additional special concern for asthmatics' peak exposures to SO_2 while performing outdoor exercise. It has been estimated that the size of the asthmatic population with peak 5–10-min exposures at concentrations >0.2 ppm (520 $\mu g/m^3$) during light to moderate exercise, who may exhibit a bronchoconstrictive response, varies from 5000 to 50,000 individuals.

For acidic aerosols, there is a very limited ambient concentration database. Annual average acidic PM concentrations in U.S. communities in the 1980s were reported by Spengler et al. (1989). In the four Eastern U.S. communities studied, it ranged up to 1.8 $\mu g/m^3$ (as H_2SO_4). In the 1980s, levels in excess of 20–40 $\mu g/m^3$ were observed for time durations ranging from 1 to 12 h. These were associated with high but not necessarily the highest atmospheric SO_4^{2-} levels. Exposures (concentration–time product) of 100–900 $\mu g/m^3$-h were calculated for the acid concentrations that were monitored. In the 1990s mandated reductions of 50% in SO_2 emissions resulted in comparable reductions in strong acid aerosols. By contrast, studies in London in the early 1960s indicated that H_2SO_4 in excess of 100 $\mu g/m^3$ was frequently present in the atmosphere and daily cumulative exposures >2000 $\mu g/m^3$-h were possible.

Brauer et al. (1989) measured exposures to SO_x vapors and aerosols with a personal annular denuder/filter pack sampler and compared the results with those measured at a centrally located monitoring site in the metropolitan Boston, MA, area. Personal exposures to aerosol H^+ were only slightly lower than the concentrations at the central monitor, and personal SO_4^{2-} was similar to central site values. By contrast, SO_2 and nitric acid (HNO_3) vapor were much lower for personal exposures than at the central site.

Meteorology and regional transport are extremely important to acid sulfate concentrations. Keeler et al. (1991) measured elevated levels of ambient H^+ simultaneously during a regional episode at multiple sites located from Tennessee to Connecticut, and Lamborg et al. (1992) measured H^+ concentrations to investigate the behavior of regional and urban plumes advecting across Lake Michigan. Their results suggested that PM acidity could be maintained over long distances in air masses moving over large bodies of water (up to 100 km or more). The conversion of SO_2 to acidic PM takes place as the prevailing winds carry the precursors from the source region in the Midwest and northeastern United States to southwestern Canada. This type of northeasterly wind flow occurs on the backside (western side) of mid-latitude anticyclones (high-pressure systems).

Highest atmospheric acidity is associated with (1) slow westerly winds traversing westward SO_2 source areas, (2) local stagnation, or (3) regional transport around to the backside of a high-pressure system. Low acidity is associated with fast-moving air masses

and with winds from the northerly directions; upwind precipitation also played a moderating role in air parcel acidity. Much of the SO_2 and PM H^+ originated from coal-fired power plants.

Size distributions of PM H^+ and SO_4^{2-} are alike, with mass median diameters (MMD) ~0.7 μm, in the optimum range for efficient light scattering and inefficient wet/dry removal. Thus, light scattering and visual range degradation are attributable to the acidic PM SO_4^{2-}. Due to the inefficient removal of PM H^+, strong acids may be capable of long-distance transport in the lower troposphere. Water associated with the acidic PM was shown to account for much of the light scattering.

A study of acid PM and ammonia (Suh et al., 1992) found no significant spatial variation of H^+ at Uniontown, PA, a suburb of Pittsburgh. Measurements at the central monitoring site accounted for 92% of the variability in outdoor concentrations measured at various homes throughout the town. There was no statistical difference between concentrations of outdoor H^+ among five sites (a central site and four satellite sites) in Newtown, CT (Thompson et al., 1991). However, there were differences in peak values, probably related to the proximity of the sampling sites to ammonia sources. Thus, while peak values may differ significantly, long-term averages should not substantially differ across a suburban community.

Outdoor concentrations of H^+ in small suburban communities are fairly uniform, suggesting that minor differences in population density do not significantly affect outdoor H^+ or NH_3 concentrations (Suh et al., 1992). In urban areas, however, both H^+ and NH_3 exhibit significant spatial variation. Waldman et al. (1990) measured ambient concentrations of H^+, NH_3, and SO_4^{2-} at three locations in metropolitan Toronto. The sites, located up to 33 km apart, had significant differences in outdoor concentrations of H^+, with the sites with higher NH_3 having lower H^+.

An intensive monitoring study was conducted during the summers of 1992 and 1993 in Philadelphia by Suh et al. (1995) using 24-h measurements of PM acidity (H^+) sulfate and NH_3 collected simultaneously at seven sites in metropolitan Philadelphia and at Valley Forge, 30 km northeast of the city center. SO_4^{2-} was evenly distributed throughout the measurement area, but H^+ concentrations varied spatially within metropolitan Philadelphia, related to local variations in NH_3 concentrations (Fig. 25.1). The amount of NH_3 available to neutralize H^+ increased with population density, resulting in lower H^+ concentrations in more densely populated areas, while spatial variation in H^+ concentrations did not appear to depend on the overall H^+ concentration. It did, however, show a strong inverse association with local NH_3 concentrations.

An analysis of results from Harvard's 24-City Study (Thompson et al., 1991; Spengler et al., 1996), which measured acidic PM concentrations at eight different small cities across North America each year during a 3-year period, revealed that the summer H^+ mean concentrations were significantly higher than the annual means at all sites. The results showed that at the sites with high H^+ concentrations, approximately two-thirds of the PM acidity occurred from May through September.

Wilson et al. (1991) examined concentration data for H^+, NH_3, and SO_4^{2-} from the Harvard 24-City Study for evidence of diurnal variability (Fig. 25.2). There were distinct diurnal patterns for H^+ concentration and the H^+/SO_4^{2-} ratio, with daytime concentrations being substantially higher than nighttime levels. Both H^+ and SO_4^{2-} concentrations peaked between noon and 6:00 pm, but no such diurnal variation was found for NH_3. They concluded that the diurnal variation in H^+ was probably due to atmospheric mixing. Air containing high concentrations of H^+ and SO_4^{2-} mixes downward during daylight hours

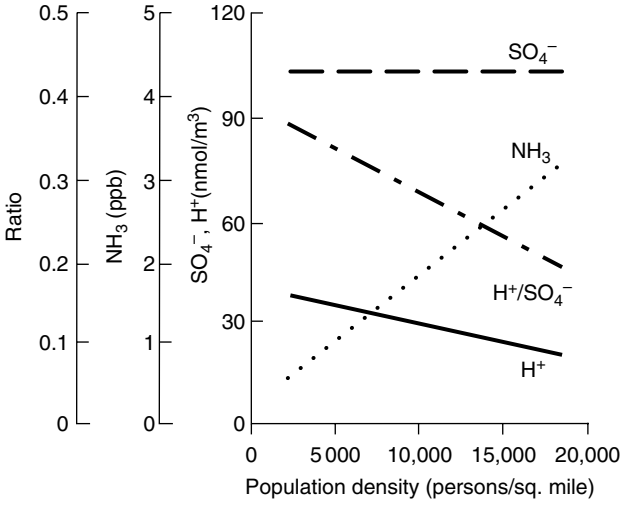

FIGURE 25.1 Mean air pollutant concentrations for days when winds were from the southerly direction, plotted versus population density. The solid line represents H^+ concentrations, the long dashed line represents SO_4^{2-} concentrations, the dashed and dotted line represents the radio of H^+ to SO_4^{2-} levels, and the dotted line represents NH_3 concentrations. All data collected in Philadelphia during the summers of 1992 and 1993. *Source*: Adapted from Suh et al. (1995).

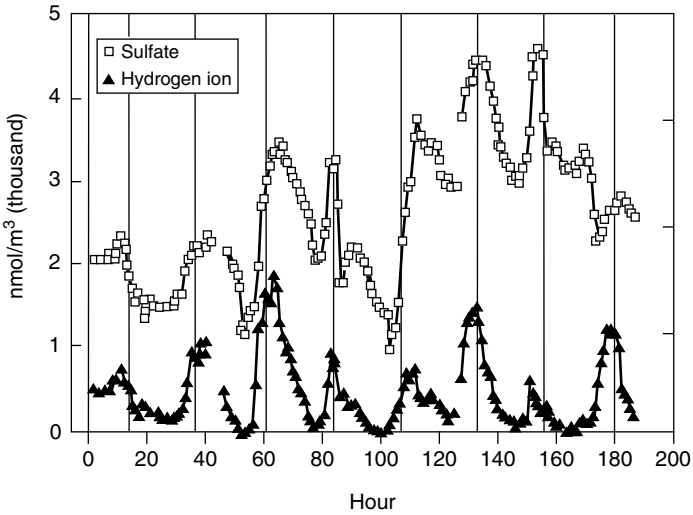

FIGURE 25.2 Diurnal pattern of sulfate and hydrogen ions at Harriman, Tennessee. *Source*: From Wilson et al. (1991).

when the atmosphere is unstable and well mixed. During the night, ammonia emitted from ground-based sources neutralized the acid in the nocturnal boundary layer, the very stable lower part of the atmosphere, but a nocturnal inversion prevented the ammonia from reacting with the acidic PM aloft. Then in the morning as the nocturnal inversion dissipates, the acidic PM mixed downward again as the process began anew. Spengler et al. (1996) also

noted diurnal variations in SO_4^{2-} and H_2SO_4 concentrations and suggested atmospheric dynamics as the cause. The diurnal variation in SO_4^{2-} was observed by other workers and discussed in terms of atmospheric dynamics by Wolff et al. (1979) and Wilson and Stockburger (1990).

25.2.3 Dosimetry

As a highly water-soluble acidic vapor, SO_2 is efficiently captured in the upper respiratory tract during inhalation, and virtually none penetrates to the lungs during normal, quiescent breathing. However, during vigorous physical activity, there is less residence time in the upper airways and, in humans, a shift to oronasal breathing involving partial flow through the less efficient oral passages. Under exercise conditions, some inhaled SO_2 can penetrate to the smaller conductive airways of the lungs and perhaps beyond them. Skornik and Brain (1990) showed that hamsters exposed to SO_2 while running had reduced pulmonary macrophage endocytosis of particles in comparison to sham-exposed animals.

25.3 HEALTH EFFECTS OF SO_2

25.3.1 Short-Term Bronchoconstrictive Effects in Humans

For asthmatics and others with hyperreactive airways exposed to SO_2 at 0.25–0.50 ppm and higher while exercising, the most striking acute response is rapid bronchoconstriction (airway narrowing), usually evidenced in increased airway resistance, decreased expiratory flow rates, and the occurrence of symptoms such as wheezing and shortness of breath. Similar responses can be produced in healthy persons, but require exposure concentrations about an order of magnitude higher and outside the range of ambient levels.

The penetration of SO_2 to sensitive portions of respiratory tract is largely determined by the efficiency of the oral or nasal mucosa in absorbing SO_2, which in turn depends on the mode of breathing (nasal, oral, or oronasal, a mixture of the two) and the rate of airflow. Controlled SO_2 exposure studies on asthmatics show that, at comparable SO_2 concentrations, bronchoconstrictive effects increase with increased ventilation rates and with the relative contribution of oral ventilation to total ventilation (Bethel et al., 1983; Roger et al., 1985). Increased oral ventilation not only allows more direct penetration of SO_2 but may also result in airway drying and changes in surface liquids, affecting SO_2 absorption and penetration. Evaporation of airway surface liquid and perhaps convective cooling of the airways caused by cold, dry air can act as direct bronchoconstrictive stimuli in asthmatics (Strauss et al., 1977; Deal et al., 1979; Anderson, 1985). The combined effect of SO_2 and cold, dry air further exacerbates the asthmatic response (Bethel et al., 1984; Sheppard et al., 1984; Linn et al., 1985a).

The bronchoconstrictive effects of SO_2 were reduced under warm, humid conditions (Linn et al., 1985b). Linn et al. (1989) compared responsiveness in volunteers classified as normals, atopics, mild asthmatic, moderate to severe asthmatics, and medication-dependent asthmatics who were exposed to SO_2 for 1 h at 0, 0.2, 0.4, and 0.6 ppm, with pulmonary function being measured at ~15 and 55 min into the exposure and at 1 week later. The normals and most of the atopics had no response to the exposures; some atopics and all of the asthmatics developed bronchoconstriction and respiratory symptoms during the 1 h exposures, but not at 1 week later.

Linn et al. (1990) exposed 21 volunteers with mild to moderate asthma to SO_2 for 10 min at 0, 0.3, and 0.6 ppm while exercising at a ventilation rate of 50 L/min. With their normal medication (theophylline, supplementable with beta-adrenergics), bronchoconstriction occurred and was exacerbated at 0.6 ppm of SO_2.

In order to determine whether bronchoconstriction induced by SO_2 can be predicted by the airway response to inhaled histamine, Magnussen et al. (1990) exposed 46 patients with asthma to air or 0.5 ppm SO_2 on 2 days. The exposure protocol consisted of 10 min of tidal breathing followed by 10 min of isocapnic hyperventilation at a rate of 30 L/min. Airway response was measured before (baseline) and after hyperventilation in terms of specific airway resistance, SR_{aw}. Exposure to air increased baseline mean SR_{aw} by 45%. Exposure to SO_2 with hyperinflation increased mean baseline SR_{aw} by 163%. When evaluated individually, 26 and 34 of the 46 patients showed an airway response to hyperventilation of air and SO_2, respectively. The airway response after SO_2 and histamine showed a weak but significant correlation ($r = -0.48$), whereas the responses to hyperventilation and SO_2 did not correlate. Thus, the mechanisms by which histamine and SO_2 exert their bronchomotor effects were different, and the risk of SO_2-induced asthmatic symptoms was poorly predicted by histamine responsiveness.

The response to inhaled SO_2 can also be exacerbated by prior exposure to ozone (O_3). Koenig et al. (1990) exposed eight male and five female adolescent asthmatics during intermittent exercise to a sequence of atmospheres, with 45 min to one, followed by 15 min to the other. The combinations were (1) air–100 ppb SO_2, (2) 120 ppb O_3–120 ppb O_3, and (3) 120 ppb O_3–100 ppb SO_2. Air–SO_2 and O_3–O_3 did not cause significant changes in function. By contrast, O_3–SO_2 produced significant changes, that is, an 8% decline in FEV_1, a 19% increase in total flow resistance, and a 15% decrease in V_{max50}.

Little time is required for SO_2 exposure to elicit significant bronchoconstriction in exercising asthmatics; exposure durations as short as 2 min at 1.0 ppm have produced significant responses (Horstman et al., 1988). Little enhancement of response was apparent on prolonged exposure beyond 5 min, although some suggestion of an increase was seen with continuous exercise between 10 and 30 min (Kehrl et al., 1987). Following a single SO_2 exposure during exercise, airway resistance in asthmatics appeared to require a recovery period of 1–2 h (Hackney et al., 1984).

The magnitude of response induced by any given SO_2 concentration was variable among asthmatics. Exposures to SO_2 concentrations of 0.25 ppm or less, which did not induce significant group mean increases in airway resistance, also did not cause symptomatic bronchoconstriction. On the other hand, exposures to 0.40 ppm SO_2 or greater (combined with moderate to heavy exercise), which induced significant group mean increases in airway resistance, also caused substantial bronchoconstriction in some individual asthmatics. This bronchoconstriction was often associated with wheezing and the perception of respiratory distress, sometimes necessitating the discontinuance of the exposure and the provision of medication. Thus some SO_2-sensitive asthmatics were at risk of experiencing symptomatic bronchoconstriction requiring termination of activity and/or medical intervention when exposed to SO_2 concentrations of 0.40–0.50 ppm (1040–1300 $\mu g/m^3$) or greater when this exposure was accompanied by at least moderate activity. These concentrations can occur downwind of point sources as 10-min averages.

Qian et al. (2009) studied that 154 patients with persistent asthma who ranged from 13 through 63 years of age were participating in the 16-week Salmeterol Off Corticosteroids Study trial. Mean 24-h concentrations of SO_2, NO_2, and $PM_{2.5}$ and 8-h concentrations of O_3 were assigned to each residence and regressed against self-measurements of peak

expiratory flow (PEF). There were negative associations between PEF and SO_2, and triamcinolone enhanced the sensitivity to SO_2.

Studies have examined exposure–response relationships over various concentration and ventilation ranges. Some examined the influence of various subject-related and environmental factors. Since the individual studies used different conditions of airway entry, ventilation rate, concentration, and so on, it was difficult to compare directly the results from different investigations. An approach used by Kleinman (1984) and Linn et al. (1983) normalized studies according to effective oral dose rate. They showed that reasonably consistent results were derived from the various controlled SO_2 asthmatic studies when adjustments were made for differences in ventilation rates and oral/nasal breathing patterns.

25.3.2 Short-Term Effects on Mortality and Morbidity

Evidence for effects of SO_2 other than short-term bronchoconstriction is less direct. There is a considerable body of epidemiological evidence demonstrating statistically significant associations between SO_2 and rates of mortality and morbidity. However, it is less likely that SO_2 was a causal factor than that it was serving as a surrogate exposure index for other pollutants in the sulfur oxide–PM complex deriving from fossil fuel combustion.

25.3.3 Short-Term Effects in Multi-pollutant Epidemiology

Older epidemiological studies (up to about the mid-1980s) assessing the health effects of air pollution, including those caused by SO_2, have not been considered as providing reliable evidence for the independent effects of SO_2. Rather, they assessed the effects of the traditional pollutant mixture produced by fossil fuel combustion processes, which included PM and SO_2 as primary pollutants plus secondary PM, including acid aerosols.

Although epidemiological studies of air pollution exposure have the advantage of studying the populations of interest (including sensitive individuals) exposed at the usual ambient pollutant levels and monitoring relevant outcomes (transient or irreversible), they have the drawback that they inevitably study exposure to a pollutant mixture. In recent years, however, more sophisticated statistical methodology has allowed partial separation of the effects of individual pollutants via modeling. Furthermore, the large number of published studies allows an overall evaluation of the effects of SO_2 in situations with varying pollutant mixes and in particular with different levels of PM.

The Ozkaynak and Spengler (1985) reanalysis of 14 years of NYC data (1963–1976) found significant associations between excess daily mortality and airborne PM, SO_2, and temperature. Differences in the rate of change of SO_2 and PM indicators during the study period allowed estimation of their separate effects. In joint regression analysis across all years, PM indicators (coefficient of haze and visibility extinction coefficient) together accounted for significantly greater excess mortality than SO_2.

The main focus of the air pollution epidemiological studies in the past decade has been on the health effects of PM. However, numerous studies have also examined SO_2 and other gaseous pollutants as potential confounders of PM's effects. Thus, a large number of risk estimates for SO_2 accumulated and provided for a more comprehensive assessment of relative importance of the classical air pollutants. While these observational studies did not resolved the issue of confounding between SO_2 and PM or other pollutants, and did not systematically examined the synergistic effects, they were still generally useful in assessing the potential adverse health impacts of SO_2. When multiple pollutants were evaluated,

PM has tended to have stronger associations with mortality or morbidity outcomes than SO_2, but there were exceptions. The discussion focused on studies published in and after 1997. To minimize the potential influence of bias due to the software convergence issue of that confounded analyses using the generalized additive model (GAM) (Dominici et al., 2002; Ramsay et al., 2003), the discussion focuses on those studies that were unaffected or have been reanalyzed. Short-term and long-term effects are considered separately.

25.3.3.1 Short-Term Effects In the decades, there were nearly 200 mortality and morbidity time-series studies that examined short-term impacts of PM, and about 60% of these studies also examined the impacts of SO_2. There have also been several multicity studies of mortality and morbidity in Europe, the United States, and Canada. These multicity studies have advantages over a collection of single-city studies because they analyze data from many cities using consistent methodology and attempt to explain variations in the risk estimate using city characteristics (e.g., differences in weather, poverty, etc.), and this discussion focuses on the results from the multicity studies.

25.3.4 Short-Term Mortality in Multi-pollutant Studies

The APHEA project in 12 European cities examined mortality effects of air pollution. APHEA (Katsouyanni et al., 1997) reported total non-accidental mortality risk estimates for SO_2 and PM, noting that the effects of these pollutants were "mutually independent" and were stronger during the summer. The observed associations were stronger in western than in central and eastern European cities (see Table 25.1). For cause-specific mortality in a 10-city subset, the estimated risks were larger for cardiovascular and respiratory categories than those for total non-accidental mortality (Zmirou et al., 1998). Samoli et al. (2001) [reanalysis by Samoli et al. (2003a)] applied an alternative model (a more flexible smoothing model to adjust for seasonal cycles) to the 12 cities data and also conducted subset analyses for moderate SO_2 levels (<200 and 150 $\mu g/m^3$). Both the alternative model and the restriction of the data to lower SO_2 levels produced higher SO_2 risk estimates and reduced the contrast between western and central and eastern risk estimates.

The APHEA 2 project expanded the number of cities to 29, increasing the statistical power to explain possible city-to-city variations in air pollution mortality effects. Its published mortality studies' focus was either on PM indices (Katsouyanni et al., 2001; reanalysis by Katsouyanni et al., 2003; Aga et al., 2003), NO_2 (Samoli et al., 2003b), or O_3 (Gryparis et al., 2004), and no mortality risk estimates were reported for SO_2. The PM effects analyses reported that PM risk estimates were not affected by including SO_2 in the models. The PM analyses, in their second-stage regressions, also found that NO_2 was an important effect modifier of PM (i.e., the cities with higher NO_2 levels showed larger PM risk estimates) in total mortality (Katsouyanni et al., 2001, 2003) and in elderly mortality (Aga et al., 2003). While they did not report numerical results, the results implied that the difference in SO_2 levels across cities did not alter the PM risk estimates.

A Spanish multicity study (Spanish multicenter study on air pollution and mortality, or EMECAM) analyzed short-term associations between mortality and SO_2 and PM in 13 Spanish cities (Ballester et al., 2002). They examined both 24-h average and daily 1-h max SO_2 levels. The estimated mortality risks for the 24-h average SO_2 were greatly reduced when two-pollutant models with PM were performed, but the estimates for 1-h max SO_2 were not attenuated by PM. They concluded that peak rather than the daily average concentrations of SO_2 was related to mortality.

