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Chapter 1

Introduction

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I. Routes of Exposure to Pollutants

A. Inhalation

Breathing produces by far the greatest total exposure of the body to environmental contaminants. Each breath brings at least a few hundred cubic centimeters (and up to several thousand cubic centimeters) of air into intimate contact with some of the most delicate membranes (the alveolar walls) in the body. When the inhaled (tidal) volume is multiplied by the number of breaths taken per minute and by the number of minutes in a day, one obtains daily intakes of air of 10,000 *l* (for an adult who never exercises) up to over 20,000 *l* (for a person who is physically very active much of the time). Compare these volumes with the average adult's daily intake of about 1.5 to 2 *l* of food and of water (Table 1.1). Also, as shown in the table, a similar situation occurs for children. Because the levels of contaminants in air, food, and water can be similar — in terms of mass of contaminant per liter — air easily can expose a person to about 10,000 times more mass of an environmental pollutant than does food or water.

TABLE 1.1
Intakes for an Adult and Child of the Major Media that Produce Exposure to Environmental Contaminants

	Daily intake (*l*)		Daily intake (g)	
Medium	Child (6 yr)	Adult	Child (6 yr)	Adult
Air				
Sedentary	4300	10,000	5200	12,000
Active	8000	20,000	9600	24,000
Water	0.6	1.5	600	1500
Food	0.2	0.6	200	600

The potential health consequences of the large daily intake of air in relation to other routes of pollutant exposure can be appreciated when one examines the commonly encountered air contaminants. Pure air is not found in nature; nearly pollutant-free air exists in clean-rooms that are built of inert materials and are supplied with highly filtered air under positive pressure to prevent inward leaks. Typical indoor or outdoor air that is not particularly polluted contains about 10 million fine particles per cubic meter and very low concentrations of hundreds of gases (other than oxygen and nitrogen) and vapors (in addition to water vapor). The pollutant load in air can easily be increased 100- to 1000-fold if an activity such as smoking, cooking, or metalworking is taking place. The full range of types of particulate contaminants

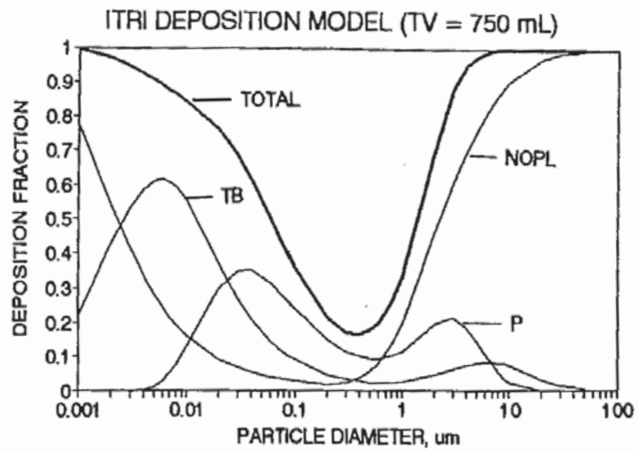

Figure 1.1
Particle deposition probabilities of unit density spherical particles inhaled by adults at a tidal volume of 750 m*l*. (Figure supplied by Dr. H.C. Yeh of the Inhalation Toxicology Research Institute, Albuquerque, NM.)

are too numerous to list completely, but the following are nearly always present in unfiltered air: viruses, bacteria, mold spores, pollen, dander (from humans and animals), insect and plant parts, soil dust, combustion products, metals and other inorganic substances, and organic compounds of various types. The expected probabilities of unit density (1 g/cm^3) particles of various sizes depositing in a normal, healthy adult's respiratory tract are shown in Figure 1.1.

