Long-Term Retention and Clearance of Particles Inhaled by Mammalian Species

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I. INTRODUCTION

Inhalation is a common route of exposure of humans to hazardous materials in the workplace and environment. For vapors and aerosols of hazardous materials, inhalation represents the most significant route of exposure. The adult human inhales about 20,000 l of air daily, and the gases and particles in the inhaled air have the opportunity to interact with about 70 m^2 of respiratory tract epithelium. Depending on their chemical composition and physical characteristics, which influence retention and tissue exposure patterns, inhaled materials may constitute an immediate or long-term threat to the health of the individual. The magnitude of the threat depends on temporal associations between tissues and the inhaled material; these associations are dictated by deposition, clearance, and, ultimately, retention patterns.

Physical and chemical descriptions of the exposure atmospheres, amounts inhaled, and the tissue retention patterns for the deposited materials are generally not available for accidental human inhalation exposures. Of necessity, studies with laboratory animals provide the exposure, dose, and biological effects data needed to evaluate the inhalation toxicity of hazardous materials potentially inhaled by humans.

Scientific understanding of the mechanisms and processes involved in deposition of particles in the respiratory tract is relatively advanced. Patterns of initial deposition and early clearance for inhaled materials have been extensively studied in humans and in several species of laboratory animals. However, only a few well-documented cases of human inhalation exposures that provide the kinds of long-term retention and clearance data required for direct risk assessments are available.

The mechanisms associated with retention and clearance of particles deposited in the respiratory tract are not well understood. Retention is a convenient expression for the time-dependent distribution patterns for materials, or their reaction products, that have deposited in the respiratory tract and have not yet been cleared. Clearance is removal of deposited materials or their reaction products from the respiratory tract. Clearance is discussed in terms of physical processes that transport particles from one place to another and dissolution-absorption processes. Some particles appear to be retained for extended periods in phagocytic cells in the alveoli. Other particles are found in cellular constituents of the interstitium. Most, or all, of the particles in the interstitium appear to be in mobile cells that have the options of remaining in the interstitium, moving to the mucociliary escalator, moving to lung-associated lymph nodes, or moving into the lymphatic or circulatory systems. Our current understanding of the factors that control or influence these options within and among species is not adequate; the options for retention and clearance must be the focus of additional research if we are to develop a better understanding of the processes involved and of the bases for species similarities and differences in these processes.

Inhalation exposures can be single events involving inhalation of only a small mass of material. Alternatively, exposures can be intermittent or chronic and may involve inhalation of substantial amounts of material over a long time period. Repeated, or chronic, inhalation exposures may produce significant cumulative lung burdens that, because of physical and/or chemical damage to tissue, can disrupt lung function and produce long-term pathological effects. Small or moderate particle burdens in lung may not represent a threat to health or respiratory tract function when relatively nontoxic materials are inhaled. However, even nontoxic materials can be problematic if large masses of the particles are accumulated in the lung.

Few human data are available on long-term retention and tissue exposure patterns for inhaled materials. In the absence of these data, scientists and regulators must rely on data from experiments with laboratory animals to provide the bases for decisions relative to human inhalation exposures. Unfortunately, differences between species make it difficult to directly extrapolate the results of animal studies to humans. Some of the more obvious similarities and differences among species relate to anatomy. Most mammalian species have the same anatomical components of the respiratory tract. The anatomical differences that influence deposition patterns include size and configuration of airways, e.g., degree of convolution of the nasal turbinates, lengths and diameters of the conducting airways, numbers of branches, branching angles, and branching patterns in the conducting airways. Other factors that influence deposition patterns include respiratory parameters such as tidal volume and respiratory frequency. The factors that result in species differences in retention of particles in the lung have not been defined, but probably include physical and functional differences among the cellular constituents of lung tissues, especially pulmonary alveolar macrophages.

This review is not intended to provide a complete discussion of deposition, retention, and clearance for all types of materials. Emphasis is on long-term retention and clearance of inhaled particles ranging from the moderately soluble to the

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latively insoluble. These types of particles can have the most significant impacts on human health because they can be retained in the respiratory tract for long times after a single exposure or accumulate with repeated exposures. This review has two major focuses: (1) long-term retention and clearance patterns for particles inhaled during acute exposures and (2) models for retention and clearance of particles inhaled during repeated exposures. Human data are discussed and included in the modeling projections where available and appropriate.

II. DESCRIPTION OF THE RESPIRATORY TRACT

A. Anatomic and Functional Differentiation into Regions

The respiratory tracts of all mammalian species can be defined in terms of three anatomical-functional regions. The three regions are identified as (1) the nasopharyngeal (N-P) region, (2) the tracheobronchial (T-B) region, and (3) the pulmonary (P) region. Figure 1 schematically depicts these three regions for the human. The anatomical divisions between the three regions of the respiratory tract are arbitrary and difficult to clearly distinguish. However, by definition, the N-P region begins at the anterior nares and includes head airways that extend to the larynx or epiglottis. The primary functions of the nasal airways can be defined as (1) olfaction, (2) heating and humidification of inhaled air, (3) filtration of airborne particles, and (4) metabolism or detoxification of deposited materials. The nasal passages are convoluted as a result of folds in the nasal turbinates. The folds represent a relatively large surface area with which inhaled air can interact. The basic structural components and function of the N-P region are the same for all mammalian species. However, there are substantial differences among species in physical sizes, shapes, and dimensions of the components of the N-P region.[1] The nasal cavity is also a dynamic structure that can change dimensions during inspiration and expiration; these changes can result in higher or lower particle deposition in the N-P region, depending on how air flow patterns and velocities are influenced. In addition to dimensional changes during nasal breathing that appear to be normal, tissue reactions to inhaled irritants may cause inflammatory responses that further alter the dimensions and shape of the nasal passages. These factors make it difficult to define a standardized N-P region for humans or for any laboratory animal species.

The T-B region is distal to the N-P region. It begins at the larynx and includes the trachea and other ciliated airways, ending with the terminal bronchioles. This represents the ducting for transport of inhaled air and aerosols into and out of the pulmonary region. In all mammals, the trachea divides into two major branches (bronchi) that supply ducting to the left and right lungs. The bronchi further divide into the smaller ducts that comprise the remainder of the T-B region.

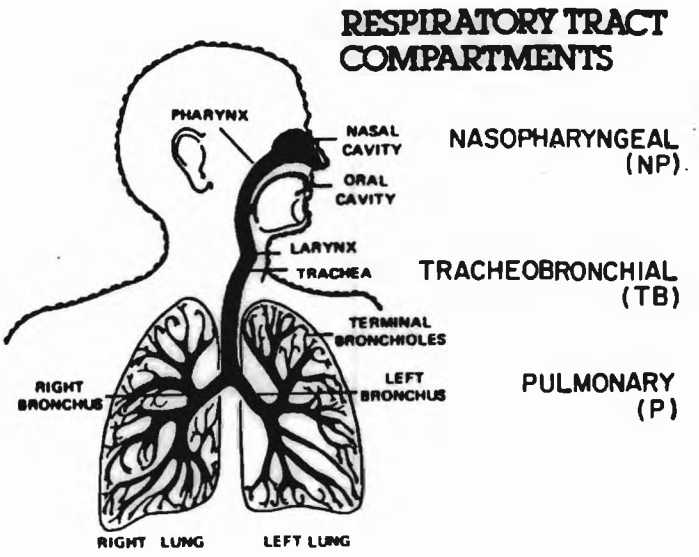

FIGURE 1. Schematic representation of the human nasopharyngeal (N-P), tracheobronchial (T-B), and pulmonary (P) regions.

The major bronchi are lined with ciliated epithelium and mucous-secreting goblet cells. Mucous glands and serous glands are also present in the major bronchi and contribute to the mucous lining of the airways. Mucus is a discontinuous blanket of viscous fluid that performs at least two important functions in the T-B region. First, inhaled materials may be trapped in the mucus and thus would have a smaller potential to cause damage to the epithelial cells or to become phagocytized. Second, the mucus overlies the ciliated bronchial epithelium and is moved toward the oropharynx by wave motion of the cilia. This movement serves to move dead cells, particles, and other debris out of the airway system, to be swallowed.

Distal to the T-B region is the P region, which has the largest surface area of the three major regions of the respiratory tract. The P region includes the respiratory bronchioles, alveolar ducts, alveolar sacs, atria, alveoli, interstitial tissues, alveolar capillaries, and pulmonary lymphatics. This is the nonciliated, gas-exchange region of the respiratory tract and is the region that dominates long-term retention of inhaled materials. By definition, the P region begins with the respiratory bronchioles and ends with the lung parenchyma. Considerable species variation in the morphology of respiratory bronchioles exists and may be the major reason for the variability observed among species in retention and clearance of inhaled materials and in biological responses to the materials.[2]

There are substantial species differences in gross and microscopic morphology of the respiratory tract.[3-6] Differences among species include lengths of airways, branching patterns, branching angles, numbers and branching generations of respiratory bronchioles and alveolar ducts, cell types, and size of alveoli. Anatomical differences are clearly important factors in initial deposition patterns for inhaled materials. However,

there are no anatomical differences that can be used to easily explain the basis for species differences in retention and clearance patterns for inhaled materials, except that the retention and fate of inhaled materials may be influenced by the initial sites of deposition. A continued effort devoted to comparative functional relationships among inhaled materials and the various structures and cell types of the respiratory tract is needed to evaluate the bases for species differences in retention and clearance of inhaled materials.

B. Cell Kinetics

The dosimetry associated with long-term retention of particles in lung tissue is dependent upon (1) the types of cells or structures the particles are associated with and (2) the length of time the particles can interact with the cells or other tissue constituents. For example, if an individual cell retaining a particle is replaced or replicates during the residence time of the particle, the exposure time will be uncertain and exposure to the particle may be shared among several cells. To understand relationships between retained particles and cell damage, it is important to determine the cell types associated with particle retention and the rate(s) at which those cells are normally replaced. Another important reason to study cell turnover times is that they may be influenced by exposures, and changes in cell turnover times may be directly linked to damage processes.

Bowden[7] provided a summary (Table 1) of published information relating to cell turnover in the lungs of rats and mice. This summary represents data from Bowden's laboratory and from other sources.[8-13] The results summarized in Table 1 are for nonmigratory cells and macrophages. Because alveolar macrophages are migratory cells, their turnover times are more properly interpreted as times for transit through the tissues of the respiratory tract. The presence of particles in the lung may influence the numbers and residence times of alveolar macrophages in the lungs.[14-16] Thus, caution has to be exercised when interpreting results of studies attempting to define the turnover times for pulmonary alveolar macrophages.

Table 1
Summary of Experimentally Determined Turnover Times for Selected Cells of Respiratory Tracts of Rats and Mice

Tissue	Turnover time (days)	
	Rat	Mouse
Tracheal epithelium	7—48	2—20
Bronchial epithelium	8—27	2—21
Bronchiolar epithelium	—	10—59
Alveolar epithelium	29	28—35
Alveolar macrophage	8—35	6—21

Data from References 7 to 13.

Another migratory cell important to normal lung function is the polymorphonuclear leukocyte (PMN). This cell accumulates in significant numbers in the lung in response to inflammatory stimuli. Some inhalation exposures result in the influx of PMN into alveoli, and PMN have been clearly implicated in physical translocation of particles.[17] Thus, when evaluating turnover times of pulmonary phagocytic cells in the lung, it is important to include PMNs.

It is clear from reviewing the literature on cell turnover studies that considerable resources need to be committed to research on cell kinetics of respiratory tract tissues. This area represents an important key to understanding retention and clearance of inhaled materials. Understanding species similarities and differences for cell turnover rates in the respiratory tract, especially for phagocytic cells, is vital to a complete understanding of the bases for species similarities and differences in long-term retention of inhaled particles.

III. DEPOSITION PATTERNS FOR INHALED PARTICLES

A. General

Deposition refers to the amount of inhaled material that deposits in the respiratory tract during an inhalation exposure. Deposition can occur during any part of the respiratory cycle. Therefore, particles not deposited during inspiration are available for deposition during exhalation.

Deposition is defined in two general ways — total deposition and regional deposition. The total amount of inhaled material deposited in the combined N-P, T-B, and P regions of the respiratory tract constitutes "total deposition". It is expressed as the ratio of the amount of material retained in the respiratory tract immediately after an inhalation exposure to the amount of material inhaled. Total deposition can range between 0 and 100% of the amount of material inhaled. However, deposition is rarely 100% because some portion of the inhaled material generally remains suspended in the air and is exhaled. Figure 2 represents predicted deposition fractions, as a function of particle size, for the three regions of the respiratory tract of nosebreathing humans. Similar deposition patterns exist for other species, with differences related to respiratory patterns and anatomical factors, as discussed later.

Regional deposition defines the initial patterns of deposition in terms of relative amounts of deposited materials present in each of the three functional regions of the respiratory tract immediately after an inhalation exposure. The deposition for each region can range between 0 and 100% and the sum for deposition in the N-P, T-B, and P regions is defined as 100%. Numerical values for regional deposition are useful for interpreting relationships between particle size and deposition, defining tissues and organs potentially at risk from inhaled materials, and evaluating species similarities and differences with respect to initial deposition patterns for inhaled materials.

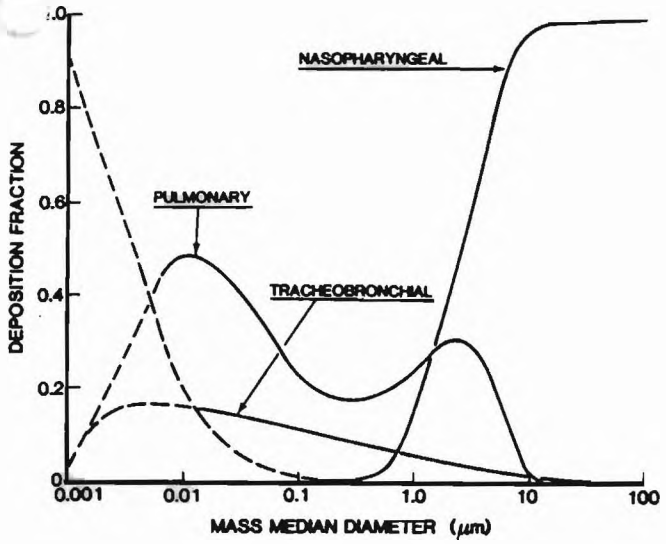

FIGURE 2. Regional deposition fractions for aerosols inhaled by humans. Solid lines are estimates based on Reference 19; dashed lines are estimates based on Reference 18. This figure presents generalizations for regional deposition patterns in all mammalian species.

Total and regional deposition are both dependent upon the particle size of the inhaled materials and upon the species. The relationships among particle size, total deposition, and regional deposition in the respiratory tract have been described in detail, with considerable discussion devoted to species similarities and differences for deposition in the N-P, T-B, and P regions of the respiratory tract. The reader is referred to References 19 to 32 as definitive reviews and summaries of the information available on this topic.

A broad range of particle sizes and types can be encountered in the home, workplace, and environment. Sizes of aerosol constituents can range from molecular dimensions to tens of micrometers in diameter. The probability of deposition of inhaled particles in the respiratory tract depends on the physical characteristics of the particles, the most important characteristics being aerodynamic equivalent size for micron-sized particles and diffusion equivalent diameter for ultrafine particles.[19,20] The site of deposition is influenced by size of the particles; anatomical features of the respiratory tract, such as diameters and lengths of airways; and breathing patterns. As a generalization, particles larger than 10 μm have a very small probability of passing the N-P region in any mammalian species that breathes through its nose. Mouthbreathing humans can deposit particles 10 μm and larger in their lungs, but most laboratory animals species are obligatory nosebreathers and cannot.

In many cases, an inhaled material may be irritating to the respiratory tract or may have unusual physical characteristics that influence its deposition. While generalizations about deposition can be made for most aerosols, caution has to be exercised for others, and it may be prudent to determine the deposition patterns for each material experimentally.

B. Factors That Modify Deposition

Most deposition data for laboratory animals come from inhalation studies in which no control of respiratory parameters was attempted. Control of respiratory parameters in animals is possible, but requires unusual conditions, such as controlled levels of sedation or the use of mechanically assisted ventilation. In contrast, it is possible to train human subjects and thus conduct inhalation exposures using carefully controlled breathing patterns. This leads to more uniformity in experimental results and allows better evaluation of inter-subject variability in deposition.

Breathing rate and tidal volume determine the flow patterns and volume of aerosol inhaled per respiratory cycle and per unit of time and also determine the relative amounts of inhaled material available for deposition in the N-P, T-B, and P regions of the respiratory tract. Table 2 presents a summary of the significant respiratory parameters for selected laboratory animals and humans, along with body weights and lung weights. There are clear differences among these species in size and relative respiratory minute volumes. Smaller animals have higher minute respiratory volumes per unit of body weight to supply their relatively larger requirements for oxygen.[33,34] The larger minute volume per unit body weight in small animals means that they inhale larger amounts of aerosol per unit body weight compared with larger animals or with humans. Thus, respiratory tract ventilation rate is an important factor contributing to the different lung burdens achieved by different species exposed to the same aerosol.

A factor that markedly influences deposition patterns is the mode of breathing. During nosebreathing, deposition of inhaled particles in the head airways can be an effective way for the respiratory system to preferentially filter large and very small particles out of the inspired air. With mouthbreathing, a potentially larger regional deposition of inhaled particles can occur in the P region. The relationships among particle size and pulmonary deposition have not been clearly established for mouthbreathing humans. Svartengren et al.[35] conducted a study using 24 nonsmoking, male human volunteers to determine the deposition efficiency for mouthbreathing of large ^{111}In-labeled Teflon particles. Alveolar regional deposition averaged 15% for 8.2-μm particles, 4% for 11.5- and 13.7-μm particles, and 1% for 16.4-μm particles. Few of these particles would have reached the pulmonary region in nosebreathing humans.