TABLE 25.1 Estimated Total (Non-accidental) Mortality Percent Excess Deaths (95% CI in Parenthesis) per 50 μg/m³ Increase in SO_2 Reported in Recent Multicity Time-Series Studies and Meta-Analyses

Study	Estimate	Comment
APHEA 1 (Katsouyanni et al., 1997), 12 European cities	Western Europe: 2.9% (2.3, 4.6) at the best lag between 0 and 3 days for each city Central eastern Europe: 0.9% (0.2, 1.5)	The effects of SO_2 and PM were "mutually independent"
APHEA 1 (Samoli et al., 2001, 2003a, 2003b), 12 European cities: using natural splines rather than sine/cosine to adjust for temporal trends	Western Europe: 2.6% (2.1, 3.1) Central Eastern Europe: 0.7% (0.0, 1.4)	Restricting data range below 150 μg/m³ or 200 increased SO_2 risk estimates
EMECAM (Ballester et al., 2002; GAM study), 13 Spanish cities	2.5% (0.3, 4.9), average of lag 0 and 1 day	
NMMAPS (Samet et al., 2000; Dominici et al., 2003), 90 largest U.S. cities	1.1% (0.5, 1.7) at lag 1 day	Adding co-pollutants reduced the estimate by ~20% and widened confidence bands
Stieb et al. (2002, 2003) meta-analyses	Non-GAM: Single pollutant (29 studies): 1.7% (1.2, 2.3) With co-pollutant(s) (10 studies): 1.6% (0.6, 2.5) GAM: Single pollutant (17 studies): 2.0% (1.3, 2.6) With co-pollutant(s) (11 studies): 1.6% (0.8, 2.4)	

The largest U.S. multicity mortality study—the National Morbidity, Mortality, and Air Pollution Study (NMMAPS)—had PM_{10} as its main focus. It also analyzed SO_2 and other gaseous pollutants in the 90 largest U.S. cities (Samet et al., 2000; Dominici et al., 2003). In their reanalysis, Dominici et al. noted that the results did not indicate significant associations for SO_2 (or for NO_2 or CO) with total mortality. These three pollutants were generally less strongly associated with mortality than PM_{10} or O_3. The combined estimates across cities for SO_2 (and for NO_2 and CO) were positive and significant at lag 1 day in single-pollutant models and remained positive [though not significant because of larger confidence intervals (CI)] with additions of other pollutants. The estimated excess total mortality risk estimate per 50 μg/m³ was smaller than those estimated in the APHEA 1 studies.

The results from a Canadian eight-city study [Burnett et al., 2000, GAM-affected; reanalyzed by Burnett and Goldberg (2003), but SO_2 and other gaseous pollutants were not reanalyzed] indicate that while SO_2 was significantly associated with total mortality in a single-pollutant model at lag 1 day, adding $PM_{2.5}$ into the regression model reduced the SO_2 risk estimates and SO_2's association with total mortality was generally weakest among the

pollutants. In a Canadian 11-city study (Burnett et al., 1998, GAM-affected and not reanalyzed), among the gaseous pollutants (PM was not analyzed), the estimated excess mortality risk for SO_2 at the mean level (~15 μg/m^3), 1.4%, was smaller than those for NO_2 (4.1%) or O_3 (1.8%).

Stieb et al. (2002, 2003, reanalysis to evaluate the impact of GAM-affected studies) conducted meta-analyses of air pollutants by extracting results from 109 time-series mortality studies conducted worldwide. For SO_2, there were 46 studies (29 non-GAM and 17 GAM studies) that reported single-pollutant estimates and 21 studies (10 non-GAM and 11 GAM studies) that reported estimates with co-pollutant(s) in the model. As shown in Table 25.1, the impact of GAM as well as an inclusion of co-pollutants appears to be small.

There are several single-city studies that have warranted attention. Hoek et al. (2000, 2001; reanalysis by Hoek, 2003) analyzed associations between air pollution and total mortality as well as deaths from specific cardiovascular causes of the entire Netherlands. PM_{10}, black smoke (BS), SO_2, O_3, NO_2, and CO were analyzed in single- and two-pollutant models in these studies. Essentially all the pollutants were significantly associated with total mortality in single-pollutant models. In two-pollutant models with SO_2 and each of the PM indices (PM_{10}, BS, SO_4^{2-}, and NO_3^-), SO_2 was more strongly associated with total mortality than the PM indices.

Wichmann et al. (2000; reanalysis by Stolzel et al., 2003) examined the mortality effects of $PM_{2.5}$ and UFP in Erfurt, Germany. The number and mass concentrations of several size ranges of UFP as well as $PM_{2.5}$, PM_{10}, TSP, SO_2, NO_2, and CO were analyzed. Among the various PM indices, the strongest associations were found for particle number concentrations in the 0.01–0.03 μm range and mass concentrations in the $PM_{2.5}$. SO_2 was associated with mortality more strongly than any of the $PM_{2.5}$, UFP, and other gaseous pollutants. In two-pollutant models with PM indices, SO_2 remained more strongly associated with mortality than the PM indices. However, the authors stated that "the persistence of the SO_2 effect was interpreted as an artifact, because the SO_2 concentration was much below levels at which effects were usually expected."

Although the time-series studies provide estimates of excess deaths from regression models, there remained the question of whether a reduction in SO_2 actually resulted in a reduction in deaths. A sudden change in regulation can provide a basis for treating the results as coming from an "intervention study." Such a situation occurred in Hong Kong, China, in July 1990 when a restriction was introduced over one weekend that required all power plants and road vehicles to use fuel oil with a S content of not more than 0.5% by weight (Hedley et al., 2002). In another recent "intervention study" in Dublin, Ireland (Clancy et al., 2002), the ban on coal sales led to 70% reduction in BS, but only a 34% reduction in SO_2. In the Hong Kong case, SO_2 levels after the intervention declined about 50%, while PM_{10} levels did not change. In the Hedley et al. study, the average annual trend in death rate significantly declined after the intervention for all-cause (2.1%), respiratory (3.9%), and cardiovascular causes (2.0%). It should also be noted that a time-series mortality study in Hong Kong (Wong et al., 2001) suggested that SO_2 was the pollutant most consistently associated with mortality, whereas PM_{10}'s association with mortality was only marginal. This appeared to support the case for SO_2, not PM, being the more influential air pollutant in this locale. Thus, the Hong Kong case suggested that a reduction in SO_2 emissions led to an immediate reduction in deaths. However, Hedley et al. showed that Ni and V, but not other elements, also had sudden and prolonged concentration drops that could have accounted for the reduction in mortality (see Chapter 9).

25.3.5 Short-Term Morbidity in Multi-pollutant Studies

The focus of air pollution acute morbidity studies in recent decades was on PM, but there were several multicity studies (mostly APHEA projects) that examined SO_2 either as a potential confounder for PM or as a pollutant of primary interest. The following summarizes the results of the multicity studies and also describes several important single-city studies.

APHEA 1 examined associations between emergency hospital admissions for asthma and BS, SO_2, NO_2, and O_3 in four European cities (Sunyer et al., 1997). Pediatric (age <15 years) and adult (age 15–64) subjects were analyzed. They found associations between asthma admissions and NO_2 in adults and SO_2 in children. These associations were reported to be independent of BS. It should be noted that the associations between SO_2 and pediatric asthma admissions were seen in London and Paris, but not in Helsinki.

Another APHEA 1 project examined associations between hospital admissions for chronic obstructive pulmonary disease (COPD) and SO_2, BS, TSP, NO_2, and O_3 in six European cities (Anderson et al., 1997). In the combined estimates across cities, SO_2 was not as strongly associated with COPD admissions as the other pollutants, but this appeared to be at least partly due to the larger heterogeneity of SO_2 estimates across cities; the SO_2 risk estimates were significantly positive in Paris, Milan, and Barcelona, but negative in Amsterdam and Rotterdam. Other pollutants showed more consistent estimates across cities, resulting in overall statistically significant estimates. Their analysis by season found that SO_2–COPD admissions associations were stronger in warm seasons.

Spix et al. (1998) summarized associations between air pollution and hospital admissions for respiratory diseases by age (15–64 and 65+) in five west European cities as part of the APHEA 1 project. In this study, the most consistent associations for both adult and elderly respiratory admissions were found with O_3. The authors concluded that "no consistent evidence of an influence on respiratory admissions was found" for SO_2. However, they also noted that the heterogeneity of estimated SO_2 effects across the cities was best explained by the number of stations providing data (i.e., larger effects for cities with more monitoring stations). Thus, the exposure estimation error associated with SO_2 may have affected the results. The combined effect estimate for elderly admissions was positive and significant.

In the APHEA 2 project, Atkinson et al. (2001; reanalysis of GAM in 2003) investigated acute effects of PM on respiratory admissions in eight European cities, but SO_2 was examined only for its influence on PM risk estimates in two-pollutant models and the risk estimates for SO_2 were not reported. Asthma (age 0–14 and 15–64 years), COPD, and all respiratory causes (65+ years) were examined. PM, especially PM_{10}, were associated with these outcomes, and O_3 was suggested as a potential effect modifier of the PM effects.

The inclusion of SO_2 in the models only modified (reduced) PM_{10}–asthma associations in the 0–14-year age group. Sunyer et al. (2003a; a GAM study) specifically examined the effects of SO_2 on the respiratory admissions in the seven APHEA 2 cities. The respiratory categories examined were the same as those analyzed by Atkinson et al. above. SO_2 was associated with asthma admissions in children, but not with other respiratory diseases in other age groups. The authors also noted that the SO_2 risk estimates were sensitive to the inclusion of PM_{10} or CO in the models. Due to relatively high correlations among these pollutants, the issue of potential confounding could not be resolved.

As part of the APHEA 2 project, Le Tertre et al. (2002; reanalysis in 2003) examined the association between PM_{10} and BS and hospital admissions for cardiovascular causes in

eight European cities. Hospital admissions for total cardiovascular, cardiovascular for age 65+, ischemic heart disease (IHD) for age 0–64, IHD for age 65+, and stroke for age 65+ were analyzed. They did not specifically estimate SO_2 effects, but examined the sensitivity of PM risk estimates when SO_2 and other gaseous pollutants were added. Adding SO_2 in the regression models did not affect PM risk estimates, but adding CO and especially NO_2 greatly reduced PM risk estimates. They concluded that the primary effect was likely attributable to diesel exhaust. Sunyer et al. (2003; a,b GAM analysis) analyzed the same outcomes as those analyzed by Le Tertre et al. (2002) in seven cities (Barcelona excluded) and provided the combined SO_2 risk estimates across seven cities. Single-pollutant models resulted in positive and significant SO_2 risk estimates for all of the cardiac outcomes except stroke. However, these estimates were reduced when CO, NO_2, BS, and PM_{10} were included in the models except for IHD admissions for ages below 65 years. They noted that SO_2 could be a surrogate of urban pollution mixtures that in some cases was more strongly associated with cardiovascular hospitalizations than PM.

The NMMAP analysis of elderly respiratory and cardiovascular hospital admissions from 14 U.S. cities focused on PM_{10} effects. SO_2 was analyzed only to examine its influence on PM_{10} risk estimates in the second-stage regression (Samet et al., 2000; reanalysis by Schwartz et al., 2003). The authors concluded that there was little evidence of PM_{10} effects being confounded by SO_2.

There were several other smaller-scale studies that suggested the roles of SO_2 in respiratory and cardiovascular outcomes. A 6-month follow-up of 84 asthmatic children in Paris found an association between air pollution and increased asthma attacks and symptoms in mild asthmatic children (Segala et al., 1998). The strongest association was for the risk of asthma attacks and SO_2 on the same day. A comparison of air pollution effects on respiratory and cardiovascular hospital admissions in Hong Kong and London found that SO_2 was associated with cardiac admissions after adjusting for other pollutants (Wong et al., 2002; a GAM analysis).

The Hong Kong "intervention" event described earlier also provided an opportunity to investigate health endpoints other than mortality. Wong et al. (1998) compared the effects of the intervention on bronchial responsiveness in primary schoolchildren living in two districts (polluted vs. less polluted) in Hong Kong. Bronchial hyperreactivity (BHR) and bronchial reactivity slope (BR slope) were used to estimate responses to a histamine challenge. They found a greater decline in both BHR and the BR slope in the polluted district than in the less polluted district. The results suggested that the reduction in SO_2 emissions was associated with reduction in bronchial hyperresponsiveness in schoolchildren.

25.4 LONG-TERM MULTI-POLLUTANT EFFECTS STUDIES

25.4.1 Population-Based Studies

Earlier studies on the chronic effects of air pollutants relied on cross-sectional comparisons that could be subject to ecologic confounding. More recent studies often involved investigations of large cohorts for which detailed individual-level information were collected to adjust for confounding. The air pollution exposure estimates in these studies were still "ecologic" in the sense that all the subjects in a community were assigned the same community average air pollution level, but the ability to adjust for potential confounders (smoking, diet, body mass index, occupational exposures, etc.) on the individual

level is a major advantage over purely ecologic studies. Hence, this type of study was called "semi-individual" (Künzli and Tager, 1997). Since these were prospective cohort studies, they require extended periods and resources, and thus, there have not been many such studies.

Krewski et al. (2009) reanalyzed two large U.S. cohort studies, the Harvard Six Cities Study (Dockery et al., 1993) and the American Cancer Society (ACS) data (Pope et al., 1995). Their replication analyses confirmed the original investigators' findings of PM effects, and their additional analyses of the ACS data reported several interesting observations. Of the gaseous pollutants examined (SO_2, NO_2, O_3, and CO), only SO_2 showed positive and significant associations with all-cause mortality. This association appeared to be robust against adjustment for other variables including $PM_{2.5}$ and SO_4^{2-}. The risk estimates for $PM_{2.5}$ and SO_4^{2-} were reduced when SO_2 was jointly included in the models. These findings were not surprising in that the high SO_2 areas overlapped the areas of high SO_4^{2-} and $PM_{2.5}$ in these U.S. data, and therefore "independent" mortality associations of these variables could not be inferred from statistical analyses alone. However, these findings suggested the impact of air pollution sources that emit SO_2.

In Pope et al.'s (2002) analysis, the follow-up data of approximately half a million subjects during 1982–1998 were linked to $PM_{2.5}$, SO_4^{2-}, and gaseous pollutant data. $PM_{2.5}$ was associated with deaths due to all, cardiopulmonary, and lung cancer causes. SO_2 was the only gaseous pollutant associated with mortality. This was consistent with Krewski et al.'s extended analysis of the original ACS data (1982–1988 follow-up period). Pope et al.'s (2004) study analyzed more specific cardiovascular causes from the 1982 to 1998 follow-up data and found associations with $PM_{2.5}$ and IHD, dysrhythmias, heart failure, and cardiac arrest, but SO_2 and other pollutants were not examined.

Another large U.S. cohort study, the Adventist Health and Smog (AHSMOG), followed a cohort of over 6000 nonsmoking Californian Seventh-day Adventists since 1977. The AHSMOG study (Abbey et al., 1999) analyzed the 1977–1992 follow-up period. PM_{10} was associated with nonmalignant respiratory disease as well as lung cancer in males. SO_2 was associated with lung cancer for both males and females. However, the number of cases for lung cancer in this study was relatively small (18 for males and 12 for female). Therefore, interpretation of these results requires caution.

25.4.2 Panel Studies

25.4.2.1 Morbidity The studies of Lawther and colleagues (Lawther, 1958; Lawther et al., 1970) showed associations between 24-h average concentrations of SO_2 of ~0.18 ppm (500 μg/m^3), in association with BS of ~250 μg/m^3, and a worsening of health status among chronic bronchitis patients in London in the 1950s and 1960s.

Schenker et al. (1983) reported that wheeze was more prevalent in nonsmoking women living downwind from mine-mouth coal-burning electric utility plants than among women in control communities with lesser exposures to the effluents. There was a significant association with SO_2, the only effluent measured, and the highest exposure group had 24-h and annual average SO_2 levels, which were between ~100 and 125% of the U.S. NAAQS.

For an acidic pollution episode in January 1985 in the Ruhr district in Germany in which average concentrations of SO_2 and PM were 800 and 600 μg/m^3, Wichmann et al. (1989) reported significant increases in deaths, hospital admissions, outpatient visits, and ambulance deliveries to hospitals in comparison with those in a less polluted control area.

Baskurt et al. (1990) studied the hematological and hemorheological effects of an air pollution episode in Ankara, Turkey, using SO_2 as a surrogate of the pollution mixture. The blood measurements were made on 16 young male military students. The mean SO_2 levels at a station proximal to the campus where the students lived were 188 and 201 μg/m^3 during first and second blood measurements, respectively. During the period between the two measurements, the mean SO_2 level was 292 μg/m^3. Significant erythropoiesis was indicated by the increased erythrocyte counts and hemoglobin and hematocrit levels. Methemoglobin percentage was increased to 2.37 ± 0.47% (mean ± standard error) from 0.51 ± 0.23%. Sulfhemoglobinemia was present in six subjects after the period of pollution, but it was not present in any student prior to this period. Significant increases in erythrocyte deformability indices were observed after the period of pollution, that is, from 1.13 ± 0.01 to 1.21 ± 0.02, implying that erythrocytes were less flexible, which might impair tissue perfusion.

25.4.3 Studies of Cohorts of Children

Other short-term responses to PM–SO_2 mixtures have been seen in children. Repeated measurements of lung function by Dockery et al. (1982) in schoolchildren in Steubenville, OH, in 1978–1980 showed statistically significant but physiologically small and apparently reversible declines of FVC and $FEV_{0.75}$ levels to be associated with short-term increases in PM and SO_2. The highest 24-h average PM and SO_2 concentrations were 422 and 455 μg/m^3, respectively. The small, reversible decrements persisted for up to 3–4 weeks after episodic exposures.

A study of the association between episodic exposures to PM and SO_2 and pulmonary function in children was conducted in the Netherlands by Dassen et al. (1986), producing results similar to those of Dockery et al. (1982). Pulmonary function values measured during an air pollution episode in which both 24-h average PM and SO_2 levels reached 200–250 μg/m^3 were significantly lower (3–5%) than baseline values measured 1–2 months earlier in the same group. Lung function parameters that showed significant declines included FVC and FEV_1 as well as measures of small airway function. Declines from baseline were observed 2 weeks after the episode in a different subset of children, but not after 3½ weeks in a third subgroup.

Studies of associations between chronic exposure to SO_2 and PM and long-term changes in respiratory function in children have also been performed. Arossa et al. (1987) reported on the changes in baseline lung function between 1981 and 1983 in 1880 schoolchildren living in or near Turin, Italy. During that interval, annual average SO_2 in central Turin decreased from ~200 to ~110 μg/m^3, and TSP dropped from ~150 to ~100 μg/m^3. During the same period, SO_2 in a suburban area declined from ~70 to ~50 μg/m^3. A group of 162 children from the suburban area served as controls. In the first survey, FEV_1, $FEF_{25–75}$, and MEF_{50} of children from urban areas were significantly lower, while in the second survey they were not significantly different from those of the controls. The slopes over time of FEV_1, $FEF_{25–75}$, and MEF_{50}, adjusted for sex and anthropometric variables, were closely related to the decrease of pollutant concentrations, suggesting that the decrease of air pollution produced an improvement of baseline lung function.

Ierodiakonou et al. (2015) examined the associations of lung function and methacholine (MC) responsiveness (PC_{20}) to both short- and longer-term mean concentrations of SO_2, O_3, NO_2, and CO in 1003 asthmatic children participating in a 4-year clinical trial that involved treatment with budesonide or nedocromil. Pollutant concentrations were assigned

based on residence location. For SO_2, the only significant association was for long-term mean concentration and reduced PC20.

The effects of ambient air pollution on cardiac function in recent years have focused on PM, and several research groups in North America have exposed groups of volunteer subjects to concentrated ambient air particles and they have reported effects on heart rate and heart rate variability, as discussed in Chapter 9.

25.4.4 Laboratory-Based Studies in Human Volunteers

The first controlled acute human exposure to SO_2 involving cardiac function measurements (Tunnicliffe et al., 2001) involved electrocardiogram (ECG) recordings made for 12 normal and 12 asthmatic young adults. Exposures were of 1-h duration, double blind, in random order, >2 weeks apart with clean air and 0.2 ppm of SO_2. Spectral analyses of R-R intervals were performed. The SO_2 exposures were associated with statistically significant increases in high-frequency (HF) and low-frequency (LF) power in the normal subjects and reductions of comparable magnitude in HF and LF in the asthmatic subjects. No pulmonary function changes or symptom frequency changes were observed in either group of subjects. These results suggested that SO_2 exposures at concentrations that are frequently encountered during air pollution episodes can influence the autonomic nervous system, which may help in elucidating the mechanisms involved in the induction of bronchoconstriction and the cardiovascular effects of ambient air pollution.

25.4.5 Summary of Health Effects of SO_2

In summary, the more quantitative epidemiological evidence from London suggests that effects may occur at SO_2 levels at or above 0.19 ppm (500 μg/m^3), 24-h average, in combination with elevated PM levels. Additional early evidence suggested the possibility of short-term reversible declines in lung function at SO_2 levels above 250–450 μg/m^3 (0.10–0.18 ppm). The results of multi-pollutant epidemiology studies suggested mortality and morbidity effects of SO_2 at much lower concentrations. Such effects could have been due to SO_2 alone, formation of H_2SO_4 or other PM components, or peak SO_2 values well above the daily mean, but the relative roles of these factors could not be determined. We do know that the capacity of fog particles to "carry" untransformed SO_2 is limited. Thus, it appears more likely that the role of SO_2, in the presence of smoke, involved transformation products such as acidic fine particles.

There is little evidence for associations between annual average levels of SO_2 and chronic disease endpoints. To an even greater extent than the more acute response associations, they are likely to be artifacts of colinear associations between SO_2 and $PM_{2.5}$ from combustion processes.

25.5 EXPOSURES TO AND HEALTH EFFECTS OF ACIDIC AEROSOLS

25.5.1 Deposition, Growth, and Neutralization Within the Respiratory Tract

The deposition pattern within the respiratory tract is dependent on the size distribution of the droplets. Acidic ambient aerosol typically has a MMD of 0.3–0.6 μm, while industrial aerosols can have an MMD as large as 14 μm (Williams, 1970). With hygroscopic growth in the airways, submicrometer-sized droplets can increase in diameter by a factor of 2–4

and still remain within the $PM_{2.5}$ range that deposits preferentially in the distal lung airways and airspaces. As droplet sizes increase above about 3 μm MMD, deposition efficiency within the airways increases, with more of the PM deposition taking place within the upper respiratory tract, trachea, and larger bronchi (Lippmann et al., 1980). For larger droplets, the residence time in the airways is too short for a large growth factor.