It is surprising that the respiratory tract is able to survive this constant onslaught for very long. In fact, many of our illnesses are produced or contributed to by the aforementioned air contaminants. Not only do inhaled substances lead to diseases of the respiratory system, but also other organ systems of the body may be affected when the contaminants (or their metabolic products) are distributed via the flow of blood or lymph fluid. Examples of respiratory-tract disorders produced by air contaminants include: respiratory infections (colds, influenzas, and fugal diseases); inflammatory-type disorders such as bronchitis, rhinitis, asthma, and alveolitis; and chronic diseases such as emphysema, cancer, and fibrosis. A number of nonrespiratory system diseases that also may result from inhaled contaminants include bone diseases, liver diseases, nervous system diseases, and diseases of muscles and blood vessels. The fact that we are able to live long and relatively healthy lives is a credit to the many elaborate and effective defensive systems found throughout the body, including the respiratory tract.

Understanding inhalation phenomena, including the types of pollutants inhaled and their fates, is both a major and an important challenge of modern science. Through such an understanding we can expect to extend our productive lifespans and to help establish safe new technologies that improve the quality of life. In addition, scientific understanding of inhalation phenomena has many applications in medicine, because many medicines, vaccines, and diagnostic agents can be effectively and safely delivered by inhalation.

B. Other Routes of Exposure

Other routes of exposure to pollutants include ingestion (of food, beverages, medicines, and other substances), dermal (skin) absorption, exposure via wounds or injections, and even absorption through the eyes. Although we are not directly concerned with these nonrespiratory routes of exposure, air contaminants can play important roles in each of them. Air contaminants find their way into our food via inhalation by domesticated farm animals, and fallout from the air directly contaminates foodstuffs and water supplies that are consumed by these animals. Air pollution also affects water used for irrigating crops and for washing food. Contaminants can land on our food prior to consumption, also.

II. Respiratory Tract Anatomy

A. Human

Our current understanding of the structure of the human respiratory tract is far from complete. Considerable research has been focused on generating such data on the typical, or average, healthy adult; however, even these data are based on highly simplified structural templates, such as smooth cylindrical bronchial tubes, y-shaped airway bifurcations, uniformly sized spherical or polyhedral alveoli, and an absence of variation in the number of bronchial divisions required in a pathway to reach the alveoli. Even a brief examination of a replica cast of human lungs will reveal many deviations from these assumptions. Individual bronchial airways are seen to be variable in diameter, frequently noncircular, and often curved or bent between bifurcations. The branch points themselves can be sharp, blunt, or asymmetric or lead to three daughter airways rather than the usual two. Other variations in the usual structures are also present. In addition, little is known about age-related, race-related, gender-related, or disease-related effects on respiratory tract anatomy.

The principal anatomical structures of the respiratory tract include the: (1) nose, consisting of the nares, vestibule, and nasal cavity proper (with the conchae or turbinates); (2) nasopharynx; (3) oral cavity; (4) oropharynx; (5) laryngopharynx; (6) larynx; (7) trachea; (8) bronchi; (9) bronchioles; (10) respiratory bronchioles; (11) alveolar ducts; (12) alveolar sacs; and (13) alveoli. These structures are conveniently grouped into larger regions, or compartments, for the purpose of simplification and to facilitate mathematical modeling. A variety of compartmental schemes have been proposed, but several very similar models have been particularly useful to inhalation toxicologists. The models are those of Task Groups of the International Commission on Radiological Protection,[1-2] the Ad Hoc Working Group to Technical Committee 146–Air Quality–of the International Standards Organization,[3] the Air Sampling Procedures Committee of the American Conference of Governmental

TABLE 1.2
Compartmental Models of the Human Respiratory System as Developed by the ICRP Task Group on Lung Dynamics, the International Standards Organization, and the American Conference of Governmental Industrial Hygienists (ACGIH)

Region	Anatomic structures included	Task group region	ISO region	ACGIH region
1	Nose, mouth, nasopharynx, oropharynx, laryngopharynx, larynx	Nasopharynx (NP) or (NOPL)	Extrathoracic (E)	Head airways region (HAR)
2	Trachea, bronchi, bronchioles	Tracheobronchial (TB)	Tracheobronchial (B)	Tracheobronchial region (TBR)
3	Respiratory bronchioles, alveolar ducts, alveolar sacs, alveoli	Pulmonary (P)	Alveolar (A)	Gas exchange region (GER)