Snipes et al.[36] exposed rats and guinea pigs to 3-, 9-, or 15-μm polystyrene latex microspheres. The 9- and 15-μm microspheres did not reach the pulmonary region in either species. Comparable results might be expected for most or all mammalian species exposed to these particle sizes during nosebreathing.

Table 2
Body Weights, Lung Weights, and Respiratory Parameters[a] for Selected Laboratory Animal Species and Humans

Species	Body weight (kg)	Lung weight (g)	Respiratory minute volume (l)	Respiratory minute volume per kg (l/min/kg)
Mouse	0.03	0.20	0.04	1.30
Rat	0.25	1.5	0.20	0.80
Guinea pig	0.70	4.0	0.46	0.66
Rabbit	2.4	9.1	0.62	0.26
Monkey	2.4	22	0.70	0.29
Cat	3.7	20	0.96	0.26
Dog	10	110	3.6	0.36
Human[b]	70	1000	20	0.29

[a] Approximations of body weight, minute volume, and lung weight for laboratory animals were adapted from Phalen[2] and the cumulative published and unpublished data from the Lovelace Inhalation Toxicology Research Institute. These approximations were assumed reasonable for comparative purposes, but may vary under different conditions of activity, sedation, anesthesia, or confinement.

[b] Adapted from References 32 and 236 (adult man, "light activity").

Changes in minute volume can be expected to result in proportional changes in the amount of aerosol entering the respiratory system. An example of the effects of altered respiratory parameters on deposition can be seen with anesthetized animals. Hamsters under general anesthesia inhaled and deposited less of an aerosol composed of insoluble particles having an activity median aerodynamic diameter of 0.45 μm than did unanesthetized hamsters.[37] The anesthesia reduced respiratory minute volume and thereby reduced the amount of aerosol drawn into the respiratory tract during the exposure.

If increased respiratory minute volumes are induced, the effect is to draw more aerosol into the respiratory tract and increase deposition. This was demonstrated in one study where the authors used CO_2 to cause the experimental animals to breathe more deeply than normal, thereby increasing aerosol intake and deposition.[38]

Respiratory parameters are a major source of variability. Lippmann[39] reviewed the literature available for human regional deposition and concluded that a considerable amount of inter-subject variability exists for deposition fraction, as a function of inhaled particle size, in all regions of the respiratory tract. Most of the variability was ascribed to variations in tidal volumes, flow rates, and functional residual capacity. The respiratory pattern can markedly influence regional deposition. Shallow, rapid breathing or deep, slow breathing can produce the same respiratory minute volume. However, the shallow, rapid breathing (small tidal volume) results in increased deposition in the N-P and T-B regions and decreased deposition in the P region. Slow, deep breathing results in a larger fraction of the inhaled volume reaching the P region, and thereby enhances deposition in the P region. Increased flow rates through respiratory airways cause increased deposition due to impaction, especially at locations where sharp angles are encountered by the inhaled particles. Small functional residual capacity allows relatively more inhaled air to mix within the pulmonary air spaces, thereby causing increased deposition in the P region; the opposite occurs with large functional residual capacity.

Inhalation of irritant substances can alter deposition patterns by changing the activity level of the subject. To some extent, this might reflect responses to physiological stress. Alarie[40] discussed this subject in detail and described ways to measure and compare the irritating properties of inhaled materials.

Bruce et al.[41] reported effects of 10 ppm ozone exposure on respiratory parameters, metabolic rate, and rectal temperature in mice. Soon after initiation of the 90-min exposures to ozone, these parameters all decreased, and the decreased respiratory parameters would have resulted in decreased deposition of aerosols inhaled along with the ozone. In a similar study, Silver at al.[42] exposed mice by inhalation to acrylate esters, which are respiratory tract irritants. Minute volume quickly dropped to one third of normal resting values and body temperature decreased. Chang et al.[43] observed depressed minute volume in rats and mice exposed to formaldehyde; Medinsky et al.[44] noted the same result for methyl bromide inhalation by rats. These changes in respiratory parameters would have altered deposition of particles inhaled during the exposures to the irritant substances.

A number of studies relating inhalation of irritant substances or stimulants to deposition have been reported in recent years. An important result of these studies is that they have provided a data base demonstrating that deposition patterns are sensitive to factors that change respiratory patterns. Knowing this, investigators can use inhalation exposures to specific materials to enhance or decrease deposition of other materials in specific large regions of the respiratory tract. In addition, the information from these studies is useful for helping to predict deposition patterns for simultaneous exposures to irritants and particles.

In addition to variability caused by respiratory parameters, some variability was ascribed to genetically related differences in airway and airspace morphometry. Deposition may therefore not be constant even for the same subject measured at different times, and any factor that influences anatomy or causes respiratory parameters to change with time will influence respiratory tract deposition patterns.

C. Intra- and Inter-Species Variability

There is considerable variability in deposition of inhaled particles within and among species. Literature sources of specific data on species similarities and differences in deposition patterns for inhaled particles include References 23, 26, 29, 32, 39, and 45 to 49.

Based on deposition studies with humans, in which respiratory parameters could be controlled and the main variables influencing deposition could be limited to anatomical differences, Heyder et al.[50] concluded that differences in airway dimensions are likely to be the primary factors responsible for inter-individual variability in deposition within a species. Airway length and diameter are important factors because they influence deposition due to sedimentation of diffusion; the time required for particle deposition to occur by both mechanisms is proportional to the distance the particle must travel. Airway length can also influence local deposition patterns in the lung. Particles that have a short path length to travel between the trachea and terminal bronchioles have a higher probability of being deposited in the P region. This may be the basis for the higher relative deposition generally observed in the apical lung lobes of experimental animals. While Heyder's data came from human deposition studies, the same degree of inter-individual variability may exist in other species.

An important consideration in evaluating tissue exposure patterns for inhaled materials and in relating the exposure patterns to biological responses is that the variability among subjects may be large. Within a population of exposed individuals, the average exposure will be representative of that occurring to only a small portion of the population. If only the highest exposures produce biological responses, study results may be incorrectly interpreted. Cuddihy et al.[51] discussed this topic in detail and presented results for Beagle dogs and humans. The authors considered inter-subject variability for lung weight, deposition, and uptake or retention of an inhaled test material. Figure 3 demonstrates the relative distribution of doses to the lung or other internal organs for Beagle dogs or humans exposed to the same test material. The distribution of doses was approximately log-normal and was typical of what might be observed for exposures to a variety of respirable materials. A small fraction of the exposed population would receive a dose that would be much less than the average. It is significant that about 2% of the population would receive more than three times the arithmetic average dose for the group. Cuddihy et al.[51] concluded that, because of the variability in exposures among individuals exposed to the same material, it would be prudent (1) to allow a factor of 10 to account for the low-to-high exposure in a group of subjects, and (2) to quantitate individual exposures whenever possible to reduce the uncertainties associated with making judgments about the toxicity of test substances. The same considerations apply to groups of animals exposed to a test aerosol to determine deposition patterns. Within the population of test animals, there will be sufficient variability so that, if possible, each individual should be evaluated separately to determine its deposition patterns.

Estimates for amounts of deposited material are normally made for each animal individually if the animals are exposed one at a time. These estimates can be made by using several methods or combinations of methods, including external monitors, aerosol particle counters, and information about air concentrations of the exposure material, minute respiratory volumes of the subject, and exposure duration. If the exposure material contains a label, e.g., a radiolabel, the amount of material deposited may be determined during the exposure by using a collimated chest monitor or immediately after the exposure by counting the subjects in a suitable counting system. In many studies, the exposure material cannot be readily measured, and assumptions must be made about the amounts of the material inhaled and deposited. In such studies, when a group of animals is simultaneously exposed to the same aerosol, the assumption is made that all of the animals received the same average exposure and have the same amount of exposure material deposited internally.

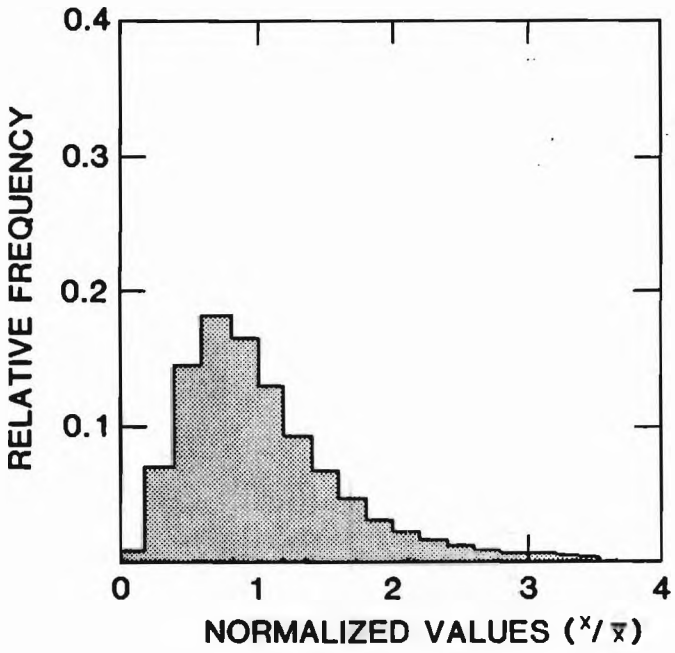

FIGURE 3. Projected internal organ dose distribution for Beagle dogs exposed to inhaled aerosols. Normalized values are the observed values divided by the group average. (Adapted from Cuddihy, R. G., McClellan, R. O., and Griffith, W. C., *Toxicol. Appl. Pharmacol.*, 49, 179, 1979. With permission.)

Deposition variability is not the same for single and repeated exposures. Single, short-term exposures of groups of animals result in a wide range of internal burdens of inhaled material. Repeated exposures tend to reduce the variability in deposition among subjects. As one example, Lundgren et al.[52] exposed Syrian hamsters once (n = 311) or seven times (n = 217) to aerosols of $^{144}CeO_2$. The coefficients of variation for lung deposition of the test material were 32 and 21%, respectively, for the single and repeated exposures. This result, where variability associated with the accumulated lung burdens exists for any single exposure but the repeated exposures provide more uniformity by averaging the results for each individual exposure, may be typical for inhalation exposures.

Variability in human inhalation studies can be reduced by conducting the study under rigidly controlled conditions. Aerosols, breathing mode, and respiratory patterns can be carefully monitored and controlled. With laboratory animals, the experimental conditions cannot be as carefully controlled. It is relatively easy to control aerosol concentrations and the chemical and physical attributes of exposure materials during animal inhalation exposures, but it is not as easy to control the respiratory parameters of the animals.

Another factor relevant to comparisons between single and repeated exposures is physiological stress, a poorly defined concept relating the physiological status of a human or animal to its environmental conditions. In essence, almost any environmental change will influence body functions of the experimental subject and thus influence the experimental results of the study. Placing experimental subjects in aerosol exposure systems may cause altered respiratory patterns and hyperactivity. Animal handling or animals' reactions to irritant or toxic aerosols may therefore influence deposition patterns and be an important source of variability among species. Conditioning to handling and to the exposure environment prior to actual aerosol exposures may reduce the magnitude of the animal's physiological responses. The adaptation inherent in a series of repeated exposures, combined with the averaging effect on deposition resulting from several individual exposures, tends to bring about more uniformity in respiratory tract deposition for repeated exposures compared with single exposures.

IV. DISPOSITION OF PARTICLES AFTER DEPOSITION IN THE PULMONARY REGION

A. General

When particles are inhaled and deposited in the P region, the initial alveolar deposition appears to be relatively random. However, several studies have indicated that this is not so. Because of the physical forces that influence particle deposition patterns, larger amounts of inhaled material are generally deposited near bifurcations and the entrances to alveolar ducts.[53-56] Therefore, even from the time of initial deposition, the distribution of particles in the lung is relatively nonuniform. These initial deposition patterns may vary substantially among species and influence subsequent distribution and clearance patterns, as well as biological responses to the deposited materials.

After deposition in the pulmonary region, particles are subjected to processes that seem to be similar among all mammalian species. The particles are rapidly phagocytized by pulmonary alveolar macrophages or other phagocytic cells.[17,57-77] Chemotactic responses are one mechanism for attracting phagocytic cells to particles deposited in the respiratory tract. If exposure levels are high, or the exposure material produces an inflammatory response, large numbers of PMNs appear in the lung and can also phagocytize particles.

The fate of specific phagocytic cells after they ingest particles can vary, depending on the physical/chemical nature of the material ingested. Some particles may be toxic to phagocytic cells; cells that ingest toxic particles may die and become debris for other phagocytes to ingest. Migration and grouping of phagocytes containing particles can lead to redistribution of the deposited particles. Understanding the interactions between phagocytes and particles is important to elucidating the mechanism(s) involved in particle retention and clearance. Also, the functional abilities, longevity, and migration and retention patterns for alveolar macrophages may be different among species and may represent the basis for differences among species in retention and clearance of inhaled materials.

There are indications that particles deposited in lung are not retained in a static mode; the particles appear to redistribute over time due to dynamic processes occurring in the lung tissue. Migration and grouping of particle-laden cells can lead to redistribution of particles in the lung and a continuously changing exposure or injury pattern that may be different among animal species and humans. There are also indications of different clearance rates from different alveolar regions within the same lung that may contribute to variation within and among species for pulmonary retention of particles.[55]

Mechanisms responsible for long-term retention of particles or their constituents after deposition in the respiratory tract are probably common to all mammalian species, including humans. Physiological conditions unique to the species, such as cell kinetics and metabolic rates, could result in differences among species in retention and clearance of materials deposited in the lung.

B. Pulmonary Alveolar Macrophages

Pulmonary alveolar macrophages are an important cell type involved in retention and accumulation of particles in the lung, as well as in clearance of particles from the lung. These are relatively large, nucleated phagocytic cells that appear to move freely in the lung. Three types of macrophages are recognized in the lung: (1) alveolar, (2) interstitial, and (3) airway.[71] These types of macrophages appear to have the same origin and are only different with regard to function. Defining macrophages in this way provides recognition of some mode(s) of communication with and among phagocytic cells in the lung that influences their dispersion patterns and function.

The movement of macrophages is like that of amoebae, they can change their shape and use diapedesis to move through small openings between cells and within spaces in lung tissue. The ability of macrophages to migrate through lung tissue or along airways improves their chances to encounter and phagocytize dead cells, inhaled particles, bacteria, and other foreign materials. Phagocytized materials can accumulate in macrophages in substantial amounts. The process of phagocytosis is a means of isolating the material in a way that makes it possible to transport the material out of the system, digest the material,

dissolve it and make it available for clearance via the dissolution-absorption process.

The numbers of macrophages in lungs of different animal species and humans have not been clearly determined. In one study, Blusse van Oud Alblas and van Furth[78] demonstrated that the total pulmonary macrophage population in SPF Swiss mice was about 2 million, of which 93% were pulmonary alveolar macrophages and about 7% were pulmonary interstitial macrophages. Macrophages normally comprise about 3% of the total alveolar cells in healthy, nonsmoking humans; a similar fraction is present in other mammalian species.[79]

The numbers of macrophages that can be washed from the lung by lavage is also quite similar among mammalian species. Brain[80] reported that the combined yields of 12 washes of the lungs of dogs, rabbits, guinea pigs, hamsters, rats, and cats provided averages of 3 to 15 million cells per gram of lung. This suggests that many mammalian species have about the same relative numbers of macrophages. While macrophages are always present in the lung, the actual number of macrophages present at any given time appears to be influenced by the presence of other phagocytes, foreign materials, or cell debris. Major influencing factors appear to be the numbers[57,81,82] and the types of particles deposited in the lungs. Small numbers of deposited particles do not appear to cause an increase in macrophage numbers, but when particle numbers are increased, macrophage numbers increase to maximum values.[71,84-87] Initially, this increase in cells is due to recruitment of blood monocytes, whose maturation is associated with migration through the pulmonary interstitium into the alveoli. However, as more and more particles deposit in the lung, the recruitment of macrophages may be supplemented by proliferation of mononuclear cells in the pulmonary interstitium.

An increase in pulmonary macrophages appears to be a generalized response to instilled[84] or inhaled particles, but the magnitude of the response depends upon particle composition,[85] number, and mass. However, the magnitude of the increase appears to be related more to the number of particles than to the total mass of particles. Therefore, equivalent masses of the same material may not produce the same macrophage response if the two masses are associated with particles having different physical sizes.[88]

Bingham et al.[89] exposed rats by inhalation to 2 mg coal dust per cubic meter for up to 4 months. The numbers of macrophages that could be lavaged from the rats were not elevated. The authors suggested that the results of most earlier studies demonstrating increased numbers of macrophages were influenced by the intratracheal instillation procedures used to produce the lung burdens. In a similar study by Castranova et al.,[90] rats were exposed by inhalation to coal dust and diesel exhaust, alone or in combination, for 2 years at air concentrations of 2 mg/m^3. None of the exposures affected macrophage viability. Exposures to diesel exhaust had no effect on numbers of macrophages recoverable from the lung by lavage, whereas the coal dust exposures did cause an elevation in the numbers of macrophages. The particle size of the coal dust was larger than that of the diesel exhaust particles, so there would have been a substantially larger number of diesel exhaust particles deposited in the lungs of these rats for the same mass of particles inhaled. If the number of particles was the most important parameter relative to changes in the macrophage response, then a larger change should have been associated with the diesel exhaust exposures than with the coal dust exposures. The opposite was observed, suggesting that size of inhaled particles is more important than numbers of particles in eliciting recruitment of macrophages. These results emphasize the points that experimental findings can be heavily influenced by exposure procedures and that biological responses are difficult to extrapolate from one material to another. The responses are clearly associated with factors that include numbers and composition of particles.

It is also possible that some inhaled particles cause a decrease in production of phagocytic cells or that there may be an inadequate number of phagocytic cells present to effectively transport the deposited particles away from the lung.[91] This may be a major factor in the ability of particles to penetrate the pulmonary epithelium and become incorporated into constituents of the pulmonary interstitium.