Some neutralization of inhaled acidic droplets can occur before deposition due to the normal excretion of endogenous NH_3 into the airways (Larson et al., 1977). Once deposited, free H^+ reacts with components of the mucus of the respiratory tract, changing its viscosity (Larson et al., 1977). Unreacted H^+ diffuses into surrounding tissues. The capacity of the mucus to react with H^+ is dependent on the H^+ absorption capacity, which is reduced in acidic saturated mucus as found in certain disease states, for example, asthma (Holma, 1985).

25.5.2 Effects on Experimental Animals

25.5.2.1 Short-Term Exposures

Respiratory Mechanical Function Alterations of pulmonary function, particularly increases in pulmonary flow resistance, occur after acute exposure. Reports of the irritant potency of various SO_4^{2-} species are variable, due in part to differences in animal species and strains and also to differences in particle sizes, pH, composition, and solubility (U.S. EPA, 1986). H_2SO_4 is more irritating than any of the SO_4^{2-} salts in terms of increasing airway resistance. For short-term (1-h) exposures, the lowest concentration shown to increase airway resistance was 100 μg/m^3 (in guinea pigs). The irritant potency of H_2SO_4 depends in part on droplet size, with smaller droplets having more effect (Amdur et al., 1978).

Animal inhalation studies by Amdur and colleagues are of interest to this discussion because they demonstrate that effects produced by single exposures at very low acid concentrations can be persistent (Amdur et al., 1986). Guinea pigs were exposed by inhalation for 3 h to the diluted effluent from a furnace that simulates a model coal combustor. Pulverized coal yields large particle mineral ash particles and an ultrafine (<0.1 μm) condensation aerosol. The core of the UFP consists of oxides of Fe, Ca, and Mg, covered by a layer containing Na, As, Sb, and Zn. Zn generally has the highest concentration on the surface. As particles cool further, there is surface formation and/or condensation of a layer of H_2SO_4. In a single 3-h exposure, it produced significant decrements in lung diffusing capacity (DL_{co}). At 1 h after exposure, there was an increase in lung permeability. At 12 h after exposure, there were distention of perivascular and peribronchial connective tissues and an increase in lung weight. The alveolar interstitium also appeared distended. At 72 h after exposure, total lung capacity (TLC), vital capacity (VC), and functional residual capacity (FRC) had returned to baseline levels, but DL_{co} was still significantly depressed. Based upon prior experience with pure SO_2 and pure H_2SO_4 exposures in the guinea pig model, Amdur et al. (1986) concluded that the humid furnace effluent effect was an acid aerosol effect because of its persistence.

In subsequent tests, 3-h exposures to the acid-coated ZnO aerosol were given on five successive days. Significant depressions of DL_{co} were produced on the second and subsequent days for 30 μg/m^3 of H_2SO_4, while 20 μg/m^3 produced significant depressions on the fourth and fifth days. The most sensitive response was a change in airway reactivity, where a significant response was produced by a single 1-h exposure to 20 μg/m^3 H_2SO_4 as a surface coating on the ZnO (Amdur, 1989).

The persistent changes in function and morphological changes following exposure to very low levels of acidic aerosol suggest that repetitive exposures could lead to chronic lung disease. However, the implications of these changes in guinea pigs to human disease remain highly speculative.

Particle Clearance Function Donkeys exposed by inhalation for 1 h to 0.3–0.6 μm H_2SO_4 at concentrations ranging from 100 to 1000 μg/m^3 exhibited slowed bronchial mucociliary clearance function at concentrations ≥200 μg/m^3, whereas, as shown in Fig. 25.3, rabbits undergoing similar exposures exhibited an acceleration of clearance at concentrations between 100 and 300 μg/m^3 and a progressive slowing of clearance at ≥500 μg/m^3 (Schlesinger, 1985).

Schlesinger (1989) examined the relative roles of concentration (C) and daily exposure (T) on H_2SO_4-induced changes in particle clearance from the gas exchange region of rabbit lungs. Exposures were for 1–4 h/day for 14 days at concentrations ranging from 250 to 1000 μg/m^3. In a follow-up study, Schlesinger (1990) extended the concentrations downward to 50 μg/m^3. The results are summarized in Fig. 25.4. The acceleration in clearance

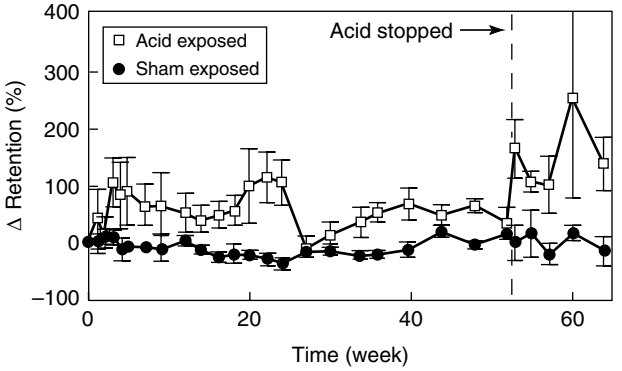

FIGURE 25.3 Mean change in % retention of tracer particles during intermittent exposure to H_2SO_4 in acid- and sham-exposed rabbits from that established in pre-exposure control tests. *Source*: From Lippmann et al. (1987).

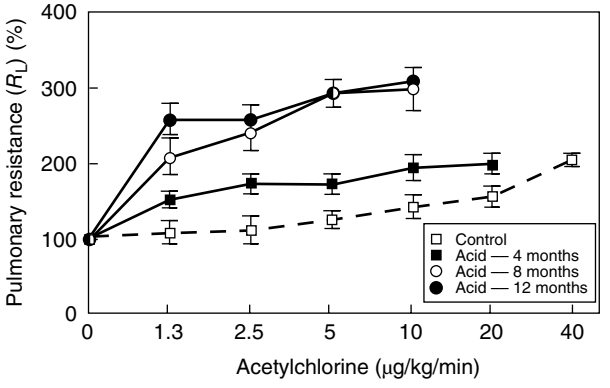

FIGURE 25.4 Effect of bronchoprovocation challenges on pulmonary resistance (R_L). The abscissa is expressed in terms of doubling doses of acetylcholine, expressed as the group mean and standard deviation in % of baseline R_L at each dose. *Source*: From Gearhart and Schlesinger (1986).

produced by 4 h at 50 μg/m^3 was essentially the same as that produced by 2 h at 100 μg/m^3 and 1 h at 250 μg/m^3, indicating that cumulative exposure, rather than concentration, governs the response, at least within the ranges of concentration and time evaluated. The results are similar to those for mucociliary clearance in the sense that relatively low levels of exposure produce an acceleration of clearance, but clearance retardation occurs at higher levels of exposure.

Cellular Function Schlesinger (1990) examined the comparative effects of exposure to the two main ambient acidic sulfates, H_2SO_4 and NH_4HSO_4, using the phagocytic activity of alveolar macrophages as the endpoint. Rabbits were exposed to 250–2000 μg/m^3 H_2SO_4 (as SO_4^{2-}) and 500–4000 μg/m^3 NH_4HSO_4 (as SO_4^{2-}) for 1 h/day for 5 days; bronchopulmonary lavage was then performed for recovery of free lung cells. Phagocytosis, measured by uptake of opsonized latex spheres *in vitro*, was altered by exposure to H_2SO_4 at concentrations ≥500 μg/m^3 and to NH_4HSO_4 at ≥2000 μg/m^3. Assessment of results in terms of the calculated hydrogen ion concentration in the exposure atmosphere showed that identical levels of H^+ produced different degrees of response depending on whether exposure was to H_2SO_4 or NH_4HSO_4. On the other hand, macrophages incubated in acidic environments *in vitro* responded similarly, regardless of whether H_2SO_4 or NH_4HSO_4 was used to adjust the pH. Thus, the response may relate more to the local pH change in the vicinity of the depositing droplet than to the total H^+ delivered.

Pollutant Interactions Osebold et al. (1980) exposed antigenically sensitized mice to 500 ppb O_3 for 3 days, with and without concurrent exposure 1 mg/m^3 of submicrometer H_2SO_4 droplets. There was an increase in atopic reactivity that was greater than that for each pollutant alone. Kleinman et al. (1989) reported that lesions in the gas exchange region of the lung of rats exposed to O_3 were greater in size in rats exposed to mixtures containing H_2SO_4 or NO_2 as well as O_3. Last (1989) reported significant increases in lung protein content in rats exposed for 9 days to 200 ppb O_3 plus 20 μg/m^3 H_2SO_4 over those in rats exposed to 200 ppb O_3 alone, as well as a trend toward increased protein in rats exposed to 200 ppb O_3 and 5 μg/m^3 H_2SO_4.

25.5.2.2 Subchronic Exposures

Particle Clearance Function Donkeys exposed for 1 h/day (5 day/week) for 6 months to an aerosol (0.3–0.6 μm) of H_2SO_4 at a concentration of 100 μg/m^3 developed highly variable clearance rates and a persistent shift from baseline rate of bronchial mucociliary clearance during the exposures and for 3 months after the last exposure. Two animals had much slower clearance than their baseline during the 3 months of follow-up, but two had faster than baseline rates (Schlesinger et al., 1979). Rabbits exposed for 1 h/day (5 day/week) for 4 weeks to 0.3 μm H_2SO_4 at 250 μg/m^3 developed variable mucociliary clearance rates during the exposure period, and their clearance during a 2-week period following the exposures was substantially faster than their baseline rates (Schlesinger et al., 1983). For a group of rabbits undergoing daily exposures via the nose at 250 μg/m^3 for 1 year, Fig. 25.3 shows that bronchial mucociliary clearance was consistently slowed after the first few weeks and became even slower during a 3-month period following the end of acid exposures (Gearhart and Schlesinger, 1988).

During the course of a 1-year series of 1 h/day, 5 day/week nasal exposures to submicrometer H_2SO_4 at 250 μg/m^3, groups of rabbits were exposed on three occasions to ^{85}Sr-tagged latex aerosols for determination of the rates of clearance from the non-ciliated alveolar region (Schlesinger and Gearhart, 1986). The latex aerosols were inhaled on days 1, 57, and 240 following the start of the H_2SO_4 exposures, and particle retention was

followed for 14 days after each latex administration. As compared to baseline rates of clearance in control animals, early alveolar clearance was accelerated to a similar degree in all three tests performed during the chronic H_2SO_4 exposures.

Airway Hyperresponsiveness The effects of daily 1-h exposures of rabbits to 250 μg/m^3 of H_2SO_4 on bronchial responsiveness was assessed at the end of 4, 8, and 12 months by *in vitro* administration of doubling doses of acetylcholine and measurement of pulmonary resistance (R_L), as shown in Fig. 25.4 (Gearhart and Schlesinger, 1986). Dynamic compliance (C_{dyn}) and respiratory rate (f) were also measured following agonist challenge. Those animals exposed for 4 months showed increased sensitivity to acetylcholine (i.e., the dose required to produce a 150% increase in R_L), and there was an increase in reactivity (i.e., the slope of dose vs. change in R_L) by 8 months, with a leveling off of the response after this time. No changes in C_{dyn} or f were noted at any time. Thus, repeated exposures to H_2SO_4 resulted in the production of hyperresponsive airways in previously healthy animals. This has implications for the role of nonspecific irritants in the pathogenesis of airway disease.

Histology In the study of Schlesinger et al. in which rabbits were exposed to 250 μg/m^3 for 4 weeks and sacrificed 2 weeks later, histological examination showed increased numbers of secretory cells in distal airways and thickened epithelium in airways extending from midsized bronchi to terminal bronchioles (Schlesinger et al., 1983). There were no corresponding changes in the trachea or other large airways. In the follow-up study, in which rabbits received daily exposures for 1 year via the nose at 250 μg/m^3, the secretory cell density was elevated in some lung airways at 4 months and in all lung airways at 8 months (Gearhart and Schlesinger, 1986, 1988; Schlesinger and Gearhart, 1986). At 12 months, the increased density remained in small and midsized airways, but not large airways. Partial recovery was observed at 3 months after the last exposure.

In a study in which dogs were exposed daily for 5 years to 1100 μg/m^3 SO_2 plus 90 μg/m^3 H_2SO_4 and then allowed to remain in unpolluted air for 2 years, there were small changes in pulmonary functions during the exposures, which continued following the termination of exposure. Morphometric lung measurements made, at the end of a 2-year post-exposure period, showed changes analogous to an incipient stage of human centrilobular emphysema (Stara et al., 1980).

25.5.3 Effects on Humans

25.5.3.1 Short-Term Effects of Controlled Exposures

Respiratory Mechanical Function H_2SO_4 and other sulfates affected both sensory and respiratory function in humans. Respiratory effects from exposure to H_2SO_4 (350–500 μg/m^3) included increased respiratory rates and tidal volumes (Amdur et al., 1952; Ericsson and Camner, 1983). However, other studies of pulmonary function in nonsensitive healthy adult subjects indicated little effect on pulmonary mechanical function when subjects were exposed to submicrometer H_2SO_4 at 10–1000 μg/m^3 for 10–120 min. In one study, the bronchoconstrictive action of carbachol was potentiated by 0.8 μm H_2SO_4 and by other sulfate aerosols, more or less in relation to their acidity (Utell et al., 1984). Asthmatics are substantially more sensitive in terms of changes in pulmonary mechanics than healthy people, and vigorous exercise potentiates the effects at a given concentration. The lowest-demonstrated-effect level was 68 μg/m^3 of 0.6 μm H_2SO_4 via mouthpiece inhalation in exercising adolescent asthmatics (Koenig et al., 1989) with somewhat greater responses

at 100 μg/m^3 (Koenig et al., 1983a). The effects disappeared within about 15 min. In adult asthmatics undergoing exposure to 0.8 μm H_2SO_4 for 2 h, the lowest-observed-effect level was 75 μg/m^3 (Bauer et al., 1988). Spengler et al. (1989) concluded that these results are consistent when the exposure metric is total amount of H_2SO_4 inhaled rather than the concentration.

By contrast, Avol et al. (1988) found no significant functional responses to H_2SO_4. They exposed 32 asthmatic volunteers, 8–16 years of age, in a chamber to clean air and to H_2SO_4 at 46 ± 11 μg/m^3 and at 127 ± 21 μg/m^3. The MMDs were near 0.5 μm with geometric standard deviations near 1.9. Temperature was 21°C, and relative humidity was near 50%. Subjects were exposed with unencumbered oronasal breathing for 30 min at rest, plus 10 min at moderate exercise (ventilation rate ~20 L/min m^2 of body surface). A subgroup (21 subjects) was exposed similarly to clean air and to 134 ± 20 μg/m^3 with 100% oral breathing. Increased symptoms and bronchoconstriction were found after exercise under all exposure conditions. For the group, symptom and lung function responses were not statistically different during control and during acid exposures with unencumbered breathing or with oral breathing.

Aris et al. (1990) exposed nonsmoking adult volunteers in chambers for 1 h to fogs containing hydroxymethanesulfonate (HMSA), the bisulfate adduct of formaldehyde, and a common constituent of California acid fogs. The droplet size was 7 μm, the HMSA concentration was 260 μg/m^3, and the H_2SO_4 content of the aerosol was 1.1 μg/m^3. A control exposure was to H_2SO_4 only. Both acid fogs produced slight increases in respiratory symptoms, but no changes in airway resistance. Thus, HMSA did not produce a specific bronchoconstrictor effect at a concentration about three times greater than the highest ambient measurements.

The effects of acid fog droplets on respiratory function and symptoms were studied by Avol et al. (1988) with exposures of both normal and mild asthmatic adult volunteers for 60 min to 8 μm MMAD fog droplets containing 0, 150, and 680 μg/m^3 of H_2SO_4, with alternating 10-min periods of rest and heavy exercise. Both normal and asthmatic subjects reported more symptoms with increasing concentration, and the asthmatics showed an increase in airway resistance at the higher acid concentration. There were no significant differences in either forced expiratory function or airway reactivity to MC between the sham and acid exposures.

Linn et al. (1989) exposed both healthy and asthmatic volunteers for 1 h with intermittent exercise to H_2SO_4 at 2000 μg/m^3, with droplet sizes of 1, 10, and 20 μm. Healthy subjects had no significant changes in lung function or bronchial reactivity to MC, but did show irritant symptoms with the 10- and 20-μm aerosols. By contrast, the asthmatics had significant decreases in function, increases in airway resistance, and increases in symptoms for all three of the droplet sizes.

Raizenne et al. (1996) examined the effects of exposure to acidic PM on respiratory function among 8–12-year-old children living in 22 communities in the United States and Canada. Air quality and meteorology were measured in each community for the year preceding the pulmonary function tests. FVC and $FEV_{1.0}$ of 10,251 white children, adjusted for age, sex, height, weight, and sex–height interaction, were examined. A 52 nmol/m^3 difference in annual mean particle strong acidity was associated with a 3.5% (95% CI, 2.0–4.9) decrement in adjusted FVC and a 3.1% (95% CI, 1.6–4.6) decrement in adjusted $FEV_{1.0}$. The FVC decrement was larger, although not significantly different, for children who were lifelong residents of their communities (4.1%, 95% CI, 2.5–5.8). The relative odds for low lung function (i.e., measured FVC less than or equal to 85% of predicted) was

2.5 (95% CI, 1.8–3.6) across the range of particle strong acidity exposures. These data suggested that long-term exposure to ambient particle strong acidity may have a deleterious effect on lung growth, development, and function.

Particle Clearance Function In healthy nonsmoking adult volunteers exposed to 0.5 μm H_2SO_4 at rest at 100 μg/m^3 for 1 h, there was an acceleration of bronchial mucociliary clearance of tracer particles (7.6 μm), which deposited primarily in the larger bronchial airways, and a slowing of clearance when the exposure was raised to 1000 μg/m^3 (Figs. 25.3 and 25.5) (Leikauf et al., 1981). For tracer particles (4.2 μm), which deposited primarily in midsized to small conducting airways, there was a small but significant slowing of clearance at 100 μg/m^3 H_2SO_4 and a greater slowing at 1000 μg/m^3 (Leikauf et al., 1984). These changes are consistent with the greater deposition of acid in midsized to smaller airways. Exposures to 100 μg/m^3 for 2 h produced slower clearance than the same exposure for 1 h, indicating a cumulative relationship to dose (Spektor et al., 1989).

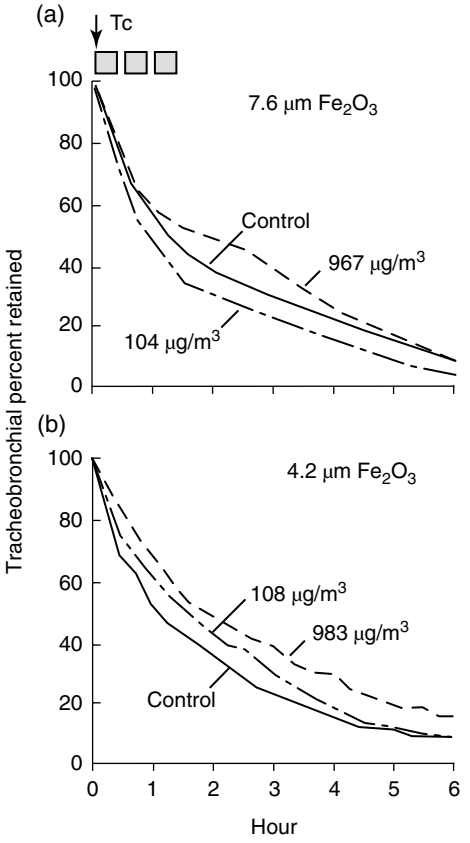

FIGURE 25.5 Tracheobronchial retention of ^{99m}Tc-tagged Fe_2O_3 microspheres in healthy adult volunteers as a function of time after a 1-min tagged aerosol inhalation for particles with aerodynamic diameter of (a) 7.6 μm or (b) 4.2 μm. Submicrometer-sized droplets of H_2SO_4 are inhaled via nasal mask during three 20-min intervals as indicated by cross-hatched boxes. The solid line indicates retention for sham exposure, the long dash–dot line for about 100 μg/m^3 H_2SO_4, and the short dash line for about 1000 μg/m^3 H_2SO_4. *Source*: From Leikauf et al. (1984).

The results of these studies were used by Yu et al. to construct a model for the effects of surface deposition of acidic droplets on mucus transport velocity along the tracheobronchial airways (Yu et al., 1986). Based on this model, mucous velocities are increased when less than about 10^{-7} g/cm^2 of H_2SO_4 is deposited, while clearance is retarded when the acid deposition exceeds this limit.

The effects of a 1-h inhalation of submicrometer H_2SO_4 aerosols via nasal mask on tracheobronchial mucociliary particle clearance and respiratory mechanics were studied by Spektor et al. (1985) in subjects with histories of asthma. A brief inhalation of tagged aerosol preceded the 1 h H_2SO_4 or a sham exposure. Respiratory function was measured before and 15 min and 3 h after the H_2SO_4 or sham exposure. After exposure to 1000 μg/m^3 of H_2SO_4, the six subjects not on routine medication exhibited a transient slowing of mucociliary clearance and also decrements in specific airway conductance (SG_{aw}), FEV_1, midmaximal expiratory flow rate (MMEF), and flow rate at 25% of TLC (V_{25}) ($p < 0.05$) in both sets of measurements. The four asthmatics on daily medication exhibited stepwise mucociliary clearance that was too variable to allow detection of any H_2SO_4 effect on clearance. Mucociliary clearance rates in both groups in the sham exposure tests were significantly slower than those of healthy nonsmokers studied previously by Leikauf et al. (1984) using the same protocols. The extent of mucociliary clearance slowing following the 1000 μg/m^3 exposure in the non-medicated subjects was similar to that in the healthy nonsmokers. This similar change, from a reduced baseline rate of clearance, together with the significant change in respiratory function, indicates that asymptomatic asthmatics may respond to H_2SO_4 exposures with functional changes of greater potential health significance than healthy nonsmokers.

25.5.3.2 Effects of Ambient SO_2 Exposures in Population Studies There are numerous studies of associations between SO_4^{2-} and various health effect indices in polluted communities and a more limited number of studies with measurements of H^+.

