3. DOSIMETRY OF DIESEL PARTICULATE MATTER

3.1. INTRODUCTION

Clearly, animals and humans receive different internal doses when breathing the same external concentrations of airborne materials such as diesel particulate matter (DPM) (Brain and Mensah, 1983; Schlesinger, 1985). The dose received in different species differs from the aspects of the total amount deposited within the respiratory tract, the relative distribution of the dose to specific regions in the respiratory tract, and the residence time of these materials within the respiratory tract, i.e., clearance. Using an external concentration breathed by laboratory animals as a basis for any guidance for human exposure to DPM would then be an inadequate approximation of the total and regional dose that humans may receive. The objective of this chapter is to evaluate and address this issue of interspecies dosimetric differences through:

- A general overview of what is known about how particles like DPM are deposited, transported to, and cleared from the respiratory tract. Information on both laboratory animals (mainly rodents) and humans will be considered and interspecies similarities and differences highlighted.
- An overview of what is known about the bioavailability of the organic compounds adsorbed onto DPM from information in humans, animals, and in vitro studies, and from model predictions.
- An evaluation of the suitability of available dosimetric models and procedures for DPM to perform interspecies extrapolations whereby an exposure scenario, conditions, and outcome in laboratory animals are adjusted to an equivalent outcome in humans via calculation of an internal dose.

The focus in this chapter will be on the particulate fraction of diesel emissions, i.e, DPM. Although diesel engine exhaust consists of a complex mixture of typical combustion gases, vapors, low-molecular-weight hydrocarbons, and particles, it is the particle phase that is considered to be of major health concern. The major constituents of diesel exhaust and their atmospheric reaction products are described here (Chapter 2).

As will be deduced in Chapter 5, pulmonary toxicity and carcinogenicity is the major focal point of diesel toxicity and of DPM deposition. Therefore, dosimetric considerations are limited to the lung. Aspects of respiratory tract dosimetry to be considered in this chapter include the characteristics of DPM, deposition of DPM in the conducting airways and alveolar regions, normal DPM clearance mechanisms and rates of clearance in both these regions, clearance rates

during lung overload (in rats), elution of organics from DPM, transport of DPM to extra-alveolar sites, and the interrelationships of these factors.

The overall goal in this chapter follows from the objective—to judge the feasibility and suitability of procedures allowing for derivation of an internal dose estimate of DPM for humans, i.e., of a human equivalent concentration to exposure concentrations and conditions used in animal studies. This goal is of significance especially in the quantitative dose-response analysis of DPM effects proposed in Chapter 6.

3.2. CHARACTERISTICS OF INHALED DPM

The formation, transport, and characteristics of DPM are considered in detail in Chapter 2. DPM consists of aggregates of spherical carbonaceous particles (typically about 0.2 μm mass median aerodynamic diameter [MMAD]) to which significant amounts of higher-molecular-weight organic compounds are adsorbed. DPM has an extremely large surface area that allows for the adsorption of organic compounds. The organic carbon portion of DPM can range from at least 19% to 43% from highway diesel engines; no data are available to characterize the organic content of DPM from nonroad engines. The toxicologically relevant organic chemicals include high-molecular-weight hydrocarbons such as the polycyclic aromatic hydrocarbons (PAHs) and their derivatives (Section 2.2.8).

3.3. REGIONAL DEPOSITION OF INHALED DPM

This section discusses the major factors controlling the disposition of inhaled particles. Note that disposition is defined as encompassing the processes of deposition, absorption, distribution, metabolism, and elimination. The regional deposition of particulate matter in the respiratory tract is dependent on the interaction of a number of factors, including respiratory tract anatomy (airway dimensions and branching configurations), ventilatory characteristics (breathing mode and rate, ventilatory volumes and capacities), physical processes (diffusion, sedimentation, impaction, and interception), and the physicochemical characteristics (particle size, shape, density, and electrostatic attraction) of the inhaled particles. Regional deposition of particulate material is usually expressed as deposition fraction of the total particles or mass inhaled and may be represented by the ratio of the particles or mass deposited in a specific region to the number or mass of particles inspired. The factors affecting deposition in these various regions and their importance in understanding the fate of inhaled DPM are discussed in the following sections.

It is beyond the scope of this document to present a comprehensive account of the complexities of respiratory mechanics, physiology, and toxicology, and only a brief review will be presented here. The reader is referred to publications that provide a more in-depth treatment of

these topics (Weibel, 1963; Brain and Mensah, 1983; Raabe et al., 1988; Stöber et al., 1993; U.S. EPA, 1996).

The respiratory tract in both humans and experimental mammals can be divided into three regions on the basis of structure, size, and function (International Commission on Radiological Protection, 1994): the extrathoracic (ET), the tracheobronchial (TB), and the alveolar (A). In humans, inhalation can occur through the nose or mouth or both (oronasal breathing). Many animal models used in respiratory toxicology studies are, however, obligate nose breathers.