Phagocytic cells can engulf a broad range of particle types and sizes ranging up to tens of micrometers in diameter. The rate and efficiency of phagocytosis appear to depend on properties of the deposited particles that include size, chemical composition, shape, mass, and degree of opsonization. In one study with spherical aluminosilicate particles in the size range 0.3 to 2.2 μm, macrophages phagocytized larger particles more rapidly and in greater numbers than they phagocytized smaller particles; the investigators suggested that 2-μm particles of this type were near the optimum size for phagocytosis by pulmonary alveolar macrophages.[92] In an earlier *in vitro* study, fewer 6-μm particles were phagocytized than 3- or 1.5-μm particles.[93] Results of these two studies suggest that the optimum size of particles for phagocytosis by pulmonary alveolar macrophages may be in the range of 1.5- to 3-μm geometric diameter.

The physical attributes and chemical composition of particle surfaces appear to be important for the rate of phagocytosis. Lundborg and Holma[94] compared rates of phagocytosis for fungal spores and polystyrene particles having about the same physical size. The *in vitro* rate of phagocytosis for the fungal spores was greater than for the polystyrene particles. Camner et al.[63,64] and Camner and Lundborg[95] found differences *in vitro* in the ability of alveolar macrophages to phagocytize 4-μm Teflon particles coated with carbon or with metals. For example, particles coated with carbon or aluminum were phagocytized at a faster rate than particles coated with silver. These differences were seen clearly only when autologous serum was used, suggesting that the differences may have been related to the presence of serum constituents that helped in some way

to mark the particles for phagocytosis. An interesting observation was that clearance rates for these particles were not affected for 8 d following inhalation, in spite of the differences in rates of phagocytosis of these particles *in vitro*.

It is clear from several *in vitro* and *in vivo* studies that alveolar macrophages can ingest large particles. However, it is not clear what the maximum size of a transportable particle is. In addition to particle size, particle shape may prohibit its transport. For example, some fibers may be ingested by macrophages, but the fibers may alter the mobility of the macrophages, making them immobile. Asbestos, for example, may be cleared slowly because of its fibrous shape. An alternate possibility, however, is that asbestos fibers induce biochemical changes that are toxic to phagocytic cells attempting to transport the fibers.[96,97]

After an alveolar macrophage has ingested a particle, the particle appears to remain with the macrophage as long as the macrophage is viable. The particles are subjected to dissolution and digestion processes within the host macrophage. If this macrophage dies, its debris is ingested by another macrophage, along with the particle or particles contained within the debris. Macrophages may remain stationary in the lung, may migrate within the lung (and ingest additional particles as they migrate), or may exit from the lung. Particles that move into the interstitium and remain there for long periods of time appear to find their way into subpleural and paraseptal positions, and into perivascular positions around pulmonary arterioles and venules associated with bronchioles. The peribronchiolar sites appear to be prominent, long-term storage sites for particles retained in the lung.[62]

The pulmonary retention sites and cellular constituents of the pulmonary region involved with long-term retention can be readily seen in animals exposed to large amounts of particulate materials. Large aggregates of particle-laden macrophages have been observed in alveoli near terminal bronchioles and within alveolar and peribronchial interstitial tissues in rats exposed repeatedly to diluted diesel exhaust.[98-111] These aggregates of diesel soot were associated with focal inflammation, Type 2 pneumonocyte proliferation, and fibrosis, depending on the diesel soot exposure concentration and length of exposure. Similar responses were seen in rats exposed repeatedly to TiO_2[112] and to fly ash.[113,114]

These observations on particle accumulations in lung tissue were not limited to small animal species. MacFarland et al.[115] repeatedly exposed cynomolgus monkeys, 23.5 h/d for 18 months, to 0.16 or 0.46 mg fly ash per cubic meter. The authors noted accumulations of the particles in alveolar macrophages, adjacent walls, and peribronchiolar lymphoid follicles. In a later study, MacFarland et al.[116] exposed rats and monkeys to respirable dusts of raw and processed shale and noted accumulations of pigment and particle-laden macrophages in lung tissues of both species.

The retention sites for particles retained in the pulmonary region appear to be the same for small and large quantities of most types of particles. Accumulations of particle-laden macrophages can cause damage to epithelial barriers and contribute to progressive lung disease by releasing enzymes and other chemicals.[117]

Considerable work is being done to understand the origin, longevity, function, and fate of pulmonary alveolar macrophages. There is a critical need for further work, with emphasis on species similarities and differences because pulmonary macrophages have such important roles in retention and clearance of inhaled materials. To speculate, differences in longevity and function of pulmonary macrophages may be the basis for most or all of the differences in retention and clearance of particles observed among laboratory animal species and humans.

C. Type 1 and Type 2 Cells

After inhalation exposures, Type 1 cells contain foreign particles, presumably for long periods of time. However, it may be that the particles were in transit through the Type 1 epithelium. Sanders and Adee[61] noted that after inhalation of plutonium dioxide by rats plutonium particles were engulfed by alveolar macrophages and Type 1 alveolar epithelial cells. They did not determine whether or not the Type 1 cell represented a retention site for the particles. In addition, Barnhart et al.[101] noted the presence of diesel exhaust particles in Type 1 cells of rats. Adamson and Bowden[84] observed the presence of carbon particles in the cytoplasm of Type 1 pneumonocytes and in cells of the pulmonary interstitium, including interstitial macrophages. Brody et al.[118] and Sorokin and Brain[55] found inhaled particles within pulmonary alveolar macrophages, Type 1 pneumonocytes, and in interstitial locations within a few hours after exposure.

Kyono et al.[119] studied localization patterns for lead particles in the lungs of rats after inhalation exposures to a lead fume having an activity median diameter of 1.1 mm. They noted the usual accumulations of lead in alveolar macrophages, but there were also lead particles in Type 1 and Type 2 pneumonocytes. For every type of cell containing lead particles, phagosomes and lysosomes were the main site of retention within the cell.

These observations of particles in Type 1 and Type 2 pneumonocytes have all been from studies with rodents. It is not clear if this is a species-related phenomenon or if similar results pertain to other species, including humans.

D. Interstitium

While phagocytosis and transport of particles in macrophages and other phagocytic cells appear to be the dominant methods of trapping particles in lung tissue, some particles may directly enter the alveolar interstitium by endocytosis, or by passing between cells lining the interstitium. Phagocytosis of particles appears to occur within a few hours of their deposition in the lung. During the time the particles are uningested,

; have the potential to penetrate into the interstitium. Whether or not there is penetration may depend upon the integrity of the alveolar lining cells. In addition, there have been several reports indicating that both the ability to penetrate and the probability of penetrating the pulmonary epithelium depends on the amount of material deposited, or the mass loading.[55,120,121] The transepithelial passage of free particles may be related to the number of alveolar macrophages available to ingest the deposited particles; with an increased particle load, a level exceeding the saturation point for increasing macrophage numbers may be reached.[67,84,85,122,123] In such a situation, the probability of particle penetration may increase.

Transepithelial transport of fine particles has been demonstrated for a variety of materials including carbon, latex, bacteria, silica, and asbestos.[85,120,124,125] The higher the number of particles, the greater the possibility that some of them will reach the interstitium where they may be phagocytized by interstitial macrophages.[85]

Bowden[16] provided a descriptive summary of the role of the macrophage in keeping the lung free of foreign particles and of the consequences, particularly as they would affect the pulmonary interstitium, of perturbing this defense system. Adamson and Bowden[122] irradiated mice to deplete monocytes and reduce the ability of the mice to produce macrophages. When the lung was then intratracheally instilled with carbon ticles, it was unable to recruit adequate numbers of macphages to respond to the challenge. This resulted in a decreased capacity for phagocytosis and the number of free carbon particles in the alveoli increased. The likelihood that some of the carbon particles would cross the alveolar epithelium and accumulate in interstitial macrophages increased, and an increased number of carbon particles translocated to lung-associated lymph nodes, suggesting an association between the accumulation of particles in the interstitium and translocation of particles to the lung-associated lymph nodes. Similar results might be seen if the ability of the lung to provide sufficient numbers of phagocytes to respond to the particle load was overloaded, or if there was an inadequate cellular response to a limited number of particles.

Although particles may enter the pulmonary interstitium by direct passage across the cytoplasm of the Type I pneumonocyte cell,[67] the possibility of macrophage transport of particles from the alveolus to the interstitium exists. One recent study concluded that alveolar macrophages can return to the pulmonary interstitium and transport phagocytized particles to lung-associated lymph nodes.[72] This issue deserves additional attention, and resolving it will significantly advance our understanding of the mechanisms involved in particle transport within lung tissue and in retention of particles in specific pulmonary tissues.

Particle Redistribution

The initial dispersion of deposited particles in the lung changes with time. After clearance of a portion of the deposited particles, the distribution of the remaining particles becomes less uniform. Changes in retention and distribution patterns for inhaled particles can have important implications for the subsequent biological effects of inhalation exposures. Particle movement in the lung will cause a changing pattern of cells at risk. Particle clumping could increase the risk of damage to cells proximate to the clumped particles (if the particles are toxic), it could reduce the dissolution-absorption rate, and it could decrease the availability of the particles for physical clearance. In some cases, the long-term retention and clearance patterns of the particles might be markedly influenced by particle redistribution and clumping. Thus, the phenomena associated with redistribution and clumping need to be addressed to help understand the long-term fate of particles deposited in the pulmonary region, and to determine if the temporal changes are species-dependent.

In one study addressing this issue, Diel et al.[126] evaluated distribution patterns for monodisperse $^{238}PuO_2$ particles inhaled by Syrian hamsters. Particle movement was not measured directly, rather it was inferred from the relative distributions of the $^{238}PuO_2$ particles in lung tissue. Temporal distribution of the particles with respect to specific anatomic structures in the lung was measured. These measurements were used to calculate the distributions of particles relative to their distance from the lung periphery, airways, foci of damage, and other particles in the lung. The inhaled particles deposited preferentially in the central regions of the lung. With time, the particles appeared to migrate toward the periphery and were cleared from all regions of the lung with equal efficiency, unless the lung was damaged as a result of the presence of the radioactive particles. Migration of particles over time resulted in a less uniform distribution in the lung. These results were confirmed in a study of rat lungs after inhalation of $^{239}PuO_2$.[127]

In a comparative study using hamsters and rats, Rhoads et al.[128] evaluated $^{239}PuO_2$ distribution patterns in lung tissue after inhalation exposure. Again, changes in lung distribution of the $^{239}PuO_2$ particles were noted over the course of the study. In both species, the relative concentration of particles in the subpleural region increased compared with the concentration in the parenchymal region. There was particle clumping in both species, but particles in the lungs of the rats tended to have a greater degree of clumping with time than those in guinea pig lungs. The relative particle concentration in the peribronchial region of the rats was low initially, then increased with time; the pattern showed no temporal trend in hamsters. This difference in patterns for clearance and redistribution of particles near small airways represents a species difference in the long-term retention patterns for inhaled particles.

Although the previous discussion involved radioactive particles, particle movement and redistribution in the lung appears to be similar for all types of particles. Both inert and toxic particles move toward the peribronchial and perivascular con-

nective tissues.[84,124,125] The process appears to be common to all mammalian species, but that has not been clearly demonstrated. Even if the process is common to humans and other mammalian species, differences in rates and degree of particle redistribution and particle clumping could still result in species differences in retention patterns and biological responses to retained particles.

V. PULMONARY LYMPHATIC SYSTEM

Leak[129] provided an excellent review of the pulmonary lymphatic system and its function. While the basic components of the system appear to be common to all mammalian species, there are significant quantitative differences among species that probably influence the functional effectiveness of their lymphatic transport systems. Leak and Jamuar[130] summarized known similarities and differences in the distribution of lymphatic vessels in humans and several animal species. The only substantive differences noted are that humans and large animal species have thick pleura with many interlobular septa that form an extensive network of lymphatic vessels and collecting vessels throughout the connective tissue. The pleura is generally thin in small animals and there is a sparse distribution of lymphatic vessels within the pleura.

Lymphoid tissue has been recognized as a constituent of lung tissue for over 100 years. Some of the lymphoid tissues are easily seen because they are external to the lung and are generally found in the mediastinum or close to the tracheobronchial bifurcation. These lymph nodes are generally referred to as tracheobronchial lymph nodes (TBLN) or lung-associated lymph nodes (LALN). The lymph nodes physically separated from lung parenchyma have been studied extensively and are recognized as structures important to lung defenses and systemic immune responses. Lymphatic tissue integrally associated with the lung interstitium has been recognized and described morphologically, but its role in lung function has not been clearly defined. Lymphoid tissue enclosed within the lung is described as "bronchus-associated lymphoid tissue" (BALT).

Brundelet[131] included a review of experimental results from studies and observations about BALT in his excellent review of the dust clearance mechanisms in the lung. Studies dating from the 1880s included observations and discussions about the possible role of BALT in the lung. Chamberlain et al.[132] and Bienenstock et al.[133,134] extended observations of earlier investigators and summarized contemporary conclusions relative to the function of BALT in lung tissue. Chamberlain et al.[132] described the ultrastructure of BALT in rat lungs as being similar to that of a lymph node, composed predominently of lymphocytes. Cytokinetic studies indicate that both locally derived, replicating cells and recirculating cells form the pool of thymus-derived lymphocytes that constitute BALT.[135] As described by Chamberlain et al.,[132] the following are significant differences between BALT and lymph nodes: (1) BALT is always within the lamina propria; (2) BALT does not have clearly defined borders; (3) BALT does not have organized capsules; and (4) BALT has no marginal lymphatic sinus. The BALT forms aggregates in the walls of bronchi and bronchioles, interstitial connective tissue, and pleural connective tissue. The most prominent aggregates of BALT are associated with bronchi and bronchioles. Aggregates of BALT range from small collections of mononuclear cells limited to the lamina propria of airways, to large nodules that extend through the muscularis and merge into the peribronchial and peribronchiolar connective tissue or the walls of adjacent alveoli. In every case, the nodules appear to be in direct contact with the bronchial or bronchiolar epithelium. All classes of blood vessels can be seen within the BALT; they do not exhibit any constant or specific pattern or show any unusual ultrastructural characteristics. Postcapillary venules identical to those of lymph nodes are associated with the BALT. The lymphoepithelium overlying BALT shows distinct morphologic differences compared with the surrounding epithelium. Epithelium covering the BALT in rats has ciliated and nonciliated cells.[132] In rabbits, the lymphoepithelial cells are flattened and have irregular microvilli, but no cilia.[136]

Bienenstock et al.[133] described morphologic characteristics of BALT in bronchial mucosa of rabbits, Syrian hamsters, humans, mice, and rats. The most extensive observations were reported for rabbits. Additional data were presented by Bienenstock and Johnston[137] and by Gregson et al.[138] The cumulative results from these references are presented in Table 3. No observations of BALT in dogs or monkeys have been reported.

The amount of BALT present in the lung and its function may depend on the environment in which the animal lives. As summarized by Chamberlain et al.[132] and Bienenstock et al.,[135] two main functions have been attributed to BALT. The first function is related to retention of particles in the lung, the second to immune responses. Some particles deposited in the lung are incorporated into BALT and may be retained there indefinitely. BALT might also represent a holding site for particles that are eventually translocated to other lymphatic tissues or excreted onto the mucociliary escalator of the bronchiolar epithelium. Fournier et al.[139] injected 0.8-μm latex particles into the tracheas of rats to determine if any of the particles would be incorporated into BALT. Approximately 700,000 particles were used per rat; none of the latex particles were later found to be associated with BALT. In contrast, ferritin molecules injected into the tracheas of rats were found in BALT. The authors concluded that the relatively small number of latex particles could have influenced the results and that lymphoepithelial nodules are structures capable of uptake and transport of certain kinds of particles across the basement membrane.

In experiments using rats intratracheally instilled with trypan blue and carmin red, Brundelet[131] described the migration

Table 3
Summary of Morphologic Characteristics of Bronchus-Associated Lymphoid Tissue (BALT) for Selected Species

Species	Observations
Rabbit	Follicles often seen immediately underlying the bronchial epithelium and in deeper structures
	Blood vessels not observed inside the follicles, but typically seen bordering BALT on side farthest away from airway lumen
	Majority of BALT cells appear to be lymphocytes
	No plasma cells in the BALT follicles
	No germinal centers observed
	No BALT observed in newborn rabbits
Syrian hamsters	No BALT observed in this species
Humans	Similar to rabbits, but mainly diffuse collections of subepithelial lymphoid tissue in bronchi
Rats	BALT found in all adult rats examined, but less BALT present in germ-free rats
Mice	BALT present in fetal and adult lungs

From References 133, 137, and 138.

pathways of labeled alveolar macrophages into BALT. Some dye-laden macrophages apparently crossed alveolar walls to penetrate directly into the BALT; most of the dye-laden macrophages appeared to penetrate into the connective tissue of the inter-alveolar walls or of the pulmonary septa. Once in the connective tissue septa, the dye-laden macrophages could migrate to a peribronchial lymphoid tissue focus and exit the BALT directly into the bronchial lumina. Similar observations by Green[140] of the transport of coal dust-laden nodules at the alveolobronchiolar junction area of the lung demonstrated that particle-laden macrophages passed through these lymphoid nodules and out onto the ciliated mucosa present at the start of the terminal bronchioles. From this point, the macrophages could be carried out of the lung via the mucociliary escalator.

In his review of alveolobronchiolar transport, Green[62] suggested that BALT may be a key factor in the processes or mechanisms by which liquid and phagocytic cells pass into and out of the pulmonary interstitium. Alveolobronchiolar transport appears to be a means of getting particles from the interstitium to the bronchial epithelium. The BALT was evaluated as a dynamic kind of tissue that could function to absorb or exude liquid and could allow phagocytic cells to pass back and forth between the interstitium and bronchiolar lumen. In essence, the BALT could represent an excretory pathway for particles or phagocytic cells that enter the interstitium via the alveoli and exit onto the bronchial epithelium. Whether or not BALT is involved in particle clearance from the P region is still an unanswered issue that deserves additional attention. BALT could represent an important type of lung tissue with functions relevant to species differences in physical clearance of particles deposited in the P region.