The earliest direct association between measured acidity and human health was Gorham's highly significant ($p < 0.01$) correlation between mortality rates for bronchitis in 53 U.K. metropolitan areas in the period 1950–1954 and the pH of winter precipitation in these areas (Gorham, 1958). There was also a correlation with SO_4^{2-} at the 5% level of significance. When multiple regression analyses were performed, pH remained significant at the 1% levels, but SO_4^{2-} lost its statistical significance.

An association based on some limited but direct measurements was reported by Kitagawa who identified H_2SO_4 as the probable causal agent for approximately 600 cases of acute respiratory disease in the Yokkaichi area in central Japan between 1960 and 1969 (Kitagawa, 1984). The patients' residences were concentrated within 5 km of a titanium dioxide plant with a 14-m stack that emitted from 100,000 to 300,000 kg/month of H_2SO_4 in the period 1961–1967. The average concentration of SO_3 in February 1965 in Isozu, a village 1–2 km from the plant, was 130 μg/m^3, equivalent to 159 μg/m^3 of H_2SO_4. Kitagawa estimated that the peak concentrations might be up to 100 times as high with a north wind. Electrostatic precipitators were installed to control aerosol emissions in 1967, and after 1968 the number of newly found patients with "allergic asthmatic bronchitis" or "Yokkaichi asthma" gradually decreased. Although Kitagawa's quantitative estimates of exposure to H_2SO_4 and the criteria used to describe cases of respiratory disease may differ from current methods, the unique aspect of this report is the identification of H_2SO_4 as the likely causal agent for an excess in morbidity.

In an independent analysis of mortality from asthma and chronic bronchitis associated with changes in SO_2 air pollution in Yokkaichi from 1963 to 1983, Imai et al. correlated mortality with sulfation index (lead peroxide candle measurements) and focused on reductions in SO_x emissions from a petroleum refinery in the harbor area in 1972 (Imai et al., 1986). Thus, it is not clear, from their analysis, what the SO_2 or H_2SO_4 exposures to the population from these emissions were. In any case, mortality rates for bronchial asthma were significantly elevated in Yokkaichi in the period 1967–1970, and the mortality rates due to chronic bronchitis were significantly elevated for the periods 1967–1970 and 1971–1974. There was a greater lag between the reduction in SO_x pollution and reduction in mortality rate for chronic bronchitis than for bronchial asthma.

While H_2SO_4 was believed, by some, to be a likely causal factor for excess mortality and morbidity during and following the December 1952 smog episode in London, the only air quality available for that time was for BS and SO_2. During the late 1950s, an air monitoring method was developed by Commins and Waller (1963) to measure H_2SO_4 in urban air, and they used it to make daily measurements of H_2SO_4 at St. Bartholomew's Hospital in Central London during the December 1962 episode. As shown in Chapter 9 (Fig. 9.2), the airborne H_2SO_4 rose rapidly during the 1962 episode, with a greater relative increase than that for BS or SO_2.

Using the method of Commins and Waller (1963), daily measurements of H^+ were made at a Central London site (St. Bartholomew's Medical School) between 1965 and 1972. The December 1962 London fog episode was the last to produce a clearly evident increase in the number of daily deaths, albeit a much smaller one than December 1952. The U.K. Clean Air Act of 1956 had led to the mandated use of smokeless fuels, and annual mean BS had declined by 1962 to about one-half of the 1958 level. The annual average SO_2 concentrations had not declined by 1962, but dropped off markedly thereafter, along with a further marked decline in BS levels. For the period between 1964 and 1972, the measured levels of H_2SO_4 followed a similar pattern of decline. The daily concentration data have been correlated with concurrent daily records of mortality in several studies. Based on an initial time-series analysis of the winter data (Thurston et al., 1997), H^+ appeared to be more strongly associated with total daily mortality than either BS or SO_2. However, a more detailed analysis of the full-year data set by Ito et al. (1993), involving statistical "prewhitening," did not indicate that H^+ had a greater degree of association with daily mortality than BS or SO_2. In the Ito et al. (1993) analysis, temperature had the greatest influence in all seasons, and all three of the pollution variables (same day and lagged 1 or 2 days) were significantly associated with daily mortality. However, there were limitations imposed on the analysis in terms of the limitation of the H^+ data to only one monitoring site, the limited precision of the measurement of H^+, and the selection of filters for controlling the confounding long-wave influences.

In a further exploratory further analysis of the Central London data set, Lippmann and Ito (1995) developed an alternate approach for separating the effects of season and temperature on daily mortality from those of pollution. They analyzed the data for each season separately, and within each season, they restricted analyses to those days in which ambient temperature had little, if any, influence on mortality. Regressions were performed for BS, SO_2, and aerosol H^+. For the winter period (November to February), mortality was most closely correlated with H^+, while for the rest of the year, it was most closely correlated with SO_2. In all seasons, the correlation was poorest for BS. The results for winter and summer are illustrated in Figs. 25.6 and 25.7.

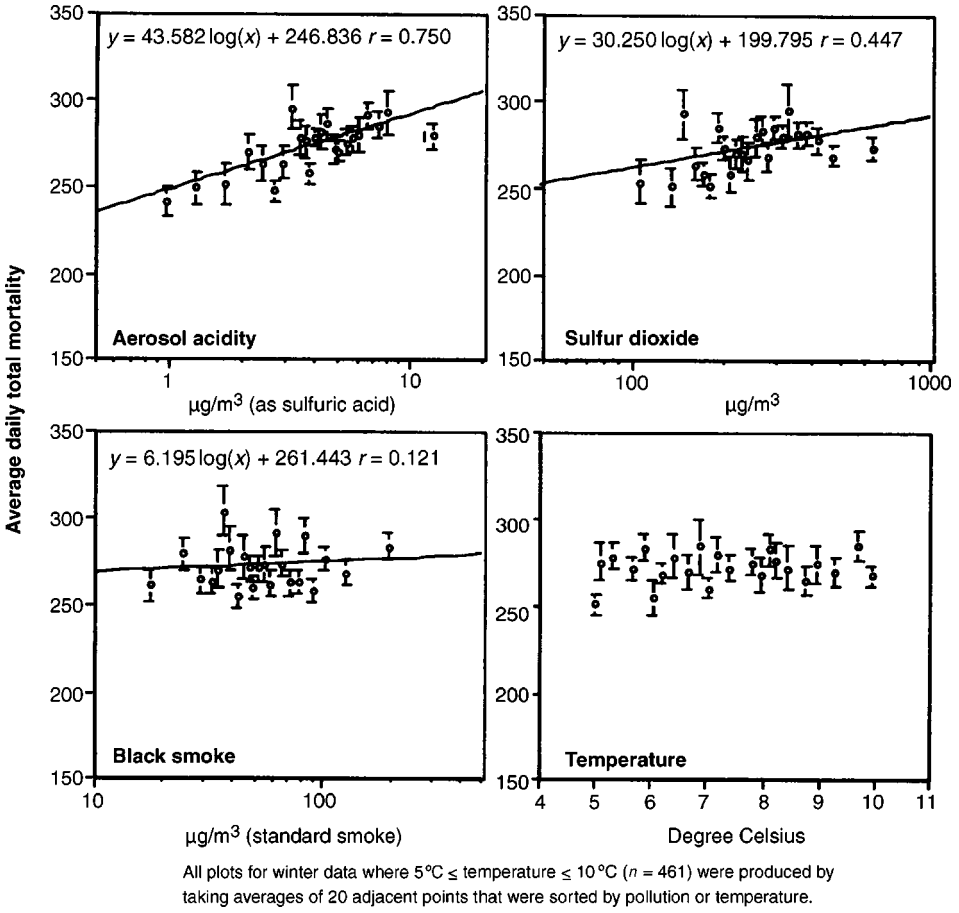

FIGURE 25.6 Mortality in London: 1965–1972, winter days with temperatures between 5 and 10°C. *Source*: From Lippmann and Ito (1995).

Ostro et al. (1989, 1991) correlated data on aerosol H^+, SO_4^{2-}, NO_3^-, and $PM_{2.5}$, as well as gaseous SO_2 and CO with daily symptom, medication usage, and other variables for a panel of about 200 adults with moderate to severe asthma in Denver, CO, between November 1987 and March 1988. The H^+ concentrations ranged from 2 to 41 neq/m^3 (0.04–0.84 μg/m^3 of H_2SO_4 equivalent) and were significantly related to both the proportion of the survey respondents reporting a moderate or worse overall asthma condition and the proportion reporting a moderate or worse cough. Of all the pollutants considered, H^+ displayed the strongest association with asthma and cough. The magnitudes of the effects were compared by using elasticities, that is, the percent change in the health effect due to a given percent change in the pollutant. Using asthma as an example, the results indicate elasticities with respect to SO_4^{2-}, $PM_{2.5}$, and H^+ of 0.060, 0.055, and 0.096, respectively (Ostro et al., 1989). This indicates that a 10% change in the concentrations of H^+ could increase the proportion reporting a moderate or worse asthma condition by 0.96%.

In their follow-up report on this study, Ostro et al. (1991) examined evidence for lagged effects and concluded that contemporaneous measures of H^+ concentration provided

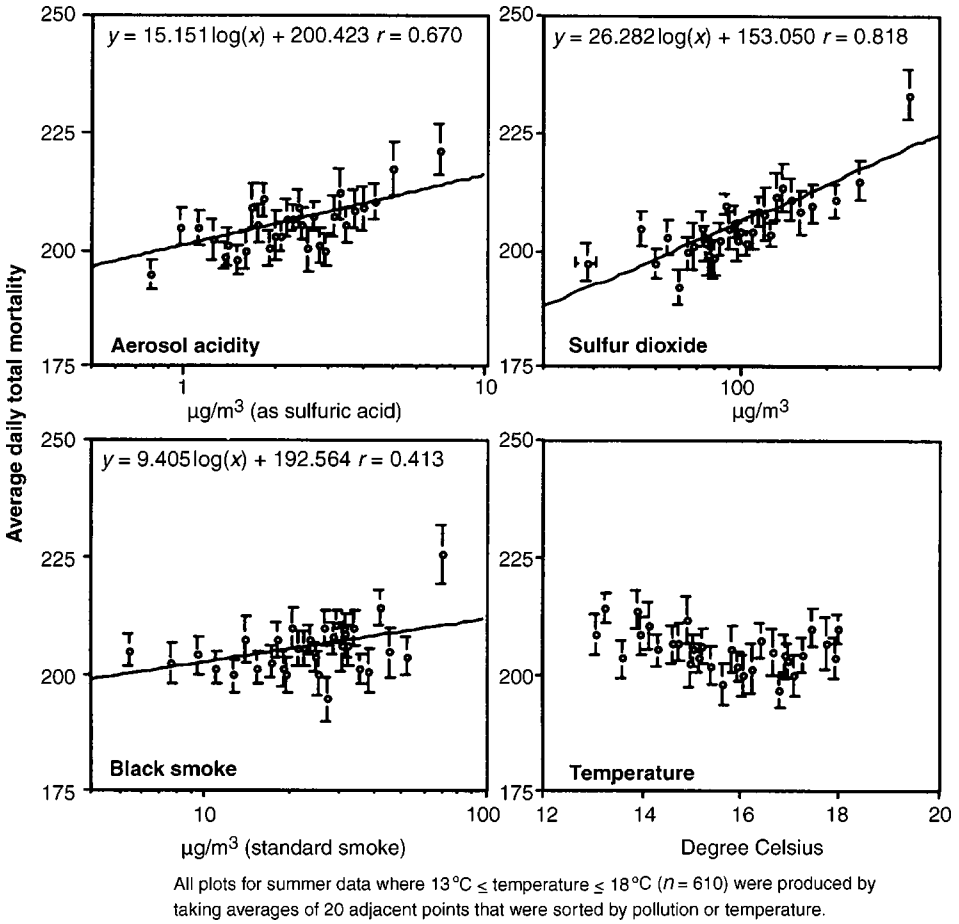

FIGURE 25.7 Mortality in London: 1965–1972, summer days with temperatures between 13 and 18°C. *Source*: From Lippmann and Ito (1995).

the best associations with asthma status and that meteorological variables were not associated with the health effects reported. They also examined the effects of exposure to H^+, adjusting for time spent outdoors, level of activity, and penetration of acid aerosol indoors. Based on the adjusted exposures, the effect of H^+ on cough increased 43%, suggesting that dose–response estimates that do not incorporate behavioral factors affecting actual H^+ exposures may substantially underestimate the impact of the pollution.

Some morbidity studies have utilized daily measurements of H^+ concentrations. In the six-city study (Speizer, 1989; Damokosh et al., 1993), aerosol H^+ had a closer association with parent-reported bronchitis symptoms in children than any other PM or gas-phase components. This was also true in the Harvard–Health Canada study in 22 North American towns (Dockery et al., 1996), and in this study aerosol H^+ was also the most closely related pollutant to baseline lung function (Raizenne et al., 1996). While pulmonary function differences in the six-city study were not statistically significant, the direction and magnitude of the differences were consistent with the results reported by Raizenne et al. (1996).

In a supplemental acute response study in Uniontown, PA, one of the 22 communities in the Harvard–Health Canada study, Neas et al. (1995) had a stratified sample of 83 children report twice-daily PEFR measurements on 3582 child-days during the summer of 1990. Upon arising and before retiring, each child recorded PEFR and the presence of cold, cough, or wheeze symptoms. The session-specific average deviation was then calculated across all of the children. A 12-h H^+ exposure to a 125-nmol/m^3 increment was associated with a −2.5 L/min deviation in the group mean PEFR (95% CI −4.2 to −0.8) and with increased cough incidence [odds ratio (OR) = 1.6, 95% CI 1.1–2.4].

Studnicka et al. (1995) studied three consecutive panels of children participating in a summer camp in the Austrian Alps. For 47, 45, and 41 subjects, daily FEV_1, FVC, and peak PEFR were recorded, with 15, 11, and 5% of participants, respectively, reporting current asthma medication. Mean levels of ambient pollutants were approximately 15% higher for the first panel than for the other two panels, but the H^+ component was twice as high for panel 1. The maximum H^+ exposure during panel 1 was 84 nmol/m^3 (4 μg/m^3 H_2SO_4 equivalent). For FEV_1 in panel 1, a significant decrease of −0.99 mL per nmol/m^3 H^+ ($p = 0.01$) was observed. For panel 2, the FEV_1/H^+ coefficient was found to be similar (−0.74 mL per nmol/m^3 H^+; $p = 0.28$), while for panel 3 it was in the opposite direction (0.10 mL per nmol/m^3 H^+; $p = 0.83$). The decrease in FEV_1 observed in panel 1 was more pronounced when the mean exposure during the previous 4 days was considered (−2.99 mL FEV_1 per nmol/m^3 H^+; $p = 0.004$).

Thurston et al. (1997) studied that 52, 58, and 56 children (ages 7–13) attending a summer "asthma camp" in the Connecticut River Valley were followed during the last week of June in 1991, 1992, and 1993, respectively. Most of the subjects had moderate to severe asthma. Daily records were kept of the environmental conditions, as well as of subject medication use, lung function, and respiratory symptoms. H^+ and SO_4^{2-} were found to be significantly and consistently correlated with acute asthma exacerbations and chest symptoms. Lung function decrements were consistently associated with O_3, but not with SO_4^{2-} and H^+.

The prospective cohort mortality study of Dockery et al. (1993) reported that H^+ correlated less well with mortality than $PM_{2.5}$ or SO_4^{2-}, but they only had 9–12 months of H^+ data in each city, compared with 14–16 years of data on the other pollutant variables, and many of the daily concentrations of H^+ were below the detection limit. A similar limitation was present in the analysis of Dockery et al. (1992) of air pollution and daily mortality rates.

25.5.4 Implications: Exacerbation of Asthma and Chronic Bronchitis

The studies of Utell et al. (1982, 1984) demonstrated that brief exposures to acidic aerosols reduced airway conductance in healthy humans and that asthmatic subjects are more sensitive than healthy individuals. The lowest concentration that produced a significant response in the group as a whole was 450 μg/m^3. Koenig et al. reported a 40% increase in total airway resistance in a group of exercising asthmatic adolescents when they were exposed to 100 μg/m^3 of H_2SO_4 (Koenig et al., 1983a) and lesser but still significant effects at 68 μg/m^3 (Koenig et al., 1989). The responses were similar to those reported by Koenig and colleagues for the same protocols and kinds of subjects for exposure to 0.5 ppm of SO_2 (1300 μg/m^3) (Koenig et al., 1981, 1983b). Thus, when SO_2 is oxidized and hydrolyzed, the resulting H_2SO_4 is 10–20 times more potent. While the average increases in airway resistance were small, the populations studied were small, and some subjects had much greater

responses than the average. Also, the populations were carefully selected and did not include the more unstable, and potentially more reactive, asthmatic individuals within the population.

Moderate exercise appears to enhance the response by increasing the dose of irritant delivered to epithelial surfaces. With increasing exercise, more pollutant is inhaled. The greater inspiratory flow rates also act to increase the percentage of highly soluble SO_2 vapor that can penetrate beyond the upper airways into those bronchial airways where reflex responses are most likely to be initiated. The greater flow rate also produces a thinner boundary layer around the airway bifurcations, enhancing "hot spots" of deposition of particles and vapors from the airstream. Thus, exercise results in increased deposition in this region for the submicrometer-sized H_2SO_4 droplets that would have minimal deposition in such airways at lower flow rates.

The irritant dose delivered to the larger bronchial airways is greater in patients with asthma and bronchitis than in healthy individuals because the former groups have airways with smaller diameters. This may account for some, or perhaps all, of the greater responsiveness of asthmatics to inhaled irritants. They may also have a greater responsiveness at the sites of deposition to the delivered dose, but this has not been clearly established in *in vivo* tests. In any case, an irritant-induced narrowing of the conducting airways of the lung can increase the surface deposition of subsequently inhaled irritant, resulting in further airway constriction.

The subjective responses to inhaled acid aerosols may include a feeling of chest tightness, and the work of breathing is increased. For individuals with chronic respiratory disease, any increment of work in breathing may be considered an adverse effect. For asthmatic individuals, the major concern is the induction of bronchospasm. The few clinical laboratory studies on carefully selected asthmatics cannot be expected to generate data on the exact conditions that provoke bronchospasm and acute respiratory insufficiency. It would be highly desirable to have an animal model for bronchial asthma so that this important issue could be systematically studied.

25.5.5 Implications: Mucociliary Clearance in the Pathogenesis of Chronic Bronchitis

Schlesinger et al. (1983) studied the effects of concentration and duration of exposure to submicrometer-sized H_2SO_4 aerosols on tracheobronchial and alveolar rates of particle clearance in rabbits and found that the effects increased with duration and concentration. Spektor et al. (1989) reported a similar finding for tracheobronchial particle clearance in humans.

The altered clearance rates during and after the exposure period may be an adaptive response of the mucociliary system to acid exposures. On the other hand, they may be early stages in the progression toward more serious dysfunctions, for example, those found in chronic bronchitis, which may result from continued irritant exposures.

A mechanistic basis for the linkage between chronic exposure to H_2SO_4 and the pathogenesis of chronic bronchitis lies in the series of studies involving chronic exposures of animals and persistent histological alterations in lung structure. As noted by Lippmann et al. (1987), these structural changes in the rabbit model have correlates in terms of clearance function changes. These, in turn, are indicative of changes in mucus secretion leading to mucus stasis, a hallmark of bronchitic disease.

The animal studies can be related to human responses in two ways. One is the coherence in functional and morphometric responses of animals to H_2SO_4 and cigarette smoke, a known causal factor for human chronic bronchitis. The other is that humans, rabbits, and donkeys all have essentially the same transient mucociliary clearance function responses to single 1-h exposures to H_2SO_4. The fact that daily 1-h H_2SO_4 exposures in rabbits and donkeys produce persistent changes in clearance function makes it highly likely that humans would also show these effects if similarly exposed. Furthermore, a comparison of the human and rabbit responses to single exposures indicated that humans respond at lower concentrations than rabbits (Schlesinger, 1986).

The effects of H_2SO_4 on the airways are very likely to be cumulative during each exposure day, at least in part. Thus, the daily 1-h exposures at 250 $\mu g/m^3$ in the rabbits may be equivalent to <50 $\mu g/m^3$ for a 7–8-h day and to a still lower concentration for equivalent effects in humans. On the other hand, the effects produced by the 1-year series of exposures in the rabbits were less severe than the condition corresponding to a clinical diagnosis of chronic bronchitis in humans.

Unfortunately, there are few data concerning the response of the human mucociliary clearance system under prolonged insult by potentially harmful pollutants such as H_2SO_4. The most direct evidence for an association between chronic bronchitis and exposure to H_2SO_4 comes from occupational exposures, but these were at high levels. Williams observed an excess incidence of chronic bronchitis in workers occupationally exposed to H_2SO_4 levels above 1 mg/m^3 (probable diameter = 14 μm); however, the excess was actually in increased incidence of episodes in affected workers rather than an increase in the number of workers affected (Williams, 1970).

Although available evidence suggests that exposure to H_2SO_4 may exacerbate disease, it has not been clearly established that it can initiate it. Some limited evidence indicates that it can. For example, in two previously healthy human subjects, Sim and Pattle (1957) found the development of what appeared to be long-lasting symptoms of bronchitis as a result of repeated exposure to H_2SO_4 lasting 1 h and given no more than twice a week, with at least 24 h between exposures. Concentrations were, however, high, ranging from 3 to 39 mg/m^3.

The suggestion for a role of H_2SO_4 in the development of chronic bronchitis is given added strength when results of studies of submicrometer H_2SO_4 or whole fresh cigarette smoke exposures, both conducted at NYU Medical Center with laboratory animals and humans, are compared (Lippmann et al., 1982). Cigarette smoke is an agent known to be involved in the etiology of human chronic bronchitis, and as shown in Fig. 25.8, the effects of smoking two cigarettes on the mucociliary clearance of tracer particles are essentially the same in humans and donkeys in terms of a transient acceleration of clearance in single low-dose exposures. Both agents produce a transient slowing of mucociliary particle clearance following single high-dose exposures (Lippmann et al., 1982). Furthermore, alterations in clearance rates persist for several months followed multiple exposures to both agents (Figs. 25.3 and 25.9). Thus, although direct evidence for an association between intermittent low-level exposures to H_2SO_4 and chronic bronchitis is lacking, the similarity in response between H_2SO_4 and cigarette smoke exposures suggests that such an association is likely.