Industrial Hygienists,[4] and the Task Group of the National Council on Radiation Protection.[5] These compartmental systems, which are largely based on the pioneering morphometric work of Ewald Weibel,[6] are shown in Table 1.2 and Figure 1.2. Further information on the human respiratory tract models may be found in several references.[7-9]

B. Rat

The laboratory rat has structures similar to those of the human, except that the oral region need not be considered, as rats appear to nose-breathe only. Also, the transition from bronchioles to fully alveolarized ducts is so abrupt that the respiratory bronchioles are essentially absent. The airways of the rat differ from those of the human in several other ways, as well. The nasal region of the rat is much more complex due to the large posterior olfactory apparatus. The airway branching is much more asymmetric, and the number of generations of airways is much smaller. The airways of the rat are depicted in Figure 1.3. A quantitative model of the airway anatomy of the rat has been published by Yeh, Schum, and Duggan.[10]

Although this book is largely limited to methods that employ the laboratory rat, a great variety of other animals is used in inhalation studies. A special August 1983 issue of the *American Review of Respiratory Disease*, Vol. 128, No. 2 (Suppl.), entitled "Comparative Biology of the Lung", contains over 20 useful papers comparing several species.

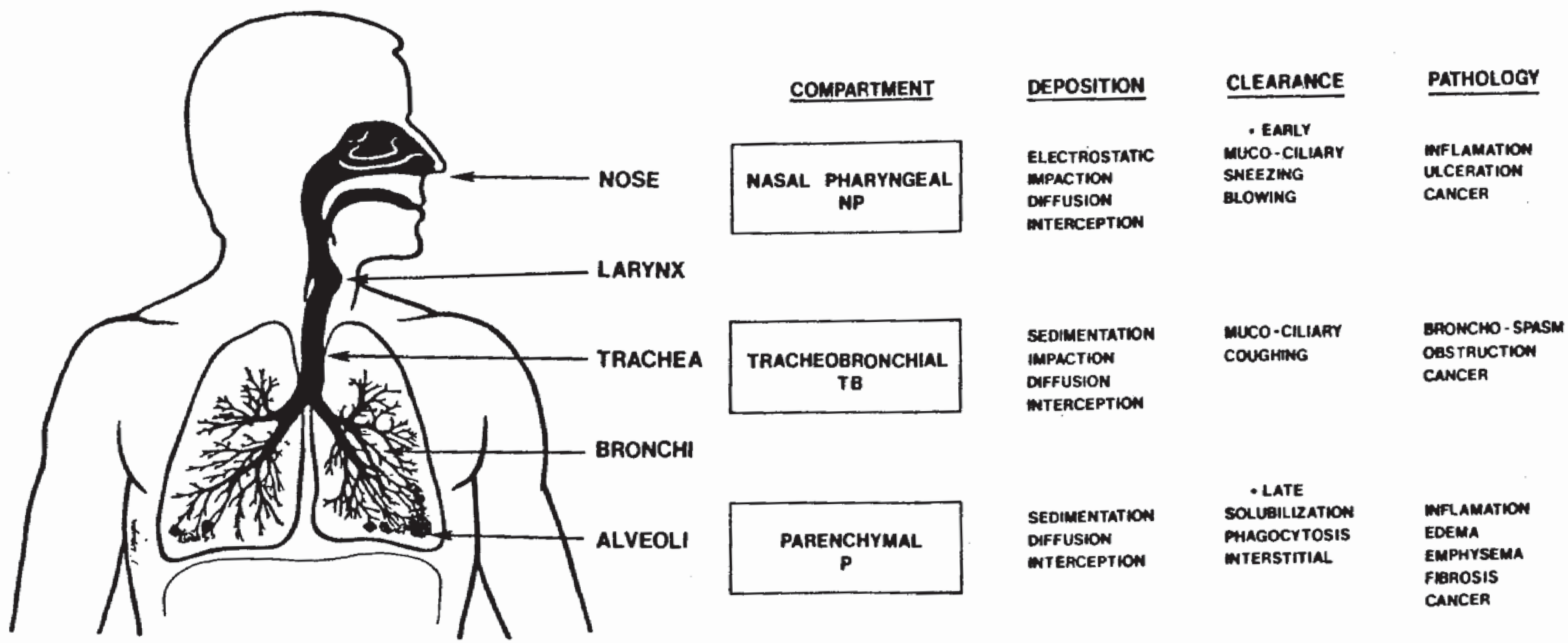

Figure 1.2
Compartmental model of the human's respiratory tract airways as defined by the Task Group on Lung Dynamics of the ICRP.[1]

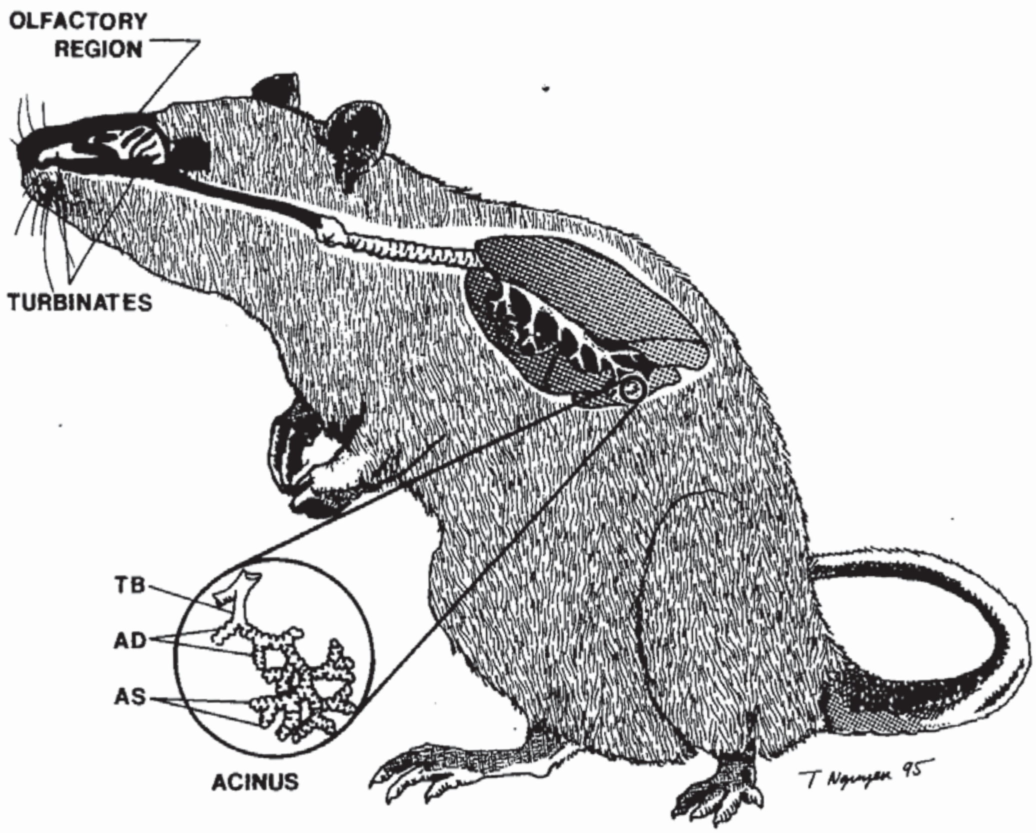

Figure 1.3
Airways of the laboratory rat. Location in the body is only approximate.