The relationship between BALT and systemic immune responses is more speculative. These lymphoid nodules along transport pathways may provide a site for intrapulmonary antigen processing and antigen production.[62] Experimental evidence suggests that both B and T cells comprise at least part of the cell constituents of BALT.[135] In general, the BALT might be part of a broad mucosal immunologic system, and its presence and function in the lung may not be unique.[135]

VI. CLEARANCE OF PARTICLES FROM THE RESPIRATORY TRACT

A. General

Clearance is defined for the purposes of this paper as removal of debris from the respiratory tract. The debris can include foreign particles, dead or living phagocytic cells, bacteria and other microorganisms, and soluble material. Descriptions of the basic processes and pathways associated with clearance of the respiratory tract are available in the scientific literature. Information from References 19, 20, 24, 30, 31, 56, and 141 through 146 was used to make generalizations about long-term respiratory tract retention and clearance of deposited particles. In addition to understanding the clearance processes that remove materials from the body, it is also important to understand processes and mechanisms associated with translocation of particles or dissolved constituents of the particles to other organs because the respiratory tract can be the portal of entry for toxic agents that have effects on other organs of the body.

Figure 4 depicts proposed pathways by which materials can physically move within and out of the respiratory tract. Transport processes cause particles to be removed from the respiratory tract and excreted in feces or transported to thoracic lymph nodes. Some particles may be absorbed directly into the circulatory system. Another important process in clearance is absorption of dissolved constituents of particles. In addition to complete dissolution of particles, constituents of particles may leach from the surfaces or interiors of the particles, leaving the core particle in its original state, except that it is devoid of specific constituents.

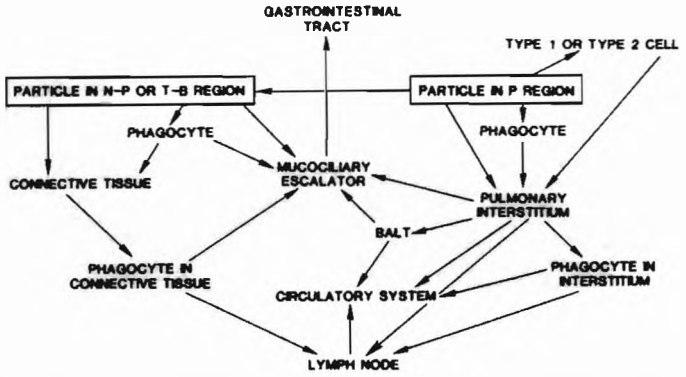

FIGURE 4. Biological fate of particles deposited in the respiratory tracts of mammalian species.

Discussions about clearance of materials from the respiratory tract may focus on biological clearance or on effective clearance. Biological clearance refers to the net result of biological activities within the respiratory tract that act to remove the deposited material, its dissolved constituents, and its metabolic products from the respiratory tract. Effective clearance applies to radioactive materials, where the inhaled material is physically decaying in addition to being cleared by biological processes.

It is important to make the distinction between dissolution and absorption in the respiratory tract. The term dissolution includes the net result of processes in cells and body fluids that cause constituents of particles to dissociate. These processes may involve solubilization of the particles or elution of constituents from the matrix of the particles. In either case, constituents of the particles become available for absorption into the lymphatic or circulatory systems, for metabolism, or for chemical reactions with, or adsorption to, tissue constituents. The solubilized constituents of the particles may remain associated with tissue constituents proximate to the particles, may be translocated and deposited elsewhere in the body, or may be excreted into urine or feces. Absorption refers to transport phenomena that cause a net transfer of material from the respiratory tract to the lymphatic or circulatory systems. The term absorption does not, by itself, imply a mechanism, and the absorbed material may be in ionic, molecular, or particulate form.

Factors that affect the solubilization of particles in physiological fluids and absorption of their constituents are not fully understood. Particle solubility is known to be influenced by the surface-to-volume ratio and other surface properties of particles.[147] The rates at which dissolution and absorption processes occur are influenced by the chemical composition of the particles and by other factors, such as temperature history. The temperature history of particles may be one of the most important considerations for oxides. Usually, in controlled laboratory environments, the solubility of oxides decreases when they are produced at higher temperatures.

Mercer[148] analyzed pulmonary clearance of particles on the basis of particle dissolution rate compared with physical translocation rate. Particle deposits in the lung were assumed to have log-normal size distributions and dissolution was a function of surface area of the particles. The smaller particles of the distribution would dissolve and leave the lung faster than the larger particles. Mercer concluded that (1) for monodisperse particles, if the dissolution rate constant [k] is known for a material, the time required to dissolve half the mass of particles can be calculated; and (2) when the dissolution half-times are much shorter than the half-times associated with the physical translocation of particles, dissolution will dominate clearance characteristics. Therefore, dissolution-absorption can be the dominant factor in clearance, even for materials having intermediate or long dissolution half-times. This is significant for many so-called insoluble particles potentially inhaled by humans. Physical clearance rates for the particles are slow, so even a modest rate of dissolution can make a significant difference in effective clearance rates.

In many cases, it is possible, on the basis of general chemical considerations, to make accurate projections about the dissolution rate for a material in question and also to accurately project its clearance rate from the respiratory tract. However, for other materials, especially those containing multivalent cations or anions, it may not be possible to project their *in vivo* dissolution and absorption rates on the basis of their chemical composition.

Weathering of particles, or allowing them to interact with chemicals, will change their surface characteristics and influence solubility. In an *in vitro* dissolution study with relatively insoluble particles of PuO_2, it was possible to markedly alter the dissolution characteristics of the particles by alternately drying and wetting them.[149] Each wet-dry cycle resulted in slight changes in the surface properties of the particles that, in turn, resulted in significantly altered dissolution characteristics. While this process has no direct implications for particles deposited in the human lung, the results point out the need to recognize that environmental weathering may alter the surface characteristics of materials that later may be aerosolized. The particles may then dissolve in the lung at unusual rates relative to what might be predicted based simply on a physical description of the material.

Phagocytic cells, primarily macrophages, clearly play a role in dissolution-absorption of particles retained in the respiratory tract. Dissolution of some types of particles is believed to occur within the phagosomes due to the acidic milieu in those organelles.[150,151] It seems clear that pH is an important factor in dissolution of particles, particularly for metallic particles. However, difficulties associated with evaluating the factors that influence dissolution-absorption rates for inhaled particles are compounded by the fact that it is difficult to precisely predict or measure pH in locations where particles are retained. Then too, while particles may be more soluble

phagosomes, the dissolved material may not diffuse or be transported away from the dissolution site.

Snipes et al.[152] concluded, from simulation modeling, that mice, rats, and dogs all showed the same *in vivo* dissolution-absorption rates for the same-sized, relatively insoluble, fused aluminosilicate particles. The same result was demonstrated for relatively soluble aerosols of cerium oxalate and cerium chloride inhaled by mice, hamsters, rats, and dogs.[153] A similar result might be expected for other kinds of particles and is an important factor to consider in species comparisons of effective clearance for inhaled materials. Overall, the data suggest that there are no significant differences among laboratory animal species and humans with regard to *in vivo* dissolution rates for any materials deposited in the respiratory tract. Differences in dissolution-absorption, if they exist, appear likely to be associated with the fate of the material after dissolution. In other words, the material may dissolve to the same extent in all species, but differences in local tissue reactions or metabolism may result in retention of the solubilized material or metabolites near the site of deposition or reaction.

In one study related to the issue of species differences in dissolution-absorption, Oberdörster et al.[154] studied clearance of ^{109}Cd from the lungs of rats and monkeys after inhalation of ^{109}Cd-labeled aerosols of $CdCl_2$ and Cd-oxide. The inhaled cadmium was cleared ten times faster from the lungs of the s than from the lungs of monkeys. The Cd was retained very tenaciously in the lungs of the monkeys as a result of binding with tissue constituents, but the tissue binding did not occur in the rats, or occurred to a lesser extent. Bailey et al.[155] are conducting a study that includes an interspecies comparison of the translocation of ^{57}Co from lung to blood after inhalation of ^{57}Co-oxide, the results of which should provide additional information about species similarities and differences with regard to dissolution and absorption of materials deposited in the lung.

There are other reports indicating that presumably soluble constituents of inhaled particles are retained for longer periods of time in the lung than would be predicted on the basis of their solubility. Examples of such inorganic materials are beryllium[156] and americium.[157] The authors' interpretation of the prolonged retention of these materials in the lung was that the particles did dissolve, but their constituents adsorbed to, or chemically reacted with, tissue components. Therefore, the deposited materials, or some form of the materials, were retained near the site where deposition occurred. This same phenomenon occurs with organic materials. For example, covalent binding of benzo(a)pyrene or metabolites to cellular macromolecules resulted in an increased pulmonary retention time for that compound after inhalation exposures of rats.[158] Certain chemical dyes[159] are also retained in the lung, where they may dissolve and become associated with lipids or react with other constituents of lung tissue. Understanding these phenomena and recognizing species similarities and differences are important for evaluating lung retention and clearance processes and interpreting results of inhalation studies.

Some chemical compounds deposited in the lung in particulate forms are mobilized faster than can be explained by their known chemical properties at the normal lung fluid pH of about 7.4.[160] In addition to *in vivo* chemical forces acting to dissolve particles, physical forces may influence the dissolution rate indirectly by altering the form of the particles and the surface area available for dissolution. Raabe et al.[161] reported a higher *in vitro* solubility for $^{238}PuO_2$ particles than for $^{239}PuO_2$ particles. Park et al.[162] noted that $^{238}PuO_2$ particles changed physical characteristics when they were stored in water compared to when they were in biological tissues. The change was quite rapid in water suspension, where radiation exposure damaged the particles and caused physical disruption of the particle matrix; this damage culminated in radiolytic fragmentation of $^{238}PuO_2$. The result was smaller, more soluble particles of $^{238}PuO_2$. Diel and Mewhinney[163] noted the same phenomenon for particles of $^{238}PuO_2$ in lung tissue.

B. Determining Clearance

Most experiments designed to study clearance actually study retention of a test material. Clearance rate projections are inferred from measurements of the amounts of material present in the respiratory tract as a function of time after deposition. An example of a noninvasive experiment to study clearance is one in which the subject is exposed to a radioactive test aerosol that allows external counting of the subject by using a whole body counter or a suitable collimated detector. Several measurements of radioactivity in the subject are made over time. The time-dependent change in the amount of the radioactive test material remaining in the experimental subject is used to determine the clearance rate of the material.

It is possible to conduct serial sacrifice experiments with laboratory animals to evaluate retention and clearance as they relate specifically to pulmonary tissue and the LALN. In most studies, the T-B and P regions are not segregated, so the results reflect lung clearance, where lung is defined as the combination of the P region and that portion of the T-B region from the major bronchi to the terminal bronchioles. Referring to lung retention and clearance as retention and clearance of the P region results in only a small error because most of the material retained in the lung after a few days is associated with the P region.

Figure 5 shows generalized retention patterns for many respirable-sized particles inhaled by rats and dogs in a single exposure. Both curves originate at 100% on the vertical axis. Immediately after deposition, particles that deposit in the N-P and T-B regions start to clear, and clearance of those two regions is essentially complete within a day or so. Particles retained in the respiratory tract longer than a few days are mostly associated with the P region, with some exceptions discussed later. The shapes of the retention curves presented

in Figure 5 emphasize the fact that, in the rat, 80 to 90% of the particles that deposit in the respiratory tract are found initially in the combined N-P and T-B regions; the other 10 to 20% are found in the P region. In the dog, about 50% of the initially deposited particles are found in the combined N-P and T-B regions and about 50% is found in the P region. The pattern demonstrated by the dog is similar to what is observed for nosebreathing humans.

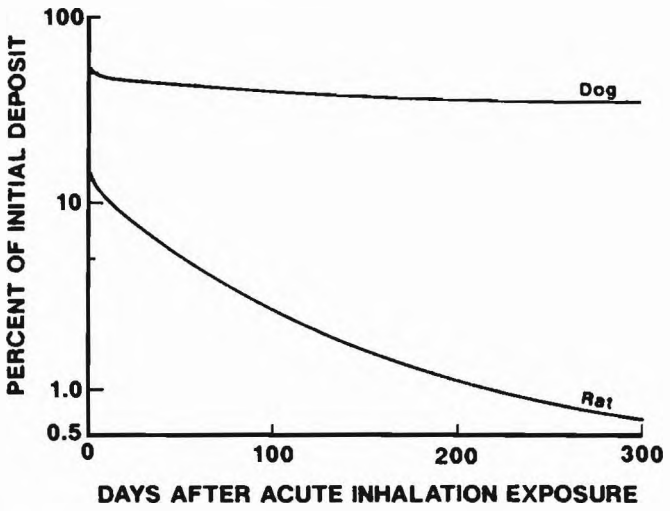

FIGURE 5. Respiratory tract retention patterns for inhaled particles.

C. Clearance Patterns for the Nasopharyngeal Region

While most particles that deposit in the N-P region clear rapidly in all mammalian species, a small percentage of the particles is retained for long time periods. Long-term retention of inhaled particles in the N-P region has been reported by Stuart,[164] Snipes et al.,[36,152] and Whaley et al.[165] Stuart concluded that particles were retained for relatively long times in the heads of Beagle dogs. He based his conclusion on analyses of longitudinal scans of the dogs that showed long-term retention of promethium oxide particles. The study by Snipes et al.[152] included mice, rats, and dogs, all exposed to monodisperse or polydisperse ^{134}Cs-labeled fused aluminosilicate particles. In all three species, 0.001 to 1% of the initial internally deposited burden of particles was retained in the N-P region and was removed only by dissolution and absorption of the particles. Particles were retained at sites in close proximity to the basement membrane of nasal airway epithelium, as seen in autoradiograms.

In the other study by Snipes et al.,[36] 3-, 9-, and 15-μm latex microspheres were inhaled by rats and guinea pigs. About 1 and 0.1% of all three sizes of microspheres were retained in the head airways of the rats and guinea pigs, respectively. For rats, the 9- and 15-μm microspheres cleared with half-times of 23 d; for guinea pigs, the same microspheres cleared with half-times of about 9 d. The 3-μm microspheres were cleared from the head airways of the rats and guinea pigs with biological half-times of 173 and 346 d, respectively. The smaller particles are apparently more likely to penetrate the epithelium and reach long-term retention sites.

Whaley et al.[165] studied retention and clearance of radiolabeled, 3-μm polystyrene latex particles instilled onto the epithelium of the maxillary and ethmoid turbinates of Beagle dogs. Retention of the particles at both sites after 30 d was about 0.1% of the amount initially deposited. Autoradiographs of turbinate tissue indicated that the particles were retained in the epithelial submucosa of both regions.

D. Clearance Patterns for the Tracheobronchial Region

The reader is referred to a review by Lippmann and Schlesinger[29] that provides considerable detail on T-B clearance in humans and experimental animals. This review emphasizes observations made in recent years relative to long-term retention of particles in and beneath the epithelium of the T-B region.

While it is accepted that most material that deposits in the T-B region clears within hours or days, in recent years there have been a number of studies demonstrating that small portions of the materials that either deposit in the T-B region or are cleared through the T-B airways are retained with retention half-times on the order of weeks or months. Patrick and Stirling[166] noted that about 1% of barium sulfate particles instilled intratracheally into rats remained in the bronchial tissue for at least 30 d. In a followup study, Stirling and Patrick[167] used autoradiography to demonstrate the temporal retention patterns for the retained $^{133}BaSO_4$ particles in T-B airways. Particles of barium sulfate were retained within macrophages in the tracheal wall for at least 7 d after intratracheal instillation of $^{133}BaSO_4$. By 2 h after instillation, some of the particles were buried in the tracheal wall. After 24 h, when most of the initial deposition of particles had cleared, 74% of $^{133}BaSO_4$ particles located on autoradiograms were located in macrophages proximate to the basement membrane. After 7 d, practically all of the remaining particles were incorporated into the walls of the airways. The authors did not determine the mechanisms by which the particles were moved into the airway epithelium. However, the rapid phagocytosis of particles deposited on the airway surfaces after inhalation or other means of exposure suggests that macrophages could be involved in particle transport into airway epithelium. It was not clear whether the intratracheal instillation procedures influenced the results of these studies.

Gore and Thorne[168] exposed rats by inhalation to polydisperse aerosols containing 45 or 87 mg UO_2/m^3. At 2, 4, 7, and 35 d after inhalation of the UO_2, autoradiography was used to determine the locations of particles retained in the T-B and P regions. The authors did not report seeing particles of UO_2

tained in the airways, but did note two phases of clearance. The first phase was associated with a clearance half-time of 1.4 d, the second phase with a clearance half-time of about 16 d. The faster clearance was presumably associated with particles deposited on the conducting airways during the inhalation exposure; the longer-term clearance was associated with clearance of UO_2 particles from the P region. In a separate study, Gore and Patrick[169] evaluated the distribution of UO_2 particles in the upper respiratory tracts of rats for up to 14 d after inhalation of aerosols similar to those used by Gore and Thorne.[168] Retention of UO_2 at airway bifurcations was noted, as was retention of particles in the trachea.

In another study, Gore and Patrick[170] also compared the retention sites of inhaled UO_2 particles and intratracheally instilled barium sulphate particles. Both types of particles were found in macrophages at sites near the basement membrane of the airways of the T-B region. The macrophages appeared to have engulfed the particles in the airways, then passed through the airway epithelium and remained in the vicinity of the basement membrane. About 4% of the UO_2 in lungs of rats was associated with intrapulmonary airways.[171,172]

Retention of inhaled or instilled particles in conducting airways has been observed by other investigators. Watson and Brain[173] observed similar results with aerosols of gold colloid and iron oxide. Both types of particles were found in bronchial epithelium, but more of the iron oxide was observed, suggesting a possible particle size effect or a relationship between the process of material uptake and chemical composition of the material. Both types of particles were found in bronchial epithelial cells, but neither gold nor iron oxide particles were seen in interstitial macrophages.