Human chronic bronchitis is a clinically diagnosed disease, but one that is characterized by certain morphological changes associated with these clinical symptoms (Reid, 1963; Mitchell, 1967; Lourenco, 1969; Suh et al., 1969; Jefferey, 1982). One of the basic stigmata is an increase in the number and/or size of epithelial mucus secretory cells in both

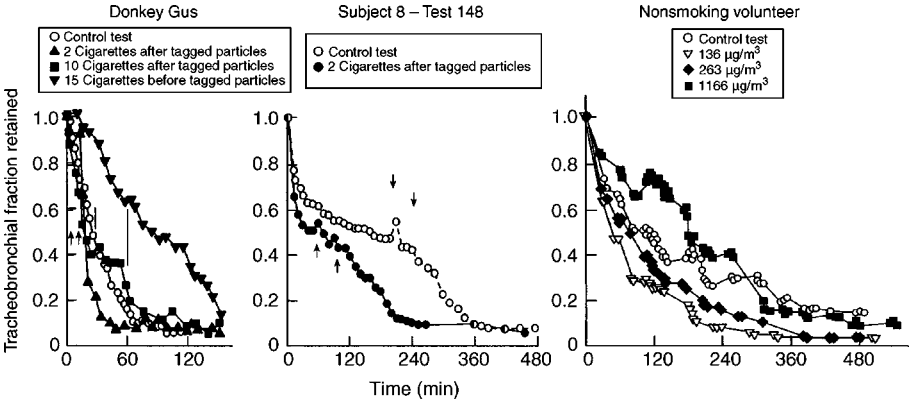

FIGURE 25.8 (left panel) Tracheobronchial particle retention versus time for donkey Gus in a control test and in tests involving exposure to whole fresh cigarette smoke from the indicated number of cigarettes. FIGURE 25.8 (center panel), Tracheobronchial particle retention versus time for a 38-year-old nonsmoking man for two tagged aerosols inhaled 2.5 h apart on the same day. Figure 25.8 (right panel). Smoke from two cigarettes, inhaled beginning about 1 h after the inhalation of the second tagged aerosol, accelerated the clearance of both. *Source:* From Lippmann et al. (1982).

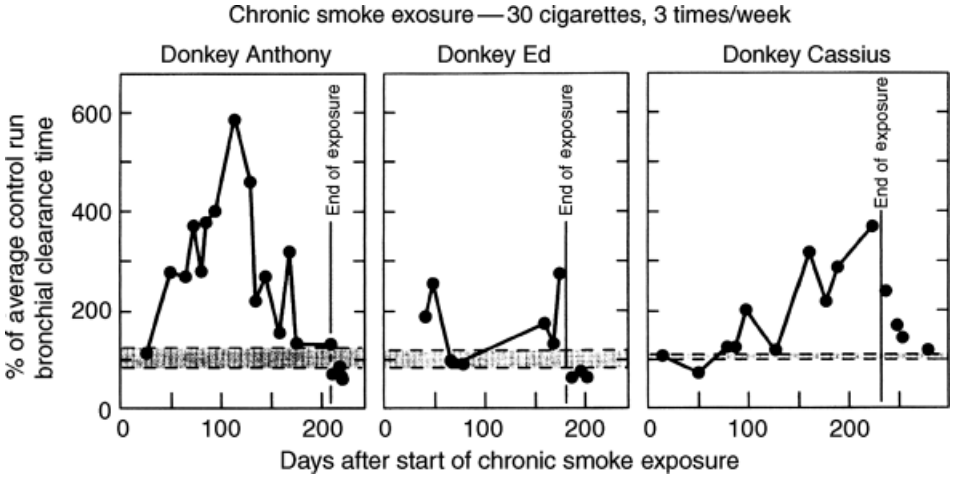

FIGURE 25.9 Effect of exposures to the whole fresh smoke from 30 cigarettes, three times per week, on the mean residence time for tagged particles on the tracheobronchial airways in three donkeys. The dashed lines indicate the range of the three control tests for each animal, which preceded the smoke exposures. *Source*: From Lippmann et al. (1982).

proximal bronchi and peripheral airways where such cells are normally absent or few in number; this change is accompanied by an increase in the volume of secretion (Reid, 1963). In the subchronic rabbit studies as well as the chronic studies, an increase in epithelial secretory cell proportions in smaller airways was noted (Schlesinger et al., 1983; Gearhart and Schlesinger, 1989).

The appearance of persistently increased secretory cell number in peripheral airways as a result of H_2SO_4 is a finding of major importance, since excessive mucus production in small airways, which is consistent with an increase in the propagation of secretory cells,

may be an early feature in the pathogenesis of bronchitis (Hogg et al., 1968). Furthermore, it demonstrates an underlying histological change consistent with the observed physiological effects of the H_2SO_4, that is, altered mucociliary clearance.

In addition to a change in the relative number of secretory cells in different airway levels of acid-exposed rabbits, two other changes were noted after H_2SO_4 exposures. There were an increase in epithelial thickness and a decrease in airway diameter. A significant increase in epithelial thickness of small bronchi and bronchioles occurred in rabbits exposed orally at approximately $250\,\mu g/m^3$ and nasally at approximately $500\,\mu g/m^3$. In addition, in the oral exposure series, the lumen diameter of the smallest airways was significantly less than in the sham controls.

In human chronic bronchitis and in experimental bronchitis in laboratory animals, an initial change in secretory cell number or size is followed by intrabronchial narrowing, especially in small bronchi and bronchioles, in part due to a thickening of the bronchial wall (McKenzie et al., 1969; Matsuba and Thurlbeck, 1973).

In summary, the first stage of effect of acid exposure may be a change in secretory cell proportions in the airways, and thickening of the epithelium may occur later. Thus, the studies of Schlesinger et al. (1983) and Gearhart and Schlesinger (1989) provide further support for the role of H_2SO_4 in the pathogenesis of chronic bronchitis via effects on the mucociliary clearance system. However, the progression of clearance dysfunction in the pathogenesis of chronic bronchitis is not known.

The Albert et al. (1973) schema describing the pathogenesis of chronic bronchitis in man from cigarette smoking is illustrated in Fig. 25.10. It may also apply to repeated exposures to other irritants such as H_2SO_4. According to this schema, irritant inhalation initially results in a tendency toward some acceleration of clearance, as excess mucus is

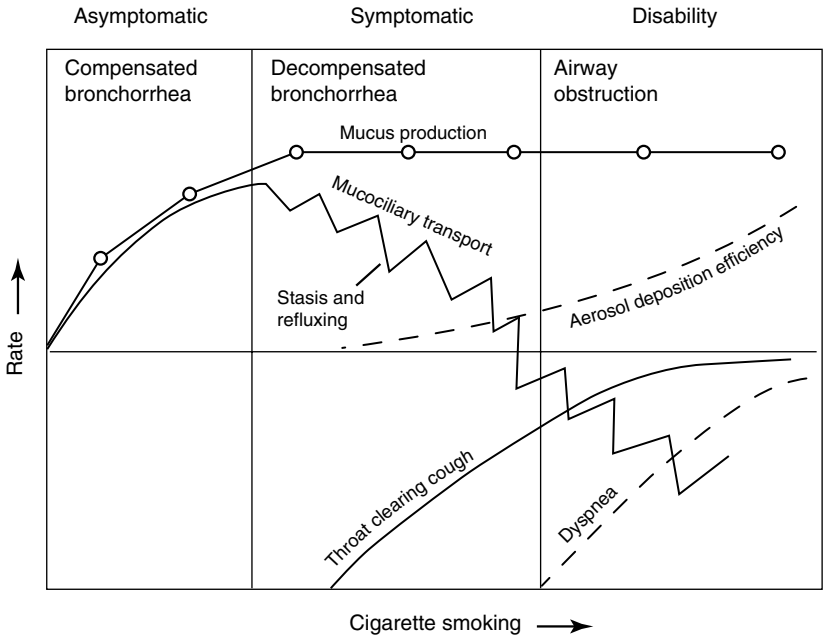

FIGURE 25.10 Tentative schema for the pathogenesis of obstructive lung disease resulting from exposure to inhaled irritants. *Source*: From Albert et al. (1973).

produced but mucosal damage has not occurred. The H_2SO_4 dose delivered to the rabbit in nasal breathing at 250 μg/m^3 may have been enough to initiate this first stage. An increase in the number of airways containing epithelial secretory cells is consistent with increased mucus production. However, the degree of clearance rate change could vary with the individual rabbit and with the time after exposure at which the clearance was measured. This may account for the fact that a significant acceleration was often observed when clearance was measured immediately after H_2SO_4 exposure, whereas retardation was more commonly observed when clearance was measured 1 day after the last H_2SO_4 exposure (Gearhart and Schlesinger, 1988).

In the next stage of the Albert et al. (1973) schema, a further increase in the level of secretion, coupled with some mucosal damage, results in an overloading of transport mechanisms; the result is a retardation of clearance. Such a retardation was observed in the study by Schlesinger et al. (1979) involving 6 months of daily 1-h H_2SO_4 exposures at 100 μg/m^3 in donkeys and in the study of Gearhart and Schlesinger (1988) involving daily 1-h H_2SO_4 exposures in rabbits for 1 year.

Since H_2SO_4 produces essentially the same sequence of effects on mucociliary bronchial clearance as cigarette smoke following both short-term and chronic exposures, it may be capable of contributing to the development of bronchitis. But the question still remains whether variable clearance rates and persistent clearance rate changes merely predispose to chronic bronchitis or are the actual initiating events in a pathogenic sequence leading to its development. Furthermore, the response of the mucociliary clearance system observed in the rabbits may be adaptive rather than pathological. Many irritants may stimulate clearance at low doses or after exposure for a short time and then retard it at higher doses or with prolonged exposures (Wolff et al., 1981). An increase in secretory cell proportions is consistent with hypersecretion. Thus, low-level exposures may initially increase secretion, which can be coped with, and may even be protective. However, pathological changes appear when adaptive capacity is overloaded. Thus, with increasing exposure time or dose, the degree of enhanced secretion may be too great, resulting in overwhelming of clearance and leading to retardation and, eventually, bronchitis (Schlesinger et al., 1983).

25.5.6 Implications: Airborne Acidity and Cancer

There is a possibility that exposure to acidic aerosols may play a role in carcinogenesis. Soskolne et al. (1989) published a review that examined a broad array of epidemiological and toxicological literature. They concluded that there was support for the hypothesis that acidic pollutants contribute to carcinogenesis in humans. They examined possible biologic mechanisms for such a contribution, including pH modulation of toxicity of xenobiotics and pH-induced changes of cells involving mitotic and enzyme regulation.

The strongest epidemiological evidence for an effect of acid mists on lung cancer comes from a follow-up study through 1986 of 1165 steelworkers exposed to acid mists at steel-pickling operations at concentrations on the order of 200 μg/m^3 (droplet size not specified). The results are summarized in Table 25.2. Since the H_2SO_4 concentrations in ambient air are about two orders of magnitude lower, there is no strong evidence at this time to link ambient air exposures to carcinogenesis. Public health authorities should consider the Soskolne et al. hypothesis as a margin-of-safety factor in the establishment of air quality guidelines and/or standards based on the more clearly established health effects associated with exposure to acidic aerosols.

TABLE 25.2 Lung Cancer and Acid Mists

Disease	Standardized Mortality Ratio[a]	
	Overall	20 Years Since First Exposure
Lung cancer (without adjustment for smoking, $n = 1156$)	1.55 (1.12–2.11)	1.72 (1.21–2.39)
Lung cancer (with adjustment for smoking, $n = 752$)	1.36 (0.97–1.84)	1.50 (1.05–2.27)
Heart disease ($n = 1156$)	0.92 (0.77–1.09)	
All causes ($n = 1156$)	0.92 (0.83–1.01)	

Source: From Steenland and Beaumont (1989).
[a]With 95% confidence intervals.
Note: The cohort was 1156 male steelworkers. Exposure was to an average H_2SO_4 concentration of 0.2 mg/m^3 based on 49 air samples collected in the late 1970s. Average duration of employment (and exposure) was 8.8 years.

25.5.7 Summary of Health Effects of Acidic Aerosols

The human health effects of major concern with respect to the inhalation of acidic aerosols are bronchospasm in asthmatics and chronic bronchitis in all exposed persons. The former relates to acute exposure, whereas the latter can be related more closely to chronic or cumulative exposures. In either case, the effects are produced by droplets depositing on the surface of the conductive airways of the lungs.

Studies related to the provocation of bronchospasm show evidence for increased airway resistance in exercising mild to moderate asthmatics. Koenig et al. (1983a, 1989) reported increased airway resistance following H_2SO_4 exposures for 30 min at rest and 10 min of exercise for adolescents at 68 or 100 μg/m^3 (0.6-μm-diameter droplets). Bauer et al. (1988) saw similar effects in adults exposed for 2 h at 75 μg/m^3 while exercising or for 16 min at 450 μg/m^3 while at rest (0.8 μm). Koenig et al. (1989) also showed that the effects previously seen at 68 μg/m^3 were increased when there was co-exposure to 0.1 ppm (260 μg/m^3) of SO_2.

Evidence for synergism is also to be found in the work of Amdur and Chen (1989), who showed that sulfuric acid (1 h at 20 μg/m^3) on ultrafine ZnO particles that simulate coal combustion effluent, when present in a mixture with SO_2, produces increased lung reactivity responses about 10-fold greater than those produced by pure droplets of H_2SO_4 of comparable size.

A causal basis for the historic association between acidic aerosols and the prevalence of bronchitis in humans has been established, at least in part, by the acute and chronic exposure studies in which rates of particle clearance from the lungs have been measured. The short-term effects are cumulative over at least several hours, and total exposures at the equivalent of current peak ambient levels produce similar transient changes in mucociliary clearance rates in rabbits, donkeys, and humans. Repetitive daily exposures of rabbits and donkeys, at comparable rates of exposure, produce both transient and persistent clearance abnormalities that are essentially the same as those produced by whole fresh cigarette smoke, a known causal factor for chronic bronchitis in humans.

Conclusive evidence for human health responses to ambient acidic aerosols is lacking, and inferences have had to be drawn from associations between health and SO_4^{2-}. Of particular interest are the studies of Dockery et al. (1982) and Dassen et al. (1986) showing the effects of episodic overexposures to ambient mixtures containing concentrations of SO_2

and PM close to current U.S. NAAQS. These exposures produced an apparent continuum of response, with a substantial fraction (perhaps 25% or more) of children having at least a small loss of lung function persisting for at least several weeks. A smaller percentage may have persistent functional decrements exceeding 10%. At concentrations that are about twice the current U.S. NAAQS, an episode in western Germany in 1985 produced increases in deaths, hospital admissions, outpatient visits, and ambulance deliveries to hospitals (Wichmann et al., 1989).

More direct semiquantitative evidence for a causal role for acidic aerosols comes from the studies of Kitagawa (1984) and Thurston et al. (1997). Kitagawa showed an association between acid aerosols and morbidity in Japan, and Lippmann and Ito (1995) showed that total daily mortality in London in the period 1965–1972 was more closely associated with daily measured acid aerosol than with BS or SO_2.

Epidemiological studies that directly address the role of acidic aerosols on human health are beginning to produce results. The reports of Raizenne et al. (1996) and Ostro et al. (1989, 1991) are both encouraging and dismaying. They are encouraging in that they directly address serious public health concerns. The initial findings are, however, quite disturbing. Dockery et al. (1996) showed that the prevalence of bronchitic symptoms in schoolchildren varies from about 3.5 to 10% as annual average H^+ concentrations (expressed as H_2SO_4 equivalent) varied from ~0.4 to 1.8 μg/m^3. Ostro et al. (1989, 1991) reported that responses among adult asthmatics were more closely associated with H^+ than with any other pollution variable for concentrations (expressed as H_2SO_4 equivalent) ranging from 0.04 to 0.84 μg/m^3. These are commonly encountered levels in the United States and well below historic ambient levels in the United States and Europe. More direct measurement data need to be made and coupled to health effects studies. The implications of the preliminary data to public health and, ultimately, to the health costs of fossil fuel consumption need to be much better documented.

25.6 AMBIENT AIR QUALITY STANDARD

The initial primary (health-based) NAAQS for SO_2 was established by the U.S. EPA in 1971, with a concentration of 365 μg/m^3 (140 ppb) as a 24-h maximum not to be exceeded more than once per year and with 80 μg/m^3 (30 ppb) as an annual arithmetic mean. EPA initiated a review of the 1971 SO_2 NAAQS in 1980 and reaffirmed the levels in 1987. A further revision of the primary NAAQS for SO_2, established in 2010, specified that the 99th percentile of 1-h maximum concentration averages, averaged over 3 years, of 75 ppb (195 μg/m^3), shall not to be exceeded.

The World Health Organization (WHO) has developed air quality guidelines for SO_2 to assist member states in establishing their own standards (WHO, 2006).

25.7 WHO GUIDELINES

25.7.1 Short-Term Exposures

Controlled studies with exercising asthmatics indicate that some of them experience changes in pulmonary function and respiratory symptoms after periods of exposure as short as 10 min. Based on this evidence, it was recommended that a value of 500 μg/m^3

(0.175 ppm) should not be exceeded over averaging periods of 10 min. Because exposure to sharp peaks depends on the nature of local sources and meteorological conditions, no single factor can be applied to this value in order to estimate corresponding guideline values over somewhat longer periods, such as an hour.

25.7.2 Exposure over a 24-h Period and Long-Term Exposure

Day-to-day changes in mortality, morbidity, or lung function related to 24-h average concentrations of sulfur dioxide are necessarily based on epidemiological studies in which people are in general exposed to a mixture of pollutants, with little basis for separating the contributions of each to the effects, which is why guideline values for SO_2 were linked before 1987 with corresponding values for PM.

There is still considerable uncertainty as to whether SO_2 is the pollutant responsible for the observed adverse effects or, rather, a surrogate for ultrafine particles or some other correlated substance, in consideration of (1) the uncertainty of SO_2 in causality and (2) the practical difficulty of reaching levels that are certain to be associated with no effects.

25.7.3 Guideline: 500 µg/m^3

A country could move toward guideline compliance by controlling emissions from one major source at a time, selecting among motor vehicle sources, industrial sources, and power sources, for the greatest effect on SO_2 at the lowest cost and monitor public health and SO_2 levels for health effect gains. Demonstrating health benefits will provide an incentive to mandate controls for the next major source category. These recommended guideline values for SO_2 were not linked with guidelines for PM.

25.8 OVERALL DISCUSSION

Due to regulatory requirements, and their impacts on emission controls, there is a much more robust literature on the health effects of SO_2 than on the other SO_x compounds that formed downstream from the SO_2 emissions to ambient air. The strong acids, H_2SO_4 and NH_4HSO_4, are far greater irritants to respiratory tract airways than their SO_2 precursor, but have shorter half-times in the air than SO_2, while $(NH_4)_2SO_4$, a weak acid, has the longest half-time. Aside from the their own irritancy to the airways, the strong acid SO_x compounds also react with the trace metals in the ambient air PM, forming soluble ionic forms that can readily migrate in the circulating bloodstream to other critical organs, such as those in the cardiovascular and nervous systems. As discussed in the chapter on ambient air PM in this volume, there are many epidemiological studies of the much greater statistically significant associations of both short- and long-term health-related responses of SO_4^{2-} and trace metals, such as Cu, Ni, and V, than those for $PM_{2.5}$, PM_{10}, and black carbon.

Since it is unlikely, in the foreseeable future, to have substantially more literature to illuminate the specific health-related responses to H^+ and trace metals in ambient air PM, the primary NAAQS for SO_x will continue to rely on SO_2 as an appropriate indicator chemical, and the implementation of the NAAQS will reduce the health impacts of both the SO_2 and other PM precursor emissions.

25.9 CONCLUSIONS

1. Since short-term inhalation exposures to SO_2 in laboratory-based exposures of healthy adult human volunteers have produced short-term decrements in pulmonary function comparable with those observed peak exposures in ambient air in short-term epidemiological studies, research discussed herein provides direct support for the current short-term primary SO_2 NAAQS.
2. SO_2 is the precursor of other acidic SO_x compounds that may also produce short-term decrements in pulmonary function.
3. The acidic compounds derived from SO_2 emissions react with PM in the ambient air to produce ionic forms of trace metals that have been shown to be statistically significantly associated with both short- and long-term health-related responses to PM, providing indirect support for (1) the current short- and long-term primary PM NAAQS and (2) for the primary SO_2 NAAQS.

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26

WORLD TRADE CENTER (WTC) DUST

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26.1 INTRODUCTION

On the morning of September 11, 2001, two passenger jet planes separately impacted on the 100-story WTC North and South Towers in New York City (NYC), and both towers collapsed within just 2 h. As a result, dense clouds of dust were generated by successive collisions of collapsing concrete floor slabs, with a resultant crushing of concrete, wallboard, and glass/slag-wool insulation into dense clouds of airborne particles that were deposited over wide areas of southern Manhattan Island (south of Canal Street), as well as portions of westernmost Brooklyn and northernmost Staten Island. In the vicinity of the towers, dust layers on the streets/sidewalks and within buildings with shattered/open windows reached, in some cases, inches in thickness. Ultimately, due to dust resuspension by air currents or mechanical disturbance during on-site/local activities, there were additional exposures of rescue/recovery workers, clean-up workers, office/various other types of workers in affected buildings and of local residents. These dusts generated by the collapse of the WTC Towers, and subsequently resuspended, designated herein as "WTC Dust," were unique in terms of particle size distribution and composition. Of the total mass in the settled WTC Dust, <1% was deemed fine (<2.5 μm in aerodynamic diameter), and >90% was coarse/supercoarse (2.5–100 μm) (Lioy et al., 2002, 2006; McGee et al., 2003; Yiin et al., 2006).

Compositionally, WTC Dusts differed from normally encountered (in Eastern United States) ambient particulate matter (PM) in that they did not originate from organic/inorganic vapor-phase pollutants, were not primarily carbonaceous, and were neither strongly acidic nor hydroscopic. Interestingly, WTC Dusts also differed from typical building construction and demolition site dusts; the latter are generally composed of much larger particles and are not as readily resuspended into the air or as rapidly dissoluble in aqueous surface fluids (e.g., in the respiratory and/or gastrointestinal [GI] tracts). The WTC Dusts were of a more uniform chemical composition, with 80–90% being composed of a

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well-blended mixture of concrete, gypsum, and synthetic vitreous fibers (SVFs). Furthermore, it is important to note that the WTC Dusts deposited out of doors in the initial period after the disaster differed from those encountered indoors after September 14 when a heavy rain washed away some of it and changed the chemical composition of the remainder (i.e., by removal of soluble components). Similarly, dusts found in outside locations—while not likely compositionally different—did differ from indoor WTC Dust in terms of physicochemical characteristics (i.e., primarily with respect to median particle sizes and pH).