3.3.1. Deposition Mechanisms

This section provides an overview of the basic mechanisms by which inhaled particles deposit within the respiratory tract. Details concerning the aerosol physics that explain both how and why particle deposition occurs as well as data on total human respiratory tract deposition are presented in detail in the earlier PM Criteria Document (U.S. EPA, 1996) and will only be briefly summarized here. For more extensive discussions of deposition processes, refer to reviews by Morrow (1966), Raabe (1982), U.S. EPA (1982), Phalen and Oldham (1983), Lippmann and Schlesinger (1984), Raabe et al. (1988), and Stöber et al. (1993).

Particles may deposit by five major mechanisms (inertial impaction, gravitational settling, Brownian diffusion, electrostatic attraction, and interception). The relative contribution of each deposition mechanism to the fraction of inhaled particles deposited varies for each region of the respiratory tract.

It is important to appreciate that these processes are not necessarily independent but may, in some instances, interact with one another such that total deposition in the respiratory tract may be less than the calculated probabilities for deposition by the individual processes (Raabe, 1982). Depending on the particle size and mass, varying degrees of deposition may occur in the extrathoracic or ET (or nasopharyngeal), tracheobronchial (TB), and alveolar regions of the respiratory tract.

Upon inhalation of particulate matter such as that found in diesel exhaust, particle deposition will occur throughout the respiratory tract. Because of high airflow velocities and abrupt directional changes in the ET and TB regions, inertial impaction is a primary deposition mechanism, especially for particles $\geq 2.5\ \mu m$ d_{ae} (aerodynamic equivalent diameter). Although inertial impaction is a prominent process for deposition of larger particles in the tracheobronchial region, it is of minimal significance as a determinant of regional deposition patterns for DPM, which have a $d_{ae} \leq 1\ \mu m$.

All aerosol particles are continuously influenced by gravity, but particles with a $d_{ae} > 0.5\ \mu m$ are affected to the greatest extent. A spherical compact particle will acquire a terminal settling velocity when a balance is achieved between the acceleration of gravity acting on

the particle and the viscous resistance of the air; it is this velocity that brings the particle into contact with airway surfaces. Both sedimentation and inertial impaction cause the deposition of many particles within the same size range. These deposition processes act together in the ET and TB regions, with inertial impaction dominating in the upper airways and sedimentation becoming increasingly dominant in the lower conducting airways, especially for the largest particles that can penetrate into the smaller bronchial airways.

As particle diameters become <1 μm, the particles are increasingly subjected to diffusive deposition because of random bombardment by air molecules, which results in contact with airway surfaces. A d_{ae} of 0.5 μm is often considered a boundary between diffusion and aerodynamic (sedimentation and impaction) mechanisms of deposition. Thus, instead of having a d_{ae}, diffusive particles of different shapes can be related to the diffusivity of a thermodynamic equivalent size based on spherical particles (Heyder et al., 1986). Diffusive deposition of particles is favored in the A region of the respiratory tract as particles of this size are likely to penetrate past the ET and TB regions.

Electrostatic precipitation is deposition related to particle charge. The electrical charge on some particles may result in an enhanced deposition over what would be expected from size alone. This is due to image charges induced on the surface of the airway by these particles, or to space-charge effects whereby repulsion of particles containing like charges results in increased migration toward the airway wall. The effect of charge on deposition is inversely proportional to particle size and airflow rate. A recent study employing hollow airway casts of the human tracheobronchial tree that assessed deposition of ultrafine (0.02 μm) and fine (0.125 μm) particles found that deposition of singly charged particles was 5-6 times that of particles having no charge, and 2-3 times that of particles at Boltzmann equilibrium (Cohen et al., 1998). This suggests that within the TB region of humans, electrostatic precipitation may be a significant deposition mechanism for ultrafine and some fine particles, the latter of which are inclusive of DPM. Thus, although electrostatic precipitation is generally a minor contributor to overall particle deposition, it may be important for DPM.

Interception is deposition by physical contact with airway surfaces and is most important for fiber deposition (U.S. EPA, 1996).

3.3.1.1. *Biological Factors Modifying Deposition*

The available experimental deposition data in humans are commonly derived using healthy adult Caucasian males. Various factors can act to alter deposition patterns from those obtained in this group. The effects of different biological factors, including gender, age, and respiratory tract disease, on particle deposition have been reviewed previously (U.S. EPA, 1996, Section

10.4.1.6). In general, there appears to be an inverse relationship between airway resistance and total deposition.

The various species that serve as the basis for dose-response assessment in inhalation toxicology studies do not receive identical doses in a comparable respiratory tract region (ET, TB, or A) when exposed to the same aerosol or gas (Brain and Mensah, 1983). Such interspecies differences are important because the adverse toxic effect is likely more related to the quantitative pattern of deposition within the respiratory tract than to the exposure concentration; this pattern determines not only the initial respiratory tract tissue dose but also the specific pathways by which the inhaled material is cleared and redistributed (Schlesinger, 1985). Differences in patterns of deposition between humans and animals have been summarized (U.S. EPA, 1996; Schlesinger et al., 1997). Such differences in initial deposition must be considered when relating biological responses obtained in laboratory animal studies to effects in humans.