In a recent inhalation study, Briant and Sanders[174] exposed rats to chain-aggregate aerosols of U-Pu. These authors observed retained particles of U-Pu in the larynx, trachea, carina, and bronchial airways throughout the course of the 84-d study. The amounts retained varied, but were at any time approximately 1% of the concurrent lung burden. The lung cleared the U-Pu with a biological half-time of 100 d, and the relative amounts of U-Pu in the airways suggested comparable particle clearance rates from the airways. Particles of U-Pu retained in the airways were located in epithelial cells.

These demonstrations of long-term particle retention in the conducting airways of the respiratory tract have important implications for exposure, dose, and response patterns for inhaled materials. Because long-term retention of particles in respiratory airways has been observed in several animal species, it is possible that it occurs in all mammalian species. If also true for humans, particle retention in tissue constituents of the T-B region has important implications for human respiratory tract dosimetry of inhaled, relatively insoluble particles. Exposure and damage to sensitive cells of the T-B epithelium, consequent to inhalation of toxic particles, could be much higher than previously predicted.

There have, in fact, been a few reports indicating that relatively insoluble particles associated with cigarette smoke are retained in the epithelium of the tracheobronchial tree of humans.[175-177] These reports strongly suggest that the processes associated with particle retention in airway epithelium of the respiratory tract are similar among mammalian species.

E. Clearance Patterns for the Pulmonary Region

For most inhaled materials, retention time in the P region is sufficiently long that both physical translocation and dissolution-absorption can be important factors in clearance. These two processes are the key elements of long-term particle retention and clearance for the P region of the respiratory tract. Physical translocation of particles from the P region takes place predominantly through (1) the mucociliary escalator and (2) transport to the LALN. Dissolution makes constituents of the particles available for absorption or for chemical reactions, including metabolism within the tissues of the respiratory tract.[178]

Because a broad range of particle sizes may be inhaled, it is relevant to know whether or not particle size is an important factor in physical clearance processes. Morrow et al.[179] suggested that clearance is not particularly dependent on particle size. Most of the retention-clearance data have been difficult to interpret because of uncertainties regarding dissolution and absorption of the test particles and the relationships between particle size and physical clearance processes. In the late 1960s, monodisperse aerosols became available for use in basic studies designed to determine relationships between aerosol particle size and clearance patterns from the pulmonary region. Snipes et al.[152] exposed mice, rats, and dogs to monodisperse, fused aluminosilicate particles radiolabeled with ^{134}Cs. These relatively inert particles were in the size range of 0.7- to 2.8-μm aerodynamic diameter and were physically cleared to both the LALN and to the gastrointestinal tract at the same rate within a species. While the study used a relatively narrow size range for inhaled particles, the results demonstrated that particle size, at least within the range commonly encountered in the workplace and environment, did not appear to affect physical clearance of deposited particles.

The continuous movement of macrophages out of the lung via the mucociliary escalator provides the opportunity to clear significant amounts of material from the lung each day. LaBelle and Brieger[57] demonstrated that phagocytic cells are involved in particle clearance from the lung. They noted that the rates of phagocyte movement out of the lung were almost identical to the rates of elimination of particles from the lung. The rate of macrophage movement out of the lung via this pathway was estimated by Spritzer et al.[180] to be 1.24 to 2.47 million per hour for rats. Lehnert and Morrow[74] suggested that the number of alveolar macrophages leaving the lung daily was 0.28 million in rats. Brain[80] estimated that macrophages leave the lungs of cats at the rate of 2.05 million per hour. Rates for macrophages leaving the lung via this pathway have not been reported

for other species, and it is possible that considerable variability exists within and among species. Significant variability within a species would make it difficult to reach conclusions about the relationship between numbers of macrophages leaving the lung per day and clearance. There is no question that macrophages are important in physical clearance of particles from the lung, but rates for physical clearance appear to be controlled by factors other than numbers of macrophages leaving the lung per day.

No one has clearly defined the mechanism(s) by which phagocytic cells travel to the mucociliary escalator. Some phagocytic cells migrate from the pulmonary alveoli to the ciliated airways. However, the limited amount of data available that relates to this issue suggests that access to this clearance pathway can also be gained through the interstitium.[181-183] As discussed in the section of this review concerning BALT, movement of macrophages onto the mucociliary escalator may be facilitated by the presence of BALT and of pores or openings in the lymphoepithelium that allow what appears to be a one-way transport of particles to the mucociliary epithelium starting at the bronchioles.

Both retention and clearance, of at least part of an inhaled burden of particles, are associated with the interstitium and interstitial macrophages. Some phagocytic cells that follow interstitial clearance pathways appear to be resident interstitial macrophages that have ingested particles passing through the alveolar epithelium. It is known that a variety of free particles may be transported across the epithelium.[84,85,120,125,184] The likely mechanism for this process is endocytosis by Type 1 pneumocytes, followed by transfer of the particle into the interstitium. Interstitial macrophages might remain where they are in the interstitium and degrade phagocytized materials, or they might move within the interstitium. Their movement may take them to a lymphatic channel, where they can further translocate to regional lymph nodes. Or, as discussed earlier, interstitial macrophages can presumably also find their way to the mucociliary escalator.

Some investigators do not agree that an interstitial transport of particles, or macrophages, from the alveoli to the bronchi can occur. Sorokin and Brain[55] studied histological sections of mouse lungs obtained at different times after exposure of the mice to an iron oxide aerosol. They reported that both free particles and particles inside macrophages were carried from the alveolar lumen into the bronchiolar lumen and onto the mucociliary escalator. It is generally accepted that particles deposited in the alveoli can reach the mucociliary escalator as free particles or as phagocytized particles. However, particles are rapidly phagocytized after deposition in the P region and most clearance appears to be macrophage mediated. The question as to whether or not interstitial transport of particles from alveoli to bronchi can occur has not been conclusively answered and represents an area where additional research is needed.

Understanding how particles are cleared from the lung to LALN is important to a basic understanding of how the lung defense mechanisms function in normal or altered states. There are proponents of a mechanism for free particle transport from the pulmonary region to LALN. For example, Lehnert et al.[77] suggested that free particles are transported to the lymph nodes where they may be engulfed by resident mononuclear phagocytes. It has been suggested that bronchoalveolar macrophages migrate daily, in significant numbers, to hilar lymph nodes;[185] this would provide the means of transporting particles from portions of the lung to those lymph nodes. It seems clear that at least some, if not most or all, particles translocated from the lung to lymph nodes are in phagocytic cells during the transport process.[17,72] Few particles remain in the lung for very long as free particles, and there have been no studies whose results clearly indicated that free particles migrated across the alveolar epithelium and into lymphatic vessels. More effort is needed to clearly define the mechanisms for transport of particles from the lung to LALN and to determine the extent to which the mechanisms are the same or dissimilar among all mammalian species.

The rates of particle translocation from lung to LALN appear to vary considerably among species. Rats and mice have particle translocation rates from the P region to LALN that are quite different from those of guinea pigs, dogs, and possibly humans. After a few days following a single inhalation exposure, transport of particles from the P region to LALN appeared to be negligible in mice and rats,[152] but continued at a constant rate in guinea pigs and dogs.[152,186] No experimental information is available about the rates of translocation of particles from the P region to LALN in humans. However, data for amounts of particles accumulated in the lungs of humans exposed repeatedly to dusty environments[187-190] suggest that relatively insoluble particles accumulate in LALN of humans at rates that may be comparable to those observed for guinea pigs, dogs, and monkeys.

The translocation rates of particles from the P region to LALN are not constant for all types and amounts of particles deposited in the lung. Klosterkötter and Bünemann[191] noted that increased lung burdens of particles caused increased translocation to LALN in rats. Rates of elimination from lung were maximal during the first month, then became increasingly slower. There were considerable differences in lymphatic transport for various types of particles and also for individual animals. Only small amounts of inert and fine-grained dusts of titanium dioxide and gamma-aluminum oxide penetrated into the lymph passages, except when large amounts were deposited. In contrast, quartz exhibited an affinity for the lymph passages. In some of the tests, over 50% of the amount of silica eliminated from the lung was found in the mediastinal lymph nodes. Klosterkötter and Bünemann[191] concluded that the lymphatic clearance route is used mainly for pulmonary clearance of toxic substances (e.g., quartz), while its role is negligible in the case of inert substances such as TiO_2. In a

er report, Klosterkötter and Gono[192] noted that concurrent exposure to quartz and TiO_2 led to a threefold increase in the quantity of TiO_2 observed in the lymph nodes of exposed rats compared with the amounts observed after exposure to only TiO_2. This indicates that the accelerated lymphatic clearance was not specific for translocation of quartz.

Exposures to other types of particles have resulted in increased translocation rates from lung to LALN. Kilpper et al.[193] noted an increased clearance of tantalum to regional lymph nodes for large lung burdens of insufflated tantalum. Adamson and Bowden[122] noted an increased accumulation of carbon particles in hilar lymph nodes of mice under conditions where macrophage response to the burden of particles was reduced and interstitial burdens of the particles were elevated. Adamson and Bowden attributed their results to an increased direct penetration of the carbon particles into the interstitium, followed by transport to the lymph nodes. Vostal et al.[194] reported elevated lymph node burdens of diesel exhaust particles in LALN in rats and guinea pigs that were exposed for long periods of time to relatively high concentrations of diluted diesel exhaust.

Ferin[123] concluded that low exposure concentrations favor a primary removal pathway for insoluble, particle-laden macrophages via the mucociliary escalator in rats; with increasing exposure levels, there is an increase in the translocation of free particles to the pulmonary lymphatic system. Even with low ticle burdens, however, particles may be found in the lymphatics soon after exposure. Vincent et al.[195] reached a similar conclusion in a more recent study in which rats were chronically exposed to TiO_2 or quartz dust at concentrations ranging from 0.01 to 90 mg/m^3. Exposure times ranged up to 222 d. Similar results for accumulations of particles in LALN were observed for both types of mineral dusts. Lymph node burdens were negligible or nonexistent when exposure concentrations of the dusts were low or when lung burdens of the mineral dusts were low. However, for exposures to the higher concentrations of the dusts, or after lung burdens of the dusts accumulated to masses involving several milligrams, measurable amounts of the dust particles were noted in the lymph nodes. The limits of detection for TiO_2 and quartz were estimated to be 12 and 10 μg, respectively. On the order of 1 mg or more of either type of dust was accumulated in lungs before detectable amounts of the dusts were found in LALN. One interpretation of this result is that both dusts were accumulating in LALN for all exposure conditions, but that the amounts could not be quantified. Numerous studies with a variety of radioactive particles have demonstrated that particles are transported to LALN under conditions where microgram quantities of the particles were deposited in the lungs. While the results for translocation to LALN may have been subject to detection limitations, these authors clearly demonstrated that TiO_2 and quartz accumulated in LALN and that accumulation occurred at faster es under exposure conditions where substantial lung burdens accumulated.

The specific clearance routes and amounts of deposited material cleared from the pulmonary region may also be influenced by the mode of exposure. As one example, Ferin[123] and Ferin and Feldstein[121] exposed rats to TiO_2 by inhalation or intratracheal instillation. By 25 d after exposure, the contents of the lymph nodes accounted for less than 1% of the total lung burden, which ranged between 0.1 and 1 mg. A substantial increase in the TiO_2 content of the hilar lymph nodes was observed for lung burdens greater than 1 mg. With lung burdens in the range of 10 mg, about 4% of the TiO_2 was found in the lymph nodes. The rate of translocation of TiO_2 particles from the lung to the hilar lymph nodes was almost directly proportional to the lung burden of TiO_2. Ferin and Feldstein[121] concluded that, with high lung burdens, macrophages could no longer contain all of the particles and thus the probability that particles would enter the interstitium and translocate to lymph nodes was increased. Fewer TiO_2 particles were found in lymph nodes after inhalation than after intratracheal instillation. The authors concluded that one reason for this might be that the carrier fluid facilitated particle penetration. However, intratracheal instillation procedures generally cause an influx of phagocytic cells into the lung, which could also be a cause for the increased translocation of particles.

Clearance rates for one material may be influenced by the presence of other materials. LaBelle and Brieger[57,81] noted a faster pulmonary clearance of intratracheally injected uranium dioxide particles in rats when the uranium dioxide particles were injected together with other particles. The total number of particles was sufficient to increase the number of phagocytic cells that could be lavaged from the lung. In a similar study, Ferin et al.[58] found that clearance of SiO_2 particles was faster when macrophages were stimulated by intratracheal injection of trypan blue or by inhalation of TiO_2. Clearance was slower when the rats were pretreated with X-ray irradiation. In this study, the stimulation of macrophages was offset by the radiation damage that reduced the ability of the lung to recruit additional phagocytic cells.

A large number of papers published over the last 3 decades contain information relevant to understanding translocation of materials from the lung to LALN. Whether or not clearance from the P region to the lymphatic system occurs most significantly for cytotoxic particles, the process of lymphatic transport is common to all mammalian species, and all types of particles appear to be cleared from the P region to local lymph nodes. However, the rates of transport and amounts of particles cleared to the lymph nodes depend, to a significant extent, on factors that include the animal species, physicochemical properties of the particles, occurrence of biological reactions to the particles, and the amounts of particles inhaled or injected into the lung.

Thomas[141,196,197] discussed the implications of particle translocation from the lung to LALN when the particles contain specific radionuclides, but he presented information that is

relevant to all types of particles. Since the LALN represent a trap for particles cleared from the lung, particles can accumulate to high concentrations in LALN. Thomas[141,196] noted that the ratio of LALN burden to lung burden for relatively insoluble particles clearly increases with time after exposure for dogs and monkeys and may have the same pattern for rats and humans. By about 100 d, the concentrations of particles in LALN and lung are approximately equal; thereafter, the ratio of concentrations for LALN/lung increases and may reach 100 or more, depending on the life span of the individual and retention characteristics of the particles in the lymph nodes and lung. Comparable results were demonstrated for single or repeated exposures.

The movement of macrophages from the lung to the LALN affords the opportunity to transport particles out of the lung, but the result is to sequester, or trap, the particles in what is generally perceived to be a dead-end compartment. Some reports have suggested that particles may appear in post-nodal lymph, from which they enter the blood and are translocated to other sites. Transmigrated particle-laden macrophages, as well as free particles, have been found in various extrapulmonary organs.[198-203] One interpretation of these results is that particles normally leave the LALN only as a result of dissolution-absorption processes. However, the LALN may have been damaged to the extent that the macrophages, or the particles delivered to the LALN, could pass through the nodes and enter the circulatory system. The consequence of particles passing into the bloodstream is that the particles may become trapped in the reticuloendothelial system of organs such as liver, spleen, kidney, and skeleton. LeFevre et al.[198] reported accumulations of particles in the livers and spleens of coal miners. The results may have been related to relatively large lifetime accumulations of particles in the lung that influenced the release of a portion of the particles into the general circulation. Radioactive particles have been observed in liver[199,200] and spleen[200] of dogs after inhalation exposures. This could have occurred as a result of direct transport of particles. However, the particles were large relative to the sizes of particles that might pass directly through pulmonary cell membranes. Another possibility is that translocation of particles from lung to lymph nodes caused accumulations of particles of $^{239}PuO_2$ in the nodes; the accumulations of particles then caused sufficient radiation damage to the lymph nodes to allow direct passage of the particles into the circulatory system. Gearhart et al.[199] observed particles of plutonium oxide in the livers of only those dogs that had been exposed to relatively large amounts of $^{238}PuO_2$ and not until more than 1350 d after a single inhalation exposure. This would have allowed ample time for accumulation of $^{238}PuO_2$ particles in the lymph nodes as a consequence of normal pulmonary clearance processes, radiation and chemical damage to the nodes, and release of a portion of the lymph node burden to the circulatory system.

Macrophages may phagocytize particles, then transport the particles directly into blood vessels.[201,204] However, if this process occurs, it is probably a rare phenomenon because particles transported from the lung in phagocytic cells appear to be exclusively transported to the lung-associated lymphatic tissues. Entry of macrophages into the lymphatic system is more likely than entry into blood capillaries since lymphatic vessels have looser endothelial cell connections and wider intercellular junctions than capillaries.[67] Also, there may be movement of particles directly from the interstitium into the circulatory system. Gross and Westrick[205] hypothesized that very small particles could pass directly from lung tissue into the circulatory system, but gave no indication about what they considered to be particle size limitations for this process. This hypothesis has been pursued in several studies, and evidence has been presented to support the idea that particles can pass directly from lung parenchyma into blood vessels (and lymphatic vessels) without having been carried by mobile cells. In one study, Stradling et al.[206] noted that a suspension of $^{238}PuO_2$, having particle sizes <5 μm, was substantially converted by fragmentation to a suspension of particles in the size range of 0.001 to 0.025 μm in 32 weeks. Most of the small particles were 0.001-μm diameter, and the authors suggested that very small particles of PuO_2 could pass directly from lung tissue into the circulatory system. In a similar study, Raabe et al.[207] noted an unusually high apparent *in vivo* dissolution-absorption rate for highly, insoluble weapons-grade PuO_2. In agreement with the results of Stradling et al.,[206] Raabe et al.[207] concluded that the apparent high degree of dissolution-absorption resulted from fragmentation of the PuO_2 into particles small enough to move readily into the blood rather than being dissolved. Diel and Mewhinney[163] had a different interpretation of results from a similar study. They quantitated the enhanced *in vivo* fragmentation of $^{238}PuO_2$ particles in canine lung tissue and concluded that the alpha decay of Pu damaged the crystalline lattice of the particles, thereby providing a larger surface area for the particles in lung tissue. The larger surface area of the $^{238}PuO_2$ resulted in a more rapid rate of particle dissolution, which in turn allowed for an increased rate of absorption of the solubilized Pu.