In addition, the composition of the outdoor WTC Dusts was changing with time due to site-related activities, such as the generation of fumes generated by metal-cutting torches and by the disturbances caused by the continuing movement into and out of the Ground Zero area of diesel-powered trucks and other heavy equipment, as well as of the ever-shifting composition of the effluents of the ongoing fires on and within the debris pile at Ground Zero, which were not completely extinguished until December of 2011. Thus, when one speaks of WTC Dusts, one must consider various temporal and spatial aspects affecting their distributions of particle size and composition.

26.2 POST-COLLAPSE HUMAN INHALATION EXPOSURES TO WTC DUSTS

26.2.1 Temporal Sequence

In the aftermath of the disaster, many inhalation exposures scenarios confronted the residents of Lower Manhattan, Ground Zero rescue and recovery workers, and cleanup workers:

1. On 9/11, most people living/working in Lower Manhattan were required to undergo mandatory evacuation from the vicinity of the WTC site. Many evacuees ended up being caught in both the aftermath of the building collapse and resuspended dust clouds and as a result became both covered with dust and underwent significant WTC Dust inhalation exposures. Even greater acute inhalation exposures befell firefighters, police personnel, and volunteers who had arrived at the WTC to help rescue survivors of the original airplane impacts. Many of these individuals did so without access to/proper use of personal protective equipment (PPE), that is, respirators.
2. On 9/12 and 9/13, while acute inhalation exposures of evacuees were much lower than on 9/11, increasing numbers of newly arrived rescue and recovery workers were highly exposed as their activities on and around Ground Zero resulted in resuspension of settled dusts. Even at this point, only a small fraction of the workers had respirators, and, of these, only some used them. The reasons were multiple, that is, apart from the high ambient temperatures on those days/nights in combination with heat generated from fires at Ground Zero and from constantly working engines of trucks/moving equipment, PPE respirator filters quickly became clogged with dust, making the work of breathing through them too great.
3. During succeeding days, weeks, and then months, inhalation exposures of outdoor workers were lowered due to rains that removal of residual settled dusts and debris was also continuing. However, increasing numbers of workers (and later, residents)

became engaged in indoor dust removal, and their potentials for exposures increased. While many of the workers that were engaged to clean contaminated commercial buildings were asbestos remediation workers trained in use of negative pressure respirators for which they had been fit-tested, their filters often became clogged within the first few hours of a standard workday, and as a result, their inhalation exposures later in a given workday could become high.

4. Exposures of residential and commercial office evacuees remained low, as long as they stayed away from areas abutting Ground Zero. In fact, most residents were not permitted to return to their buildings until the site was certified as clean. For most residents, reoccupation took place over many weeks. In the cases of workers who had left offices that had become contaminated with WTC Dusts, many were back at work within several weeks. However, the adequacy of initial cleanings was often disputed, leading to many second-stage cleanings because building owners and managers lack experience with this type of unprecedented disaster. While government agency mandates and guidelines for minimizing inhalation exposures to WTC Dust were generally followed, these guidelines were in many cases inadequate with respect to health protection.

Many government agencies were sufficiently concerned about potential health effects from exposures to WTC Dusts to undertake collection and analyses of samples of both settled and airborne dusts in the days and weeks after the disaster to clarify the nature and extent of ongoing toxicant exposures. However, these efforts relied primarily on analyses of ambient air samples of $PM_{2.5}$ collected by fixed-site monitors located at the perimeter of the WTC Ground Zero and at various other sites in Lower Manhattan and surrounding areas. Beyond characterizing the mass concentrations of $PM_{2.5}$ (and, in some cases, their physicochemical characteristics), these analyses were relied upon to help estimate potential inhalation exposures and human health risk incurred by those working at/in the vicinity of the WTC. The data were also utilized to help evaluate similar parameters for individuals (local residents/office workers) who were exposed to the WTC Dust generated by the initial collapse and who faced dust-filled apartments when the quarantine was lifted.

26.2.2 What Was Measured

The ambient air ($PM_{2.5}$) monitoring activities primarily focused on overall mass concentrations and specifically the concentrations of individual constituents such as lead (Pb), chromium (Cr), and nickel (Ni), polychlorinated biphenyls (PCBs), dioxin-like compounds, asbestos fibers, and volatile organic compounds (VOCs). Some of these choices were based upon factors associated with the buildings themselves and their contents, that is, Pb and asbestos were part of the original materials used when WTC construction began in the 1960s and PCBs were used as dielectric fluid in transformers and capacitors. Others were selected as they were related to the disaster itself, that is, dioxin and VOCs produced as a result of fuel combustion/volatilization. As noteworthy as the agents whose levels were monitored were those that were not, especially the dust components that contributed to 80–90% of the mass of settled WTC Dusts, that is, crushed concrete, gypsum, and SVF.

Significant elevations of the concentrations of these evaluated contaminants were found in/near Ground Zero for a short period immediately after September 11, with

elevations denoting concentrations higher by a factor of 10 or more and often by factors of 100 or 1000, compared with other measures of contaminants taken post-September 14. Many of the elevated values occurred in "restricted zones," that is, access limited to emergency management and rescue personnel and to other credentialed people. Though ambient air $PM_{2.5}$ levels for all these substances decreased to background concentrations (characteristic of pre-September 11 levels in NYC metro-area by February 2002), regulators still concluded that (1) persons exposed to extremely high levels of ambient $PM_{2.5}$ (i.e., including small fractions of WTC Dusts) and its components during the collapse and over several hours afterwards were at risk for immediate acute (possibly chronic) respiratory and other types of symptoms [e.g., cardiovascular (CV)]; (2) because the first measures were taken on September 14 and those of other contaminants not until September 23, and as levels in/near Ground Zero were highest in the first few days after the disaster, exposures and potential health impacts could not be evaluated with certainty; and (3) except for exposures on September 9/11 and possibly during the next few days, persons in the surrounding community were judged, by the public agencies, to be unlikely to suffer short- or long-term adverse health effects caused by exposure to elevations in ambient air concentrations of the contaminants that were evaluated.

Despite their limited scope, the analyses did prove revelatory. The EPA provided summaries of findings for each contaminant/class of contaminants that it selected as a likely, or at least a possible, risk factor.

26.2.3 Particulate Matter (PM) in Terms of Mass Concentration

People caught in the initial dust/smoke cloud were briefly exposed (4–8 h) airborne $PM_{2.5}$ in the mg/m^3 range (thousands of $\mu g/m^3$). During the first several days post-disaster, levels at the WTC perimeter exceeded the EPA National Ambient Air Quality Standards (NAAQS) for PM2.5 (65 $\mu g/m^3$, 24-h); levels at other nearby Lower Manhattan sites exceeded the 40 $\mu g/m^3$ 24-h air quality index (AQI) level of concern for susceptible subgroups in the general population. By mid-late October, $PM_{2.5}$ levels in the region had largely returned to levels typical of NYC and other urban areas; only a few WTC/nearby sites occasionally approached or exceeding the AQI level of concern.

26.2.4 Lead (Pb), Chromium (Cr), Nickel (Ni), and Other Metals

Persons caught in the initial WTC-related dust cloud experienced brief exposures to high Pb levels based on analyses of settled dust samples. In late September 2001, airborne Pb concentrations at the WTC perimeter sites reached >1.5 $\mu g/m^3$ on some days, that is, significantly greater than urban background levels in U.S. cities. After mid-October, airborne Pb at all sites in Lower Manhattan outside Ground Zero dropped to levels comparable with background typical of NYC and other Northeast U.S. urban areas. Samples evaluated for total airborne Cr at Ground Zero/surrounding sites never exceeded the OSHA permissible exposure limit (PEL) (1 mg/m^3) or ATSDR's intermediate minimum risk level (MRL) for Cr(VI) particles (1 $\mu g/m^3$). Airborne nickel in Ground Zero/surrounding site samples never exceeded the OSHA PEL (1 mg/m^3). Levels of other elements [e.g., calcium (Ca), sulfur (S), silicon (Si), etc.] in WTC PM2.5 particles also were above typical background levels on an episodic basis at sites on or near the WTC perimeter on some days extending into late October/November 2001.

26.2.5 Polychlorinated Biphenyls (PCBs) and Dioxins

Of several hundred PCB air measurements, only one was >100 ng/m^3 (at 153 ng/m^3) and three at >50 ng/m^3. This is compared to typical urban background PCB concentrations in the 1–8 ng PCB/m^3 range. After 1 month, nearly all readings were in the range of typical urban PCB levels or not detected. There were no exceedences of any short-term occupational health benchmark. Dioxin toxic equivalent (TEQ) levels in air near Ground Zero were up to three orders of magnitude higher than typical for urban areas (0.1–0.2 pg TEQ/m^3). Levels in/near Ground Zero, starting September 23 (date of first sample) and through late November, ranged from 10 to >150 pg TEQ/m^3. Levels measured several blocks from Ground Zero were elevated above typical urban background but considerably less than in or near Ground Zero. Even these elevations soon dropped, that is, background by December.

26.2.6 Volatile Organic Compounds (VOCs)

Ground Zero samples of VOC were not representative of general air quality at the site. Most samples were collected from plumes of fires and smoldering rubble [to alert Fire Department of New York (FDNY) and contractors/union health/safety officers on-site about conditions that might pose immediate health concerns for on-site workers and volunteers]. Thus, analyses of actual Ground Zero worker VOC exposures were not conducted. Eleven VOC were evaluated at sites surrounding Ground Zero; there were no exceedances of screening benchmarks for 1,4-dioxane, ethanol, styrene, tetrahydrofuran, or xylenes. Exceedances were however noted for acetone, benzene, 1,3-butadiene, chloromethane, ethylbenzene, and toluene. Except for benzene, these exceedances occurred only in restricted zones. Also, the exceedances were all grab samples; 24-h samples of benzene, 1,3-butadiene, ethylbenzene, and toluene were about three orders of magnitude (1000 times) lower. Exceedances for benzene were more frequent; some were further from Ground Zero than the other VOC, suggesting benzene above typical background (by about a factor of 10) may have been sustained for a month or more post-September 11.

26.2.7 Asbestos Fibers

The large majority of outside measures of airborne asbestos fiber concentrations were within the range of typical background levels. The few exceedances that occurred near September 11 were in time and close in proximity to Ground Zero. A systematic study in late 2001 suggested indoor levels of asbestos fibers in WTC Dusts were slightly higher near Ground Zero as compared with indoor levels in buildings further away. Analyses performed for several residential apartments (NYCDOHMH/ATSDR) between November 4 and December 11, which included 57 apartments in Lower Manhattan as well as 5 comparison apartments in areas outside the exclusion zone, showed that airborne fibers were not detected above background levels. However, bulk settled dust samples showed that there was asbestos in 16% of the apartments (but none in more distant comparison sites). Further, SVFs (i.e., slag wool or fibrous glass) were found in both indoor and outdoor settled dust samples. Another study sampling indoor air and dust on September 18, in two locations very near Ground Zero, found asbestos in both airborne and settled dust. However, those same analyses also found there were low background concentrations of dioxins, PCBs, and metals. That levels of asbestos fibers in the WTC Dust samples were not high was not unexpected. There was chrysotile asbestos insulation within the first 20 floors of the WTC

North Tower, but none in the other 80 floors or in the adjacent South Tower. There was no amphibole asbestos insulation installed in either tower. Ultimately, very few of the airborne asbestos measures in the EPA WTC database exceeded conservative fiber count limits, even in the first few days when the fiber concentrations were "highest."

26.2.8 Other PM Properties and Their Interpretations

Other analyses, primarily dealing with physicochemical characteristics of the WTC Dust samples, also yielded important information about the potential for entrainment, retention, and subsequent health risks from exposures to the WTC Dusts in the earliest days after the disaster. Among these was pH. The issue of alkalinity of the WTC Dust, and its potential as a possible health concern for exposed individuals, was that there was a consistent particle size-related difference in pH values for the dusts. Specifically, very high pH (≥10.0) values were measured for aqueous solutions of the initial samples of settled supercoarse WTC Dusts (i.e., not leached by 9/14 rainfall).

26.2.9 Relevance of Established Concentration Standards and Guidelines

There were, and remain, problems in interpreting the WTC-related air quality monitoring. These include issues concerning the protocols that were selected for characterizing exposures related to indoor cleanup and documentation of exposure and health risks to people in Lower Manhattan buildings. One was the failure to first adequately consider (1) the unique particle size distribution/chemical composition of the WTC Dust, (2) the likelihood of excessive inhalation exposures, and (3) optimal means for preventing/controlling excessive exposures. Most importantly, there was too much focus on toxicants that were present as small mass fractions that, collectively, made up only a small percentage of the WTC Dust in air samples analyzed, that is, recall nearly all the WTC Dust was made up of >2.5 μm diameter particles, with most of that within particles >10 μm. Little consideration was given to potential toxic effects of inhaling the three major mass components in these coarse and supercoarse particles, for example, crushed concrete, gypsum, and SVF, each a known irritant. These problems were compounded by the intense initial EPA focus on asbestos fibers as the index toxicant for WTC Dust. There was a secondary focus on other trace components that were generally considered to pose health risks, that is, metals, molds, PAHs, PCBs, and dioxins. The measured airborne concentrations of these agents were also almost all below current occupational exposure limits.

Since many rescue and recovery workers did develop respiratory illnesses after exposure to WTC Dusts, and it is known that established occupational/ambient air exposure limits had seldom (and only briefly) been exceeded, it is likely that exposure components that were not measured (such as the coarse alkaline particles and SVF) were potentially the most likely to be responsible for these observed increases in incidence of adverse health effects.

26.3 POTENTIAL DOSIMETRY OF WTC DUSTS

The WTC Dust, as summarized above, differed greatly from conventional airborne dusts encountered in occupational and community settings in terms of both particle size distribution and chemical composition. Many thick deposits of WTC Dust were deposited on a

variety of outdoor surfaces in Lower Manhattan during the first day after the disaster. These settled WTC Dust deposits persisted until either the major rain of 9/14 or their removal by responders at Ground Zero. In contrast, WTC Dusts that covered indoor surfaces often remained for much longer periods of time (due to quarantines of contaminated buildings, which limited the startup of indoor cleanup).

26.3.1 Use of Particle Size-Selective Dust Samples

Teams from New York University (NYU) and Rutgers University collected and analyzed WTC Dust samples in order to characterize the chemical composition, particle size distribution, and potential for resuspension of the settled dust particles into human breathing zones, settled WTC Dust samples were collected on September 12 and 13, 2001, and aliquots were separated into size fractions through mesh. The PM_{53} fraction passing through the screens was aerosolized into an elutriation chamber and passed in a size-selective air sampler inlet with a 10-μm aerodynamic cut size (to remove/isolate $PM_{10–53}$ as well as a distinct PM_{10} fraction). The PM_{10} fraction, in turn, was aspirated through a 2.5-μm cut cyclone to remove the coarse fraction ($PM_{2.5–10}$). Any remaining airborne $PM_{2.5}$ was collected on Teflon membrane filters. Each size fraction was then subjected to physical and chemical/compositional analyses. Similar work was done on settled indoor WTC Dust samples collected on September 13 and in June 2002. For the latter, there were substantial commonalities in the findings.

Because of the shift toward coarse/supercoarse sizes among the WTC Dust particles, it would be expected that how these are handling upon entrainment would impact on how each exposed individual ultimately responded to their exposure on 9/11 or the days thereafter. Specifically, penetration of inhaled particles into the thorax is limited by deposition in the upper respiratory tract (URT) during inspiration, and this varies with particle size distribution, flow rate and tidal volume, the fraction passing through oral pathway, and *in vivo* airway dimensions. All of these can vary considerably from person to person, depending on age, transient illness, history of cigarette smoke exposure, and other short-term toxicant exposures that cause transient airway constriction, as well as elements of occupational histories associated with loss of lung function or cumulative injury.

26.3.2 Impact of Oral Versus Nasal Inhalation on Dosimetry

One must also consider that the vast majority of the inhalation exposures were by mouth breathing and less so via the nose. For particles that penetrate the lower respiratory tract (LRT) (airways within thorax), deposition patterns and efficiencies within thoracic conductive airways (trachea, bronchi, bronchioles) are mainly by impaction and sedimentation, and those of particles that deposit in smaller gas exchange airways by diffusion. In addition, some entrained particles remain airborne during tidal breath inhalation/exhalation and are not deposited. Even so, ≈15% of inhaled air remains in the deep lung over multiple breaths, and a corresponding volume of residual lung air is exhaled. Particles (or aggregates) >1 μm in diameter deposit in conductive airways. Those components that are insoluble or poorly soluble on the mucous layer of the airway lining fluid are carried proximally toward the larynx within a day, swallowed, and pass through to the GI tract. Particles that deposit in oral and ciliated nasal passages of the URT are also swallowed and pass to the GI tract. Lastly, most insoluble particles and their components >0.1 μm that reach the non-ciliated deep lung airways are phagocytosed by alveolar macrophages (AM) within weeks; these

cells and the particles within them are drawn into terminal bronchioles due to the high surface tension of the lining fluid and then cleared by mucociliary clearance and passage to the GI tract. In each scenario, it is believed that with WTC Dusts this might have contributed to increases in the incidence of both gastroesophageal reflux disease (GERD) and symptoms (GERS).

While these patterns could explain effects of conventional particle exposure, WTC Dust contained a lot of its total PM in the coarse thoracic range ($PM_{10–2.5}$), and even greater mass fractions were present in the $PM_{10–53}$ and $PM_{>53}$ size ranges. Further, alkalinity of the PM in the larger size ranges was greater than in dust $PM_{2.5}$ particles. As a result, as deposition of highly alkaline particles in the URT and in tracheobronchial airways in lower tract represent a unique challenge to the ability to clear the airways without eliciting adverse effects (such as erosion or death of airway surface epithelial cells), normal defense mechanisms in larger airways could have been overloaded in the WTC Dust-exposed individuals.

26.3.3 Disruption of Mucociliary Particle Clearance in the Airways

The disruption of mucociliary particle clearance in the airways by highly alkaline irritants could explain the excess incidences of cough and of respiratory (and GI) region pathologies seen in populations exposed to high levels of the large-diameter WTC Dust particles. Very high acute exposures/lower levels of chronic exposure to irritants can not only disrupt clearance but also destroy ciliated and mucus-secreting epithelial cells in the airways, leading to increased retention of all kinds of PM in the lungs and not just the irritant particles themselves. There is evidence that exposure to WTC Dust caused disruption of mucociliary clearance, that is, unusual ultrastructural ciliary abnormalities were noted in WTC response workers that corresponded to their respiratory and ciliary functional abnormalities. More recent studies in rat models showed extensive persistent damage to local ciliated cells and ultimately prolonged retention (i.e., ≈90%) of original WTC Dust burdens being retained for up to 1-year post-exposure [by intratracheal (IT) inhalation of WTC Dusts from 9/12 or 9/13 at levels modeling first responder (FR) exposure paradigms].

In view of the unique particle size distributions/high alkalinity of WTC Dust, standard dosimetry models and data tabulations were of little value in describing either acute or cumulative dust dosages received by individuals or distributions of doses in exposed population groups. Such estimations were further complicated by airborne WTC Dust levels that were highly variable temporally and spatially, with localized hot spots of exposure being related to human activities causing resuspension. Dose variations were also caused by use/nonuse of respiratory protection (even some used gauze masks and handkerchiefs to block inhalable particles) in the first few hours and days.

26.4 ASSOCIATIONS BETWEEN WTC DUST INHALATION AND HEALTH EFFECTS

26.4.1 Introduction

Several studies documented excesses in both acute and chronic health effects in rescue and recovery workers and volunteers heavily exposed to WTC Dust during the first few days post-9/11. Others noted effects in residents and local workers heavily exposed by the initial

dust clouds and then from more chronic exposures from resuspended dusts over longer periods. Despite differences in exposure pattern and intensity, the literature has shown associations of clinical disease with exposure to WTC Dust that demonstrate similarities in effects experienced and causality. Populations with the highest disease incidence and most severe responses included those likely exposed to quite high WTC Dust levels, that is, those at Ground Zero and FDNY employees. Less is known about local residents/workers; however, dose–response relationships for adverse effects have been shown in these populations. Clinical examinations have also been done on individuals exposed to lesser WTC Dust levels initially but had exposures extending over subsequent months, including local residents and office workers. Other population groups not given clinical exams, and whose exposures were less well defined, have been evaluated by questionnaires.

Despite different temporal patterns of WTC Dust exposure, most groups exhibited very similar clusters of specific disease categories within three specific anatomic regions, that is, upper respiratory, lower respiratory, and GI tract. One early clinical manifestation reported among those working in areas where dust contamination was substantial was "WTC cough." This "cough" was accompanied by bronchial hyperreactivity and respiratory distress. Of 332 firefighters having "WTC cough," 95% had symptoms of shortness of breath; 87% had GERD; and 54% had nasal symptoms. Beyond this "cough," many of the population groups had elevated incidences of chronic diseases that developed in the same three anatomic regions. Among the pathologies that the World Trade Center Health Program (WTCHP) was mandated to identify as related to occupational exposures to WTC Dusts were asthma, chronic cough syndrome, chronic laryngitis, chronic nasopharyngitis, chronic respiratory disorder, chronic rhinosinusitis, GERD, interstitial lung diseases, reactive airways dysfunction syndrome (RADS), sleep apnea, upper airway hyperreactivity, and WTC-exacerbated chronic obstructive pulmonary disease (COPD).