III. Major Types of Inhalation Studies

A. Toxicological

Fundamental to the practice of experimental toxicology is the principle that each route of exposure must be examined for its possible uniqueness. Many substances that are essentially harmless in food or water can produce serious illness and even death when inhaled in sufficient quantities. Among the myriad examples are many inorganic dusts; allergenic spores, pollens, and danders; several metals; certain microorganisms; and a variety of combustion products, including tobacco smoke. Also, the tissues of the respiratory system are capable of metabolizing (chemically transforming) many organic substances in ways that differ from their metabolism in the gastrointestinal tract. It is clear that inhalation studies frequently have no viable substitutes when one is investigating the consequences of breathing contaminated air.

Inhalation toxicology studies must be conducted with many types of air contaminants: natural, those produced by old or low-technology processes, and those produced by new or high-technology processes. Such studies require generating the test substance in physical and chemical forms that are as similar as is practical to the materials inhaled by humans. Also, when mixtures are present that may influence toxicity, the mixtures must be examined in inhalation studies. The journal *Inhalation Toxicology* publishes a variety of research studies that are of importance to inhalation toxicologists. Other journals that cover specialized related areas include the *American Industrial Hygiene Association Journal*, *Applied Occupational and Environmental Hygiene*, and *Toxicology and Environmental Health*.

B. Medical

Inhalation studies with medicinal substances must evaluate not only potential toxicity, but efficacy as well. In such studies, chemical and physical forms usually are intentionally varied in order to maximize the therapeutic or diagnostic effects. This additional requirement places demands on the flexibility of systems used to generate the particles and gases under study. Such demands are not to be taken lightly, as eventual approval of a medicinal agent can be limited to the exact physical forms (median particle size and size distribution, for example) that were used in the animal studies. *The Journal of Aerosol Medicine* publishes basic and applied research relating to the inhalation of medications.

C. Physiological

Inhalation studies are performed by scientists in their exploration of how living systems function. Particles in air can be used as tracers to follow clearance pathways or, alternatively, to learn how inhaled substances cross respiratory tract surfaces to gain access to the rest of the body. Physiologists also use inhalation studies to study the normal distribution of inhaled air throughout the lungs and within the nose and to examine the effectiveness of the mixing of air in successive breaths. Generally, such studies use particles and gases that, by design, have no toxic or physiologic effects of their own. The design and performance of such tracer studies have their own set of challenges. In addition to the previously mentioned journals, the *Journal of Applied Physiology* carries research papers on basic inhalation phenomena.

IV. Requirements of Inhalation Studies

From the preceding discussion it is clear that a particular study may have many unique and challenging requirements; however, some features of inhalation studies are essentially universal. An experimental atmosphere must be generated, controlled, characterized, and presented to experimental subjects. Unwanted contaminants must be effectively eliminated, and the air temperature and relative humidity must be within limits that do not stress the subjects or otherwise harm the experiment. The subjects themselves must be well characterized, appropriate for the study, and treated in an ethical manner (this is accomplished by minimizing stress, monitoring for pain or discomfort, medicating when needed, and even terminating the study, if necessary).

The implications of the requirements for successfully conducting a given inhalation study are substantial. First, a team of scientists may be required. This team typically includes a physical scientist (with aerosol or atmospheric science training), a physiologist (with pulmonary expertise), and a person who has primary expertise relating to the problem under study (toxicology, asthma, immunology, etc.). In addition, inhalation studies require specialized, sophisticated, well maintained, and, often, custom equipment. Air purification alone, which generally is essential to a modern inhalation study, can cost tens of thousands of dollars, take months of engineering design, and require a long-term commitment of funds and personnel for retrofits, maintenance, and repair. In addition, inhalation studies generally require much more space than do most biomedical investigations. Air moving equipment, animal isolator units, exposure chambers, aerosol generators, real-time gas and aerosol monitors, analytical support, and the biomedical research items may require the use of several laboratory rooms. Inhalation studies that meet today's standards usually can only be performed successfully by a well trained, experienced, and adequately funded team of specialists working in a sophisticated laboratory.