There may be preferential drainage of specific particle sizes via the lymphatic vessels or capillaries. Electron microscopic studies by Lauweryns and Baert[208] demonstrated that carbon particles (diameter of 25 nm) were cleared from the lungs of rabbits via the lymphatics, while ferritin molecules (diameter of 10 nm) were absorbed by both blood capillaries and lymphatics. Both types of particles were seen in the interstitium and the lymph capillaries within half an hour after their intratracheal administration. The quantitative role of blood capillaries and lymphatics in alveolar clearance was also evaluated by Meyer et al.,[209] who studied the removal of labeled albumin from the alveolar spaces in dogs. These authors found that, during a 4-h observation period, albumin absorption via the blood was about 11 times higher than absorption via the lym-

ıtics, emphasizing the importance of regional blood flow in absorption of pulmonary exudates. However, considering the fact that blood flow through the lung is about 400 times higher than lymph flow, these authors estimated that the "efficiency" of particle absorption via the lymphatics was about 37 times that of blood.

F. Intra- and Inter-Species Variability

Many measurements of pulmonary retention and clearance have been conducted on a variety of laboratory animal species. For some exposure materials, data from more than one laboratory are available for the same species. In many cases, at least two laboratory animal species were exposed to the same aerosolized material, so direct comparisons among species are possible. Relatively few human inhalation exposures to the same materials as used for the animal studies have occurred, so only a limited number of direct comparisons are possible between laboratory animals and humans.

Table 4 contains a summary of selected results for pulmonary retention of inhaled materials after single inhalation exposures to small masses of relatively insoluble materials. Studies of less than about 3-months duration were not considered. The variability in these results was caused by several factors. In many cases, the reported results did not allow division of the lung burden between short- and long-term clearance. Also, for most studies, solubility and amounts of absorption of the exposure materials were not known or were not reported. The broad range of particle sizes would have influenced deposition patterns, the ratios of amounts of material cleared rapidly or slowly, and dissolution-absorption rates, but probably not the physical clearance of particles from the pulmonary region.

The information shown in Table 4 was used as the basis for approximations of biological clearance rates for particles inhaled by the species listed in Table 5. In addition, approximations are included for the fractions of pulmonary burdens initially deposited in the P regions that were subjected to short- or long-term clearance. These approximations are not arithmetic averages; rather, they represent estimates of trends depicted in the data in Table 5. These approximations clearly will not apply to all types of inhaled particles. For example, in some cases, deposition and clearance may be influenced by the physicochemical and/or biological characteristics of the inhaled material. However, the generalizations that led to Table 5 allow comparisons for the consequences of chronic inhalation exposures among these animal species and humans that might not otherwise be possible.

Figure 6 demonstrates pulmonary retention curves based on the data in Table 5. These patterns clearly demonstrate similarities and differences in pulmonary retention and clearance among these species that might be applicable to a variety of inhaled materials. The net retention and clearance patterns for the pulmonary burdens of material were similar for guinea pigs, monkeys, dogs, and humans. For these species, about 20 to 30% of the initial burden of particles cleared with a half-time on the order of 1 month; the balance cleared with a half-time of several hundred days. Rats and mice cleared about 90% of the deposited material with a half-time of about 1 month and 10% with a half-time greater than 100 d. The relative division of the pulmonary burden between short- and long-term clearance represents a significant difference between most rodents and larger mammals and has considerable impact on long-term patterns for retention of material acutely inhaled, as well as for accumulation patterns for materials inhaled in repeated exposures.

VII. MODELING PULMONARY RETENTION AND CLEARANCE OF PARTICLES

A. Models for Effective Lung Retention and Clearance

A widely used method for expressing effective clearance and retention of lung burdens of materials is to fit the data to a single- or multi-component exponential function. This procedure does not differentiate between physical clearance of particles and absorptive processes, but assumes that the effective reduction of the lung burden occurs in a way that can be mathematically expressed as a single, first-order process or as the sum of two or more first-order processes. In this procedure, the decrease in the lung burden of particles is assumed to be proportional to the lung burden. The time-dependent decrease in the lung burden is

$$d(L) = -k(L)dt \quad (1)$$

and

$$L(t)/L_0 = \exp(-kt) \quad (2)$$

where L(t) represents the lung burden of particles at time t and L_0 represents the initial lung burden of particles. Because 100% of the initial lung burden is in a single compartment in this simple case, the expression becomes

$$L_t = L_0 \exp(-kt) \quad (3)$$

More generally, the form of the expression is

$$L_t = L_0 \Sigma A_i \exp(-k_i t) \quad (4)$$

where A_i = fraction number i of the lung burden; k_i = clearance rate for fraction number i; and, t = time, usually expressed in days after inhaling the particles.

For repeated exposures, this simple model can be used to estimate what the equilibrium lung burdens of particles would be for lung deposition of a known mass of material per day.

Table 4
Comparative Pulmonary Clearance Data for Relatively Insoluble Particles Inhaled by Laboratory Animals and Humans

Species	Aerosol matrix	Particle size		Pulmonary burden*				Study duration (days)	Ref.
		μm	Measure	P_1	T_1	P_2	T_2		
Mouse	FAP	0.7	AMAD	0.93	34	0.07	146	850	152
	FAP	1.5	AMAD	0.93	35	0.07	171	850	152
	FAP	2.8	AMAD	0.93	36	0.07	201	850	152
	Ru Oxide	0.38	CMD	0.88	28	0.12	230	490	237
	Pu Oxide	0.2	CMD	0.86	20	0.14	460	525	237
Rat	Diesel soot	0.12	MMAD	0.37	6	0.63	80	330	238
	FAP	1.2	CMD	0.62	20	0.38	180	492	239
	FAP	0.7	AMAD	0.91	34	0.09	173	850	152
	FAP	1.5	AMAD	0.91	35	0.09	210	850	152
	FAP	2.8	AMAD	0.91	36	0.09	258	850	152
	Latex	3.0	CMD	0.39	18	0.61	63	190	36
	Pu Oxide	<1.0	CMD	0.20	20	0.80	180	350	240
	Pu Oxide	2.5	AMAD	0.75	30	0.25	250	800	241
	U_3O_8	~1—2	CMD	0.67	20	0.33	500	768	242
Guinea pig	FAP	2.0	AMAD	0.22	29	0.78	385	1100	243
	Diesel soot	0.12	MMAD			1.00	>2000	432	238
	Latex	3.0	CMD			1.00	83	190	36
Dog	Coal dust	2.4	MMAD			1.00	1000	160	244
	Coal dust	1.9	MMAD			1.00	~700	301—392	245
	Ce Oxide	0.09—1.4	MMD			1.00	>570	140	246
	FAP	2.1—2.3	AMAD	0.09	13	0.91	440	181	247
	FAP	0.7	AMAD	0.15	20	0.85	257	850	152
	FAP	1.5	AMAD	0.15	21	0.85	341	850	152
	FAP	2.8	AMAD	0.15	21	0.85	485	850	152
	Nb Oxide	1.6—2.5	AMAD			1.00	>300	128	248
	Pu Oxide	1—5	CMD			1.00	1500	280	237
	Pu Oxide	4.3	MMD			1.00	300	300	249
	Pu Oxide	1.1—4.9	MMAD		~1		400	468	250
	Pu Oxide	0.1—0.65	CMD	0.10	200	0.90	1000	~4000	251
	Pu Oxide	0.72	AMAD	0.10	3.9	0.90	680	730	252
	Pu Oxide	1.4	AMAD	0.32	87	0.68	1400	730	252
	Pu Oxide	2.8	AMAD	0.22	32	0.78	1800	730	252
	Tantalum	4.0	AMAD	0.40	1.9	0.60	860	155	253
	U_3O_8	0.3	CMD	0.47	4.5	0.53	120	127	254
	Zr Oxide	2.0	AMAD			1.0	340	128	255
Monkey	Pu Oxide	2.06	CMAD			1.0	500—900	200	256
	Pu Oxide	1.6	AMAD			1.0	770—1100	990	257
Human	FAP	1	CMD	0.14	40	0.86	350	372—533	258
	FAP	4	CMD	0.27	50	0.73	670	372—533	258
	Latex	3.6	CMD	0.27	30	0.73	296	~480	259
	Latex	5	CMD	0.42	0.5	0.58	150—300	160	260
	Pu Oxide	0.3	MMD			1.00	240	300	261
	Graphite and PuO_2	6	AMAD			1.00	240—290	566	262
	Pu Oxide	<4—5	CMD			1.00	1000	427	263
	Th Oxide	<4—5	CMD			1.00	300—400	427	263
	Teflon	4.1	CMD	0.30	4.5—45	0.60	200—2500	300	264
	Zr Oxide	2.0	AMAD			1.00	224	261	255

Note: FAP = fused aluminosilicate particles; AMAD = activity median aerodynamic diameter; MMAD = mass median aerodynamic diameter; CMD = count median diameter; MMD = mass median diameter. Some aerosols were monodisperse, most were polydisperse, with geometric standard deviation in the range of 1.5 to 4. Clearance half-times are approximations for biological clearance, the net result of dissolution-absorption processes and physical clearance processes. In some examples, the original data were subjected to a computer curve-fit procedure to derive the values for P and T.

* Pulmonary burden = $P_1e^{-(\ln 2)t/T_1} + P_2e^{-(\ln 2)t/T_2}$, where P_1 and P_2 equal fractions of the initial pulmonary deposition, T_1 and T_2 equal retention half-times in days, and t equals days after exposure.

Table 5
Average Pulmonary Retention Parameters[a] for Insoluble Particles Inhaled by Selected Animal Species, Including Humans

Species	Pulmonary retention parameters			
	P_1	T_1	P_2	T_2
Mouse	0.9	30	0.1	240
Rat	0.9	25	0.1	210
Guinea pig	0.2	29	0.8	570
Dog	0.3	30	0.7	700
Monkey[b]	0.3	30	0.7	700
Human	0.3	30	0.7	700[b]

[a] Pulmonary retention (fraction of initial deposition) $= P_1 \exp[(-\ln 2)t/T_1] + P_2 \exp[(-\ln 2)t/T_2]$, where P_1 and P_2 = fractions of pulmonary burden in fast- and slow-clearing components, respectively; T_1 and T_2 = clearance half-times (days) for P_1 and P_2, respectively; and t = time in days.

[b] Assumed the same as for dogs.

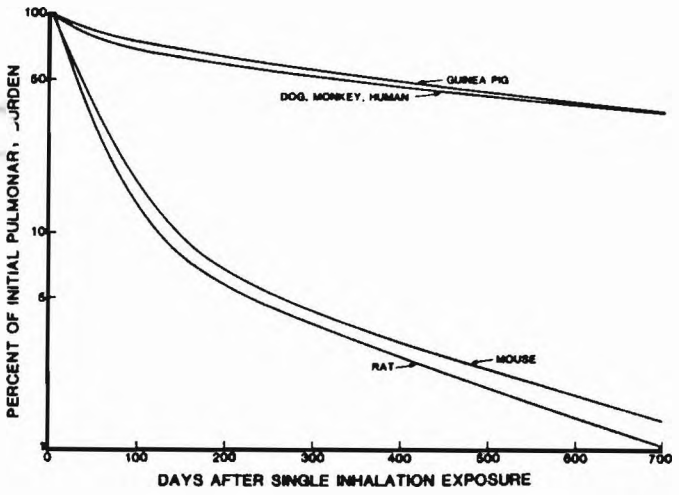

FIGURE 6. Comparative pulmonary retention of particles inhaled by selected animal species, including humans.

The procedure is to integrate the expression to infinite time. The resulting mathematical expressions can be solved to determine the equilibrium lung burdens. Where lung retention and clearance can be represented by a single- or two-component exponential expression, respectively,

$$LB = A\frac{[1 - \exp(-kt)]}{k} \times (\text{mass/day}) \qquad (5)$$

and

$$LB = \left(\frac{A_1[1 - \exp(-k_1 t_i)]}{k_1} + \frac{A_2[1 - \exp(-k_2 t_i)]}{k_2}\right) \times (\text{mass/day}) \qquad (6)$$

B. Basic Components of a Materials Balance Model

1. Anatomical Compartments

Models are useful in deposition, retention, and clearance studies to describe the fate of inhaled materials. To allow this, the model must be designed to approximate the physiological or anatomic compartments in the system being studied. The compartments generally correspond to the three major regions of the respiratory tract (N-P, T-B, and P), LALN, blood, and one or more additional internal organs such as liver, skeleton, and kidney, as well as to excreta. Provisions are made in the models for appropriate entry of the material into the system, physical removal or translocation of the material among regions or compartments of the system, and removal of the material from the system (exhaled air, urine, feces, physical decay, or chemical alteration).

An important first step in modeling, therefore, is to define the model. The model must be based on anatomical and functional organization and must produce results that can be used to mathematically describe the time-dependent fate of inhaled material. The model must also describe the time-course of movement of the inhaled material among the model compartments and provide a mathematical means of estimating exposure or dose to each compartment. Figure 7 presents an example of a simple model that can be used for relatively insoluble inhaled materials. Note that a dissolution-absorption factor is included because even relatively insoluble materials have dissolution and absorption rates that influence the retention and clearance of the inhaled material.

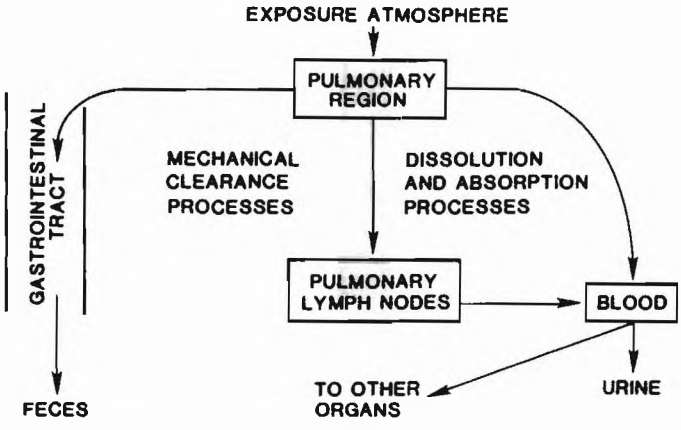

FIGURE 7. Model for simulating particle retention and clearance patterns in laboratory animals and humans.

The transport rates for moving the inhaled material between compartments and out of the system can be represented by either constants or complex mathematical expressions. The form of the expression is secondary to its utility in describing the time-dependent fate of the inhaled material. The dissolution-absorption function may be the same or different for the respiratory and gastrointestinal tracts. This can be determined experimentally and should be used in cases where unusual degrees of dissolution-absorption in the gastrointestinal tract would influence interpretation of the modeling results. An example of where this could happen is in studies conducted with a moderately soluble material that deposits in the skeleton or liver after absorption from the lung or gastrointestinal tract. In these cases, it would be important to compare the relative exposures of tissues in the respiratory tract with exposures of liver or skeleton.

2. Experimental Organ and Tissue Data

Determination of tissue and organ content of the test material as a function of time after a single exposure is critical to modeling the fate of inhaled materials. The data set has to agree with the compartments defined in the model. Typically, these would include, as a minimum, the major regions of the respiratory tract, LALN, and carcass. Blood and other organs would be defined as compartments, if needed, to clarify the fate and tissue exposure patterns produced by the inhaled material. The tissue and organ data for various times throughout the study, with the first samples collected immediately after the exposure, are evaluated and expressed in terms of percent of the initial inhaled burden. Because changes in both the model system and the body will usually occur fastest at early times after exposure, the sampling is traditionally weighted to yield more data early in the study, with sampling times becoming less frequent with increasing time after exposure.

3. Excreta Data

Solubilized and absorbed material can be redeposited in internal organs such as liver or skeleton, can be excreted in urine, or can be excreted across the gastrointestinal tract into feces by a pathway termed endogenous fecal excretion. Physical clearance of particles from all three regions of the respiratory tract results in feces being a major route for clearance of most inhaled materials.

4. Solubility-Absorbability of Test Material

To accurately assess retention and clearance patterns for single inhalation exposures and make projections for repeated exposures, it is crucial to know the solubility-absorbability of the test material. It is not possible to accurately project the magnitudes of lung burdens of materials that would accumulate under repeated exposure conditions without having an approximation for the extent to which the material would dissolve in physiological fluids and be absorbed from the respiratory tract. Unfortunately, adequate information about *in vivo* solubility is a major unknown in most inhalation studies and for most accidents involving inhalation of hazardous materials.

The advantage of using an *in vivo* procedure is that it avoids the criticisms associated with using *in vitro* test systems; the material of interest is tested under conditions where it can be dissolved in biological fluids and absorbed across biological membranes.

Morrow et al.[210] injected radiolabeled metal oxide particles into muscle of rabbits and rats, measured the retention of the test materials at the injection sites, and related retention of the intramuscularly injected material to lung retention of the material in dogs. Clearance kinetics were similar for the lung and muscle burdens of radiolabeled test materials. In a similar study, Morrow et al.[211] injected neutron-activated coal dust suspensions into muscle of rats and then monitored the injection sites. The intramuscular injection provided a clearance rate for the neutron-activated coal dust that was comparable to that observed for the inhalation studies. It appeared that removal of the radioactive constituents of the coal dust occurred as a consequence of dissolution-absorption of the coal dust particles rather than as a preferential leaching of radionuclides from the matrices of the particles.

Thomas et al.[212] used a similar intramuscular injection procedure with rats to determine the *in vivo* absorption of four chemical forms of barium. After the injection procedure, the rats were measured periodically to determine the amounts of ^{133}Ba remaining at the sites of injection. The most insoluble form of barium was barium incorporated in fused aluminosilicate particles. This form of barium cleared slowly, presumably by dissolution of the particles and absorption of the radiolabel, with a half-time of 1400 d.