26.4.2 Occupational Groups Exposed to WTC Dust with Clinical Examinations

Levin et al. (2004) reported that from a subset of 1138 of the 11,768 non-FDNY workers and volunteers during July to December 2002, a substantial proportion experienced new-onset or worsened preexisting lower and upper respiratory symptoms, with persistence for months after their specific WTC work stopped [46% worked September 11 and 84% over September 11–14 when exposures were greatest, with a median length of time worked of 966 h (range 24—4080 h)]. In that period, 21% reported using respiratory protection (i.e., full- or half-face respirators). Of 610 examinees present in Lower Manhattan on September 11, 51% reported being directly in the main dust clouds, and an additional 31% reported exposure to substantial amounts of dust. Lower and upper respiratory symptoms were reported by 60 and 74%, respectively: 40% had WTC-incident lower respiratory symptoms that persisted to the month before screening, and 50% had persistent upper respiratory symptoms. Among 851 participants with persistent symptoms, an average of 32 weeks (range: 7–63 weeks) had elapsed since they stopped working at the site. Of all participants, 46% had nasal mucosal inflammation, with other respiratory abnormalities (e.g., abnormal nasal turbinates or sinuses, rhonchi, wheezing) being less common.

Skloot et al. (2004) studied 96 male ironworkers using physical exams, spirometry, and chest radiographs. Cough was the most common symptom, upper/lower pulmonary complaints were common among almost half, and 19 had abnormal radiographs. Cough was more common among those who began work on 9/11 compared with those who arrived later (78 vs. 54%). Spirometry did not differ between smokers and nonsmokers, and those

who wore protection seemed to have less respiratory symptoms than those who did not. The analyses revealed a significant odds ratio (OR) between cough and exposure during 9/11 (OR = 3.64, 95% CI = 1.35–9.83); however, changes in lung function (tests) were not associated with exposure duration or onset.

Herbstman et al. (2005) studied 183 WTC cleanup/recovery workers, among whom 91% wore a respirator some of the time. The subjects were divided according to whether they had respiratory symptoms (64 subjects, 35%) or not (119 subjects, 65%) when they began working at the WTC site. The data showed that of the groups with existing illnesses and symptom-free, 31 and 34% developed new cough, 23 and 24% developed new phlegm, and 16 and 19% developed *de novo* wheeze. New-onset cough in subjects with no lung problems was the only symptom that followed a (length of) exposure–response pattern. Those with extant respiratory symptoms had a greater prevalence of new upper respiratory conditions (such as nasal congestion, sore throat, and hoarseness). Smoking seemed to play no apparent role in the findings.

Herbert et al. (2006) gathered background information for studies of chronic respiratory diseases resulting from exposures to WTC Dust. Eligible workers/volunteers who worked 4 h on September 11, 24 h during September 2001, or 80 h any time within September to December were examined clinically, completed a standardized medical questionnaire, had a pulmonary function assessment, and provided responses about personal health before and after 9/11. Of the 9442 study participants, all had fewer lower or upper respiratory symptoms before than after 9/11. Symptoms tended to clearly show a greater risk for those whose exposures to WTC Dust happened in the first few weeks. Still, even subjects who arrived after October 1, 2001, had a prevalence of 41% for lower respiratory symptoms (~3 times greater than prior to 9/11) and a prevalence of 59% for upper respiratory conditions (≈2 times the percentage prior to 9/11). When pulmonary function was assessed, the subjects had low forced vital capacities (FVC). Among 4641 never smokers in the cohort, there was a >2× increased prevalence of lowered pulmonary function as compared against all U.S. Caucasian males (27 vs. 13%). Ultimately, time of arrival at Ground Zero was correlated with reduced FVC, with those arriving on 9/11 having much poorer FVC than those arriving October 1 or later.

Buyantseva et al. (2007) studied 1588 NYC police officers—categorized as having heavy, moderate, or light exposure to WTC Dust—and compared adverse pulmonary effects pre- vs. post-9/11. Among the officers, prior to 9/11 there was a 4.8% prevalence of cough compared with 43.5% after 9/11. The OR, and race/sex-adjusted OR, rose for chronic cough. Among police officers who had no respiratory symptoms prior to 9/11, there was both a significant rise in early onset resolved cough ($p = 0.02$) *and* of persistent cough ($p = 0.04$) that rose in tandem with level of WTC Dust exposure. Bowers et al. (2010) examined exposure-related sarcoidosis (multisystem inflammatory disorder, unknown etiology, characterized pathologically by non-caseating granulomas). The study described two cases of sarcoidosis in rescue workers with significant exposure to the dusts and who presented with extrapulmonary rheumatologic manifestations. The first was a 33-year-old white police detective found to have sarcoidosis after evaluation of diffuse joint pain. The second was a 40-year-old African-American officer who presented with uveitis and was then diagnosed with sarcoidosis.

Kleinman et al. (2011b) compared pulmonary function within an NYPD emergency responder cohort, without history of repetitive respiratory exposures. Of 206 members who reported arrival time, exposure location, duration, smoking history, respirator mask

usage, and respiratory symptoms and who underwent clinical evaluation and follow-up spirometry in 2002 and 2007, there was a mean long-term decline (vs. baseline) in FVC of 190 mL (3.7%) in 2002 and 330 mL (6.4%) in 2007. FEV_1 was not significantly affected in 2002 but declined 160 mL (3.9%) after a further 5 years. Abnormal spirometry was observed in (5.3%) of subjects, particularly those who experienced higher exposure intensity/duration. The smokers in the group, and subjects who failed to wear protective respiratory masks, tended to display the greater declines. In 2002, 11 subjects (5.3%) exhibited mild pulmonary dysfunction (60–80% of predicted). Upon reevaluation in 2007, abnormalities had resolved in 4 of the 11, but 6 (54.5%) continued to exhibit mild pulmonary dysfunction.

Wisnivesky et al. (2011), along these longitudinal lines of analyses, evaluated a cohort of >50,000 police officers, firefighters, construction workers, and municipal workers who had participated in the rescue and recovery work phases and reported on the incidence/prevalence rates of disorders in a span of 9-year post-9/11 period. Incidence rates were also assessed by level of exposure (days worked at WTC site/exposure to dust cloud). The 9-year cumulative incidence of asthma was found to be 27.6% (number at risk: 7027), sinusitis was 42.3% (5870), and spirometric abnormalities was 41.8% (5769)—with three-quarters of these being low FVC. Incidence of disorders was highest in workers who had had greater levels of dust exposure.

Kim et al. (2012) studied risk of asthma among 20,834 participants in a cohort evaluated between July 2002 and December 2007. All subjects underwent a baseline clinical exam, with follow-up every 12–18 months from 2002 to 2007. If a respondent said they had an asthma attack in the past 12 months, they were chosen to serve as a control group for annual asthma risk. The results indicated there were exposure-related increases in lifetime asthma prevalence; pre-9/11 it was 2.9%, rose to 12.8% in 2002, and reached 19.4% in 2007. WTC FR had an age-standardized lifetime asthma SMR prevalence of 1.6 (95% CI = 1.6, 1.7) after 9/11 for all years of follow-up. Age-adjusted 12-month SMR were increased in the 2002–2005 timeframe; it was 1.7 (95% CI = 1.6, 1.8) compared with 0.3 (95% CI = 0.3, 0.4) in 2000. The increasing trend from 2002 on was the same for both women and men, and it increased for all ages (highest being for 40–49-year-olds). SMR were elevated for all occupational groups, with protective services having an SMR of 1.5 (95% CI = 1.5, 1.6) and installation, maintenance, and repair workers having an SMR of 2.0 (95% CI = 1.8, 2.2). Acute asthma SMR were elevated for all professions.

26.4.3 Firefighters/Emergency Medical Technicians

Studies on FDNY workers who had pre-9/11 health baselines that, in turn, provided ideal benchmarks by which to judge the impact of exposures to WTC Dust. Banauch et al. (2002) reported injuries and illnesses among FDNY rescue workers who had responded to the disaster. In the 48 h after the collapses, ≈90% of 10,116 FDNY rescue workers evaluated at the WTC site reported an acute cough, often accompanied by nasal congestion, chest tightness, or chest burning, but only 3 of them required hospitalization. Compared with numbers of service-connected respiratory medical leave incidents during the 11 months pre-9/11, the number increased fivefold during the 11 months post-9/11. Banauch et al. (2002) also reported that during the 6 months after the disaster, 332 firefighters and 1 EMS worker had WTC-related cough severe enough to require >4 consecutive weeks of medical leave. Despite treatment of upper and lower aero-digestive tract irritation (i.e., sinusitis,

GERS, and/or asthma), 173 (52%) showed only partial improvements. As of August 2002, a total of 358 firefighters and 5 EMS workers remained on medical leave or light-duty assignment because of respiratory illness. The high incidence of respiratory problems and related medical leave among FDNY rescue workers demonstrated a need for adequate respiratory protection.

Banauch et al. (2003) then conducted a prospective study of a representative sample of 179 FDNY fire/rescue workers to examine links between dust exposures and pulmonary hyperreactivity and WTC cough. At 1, 3, or 6 months post-9/11, subjects in both highly and moderately exposed groups showed significant declines in FVC, FEV_1, and FEV_1/FVC, compared with control workers. Bronchial hyperreactivity also showed an exposure dose–response trend at each follow-up time point. After adjusting for smoking and airflow obstruction, highly exposed workers at 1 month were 7.3 times more likely to have hyperreactive airways than controls. For moderately exposed workers, the risk was 6.3 times (at 6 months, it was 6.8 times). Among subjects who had hyperreactive airways at 6 months, respiratory symptoms were more frequent, and leave for respiratory illnesses was significantly longer (i.e., 45 vs. 12 days in nonreactive workers). Banauch et al. (2005) then reviewed aero-digestive inhalation lung injuries resulting from the WTC Dust exposures and the persistence of nonspecific bronchial hyperreactivity. Aero-digestive inflammatory injuries, like declines in pulmonary function, RADS, asthma, reactive upper airways dysfunction syndrome (RUDS), GERD, and (rare) inflammatory pulmonary parenchymal diseases, were documented. In the FDNY workers, there was persistent hyperreactivity associated with exposure intensity, independent of airflow obstruction. At 1-year post-collapse, 23% of highly exposed subjects were hyperreactive compared with only 11% of moderately exposed workers and 4% of the controls. At 1 year, 16% met the criteria for RADS.

Banauch et al. (2006) did a longitudinal study of pulmonary function in 12,079 FDNY workers employed on or before September 11, 2001. Between January 1, 1997, and September 11, 2002, a total of 31,994 spirometries were obtained, and FEV_1 and FVC were analyzed for differences according to estimated WTC Dust exposure intensity. Adjusted average FEV_1 during the first year after 09/11 was then compared with that in the 5-year period before the disaster. Median time between September 11, 2001, and a worker's first spirometry afterward was 3 months; 90% were assessed within 5 months. WTC-exposed FDNY workers experienced substantial reductions in adjusted average FEV_1 during the year post-9/11 (mean, 372 mL). The exposure-related FEV_1 decrements equaled 12 years of aging-related FEV_1 decline. Moreover, exposure intensity assessed by initial arrival time at the WTC site correlated linearly with FEV_1 reduction in exposure intensity–response gradient ($p = 0.048$). Symptoms also predicted further FEV_1 decreases. Similar findings were noted for adjusted average FVC.

Weiden et al. (2010) studied the FDNY cohort and 1720 subjects sent for pulmonary medicine evaluations, that is, including 919 PFT, 122 methacholine challenge tests, and 982 CT (computerized tomography) scans. For subjects who had a PFT pre-9/11, there were significant declines in median FEV_1 and FEV_1/FVC ratios; 59% had obstructive airways diseases (OAD) based on a variety of factors. When adjusted for age, race, gender, height, weight, and smoking, declines in FEV_1 were significantly correlated with predicted residual volume % (RV) and responses to bronchodilators. These findings were consistent with injury to the airways, including bronchial wall thickening of the large airways and air trapping, which was seen in CT scans. The Weiden et al. (2010) findings helped to explain, in part, those of Rom et al. (2010) who reported that FDNY workers had significant

respiratory symptoms characterized by cough, dyspnea, gastroesophageal reflux, and nasal stuffiness with a significant group 1-year decline in FVC and FEV_1. In these workers, bronchial hyperreactivity (methacholine challenge) correlated with bronchial wall thickening on CT scans.

Webber et al. (2011) examined physician-diagnosed asthma and other respiratory ailments in a cohort of FDNY workers [14,314 firefighters and emergency medical service (EMS) providers]. After exclusions, there were 9715 male firefighters and 1228 male EMS workers studied; there were also 863 retired firefighters (7.9% of total) who returned to work to assist in rescue efforts. Subjects with a history of OAD were excluded from employment by the FDNY. Thus, in this group, there were only 85 asthma cases diagnosed pre-9/11 and no cases of emphysema. Exposure category was determined according to time of arrival at WTC site: group 1 = morning of 9/11; group 2 = afternoon of 9/11; group 3 = after 9/12; and group 4 = days 3 and 14 after 9/11. Duration of exposure to dust was also categorized into 1–3 months and 4–11 months. Self-reported symptoms/illnesses from the most recent health survey showed 17.3% of the subjects now with sinusitis, 12.2% bronchitis, 9.3% asthma, and 4.1% COPD/emphysema. Rates were greatest among the retirees, but little difference between EMS workers and firefighters.

Those arriving early at Ground Zero reported diagnosed asthma at a rate of 14%, while those arriving later had a rate of 5.9% (OR = 3.3; 95% CI = 2.4, 4.8). This pattern was the same for all respiratory diagnoses. After adjusting for smoking, age, arrival time, and duration of exposure, retirees were 10.2 times more likely to have asthma than active men and 7.4 times more likely to have COPD/emphysema. The authors also reported a link between quintile of FEV_1% and OAD. Thus, the lowest FEV_1% predicted 41.1% of men with asthma and 50.6% with COPD/emphysema, while those firefighters in the highest FEV_1% had only 10.5% asthma and 6.8% COPD/emphysema. Among firefighters not reporting any respiratory diagnosis (i.e., those without any respiratory conditions), 23.5% were in the highest quintile, and 14.6% the lowest FEV_1%. EMS workers showed the same pattern. Sinusitis (9.7%) and asthma (8.8%) were the most common physician diagnosis, and those with the longest exposures had the most reported cases of sinusitis (11.4%) and bronchitis (10.3%). Comparing firefighters in group 1 with those in group 4, there was 11.4% asthma contrasted with 5.3%. Cough, shortness of breath, and wheeze was correlated with lowest quintile FEV_1%; in those without respiratory symptoms, an opposite pattern emerged.

Kazeros et al. (2013) hypothesized that persistent asthma-like symptoms in WTC Dust-exposed individuals would be associated with systemic inflammation characterized by peripheral eosinophils. For this study, an initial population of 2462 subjects in the WTC Environmental Health Center (WTCEHC) who had undergone standardized evaluations including answering questionnaires and complete blood count in the period of September 2005–March 2009 was identified. From this pool, individuals with preexisting respiratory symptoms/lung disease diagnoses prior to 9/11 and current tobacco use were excluded. Among the final analyzed 1517 subjects, the data showed respiratory symptoms that developed after WTC Dust exposure, including persistent included dyspnea on exertion (68%), cough (57%), chest tightness (47%), and wheeze (33%). A larger percentage of those with wheeze had elevated peripheral eosinophil levels compared with those without wheeze (21 vs. 13%). Individuals with elevated peripheral eosinophils were also found to be more likely to have airflow obstruction on spirometry (16 vs. 7%). Thus, the data was suggestive of a role for eosinophils in the lung inflammation in this population, the latter which would be consistent with the development of a WTC-related asthma.

26.4.4 Residential/Working Community Members

Several studies documented adverse health effects in the local community. According to Reibman et al. (2009), >360,000 local workers and >57,000 residents (south of Canal St.) were estimated to have had potential WTC Dust exposures after 9/11. Analysis of WTCHC registry populations demonstrated an increase in upper and lower respiratory symptoms and analysis of subgroups showed a dose–response relationship between exposure to WTC Dusts and persistence of lower respiratory symptoms/impacted lung function (Maslow et al. 2012).

Reibman et al. (2009) described how among 1898 individuals evaluated between September 2005 and May 2008 upper and lower respiratory symptoms developed post-9/11 and persisted. The most common abnormality was low FVC, a finding similar to that in rescue and recovery individuals. To clarify the mechanism for respiratory symptom with normal lung function, the population was evaluated to see symptoms in subjects that were associated with airway hyper-responsiveness (i.e., using methacholine challenge). Of those with normal lung function ($n = 68$), 51% had a $PC_{20} < 4$ mg/mL, consistent with airway hyperreactivity. The study also examined whether patients with any abnormal spirometry had improved lung function after using a bronchodilator. In these individuals, there was significant improvement in FEV_1 in patients with an "obstructed" pattern and significant but small improvements in FEV_1 in patients with a "low FVC" pattern. Both FEV_1 and FVC improved in response to bronchodilator in the "obstructed and low FVC" group. In the end, spirometry measurements below the lower limit of normal in 31% of the subjects evaluated. Thus, residents and local workers, believed to have had less exposure to WTC Dusts compared with those with work-associated exposures, also had persistent respiratory symptoms with lung function abnormalities at 5 or more years after the disaster.

As noted above, there were several populations with work-related or residential exposures to WTC Dust who were solely evaluated based upon their responses to questionnaires. For details of those studies and their findings, the Reader is directed to Lippmann et al. (2015).

26.5 STUDIES OF BIOLOGIC RESPONSES TO WTC DUSTS

26.5.1 Summary of Responses to WTC Dust Exposures in Monitored Populations

Similarities/differences in responses between individuals who underwent occupational exposures to WTC Dusts and those who had incidental exposures in residences and office environs are notable. Even with variations in terms of population size and sensitivities of assays, as well as homogeneity of dust exposures within evaluated populations, it is noteworthy that response patterns to incidental exposures have appeared to be so similar to those among individuals who had been engaged in rescue and recovery activities (and thus believed, initially, to have been more heavily exposed). This suggested that duration of exposure to undisturbed dust residues might have been as or more important than shorter-term exposures to higher dust levels. Nevertheless, temporal sequence was a critical criterion for the attribution of causation to the WTC Dust exposure. In the studies reviewed here, after adjusting analyses for smoking and age as potential confounders, asthma and other respiratory disease excesses were found to have occurred *post*-9/11; this was especially true among workers studied without respiratory ailments prior to 9/11. The upshot

from the epidemiology studies to date has been that there was a consistent causal association between WTC Dust exposure and adverse respiratory, upper digestive, and cardiac effects. Even short-term exposures were associated with increased risks of adverse conditions, with risks often increased in a dose-related fashion, with more prolonged or more intensive exposures being associated with greater risk. The plausibility of a causal link between WTC Dust and adverse health outcomes has been strengthened by the consistency of the findings over time. Overall, there is sufficient evidence that, to a reasonable degree of scientific certainty, exposure to WTC Dust is causally associated with increased risk of COPD, RADS, interstitial lung disease, restrictive lung disease, and chronic laryngitis, pharyngitis, and rhinosinusitis.

26.5.2 *In Vivo* Studies

To date, there has been little research from potential health effects from WTC Dusts following controlled inhalation exposure studies in laboratory animals. Conventional exposure studies with rodents have proven impractical, as particles with diameters >2.5 μm for the most part do not penetrate nasal passages in these obligatory nose-breathing species. For the most part, some of the earliest studies used WTC Dusts, but only in the fine mode fraction, and others used exposures by IT instillation. The earliest rodent exposure studies performed in the immediate aftermath of the disaster (Gavett et al., 2003) sought to evaluate potential respiratory health effects from inhalation exposure to a fine-sized fraction of WTC Dust. These studies hoped (at time when no information at all was available) to gain information about potential short/long-term toxicities of the dusts after a very high-dose, short-term exposure and in *in vitro* evaluations.To compare WTC Dust toxicities to previously tested PM materials, mice were exposed using respirable size particles derived by size-fractionated samples. The samples included a sample of pooled $WTC_{2.5}$ from seven individual collection sites (WTCX) and a sieved sample from another site that was size-separated for nose-only aerosol exposure (WTCb). Reference $PM_{2.5}$ samples of Mount St. Helens dust (MSH) and residual oil fly ash (ROFA) were also tested. Pooled WTCX was administered by oropharyngeal aspiration at 10, 31.6, or 100 μg/dose. Respiratory responses 24 h post-exposure were compared with those by similarly aspirated low (MSH) and high (ROFA) toxicity reference $PM_{2.5}$; vehicles received saline only. The study showed the aspirated WTCX induced significant neutrophilic inflammation (without concurrent macrophage influx) at a relatively high dose (100 μg); the effect was not as great as that from 100 μg ROFA and only slightly more than that of 100 μg MSH. However, this same dose of WTCX caused airway hyperresponsiveness to methacholine (Mch) aerosol to a greater degree than did ROFA. Mice exposed to lower (10 and 31.6 μg) WTCX doses and mice exposed nose only to WTC*b* ($10.64 \pm 3.10\,mg/m^3$, MMAD 1.05 μm) had no significant changes in Mch responsiveness or inflammation 24 h after their exposures. In a parallel study, mice were also exposed to $WTC_{2.5}$ from seven sites around Ground Zero; all mice exposed to the individual dust developed hyperresponsiveness to Mch as seen with $WTCX_{2.5}$-exposed mice. No particular response patterns were found that related to geographical location of the samples. Thus, these initial *in vivo* studies showed that high doses of $WTC_{2.5}$ could promote mechanisms of airflow obstruction in mice. No analyses on the coarse/supercoarse materials were done at the time.

The major caveat with these early studies was that airborne $WTC_{2.5}$ levels—if extrapolated to humans based on mouse doses—would have to have been quite high in the period after the building collapses when rescue and recovery efforts were in effect. Given that

$WTC_{2.5}$ only represented ≈1% of the total WTC Dust mass (and did not even take into account alkalinity of the particles >2.5 μm), the effective 100 μg $WTC_{2.5}$ dose used would have had to reflect an instantaneous total dust deposition of ≈10 mg WTC Dust to each mouse.

Based on established deposition efficiencies for fine particles in the mouse lung (10–20%), murine minute volume (0.035 LPM), and assuming an exposure equivalent to the period established for a reference FR (i.e., 4 h; Mayor's WTC Medical Working Group; personal communication), the 100 μg $WTC_{2.5}$ dose would have then reflected an atmosphere of ≈6000 mg *total dust*/m^3. Such an atmosphere would be far above the surmised upper range of Ground Zero (non-immediate cloud) air levels of ≈1000 mg *total dust*/m^3. Had that upper level been used as the basis to dose mice, this would have meant that a maximal amount of material that may have been deposited in a 4-h representative exposure would have been only ≈16.8 μg $WTC_{2.5}$. Nonetheless, at the early time post-exposure, these data were considered to be critical in terms of providing some context of potential human health effects from exposure to the WTC Dusts.