V. Problems Encountered

The problems encountered in an inhalation study are largely practical ones, and they yield to the problem-solving methods of several key scientific disciplines (Table 1.3). Maintaining healthy animal subjects, a real problem at times, requires experts in the disciplines of microbiology, nutrition, physiology, and veterinary medicine. Establishing a well characterized study atmosphere is dealt with by rigorous application of the principles of engineering, physics, and chemistry. Monitoring animals during exposures can be a challenging problem in itself. It is solved using equipment, knowledge, and techniques from the discipline of physiology. Acquiring and processing tissues, especially lung tissue, also present several challenges; the disciplines of anatomy, biology, and physiology are required to meet them. Although the problems encountered in inhalation studies are significant and often unique, they are solvable by the application of sound scientific principles that have been established over the past four decades.

TABLE 1.3
Summary of Commonly Encountered Problems and the Scientific Disciplines that Permit Their Solution

Problems	Disciplines
Maintaining healthy subjects	Microbiology, nutrition, physiology, veterinary medicine
Air cleaning and conditioning	Engineering
Aerosol and gas generation	Physics, chemistry, engineering
Characterization of the study atmosphere	Physics, chemistry
Monitoring animals during exposure	Physiology, engineering
Acquiring and processing tissue samples	Anatomy, biology, physiology

VI. Scope of this Book

A. Overview

The basic requirements that essentially all inhalation studies have in common will be the focus of this book. It is not possible for a single volume to cover all types of studies, but the foundations can be treated here. Although studies with humans and laboratory animals have similar basic requirements, this book is largely limited to animal studies (with a focus on the rat). Animal studies are not only more common than human studies, but they also are usually more expensive and more demanding. Sound ethical principles require that well conducted animal studies precede studies with human subjects. If one can conduct a good animal inhalation study, similar human studies probably will be relatively easy, but not vice versa. Several useful reference works on inhalation toxicology can be found that provide essential guidance.[11-19]

B. Focus of the Chapters

Each chapter focuses on an important aspect of laboratory animal inhalation studies. The chapters are written to be independent, in that their order is immaterial, and each can largely stand alone. Because they are written by specialists in a variety of disciplines, their organization differs somewhat. Chapter 2, by William Mautz and Michael Kleinman, relates to the health and welfare of the animal subjects and to the process of obtaining approval of a animal research protocol from an institutional review committee. In a real sense, Chapter 2 is an ethics chapter, for it describes the prevention of disease, provisions for comfortable housing, and maximizing the study benefits while minimizing the stresses. Chapter 3, by Robert Phalen, is largely an engineering exercise. It covers the specialized area of cleaning and conditioning the "throughput" air to be used. Topics such as particle and gas scrubbing, temperature and humidity adjustment, and selection of equipment are presented. Chapter 4, by Michael Kleinman and Robert Phalen, is oriented toward physics and chemistry, as well as engineering. It deals with the complex specialties of generating study atmospheres and measuring their physical and chemical properties (i.e., atmosphere characterization). Chapter 5, by Robert Phalen, deals with exposure methods. Because such methods are so varied (exposures using chambers, manifolds, helmets, masks, canulas, etc., have been performed previously), the chapter focuses on nose-only exposures — perhaps the most versatile method of inhalation exposure. Chapter 6, by William Mautz, is based in physiology; it covers methods used to monitor animals during their exposure. Such monitoring is useful both to observe effects and to estimate the inhaled "dose" of the study material. Chapter 7, by Ronald Rasmussen, describes the anatomical methods used for acquiring and preparing tissue samples for further study. Euthanasia, fixation, embedding, sectioning, staining, and other demanding techniques are described. Chapter 8, by Deepak Bhalla, deals with two very important specialized sampling methods: bronchioalveolar lavage and blood collection.

The techniques described in this book look at the respiratory tract both as a potential target, and as a portal of entry to the body for inhaled materials. Taken together, the various chapters can provide a great deal of guidance to those who are beginning to perform inhalation studies or wish to modernize their ongoing studies.

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