Using the same approach, Ferin[213] injected a suspension of TiO_2 particles into muscle of rats. Absorption of the TiO_2 was negligible over a 118-d test period. Assuming that the solubility of TiO_2 is the same in lung and muscle, results from this intramuscular injection study affirmed the author's observations that the TiO_2 was relatively insoluble in the lung and helped confirm that clearance of TiO_2 particles from the lungs of rats was predominantly by physical clearance processes.

While the intramuscular injection procedure provides data for the approximate dissolution-absorption characteristics of a material *in vivo*, intratracheal instillation of a test material can yield data about absorption of the material directly from the lung. It is important to precisely determine the amount of material placed into the lung and account for its clearance. After rapid clearance of the small portion of the test material typically deposited on tracheobronchial airways during the intratracheal instillation procedure, clearance of the lung burden occurs by physical clearance and dissolution-absorption.

Cuddihy et al.[153] used another type of approach to estimate dissolution-absorption of materials from tissue and the excreta content of the material. In their approach, the rate at which

adionuclides appeared in bone, liver, and remaining tissues or were excreted into urine was controlled by the solubilization and absorption of the materials retained in the respiratory tract. The data for tissue and urine content of the radionuclides as a function of time were used to approximate the dissolution-absorption rates directly.

In vitro test systems have been used in some instances to approximate the dissolution properties of test materials. Morrow et al.[210,214] studied pulmonary retention and clearance of several metal oxides inhaled by dogs. They simultaneously approximated the solubility of the metal oxides by using an ultrafiltration procedure, with several buffered and unbuffered biological solutions, including human or bovine serum. Results provided a ranking of ultrafiltrability for the metal oxides that corresponded to the ranking for clearance from the lungs of the dogs; the greater the ultrafiltrability, the more rapid the clearance. This provided a ranking useful for evaluating the results of *in vivo* determinations of clearance by absorption of the pulmonary burdens of the metal oxides.

Moss and Kanapilly[215] presented an overview of *in vitro* techniques used to measure the dissolution rates of selected materials. The authors made the point that *in vitro* dissolution tests are relatively simple compared with *in vivo* procedures. Although true, results from *in vitro* test systems are difficult to relate directly to *in vivo* results. Particles being dissolved *in vivo* are subject to unknown conditions relative to pH and enzyme action, both of which may not be constant over time and are difficult, at best, to simulate *in vitro*. The *in vitro* test systems serve as useful screening procedures and ways to obtain relative dissolution rates for a variety of materials, but they may not produce results directly applicable to *in vivo* dissolution-absorption rates. This results from the inability to precisely simulate *in vivo* dissolution conditions and tissue reactions with substances dissolved from particles or leached from their surfaces.

VIII. REPEATED INHALATION EXPOSURES

A. General

Respiratory tract clearance processes begin to decrease the burden of particles deposited during a single inhalation exposure. Given sufficient time, the deposited particles would be almost completely removed by these clearance processes. However, single inhalation exposures may be the exception rather than the rule. Repeated or chronic exposures produce lung burdens of the inhaled material that generally increase with time until the rate of deposition is balanced by the rate of clearance. This is defined as the "equilibrium lung burden". The accumulation patterns are unique to each animal species and are possibly unique to the inhaled material, especially if the material alters deposition and/or clearance patterns. It is important to evaluate these accumulation patterns because they dictate what the equilibrium lung burdens of material will be for repeated exposures to a specified exposure atmosphere. Alternatively, these patterns indicate the relative concentrations of exposure atmospheres that should be used to produce target lung burdens of the inhaled test substance. In studies where more than one species is exposed to a test material, the accumulation patterns can be used to predict "equivalent concentrations" of airborne materials. Equivalent concentrations are defined as species-dependent concentrations of airborne material that, when repeatedly inhaled, produce equal lung deposits of the inhaled material per gram of lung tissue during a specified exposure period.

B. Lung Overloading with Inhaled Materials

When small amounts of particles are inhaled and deposited in the lung, the lung's clearance processes are able to remove the particles at a rate sufficient to keep the lung relatively free from accumulations of the particles. However, when the air concentrations of the particles are high, or exposures long, the lung cannot clear the particles fast enough, and it tends to accumulate particles until the equilibrium lung burden is reached. If lung clearance processes are altered by the amounts of material inhaled during the exposure, or by the accumulated masses of particles, the lung burden increases beyond the equilibrium lung burden. This process will be referred to as lung overloading.

Literature on the topic of lung overloading in laboratory animals has grown markedly in recent years. Most of the experimental work has been conducted with rats exposed chronically to diesel exhaust.[99,103-105,110,111,216-219] Exposures covered a broad spectrum of conditions and exposure times, but all exposures produced significant lung burdens of diesel soot.

Impairment of particle clearance from the lung has been a common consequence of exposures of rats to large amounts of diesel exhaust. This means that lung burdens of diesel soot particles accumulated to the extent that lung clearance rates decreased relative to normal rates. The consequence was a shift in the relationship between deposition and clearance, such that the lung accumulated particles at a faster rate than occurred during the early phase of the exposure. The change was quantitated in a few studies. For example, in studies with rats exposed repeatedly to diesel exhaust, Vostal et al.[104] and Chan et al.[220] reported dose-related slowing of clearance of ^{14}C-labeled diesel particles following periods of 20-h/d exposures to concentrations of diesel exhaust up to 6 mg soot per cubic meter. Chan et al.[220] noted that the rate of alveolar clearance of diesel particles from lungs of rats was dependent upon the total diesel particle burden. Normal clearance rates occurred when lung burdens were less than 0.8 mg of diesel soot, but slowed when lung burdens were 6.5 mg of soot. This report was one of the first in a series of reports that confirmed the phenomenon now widely recognized as altered lung clearance resulting from lung overloading with relatively insoluble, particulate material. Griffis et al.[218] reported an approximate doubling of lung clearance half-times in rats following 18 weeks

of exposure, 7 h/d, to diesel exhaust at concentrations of 4.1 mg soot per cubic meter. Results of more recent studies have been summarized by Wolff et al.[111] Rats were exposed for 24 months to 0, 0.35, 3.5, or 7.0 mg diesel soot per cubic meter. Lung burdens as high as 20.5 mg soot per gram lung were produced. None of the exposures resulted in altered tracheal mucociliary clearance, but long-term pulmonary clearance was decreased by a factor of about 4 after 24 months of exposures to either 3.5 or 7.0 mg diesel soot per cubic meter.

Heinrich et al.[217] exposed hamsters and rats to 4 mg of diesel exhaust per cubic meter for 19 h/d, 5 d/week. The exposure did not alter alveolar clearance of a test aerosol of $^{59}Fe_2O_3$ inhaled by hamsters after 12 months of exposure to the diesel exhaust. However, clearance of this same test aerosol inhaled by rats was slowed by a factor of 2.5 after only 3 months of exposure. These results confirm previous findings that inhalation of diesel exhaust can alter pulmonary clearance, but also emphasizes the fact that considerable species variability may exist in terms of lung accumulations of diesel soot and biological responses associated with exposure to the same air concentration of diesel soot. Lung burdens of diesel soot were not reported, so it is difficult to judge the extent to which results were influenced by the amounts of diesel soot accumulated in the lungs of the rats and hamsters.

Progressive accumulations of particles in rodent lungs with long-term exposures have not been unique to diesel exhaust. Bolton et al.[221] and Vincent et al.[222] described an "overload" phenomenon in rats, with respect to accumulation of asbestos, when it exceeded about 1.5 mg per lung. Impairments in lung clearance have also been observed following inhalation of pulverized coal combustion dust at 38 mg/m^3 and fluidized bed combustion fly ash at 37 mg/m^3 for 4 weeks, resulting in lung burdens of about 3 mg per rat.[219] Rats exposed to coal dust appear to produce accumulations of particles in lung that have the same magnitude and pathological appearance as those in rats exposed to diesel exhaust when similar exposure conditions are used.

The lung overload effect seems to be nonspecific and may apply to all types of particles that are not very soluble and therefore are persistently retained in the lungs. The effect does not appear to involve deposition, rather it is a consequence of abnormal pulmonary clearance. The reduced clearance is accompanied by large accumulations of particle-containing macrophages in pulmonary air spaces. This phenomenon has been observed a number of times in rats, but there are no definitive data available for other species. Collectively, studies on particle overloading indicate that the phenomenon occurs when at least 1 to 3 mg of particles per gram of lung has been deposited. Particle overloading of the lung appears to be uniquely associated with specific accumulation patterns of particle-laden macrophages and cumulative lung burdens of particles.[88,223] There are morphologic changes in the lung consistent with inhibited phagocytic clearance of particles. These changes are manifest in macrophage accumulations, epithelial cell proliferation, inflammatory reactions, and increased amounts of particles in the pulmonary interstitium and LALN. The pulmonary macrophage is significantly involved with the lung overload phenomenon and may, in fact, help trigger the process; toxicological alterations in macrophage function due to the inhaled materials may be an important aspect of the immediate- and long-term lung responses to excessive lung burdens of particles.[117] Alternatively, the numbers of particles deposited in the lung simply overwhelm the functional abilities of macrophages to deal with them normally, and they tend to accumulate and initiate the sequelae typically noted in lung tissue as a consequence of lung overloading with particles.

It is not clear whether the mass of retained particles or the volume occupied by the particles is more important in initiating and perpetuating the lung overloading process. Morrow[88] suggested that the volume of particles available for phagocytosis is the significant factor in the lung overloading process and that the inability of particle-laden alveolar macrophages to translocate to the mucociliary escalator is correlated to an average composite particle volume per alveolar macrophage in the lung. When the particulate volume in a macrophage exceeds approximately 60 μm^3 per macrophage, with a uniform distribution of particles over the pulmonary pool of alveolar macrophages ($\sim 2.5 \times 10^7$ macrophages in the Fischer 344 rat), the lung overload effect appears to be initiated.

When lung burdens of particles have accumulated to the extent that normal lung clearance patterns are altered, the assumption is made that lung clearance, in general, is altered. This conclusion is justified for lung burdens in the range of tens of milligrams of test particles per gram of lung, at least in rats. It is not clear, however, that lung burdens in the milligram-per-gram lung range can produce this effect. When any quantity of test particles is inhaled, the particles are sequestered to some extent in the lung. When large quantities of particles are inhaled under repeated exposure conditions, the particles accumulate in a nonuniform pattern, mainly in accumulations of macrophages within alveoli. The accumulations of macrophages laden with particles become less available for clearance with time as a consequence of their burial in the large amounts of material being repeatedly inhaled. These large daily deposits of particles overwhelm the capacity of the lung defense mechanisms. However, prior to incorporation of the inhaled material into the sequestered lung burden, which makes it less available for physical clearance and possibly for dissolution-absorption, the deposited material may be cleared at a normal rate. This means that the lung may have the ability to clear newly deposited materials at a normal rate, in spite of the presence of a substantial lung burden of sequestered particles, if the repeated or chronic exposure is terminated.

The accumulated lung burden that triggers impaired clearance has not been precisely determined. Additional research is needed to determine the magnitudes of lung burdens of diesel

ɔt or other kinds of particles that initiate or result in altered clearance. The results should also demonstrate whether or not a relationship exists between rate of accumulation of lung burdens of particles and the extent of any changes in clearance processes or rates. In addition, it is important to evaluate the degree to which lung function can be returned to normal after the overloading phenomenon.

It is common practice to evaluate the extent to which lung clearance has been altered by using a radiolabeled test aerosol inhaled after the lung-loading process. Different opinions have been expressed as to the kind of aerosol most appropriate for this test. Using diesel exhaust as an example, one argument is that if a study is designed to determine the amount of inhaled diesel exhaust that alters clearance, then a test aerosol inhaled after the lung overloading with diesel exhaust particles should be as physically similar as possible to diesel exhaust. For other kinds of exposure materials, the test aerosol should be selected to match the physical characteristics of those test materials as nearly as possible. It is argued that using an aerosol different from the test material would yield invalid results because the two aerosols might not deposit or clear the same way.

A number of studies have determined the deposition and clearance of other types of aerosols inhaled after the lung-overloading process. The results of these studies have important implications relative to the reversibility or irreversibility of altered lung clearance resulting from the lung burden of diesel soot. Studies have been done in which lung overloading with diesel soot was followed by inhalation of a test aerosol of Cs-labeled, fused aluminosilicate particles[111] or ^{14}C-labeled diesel soot[220] to determine the effects of the lung burdens of diesel soot on the rate of lung clearance. Heinrich et al.[217] measured clearance of iron oxide particles after 18-months exposure to 3.9 mg diesel soot per cubic meter. Results of all of these studies indicated that, at some point in the lung overloading process, the lung demonstrated a diminished ability to clear particles. Thereafter, the rate of lung overloading accelerated with continued exposures.

There are no data on lung burdens of diesel particles in humans. However, data are available for other inhaled materials. One example is fumes from arc welders. Kalliomäki et al.[224] reported retained lung burdens of arc welding particles of about 0.2 g after 5- to 30-years exposure of shipyard arc welders. Another example is coal dust. Lung accumulations of coal dust on the order of 10 to 40 g have been reported for British coal miners.[225-228] Stöber et al.[187] reported lung burdens of as much as 25 g in Saar coal miners and of 50 g in Ruhr coal miners. Dobreva et al.[229] and Verma et al.[230] reported comparable amounts of particulates in lungs of hard rock miners. These lung burdens in miners represented the net lung-retained burdens after many years of repeated exposure to mine atmospheres. A lung burden of 10 to 20 g in humans corresponds to 10- to 20-mg particles per gram lung, which is similar to the lung burden of diesel soot in rats after 24 months of exposure to 3.5 or 7.0 mg soot per cubic meter. Results from humans, as they become available, will represent important data for comparing results among species.

A complicating factor with human exposures is the definition of the exposure history. A more difficult problem is relating industrial and environmental exposures to other factors. For example, the extent to which personal habits, age, smoking history, and other exposures contribute to the lung burdens of specific materials inhaled by humans in work environments is not known. McClellan[231] made the point that cigarette smoking is the dominant cause of air pollution-induced disease and, as such, limits our potential for developing an understanding about the human health effects of inhaled materials through human studies. The alternative is to use human data, but to supplement it with information from studies ranging from molecular to whole animal studies. Considerable work needs to be done to compare and contrast results from laboratory animal studies with the expanding human data base.

C. Comparative Lung (and LALN) Burdens from Repeated Exposures of Man and Animals

The following discussion presents some basic considerations and an approach to making projections for lung burdens in laboratory animals and humans exposed to the same airborne material or to concentrations of the airborne material that produce similar lung burdens. It is important to recognize these species differences when designing studies or evaluating responses to inhalation exposures.

The approach used in this example was simulation modeling. The desired result from all models is a mathematical way to evaluate, compare, and predict lung accumulation patterns for inhaled materials. The simulation approach is relatively simple and was based on the GASP-IV language.[232] The model (Figure 7) included only the pulmonary region and LALN to simplify the presentation. Particles deposited in the P region during inhalation exposure were assumed to clear by (1) physical translocation of particles from the pulmonary region [MP(t)] to the gastrointestinal tract, (2) physical translocation of particles to the LALN [ML(t)], and (3) dissolution-absorption of particles [A(t)]. The transfer rates represent the fraction of the material in each respective compartment that is removed per day. The mechanical clearance rates were all assumed to be independent of each other. The dissolution-absorption rate (percent of the total mass of particles absorbed per day) was assumed to be constant among species and to be the same for particles in the P region and in the LALN.

An important assumption was that deposition, retention, and clearance of the chronically inhaled particles were the same as for a small mass of particles inhaled during a single acute inhalation exposure. Stated another way, each increment of the repeatedly inhaled aerosols was assumed to deposit and clear the respiratory tract as if it were the only material inhaled and its deposition and clearance were not influenced by either its mass or the mass of particles already in the respiratory tract.

Transfer rates for simulation modeling of the fate of par-

ticles inhaled by mice, rats, guinea pigs, monkeys, dogs, and humans are summarized in Table 6. These represent transfer rates that yielded curves giving close approximations of the equations presented in Table 5. A dissolution-absorption rate of 0.000693 per day for the retained particles was inherent in the simulations for each species.

Respiratory minute volumes and lung weights (Table 2) appropriate for each species were used for these simulations. The aerosol concentration was arbitrarily set at 0.1-mg particles per cubic meter, with an aerodynamic diameter of 1.0 μm. The deposition fractions were established based on the information in Figures 8 to 11, which represent approximations based on data from Lippmann,[23] Raabe et al.,[26] and Schlesinger.[233] The simulated exposures were set at 8 h/d, 5 d/week, for 2 years. A summary of the exposure information input for the simulation model is presented in Table 7.

The modeling projections for accumulation of the particles in lungs and LALN of these six species are presented in Figures 12 and 13. These simulations demonstrate the patterns for accumulating pulmonary and LALN burdens of inhaled material and also make the point that the amount of material accumulated per gram of lung (specific lung burden) is different for the different species exposed to the same aerosol. Rats and mice have a high daily relative deposition for the inhaled particles. Their accumulation patterns show a rapid initial accumulation of particles. However, clearance is relatively rapid for both rats and mice, which dictates that equilibrium pulmonary burdens for this exposure material will be reached during the 2-year exposure. The other four curves reflect similar particle accumulation patterns in the other species; those patterns are dictated by smaller relative daily deposition rates and slower clearance rates for the particles inhaled by these species. According to these projections, guinea pigs, monkeys, dogs, and humans would not reach an equilibrium pulmonary burden of this exposure material during the 2-year repeated exposure. The projected pulmonary burdens among these species after 2 years of simulated repeated exposures differed by a factor of almost 12.