With time, modeling of exposures at/around Ground Zero using rodent hosts became refined. In a "realistic exposure" study, Cohen et al. (2014) used a novel system developed by Vaughan et al. (2014) to expose rats to WTC Dust using paradigms that mimicked mouth breathing exposures faced by rescue workers/other personnel (i.e., FR) at Ground Zero over the course of the first week post-disaster. Here, coarse/supercoarse WTC Dust particles (collected on-site on September 12, 2001, and September 13, 2001) were delivered directly into the lungs of rats via IT instillation. Responses in the lungs were evaluated after rat exposures to two daily 2-h regimens at dust levels extrapolated to simulate those of the FR. To understand potential changes induced by the dusts, lungs of the rats were harvested 2 h post-exposure, and total RNA was extracted for global gene expression analysis. Among the >1000 genes affected by WTC Dust [under isofluorane (ISO) anesthesia] or ISO alone, 166 were unique to the dust. In many instances, genes maximally induced by the dust exposure (relative to in naïve rats) were unchanged/inhibited by ISO only; similarly, several genes maximally inhibited in dust-exposed rats were largely induced/unchanged in rats that received ISO only.

Overall, these data showed that lungs of rats exposed to WTC Dust—after accounting for any impact from ISO—displayed increased expression of genes related to lung inflammation, oxidative stress, and cell cycle control, while several genes involved in antioxidant functions were inhibited. These changes suggested acute inflammogenic effects and oxidative stress in the lungs of WTC Dust-exposed rats. From this, it was concluded a single *very high* exposure to WTC Dusts could potentially have adversely affected the respiratory system—in terms of early inflammatory and oxidative stress processes. Subsequent studies have sought to determine if the effects might have any relevance to chronic lung pathologies that had become evident among FR who encountered the highest dust levels on September 11–13, 2001, as well as if the effects on genes were acute, reversible, or persistent and associated with corresponding histopathologic and/or biochemical changes *in situ*.

In a follow-on study, Cohen et al. (2015) investigated potential changes in particle clearance induced by the alkaline nature of the WTC Dusts. That study ascertained if entrained WTC Dust caused damage *in situ* that modulated the retention, and thus potential impacts, of the WTC Dust itself, and possibly of other major Ground Zero airborne co-pollutants, for example, metal-cutting fume particle (CFP) and diesel exhaust particle (DEP). In examining rats exposed to WTC Dust (as earlier), and then isolating their lungs

over a 1-year period post-exposure, it was found that WTC Dust induced significant decreases in levels of airway ciliated cells and increases in hyperplastic goblet cells. These changes were associated with significant prolonged dust retention (≈90–95%) over the 1-year period. These findings were in line with those of McMahon et al. (2011), who noted ultrastructural ciliary abnormalities in some Ground Zero workers that corresponded to ciliary and respiratory function abnormalities. Among the ultrastructural abnormalities was a disarray of axonemal microtubules/axonemes that were replaced by homogeneously dense cores.

26.5.3 *In Vitro* Studies of Potential Biological Mechanisms

As for *in vivo* studies, there is little information in the literature on potential mechanisms of effects of WTC Dusts based on *in vitro* studies. In an early study, Payne et al. (2004) examined potential mechanisms for how WTC Dusts might affect functions of lung cells with multiple vital roles, that is, AM and type II (TII) epithelial cells, including changes in their ability to produce/release select cytokines. $WTC_{2.5}$ and $WTC_{10–53}$ from samples collected over 9/12–9/13 by the NYU research team were tested for effects using cells isolated from healthy humans (Royal Brompton Hospital, London). Dust samples were suspended at 10 mg/mL in serum-free low protein media; sonicated; diluted to 10, 1, or 0.1 mg/mL; and applied to the cells at particle suspensions of 5, 50, or 500 μg particles/well.

Conditioned media were collected after 6 or 24 h of culture at 37°C (5% CO_2) and analyzed for TNFα, IL-6, and IL-8 (ELISA). The data showed that $WTC_{2.5}$ caused significant AM IL-8 release after just 6 h; small increases were seen only with the lower dose after 24 h. TII responses at 24 h were mostly analogous to 6-h-treated AM. $WTC_{10–53}$ particles caused small increases in AM IL-8 release (significant only after 6 h with 5 μg dose) and failed to induce TII IL-8 release. Levels of AM IL-8 release at 24 h were always greater than those at 6 h, but there were no significant particle effects. Unlike with IL-8, 50 μg $WTC_{2.5}$ consistently induced maximal AM release of IL-6 and TNFα, and these levels were always significantly greater than that induced by 5 μg dust. Increasing length of incubation also led to significant time-dependent differences in cytokine levels; this seemed to amplify the particle toxic impact, that is, maximum AM IL-6 and TNFα release achieved after 24 h with 50 μg dose increased seven- and fivefold above that at 6 h. For TII cells incubated 24 h with $WTC_{2.5}$, IL-6 release responses fell between those of 6- and 24-h-treated AM; there were no TNFα release responses at all.

When $WTC_{10–53}$ was tested, the only effect noted was a non-dose-related increase in AM IL-6 release after 24 h; TII cells only had nominal IL-6 release after 24 h. With TNFα, TII cells again *failed to respond*; AM only showed a response after 24 h, with a maximum once again at a 50 μg. In both cell types and at either exposure duration, the use of the highest (500 μg) dose of either dust fraction uniformly caused significant declines (from 50 μg levels) in cytokine release (except for TII TNFα). These results showed that lung epithelium/resident macrophage exposure to WTC Dusts could cause release of several factors that could contribute to inflammation/airway remodeling processes if they were also released in an intact lung. The data also showed that a prolonging of the period in which WTC Dust could interact with cells led to concurrent increases in these effects (and so, *in situ*, a likely worsening of situations in lung of exposed host). In the context of the Cohen et al. (2015) retention data, the latter is more likely an ongoing issue in exposed FR.

Another study by Xu et al. (2011) examined if WTC Dusts caused direct cytotoxicity to two airway cell types most directly exposed to inhaled dust, that is, airway epithelial and

smooth muscle cells. The study also evaluated if the presence of the dusts could modulate effects of cigarette smoke on these cell types as a good number of individuals who responded to the disaster were smokers. Human cultured airway epithelial (BEAS-2B) cells were exposed to 10% cigarette smoke extract (CSE), $WTC_{10–53}$ (at 0.01–0.5 μg/μL), or a combination of the two for 2–24 h. Cell viability was measured via changes in mitochondrial integrity (MTT assays) and apoptosis [poly-ADP-ribose polymerase (PARP) immunoblotting]. Conditioned cell culture media from the CSE ± WTC Dust-exposed cells were then applied to cultured human airway smooth muscle cells that, in turn, were assayed for mitochondrial integrity and ability to synthesize cyclic AMP (a regulator of airway smooth muscle constriction). The data indicated that the BEAS-2B cells underwent necrotic cell death after exposure to WTC Dust or CSE for 2–24 h, without evidence of apoptosis. Smooth muscle cells demonstrated cellular toxicity and enhanced cyclic AMP synthesis after their exposures to conditioned media from the exposed epithelia. These studies clearly showed that WTC Dust (at least its supercoarse fraction) or CSE alone exerted direct adverse effects on airway epithelial and smooth muscle cells and altered signaling properties of airway smooth muscle cells. Further, the combination of CSE + WTC Dust exerted an interactive effect on cell toxicity. Xu et al. (2011) posited if these initial cell death events might have contributed to some of the chronic lung effects associated with WTC Dust exposure among FR.

Building on the original Payne et al. (2004) studies, Wang et al. (2010) hoped to identify some potential mechanisms for the increases in cytokine release induced by fine WTC Dust. It was surmised that because activation of mitogen-activated protein kinase (MAPK) signaling pathways causes cytokine induction, these pathways were likely impacted by WTC Dust. The study used BEAS-2B cells exposed to $WTC_{2.5}$ for 5 h. Exposures to various doses of $WTC_{2.5}$ caused significant dose-related increases in *IL-6* mRNA expression, as well as in corresponding protein levels in the media. Apart from IL-6, cytokine multiplex analyses revealed that IL-8 and IL-10 formation was also elevated. Both extracellular signal-regulated kinase (ERK) and p38, but not c-Jun N-terminal protein kinase signaling pathways, were activated in the dust-exposed cells. Inactivation of ERK signaling pathways by PD98059 effectively blocked the IL-6, IL-8, and IL-10 induction; p38 kinase inhibitor SB203580 significantly decreased induction of IL-8 and IL-10. Together, these data demonstrated that activation of MAPK signaling pathway(s) likely played an important role in $WTC_{2.5}$-induced formation of several inflammatory (and, subsequently, anti-inflammatory) cytokines. Wang et al. (2010) noted the results were important in that they helped define one mechanism by which WTC Dusts may have acted to cause the documented increases in asthma/other inflammation-associated respiratory dysfunctions in Ground Zero-exposed FR.

Weiden et al. (2012), in a series of studies (see also Naveed et al., 2011a, 2011b), compared the effects of WTC Dust size on AM inflammatory cytokine/chemokine release *in vitro*. Normal adherent AM from 15 subjects without dust exposure were incubated in media alone, media with 40 ng LPS/mL, or media containing suspensions of $WTC_{10–53}$ or $WTC_{2.5}$ at 10, 50, or 100 μg/mL for 24 h. Culture supernatants were then collected and assayed for 39 chemokines/cytokines. To assess potential translatability of the findings, sera from WTC Dust-exposed subjects who developed lung injury (n = 70) were also assayed for the cytokines. In the *in vitro* studies, cytokines formed two clusters, with granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage-derived cytokine (MDC, CCL22) as a result of $WTC_{10–53}$ and $WTC_{2.5}$. GM-CSF clustered with IL-6 and IL-12(p70) at baseline, after exposure to $WTC_{10–53}$ and in sera of dust-exposed subjects with lung injury. Similarly, MDC clustered with chemokines GRO (CXCL1) and MCP-1.

$WTC_{10–53}$ consistently induced greater cytokine release than $WTC_{2.5}$ and a stronger inflammatory response than $WTC_{2.5}$. The authors concluded that as GM-CSF and MDC consistently clustered separately, these chemokines likely had a key role in the ultimate differential cytokine release seen during WTC Dust-induced lung injuries.

26.5.4 Overview of Biologic Responses

From these early and more recent *in vivo* and *in vitro* studies, it is clear that WTC Dusts could cause toxic responses in various cell types found in the lungs. Whether such changes alone gave rise to many of the observed pulmonary/CV health effects noted with still increasing frequency among FR and others exposed to WTC Dusts remains to be determined. Ongoing studies in rodents may provide critical proof that the dusts were actual causative agents for the noted pathologies. It is also possible WTC Dusts were *contributive* to rather than directly *causative* of many health effects. This too is a subject of ongoing studies using co-exposure of rodent models to WTC Dusts and key co-pollutants that were present in Ground Zero air, that is, CFP and DEP.

While definitive mechanisms of effect for the WTC Dusts are still not yet fully defined, recent studies have nevertheless built on the above-noted findings and have begun to identify biomarkers of *potential* health alterations due to exposure to the WTC Dusts. Using serum collected from—and noninvasive measures to examine—FR and others exposed at Ground Zero, novel biomarkers of *potential* lung injury, altered CV status, and dysregulated inflammation have been identified. These included significant changes in expression of matrix metalloproteinases (i.e., MMP-2, -3, -12) (Kwon et al., 2013; Nolan et al., 2014), serum immunoglobulin (IgA, IgG) (Ferrier et al., 2011), select cytokines/chemokines [IL-6, IL-8, GRO, GM-CSF, granulocyte colony-stimulating factor (G-CSF), and IFN-inducible protein-10 (IP-10)] (Nolan et al., 2012; Cho et al., 2014), and select cardiovascular disease (CVD) markers (apolipoprotein-AII, C-reactive protein, macrophage inflammatory protein-4) (Weiden et al., 2013, 2015; Schenck et al., 2014) that could be correlated with increased risks for CVD or lung injury.

In conclusion, there has been significant progress in identifying potential mechanisms of toxicity for WTC Dusts as well as of biomarkers of pulmonary/CV diseases associated with exposure to the dusts. Still, much more research into effects from *in vivo* and *in vitro* exposures to WTC Dusts is needed. Among the areas needing additional analyses, as noted, exposures to WTC Dusts did not occur in a vacuum, and so interactive toxicities/effects from co-exposures to other major co-pollutants at Ground Zero remain to be described. Also, while some immune system-related endpoints have been examined, it remains unclear if WTC Dusts were immunomodulants that allowed nascent diseases (i.e., cancers, autoimmune diseases, asthma) to flourish when they may not otherwise. Lastly, there is an unmet need to develop, identify, and validate noninvasive methods to quantitate remaining dust burdens in the lungs of WTC Dust-exposed workers and residents.

26.6 POSSIBLE ROLES OF MINOR MASS COMPONENTS AS CAUSAL FACTORS FOR OBSERVED HEALTH EFFECTS

Initial concerns about possible adverse health effects that might occur, especially in relation to chronic health effects, focused on known toxicants/carcinogens that were present at relatively low concentration levels, that is, asbestos, metals (Cd, Cr, Fe, Pb, Hg, As), combustion products (PAHs, PCBs), and ambient air $PM_{2.5}$. There were, in retrospect, two

good reasons why these initial concerns were misplaced. First, levels of these components in the WTC Dust $PM_{2.5}$ fraction were initially quite low and then declined markedly. Second, most of the excess disease incidences found in epidemiological studies were not those most closely associated with these components. In contrast, the three major mass components of settled WTC Dusts that were present in particles >2.5 μm were all known irritants, that is, highly alkaline cement and gypsum components as chemical irritants and SVF as physical irritants.

There is good reason to expect biological responses to these components based on interactions post-airway deposition. No routine/special-purpose $PM_{2.5}$ sampling filters were ever assayed for alkalinity or SVF content. Some settled dust samples collected were analyzed for their percentage of SVF and generally showed that 20–25% of the dust was SVF. From an examination of literature on associations between WTC Dust exposure and adverse respiratory/GI effects, inhalation exposures to resuspended SVF, in the presence of highly alkaline co-contaminants, cause adverse pulmonary/gastroesophageal effects in humans. There is also supporting evidence that the same kinds of effects can be seen following inhalation exposures of laboratory animal models. Thus, it appears SVF may be one causal factor as well as a signature component in WTC Dusts.

26.7 ROLES OF MAJOR MASS COMPONENTS AS POTENTIAL CAUSAL FACTORS FOR OBSERVED HEALTH EFFECTS

Zeleke et al. (2010) conducted a cross-sectional/cross-shift study at a cement factory in Ethiopia where personal "total" dust was measured in worker breathing zones and PEF before and after the shift. When a dayshift ended, acute respiratory symptoms were recorded on a 5-point scale via questionnaire. The highest (geometric) mean dust exposure was 38.6 mg/m^3 (crusher section), then 18.5 mg/m^3 (packing section), and then 0.4 mg/m^3 (guards). Prevalence of respiratory symptoms among the highly exposed workers was stuffy nose (85%), shortness of breath (47%), and "sneezing" (45%); PEF decreased significantly across the shift in the highly exposed group. Multiple linear regression showed a significant negative association between the percentage cross-shift change in PEF and total dust exposure. Number of years of work in high-exposure sections and current smoking were also associated with cross-shift decreases in PEF. Meo (2004) noted that cement particles ranged in aerodynamic diameter from 0.05 to 5.0 μm and concluded that a high level of/prolonged inhalation to cement dust could provoke clinical symptoms and inflammatory responses that may result in functional and structural abnormalities. The commonest clinical complaints among the cement mill workers in that study were chronic cough and phlegm production, impaired lung function, chest tightness, obstructive and restrictive lung diseases, conjunctivitis, headache, fatigue, and carcinoma of the lung, stomach, and colon. The particle size of the cement mill dust was not as large as the WTC Dust, but just as alkaline.

Similarly, there is data on human, animal, and cellular responses to high mass doses of other (super)coarse dusts, primarily volcanic dusts. The latter contain PM over a large particle size range, but are not alkaline or readily soluble in the lung; sometimes these dusts are acidic. Buist and Bernstein reviewed health effects by Mount St. Helen dusts and concluded "Effects of both short- and long-term exposures to the relatively low levels of airborne ash typical following such a volcanic eruption were minor, and related more to irritant effects of the ash on airways than to potential of the ash to initiate fibrotic responses."

Baxter et al. (2014) evaluated risk assessment performed for 13,000 Montserrat residents and concluded, in an absence of clinically manifest diseases, the endpoint commonly used in occupational epidemiological studies of silicosis was a radiologic one, that is, for pneumoconiosis and small opacities. Risks in the island north, where most lived, were computed by a model so small and uncertain that it could be ignored. In terms of lesser inhabited areas (central island) that received the most ashfall, the "best estimate" probability of developing early radiological evidence of silicosis was <1/1000 after 5 years of volcanic activity. This cluster of studies of the health effects of volcanic ashes, when taken in conjunction with data from comparative toxicological studies, indicates that the alkalinity of the WTC Dusts was likely to be a major determinate of its ultimate health effects.

26.8 CONCLUSIONS

The spectacular collapses of the WTC Twin Towers on the morning of September 11, 2001 posed a unique challenge to New York City (NYC) and the nation and especially in terms of the public health risks of hundreds of thousands of people who worked and/or lived in Lower Manhattan. This disaster resulted in the conversion of major fractions of the WTC Towers into an enormous cloud of dust that spread throughout Lower Manhattan, as well as a massive pile of debris at Ground Zero. Ultimately, each of these type of dust dispersion gave rise to inhalation exposures of an unprecedented nature and amount, due initially to the dust levels that were suspended in the air and then to the subsequent resuspension of dusts that had settled onto the streets and within buildings. Within the first few days, public agencies involved in occupational and environmental risk assessment and management sought to help, but they were unprepared to provide adequate public health protection in terms of abilities to (1) adequately assess the nature and magnitudes of the risks, (2) prescribe suitable methods to monitor subsequent exposures, and (3) prescribe effective means to minimize exposures. Further, this guidance was never adequately revised in light of emerging evidence of substantial unanticipated adverse health effects among workers/residents attributable to inhalation of components of the WTC Dusts.

In summary, it is our view that the key elements associated with WTC Dust exposures by various populations of workers and local residents are as follows:

1. The collapses of the WTC Towers on September 11, 2001, created a dense dust cloud that radiated out at very high velocity, creating settled dust deposits ranging from clearly visible to inches thick on the streets, building exteriors, interior building surfaces, and within air ducts throughout Lower Manhattan. WTC Dust deposition was much lower in other parts of NYC and adjacent areas. These settled dusts differed in important ways from conventional settled dusts in regard to (a) particle size distributions, (b) chemical composition, and (c) ease of redispersion into the ambient air (i.e., by air movement /physical disturbance caused by peoples' activities).
2. In terms of particle size distribution of WTC settled dusts, ~1% was <2.5 μm in aerodynamic diameter, 0.3–0.4% ranged from 2.5 to 10 μm, ~ 40% ranged from 10 to 53 μm; the remainder did not pass through a screen with a 53 μm cut-off pore size. As compared to outdoor settled WTC Dust, the deposits within buildings were depleted of particles in the upper end of the particle size range. In terms of the major components, 80–90% was attributable to a mixture of SVFs from glass- and

slag-wool insulation, gypsum from wallboards, and cement from the concrete originally within the Towers. Cellulose, attributable to paper, accounted for 9–20%, and chrysotile asbestos fibers for 0.8–3.0%, with other components having much smaller amounts. With regard to particle chemistry, the aqueous solubility of calcium oxide from the cement and the calcium sulfate from the gypsum resulted in a very high pH (9–11) for the outdoor settled dust samples collected over the first few days and even higher pH levels in indoor dusts (pH > 12) that had a smaller percentage of supercoarse dust. By contrast, the pH in particles in the fine dust fraction (<2.5 μm) was nearly neutral (pH 7–8). Thus, the particle size range of the alkaline dusts created by the collapses did not extend to ≤2.5 μm.

3. Almost all the air monitoring for PM mass concentration in Lower Manhattan after September 11, 2001, were based on determinations of total gravimetric mass of $PM_{2.5}$ or of specific components of the $PM_{2.5}$ air samples. There were very few measures of levels of major mass components generated by the buildings' collapse, that is, in particles >2.5 μm (SVF, cement, gypsum).
4. Coarse (>2.5 μm) alkaline particles that were *inhaled* via the nose were likely deposited in conductive airways in the head and LRT tracheobronchial airways, including most that were between 2.5–10 μm as well as smaller percentages of particles of ~10–30 μm that had not been deposited in the URT airways. On the other hand, for cleanup workers having higher tidal volumes and switching to mouth breathing, there was more penetration of coarse and supercoarse dust into their LRT.
5. The high pH of these coarse particles could have overwhelmed the capacity of the conductive airways to maintain the homeostasis that removes debris from airways, thereby inducing acute responses such as cough, chest pain, and other respiratory symptoms and, after clearance to the GI tract, gastroesophageal reflux. This also could have subsequently permitted prolonged retention of the WTC Dust particles themselves and, possibly, other major co-pollutants that were present in the air at Ground Zero [metal-cutting fume particle and diesel exhaust particle].
6. The epidemiological literature now shows significant excesses of adverse health effects among FR and other worker and local residents exposed to the WTC Dusts. These include several chronic diseases/pathologies, such as respiratory and gastroesophageal illness, as well as CV abnormalities, at levels greater than seen in comparison populations that lived and worked at further distances from Ground Zero. That many of these adverse outcomes began to manifest post-9/11 provides evidence of a causal association attributable to WTC Dust exposures. Because the types of responses known to have occurred in these populations have not been associated with exposures to toxicants that were monitored after 9/11, that is, asbestos fibers, trace metals, PAHs, PCBs, and dioxins, they thus had to be due to the unmonitored components spread throughout the area post-9/11, including the coarse and supercoarse particles composed of cement, gypsum and SVFs, all known irritants to large airways.

It is clear that, more than 16 years since the disaster, much remains to be done to not only identify how exposures to WTC Dusts may have contributed to diseases/adverse health effects in those who were at/near Ground Zero but also devise methodologies to ascertain who is still healthy but at risk for developing exposure-related lung, CV, and other pathologies.

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