These results represent reasonable projections using currently available information about deposition, retention, and clearance for these laboratory species and humans. It has to be emphasized that the patterns demonstrated in Figures 12 and 13 are influenced by the physical clearance parameters and dissolution-absorption characteristics of the inhaled material. In addition, if inhalation of an aerosol influences respiratory patterns, the inhaled material will affect the rates and patterns by which the aerosolized material accumulates in the lung. Likewise, translocation patterns from the P region to LALN appear to be influenced by the chemical composition of inhaled material, as well as by the mass of material. Therefore, while the patterns demonstrated in these figures may be representative for inhalation exposures to relatively low air concentrations of relatively inert materials, the patterns cannot be assumed to be definitive for all materials inhaled in any quantities by these six species.

There are only limited data available to compare with these kinds of projections for repeated exposures. In a 5-year study using uranium oxide, daily inhalation of 5 mg UO_2 dust per cubic meter caused a rapid build-up of uranium in the lungs and tracheobronchial lymph nodes of dog and monkeys, but not in rats.[234,235] Concentrations of uranium in the lungs of rats reached an equilibrium level that was nearly an order of magnitude lower than the uranium concentrations found in lungs of dogs or monkeys. Concentrations of uranium in the TBLN were also much lower for rats than for dogs or monkeys, for which the values were quite similar. These patterns observed experimentally for uranium oxide agree reasonably well with the simulated projections presented in Figures 12 and 13 for repeated inhalation of relatively insoluble particles.

In another study, reported by Wolff et al.,[111] F344 rats were exposed to diesel exhaust for 2 years. Exposures were 7 h/d, 5 d/week to 0.35 mg diesel soot per cubic meter. The data

Table 6
Transfer Factors for Modeling Pulmonary Retention and Clearance of Particles[a] Inhaled by Selected Animal Species, Including Humans

Species	Mechanical particle clearance from the pulmonary region, MP(t)	Clearance to lymph nodes, M(L)
Mouse[b]	$0.023 \exp(-0.008t) + 0.0013$	$0.0007 \exp(-0.5t)$
Rat[b]	$0.028 \exp(-0.01t) + 0.0018$	$0.0007 \exp(-0.5t)$
Guinea pig[c]	$0.007 \exp(-0.03t) + 0.0004$	0.00004
Dog[b], monkey[d], human[d]	$0.008 \exp(-0.022t) + 0.0001$	0.0002

[a] Dissolution-absorption half-time assumed 1000 d.
[b] Adapted from Reference 152.
[c] Adapted from Reference 187.
[d] Assumed the same as for dogs.

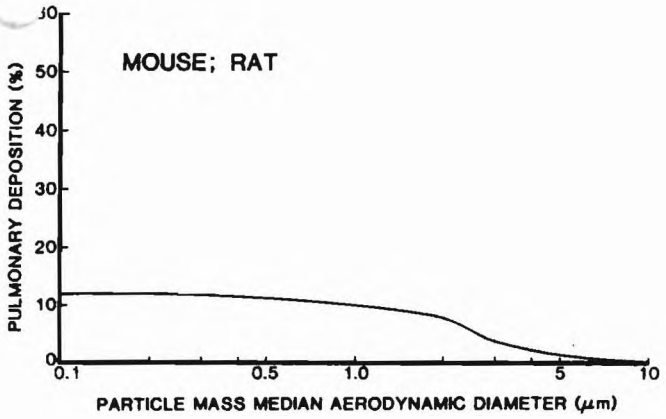

FIGURE 8. Pulmonary deposition of particles inhaled by rats and mice (fraction of the total amount inhaled).

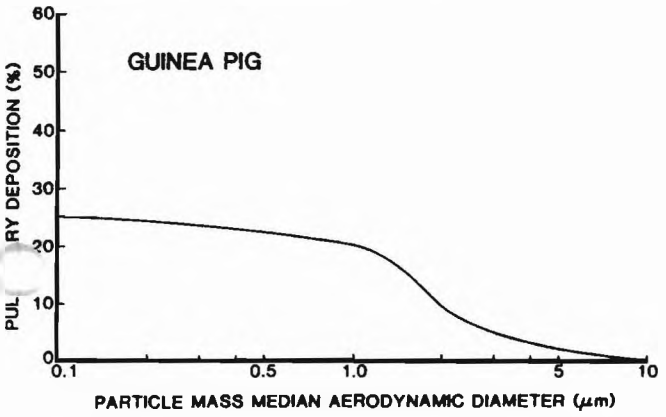

FIGURE 9. Pulmonary deposition of particles inhaled by guinea pigs (fraction of the total amount inhaled).

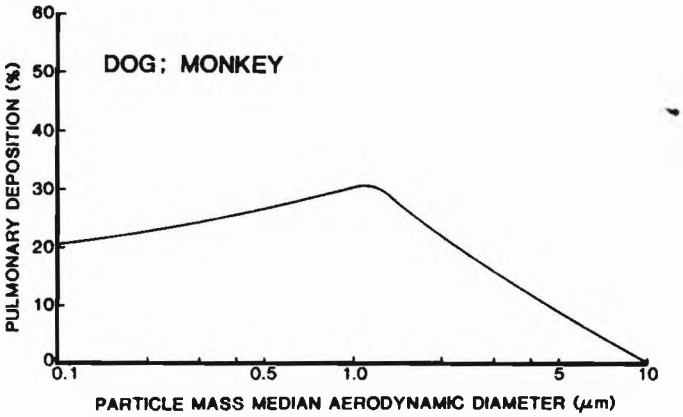

FIGURE 10. Pulmonary deposition of particles inhaled by dogs and monkeys (fraction of the total amount inhaled).

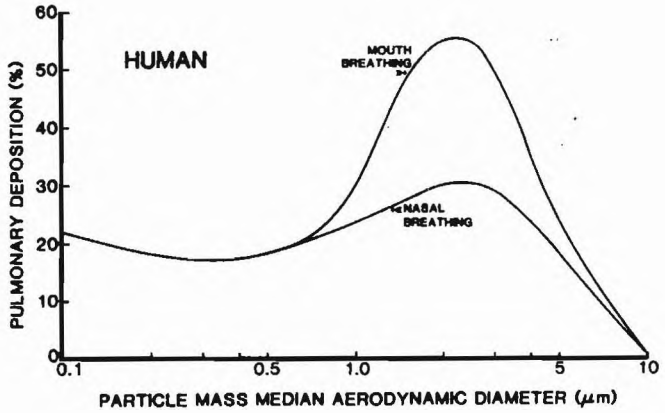

FIGURE 11. Pulmonary deposition of particles inhaled by humans (fraction of the total amount inhaled).

Table 7
Summary of Exposure Information Used for Simulating Pulmonary and Lung-Associated Lymph Node Burdens of Particles in Mice, Rats, Guinea Pigs, Dogs, Monkeys, and Humans

Common Parameters

	Values
Exposure atmosphere	0.1 mg particles per cubic meter
Mass median aerodynamic diameter	1 μm
Particle dissolution-absorption	1000 d half-time
Exposure pattern	8 h/d; 5 d/week
Duration of repeated exposure	2 years

Specific Parameters

Species	Respiratory minute volume (l)	Pulmonary deposition fraction	Particles (μg) deposited/day/g lung
Mouse	0.04	0.10	0.96
Rat	0.20	0.10	0.64
Guinea pig	0.46	0.20	1.10
Monkey	0.70	0.30	0.46
Dog	3.6	0.30	0.47
Human (mouthbreathing)	20	0.30	0.29

for lung content of the diesel soot and a simulation model[152] projection for the data are presented in Figure 14. The model was modified by (1) defining the diesel soot dissolution-absorption rate as 0.000693 per day, (2) using a value of 0.16 as the pulmonary deposition fraction, and (3) using 0.2 l/min as the respiratory minute volume for these rats. The model used the assumption that deposition, retention, and clearance

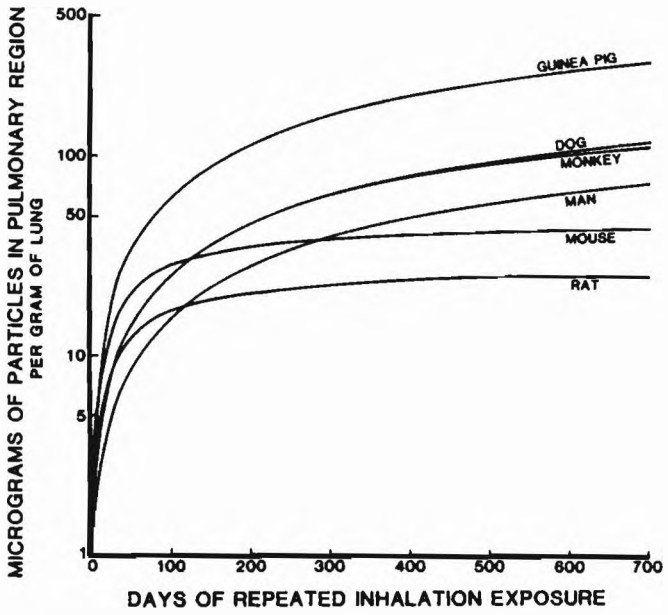

FIGURE 12. Simulation model projections for accumulation of particles in the pulmonary region of selected mammalian species, including humans, with chronic exposures 8 h/d, 5 d/week to 0.1 mg particles per cubic meter; particle dissolution-absorption half-time assumed to 1000 d. Results are presented as micrograms of particles per gram lung.

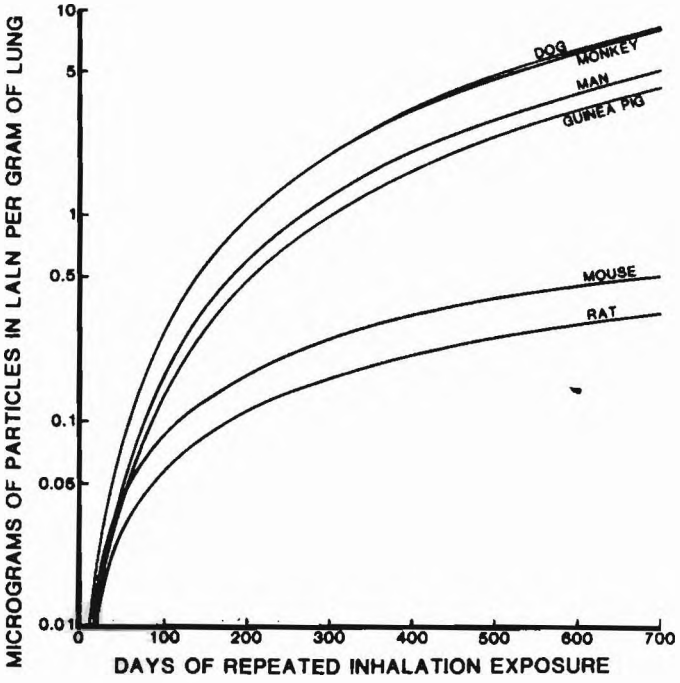

FIGURE 13. Simulation model projection for accumulation of particles in the lung-associated lymph nodes (LALN) of selected mammalian species and humans with chronic exposures 8 h/d, 5 d/week to 0.1 mg particles per cubic meter; particle dissolution-absorption half-time assumed 1000 d. Results are presented as micrograms of particles in LALN per gram of lung.

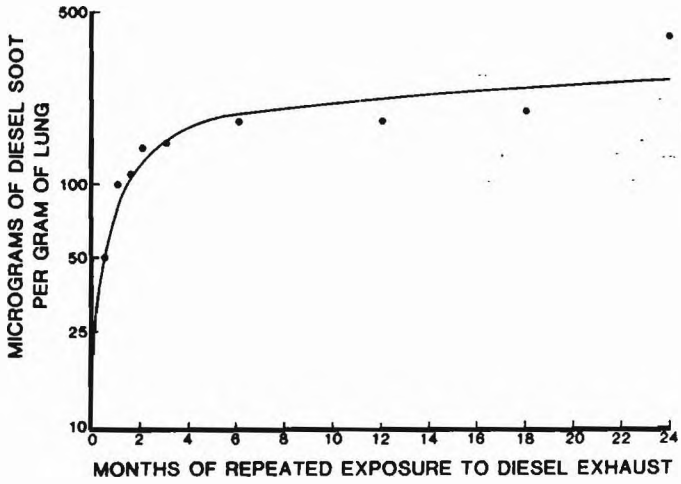

FIGURE 14. Mean values for diesel soot content of lungs (± SE) and simulation model results for diesel soot content of rat lungs for rats exposed 7 h/d, 5 d/week to 0.35 mg diesel soot per cubic meter.

of every daily intake of diesel exhaust soot were the same from the first exposure to the last exposure, 2 years later. The simulation fit the data well enough to conclude that the model was reasonable for this repeated exposure to diesel exhaust. The fact that the simulation accounted for the observed patterns of lung accumulation of diesel particles strongly suggests that the assumptions were valid for this exposure concentration and accumulated lung burden of diesel soot. However, it is clear that the assumptions are not valid for comparable exposures of rats to 3.5 or 7 mg diesel soot per cubic meter[111] where lung overloading with diesel soot occurred.

The agreement between observed and simulated patterns for lung and LALN accumulations of uranium and diesel soot particles lends a degree of confidence to the assumption that appropriate models can be used to make accurate projections, relative to lung and LALN burdens that would result from repeated exposures.

D. Equivalent Concentrations of Exposure Material

Another way to model pulmonary accumulations of inhaled particles is to select the endpoint lung burden. As an example, 75-μg particles per gram lung was chosen because it was the specific lung burden projected for humans in Figure 12. Next, the aerosol concentrations needed to reach that burden after 2 years of repeated exposure, 8 h/d, 5 d/week were computed for the other species. Figure 15 presents the results of simulations for all six species, using the equivalent air concentrations. This figure indicates (1) the relative air concentrations of this aerosol projected to produce the same pulmonary concentration of particles in the six species, and (2) the patterns that would be projected for accumulations of particles in the P region. It emphasizes the fact that different air concentrations of the test material are needed for different species to produce

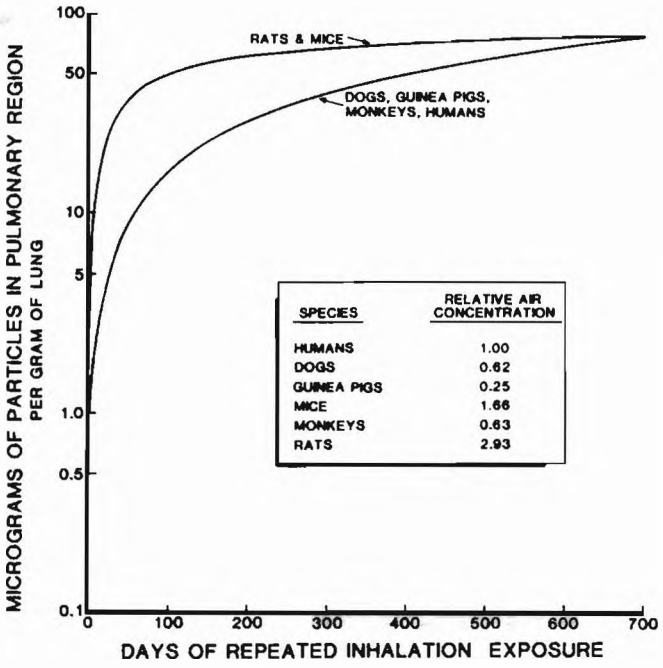

FIGURE 15. Simulation model results for pulmonary accumulation of particles in selected species exposed to achieve 75 μg particles per gram lung during a 2-year exposure 8 h/d, 5 d/week to an aerosol containing 1-μm mass median aerodynamic particles with a dissolution-absorption half-time of 1000 d. Results include relative air concentrations for these species exposed to the defined aerosol.

the same endpoint lung burden during a repeated exposure. The patterns for accumulation of particles in lung were identical for guinea pigs, monkeys, dogs, and humans because of the assumptions used in the simulations. The actual accumulation patterns may be influenced by air concentrations of exposure materials and by the chemical composition of the exposure material.

These simulation projections used the assumption that deposition and clearance of inhaled materials would be independent of the amount of material inhaled, as well as of the amount of material accumulated in the lung. Also, the potential changes in lung accumulation patterns resulting from irritation or toxic effects of the test material were not considered. Results from the literature generally indicate that these assumptions are valid, at least for nuisance dusts or materials with low toxicity, until relatively large quantities (milligrams per gram of lung tissue) of the test material have accumulated in the lung.

These examples were for a hypothetical test aerosol and commonly used laboratory animal species. The same process could be used for evaluating and making projections for a variety of test aerosols and additional animal species, including humans. The most important information necessary for these projections is (1) the deposition and clearance parameters for the species, and (2) the dissolution-absorption characteristics of the test material. These are the factors that dictate the accumulation patterns for the inhaled material.

IX. SUMMARY

This review summarizes contemporary scientific knowledge relative to the fate of particles inhaled by mammalian species. Processes involved in deposition, retention, and clearance are similar among species. There are important differences among species in amounts of materials deposited in the various regions of the respiratory tract; the bases for these differences are reasonably well understood. The bases for species differences in retention and clearance are not understood. Understanding retention and clearance patterns and the biological mechanisms associated with them is essential to a complete understanding of physiological processes in a given species and for making extrapolations among species.

The available data base for laboratory animals contains a wealth of information on single, acute inhalation exposures. The complementary data base for repeated exposures is growing, but is still relatively small. It is important that we expand and begin to understand the data base for repeated or chronic inhalation exposures since they are the most likely types of industrial and environmental exposures.

Having a means of accurately relating laboratory animal data to the sparse amount of human data now available and likely to be available in the future is vital to inhalation toxicologists and organizations that establish exposure standards.

A desired outcome of this kind of review is a statement as to which animal species is the best surrogate for humans. One aspect of the answer to this question is that there is no standard human. Considerable variability exists as a consequence of race, sex, age, size, health status, and other factors. The same considerations apply to all species. In addition, each species has its unique deposition, retention, and clearance patterns for inhaled materials, as well as potential species-dependent responses to inhaled materials or the injury patterns they produce. While no one species can adequately serve as a human surrogate, the composite data from animal studies can supplement human data to allow reasonable projections about human inhalation exposures.

ACKNOWLEDGMENTS

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