The deposition of inhaled diesel particles in the respiratory tract of humans and mammalian species has been reviewed (Health Effects Institute, 1995). Schlesinger (1985) showed that physiological differences in the breathing mode for humans (nasal or oronasal breathers) and laboratory rats (obligatory nose breathers), combined with different airway geometries, resulted in significant differences in lower respiratory tract deposition for larger particles (>1 μm d_{ae}). In particular, a much lower fraction of inhaled larger particles is deposited in the alveolar region of the rat compared with humans. However, relative deposition of the much smaller diesel exhaust particles was not affected as much by the differences among species, as was demonstrated in model calculations by Xu and Yu (1987). These investigators modeled the deposition efficiency of inhaled DPM in rats, hamsters, and humans on the basis of calculations of the models of Schum and Yeh (1980) and Weibel (1963). These simulations (Figure 3-1) indicate relative deposition patterns in the lower respiratory tract (trachea = generation 1; alveoli = generation 23) and are similar among hamsters, rats, and humans. Variations in alveolar deposition of DPM over one breathing cycle in these different species were predicted to be within 30% of one another. Xu and Yu (1987) attributed this similarity to the fact that deposition of the submicron diesel particles is dominated by diffusion rather than sedimentation or impaction. Although these data assumed nose-breathing by humans, the results would not be very different for mouth-breathing because of the low filtering capacity of the nose for particles in the 0.1 to 0.5 μm range.

For dosimetric calculations and modeling, it would be of much greater importance to consider the actual dose deposited per unit surface area of the respiratory tract rather than the relative deposition efficiencies per lung region. Table 3-1 compares the predicted deposited doses of DPM inhaled in 1 min for the three species, based on the total lung volume, the surface area of all lung airways, or the surface area of the epithelium of the alveolar region only. In Table 3-1,

the deposited dose, expressed as either mass/lung volume (M) or mass/surface area(s) (M_1), or mass/alveolar surface area (M_2) is lower in humans than in the two rodent species as a result of the greater respiratory exchange rate in rodents and smaller size of the rodent lung. Such differences in the deposited dose in relevant target areas are important and have to be considered when extrapolating the results from DPM/DE exposure studies in animals to humans. Table 3-1 indicates that the differences (between humans to animals) are less on a surface area basis (≈3-fold) than on a lung volume basis (≈14-fold). This is due to larger alveolar diameters and concomitant lower surface area per unit of lung volume in humans.

Particle deposition will initiate particle redistribution processes (e.g., clearance mechanisms, phagocytosis) that transfer the particles to various subcompartments, including the alveolar macrophage pool, pulmonary interstitium, and lymph nodes. Over time, therefore, only small amounts of the original particle intake would be associated with the alveolar surface.

3.3.2. Particle Clearance and Translocation Mechanisms

This section provides an overview of the mechanisms and pathways by which particles are cleared from the respiratory tract. The mechanisms of particle clearance as well as clearance routes from the various regions of the respiratory tract have been considered in the PM Criteria Document (U.S. EPA, 1996) and reviewed by Schlesinger et al. (1997).

Particles that deposit upon airway surfaces may be cleared from the respiratory tract completely, or be translocated to other sites within this system, by various regionally distinct processes. These clearance mechanisms can be categorized as either absorptive (i.e., dissolution) or nonabsorptive (i.e., transport of intact particles) and may occur simultaneously or with temporal variations. Particle solubility in terms of clearance refers to solubility within the respiratory tract fluids and cells. Thus, a poorly soluble particle is one whose rate of clearance by dissolution is insignificant compared to its rate of clearance as an intact particle (as is the case with DPM). The same clearance mechanisms act on specific particles to different degrees, with their ultimate fate being a function of deposition site, physicochemical properties (including any toxicity), and sometimes deposited mass or number concentration.

3.3.2.1. *Extrathoracic Region*

The clearance of poorly soluble particles deposited in the nasal passages occurs via mucociliary transport, and the general flow of mucus is backwards, i.e., towards the nasopharynx. Mucus flow in the most anterior portion of the nasal passages is forward, clearing deposited particles to the vestibular region where removal is by sneezing, wiping, or blowing.

Soluble material deposited on the nasal epithelium is accessible to underlying cells via diffusion through the mucus. Dissolved substances may be subsequently translocated into the

bloodstream. The nasal passages have a rich vasculature, and uptake into the blood from this region may occur rapidly.

Clearance of poorly soluble particles deposited in the oral passages is by coughing and expectoration or by swallowing into the gastrointestinal tract.

3.3.2.2. *Tracheobronchial Region*

The dynamic relationship between deposition and clearance is responsible for determining lung burden at any point in time. Clearance of poorly soluble particles from the TB region is mediated primarily by mucociliary transport, a more rapid process than those operating in alveolar regions. Mucociliary transport (often referred to as the mucociliary escalator) is accomplished by the rhythmic beating of cilia that line the respiratory tract from the trachea through the terminal bronchioles. This movement propels the mucous layer containing deposited particles (or particles within alveolar macrophages [AMs]) toward the larynx. Clearance rate by this system is determined primarily by the flow velocity of the mucus, which is greater in the proximal airways and decreases distally. These rates also exhibit interspecies and individual variability. Considerable species-dependent variability in tracheobronchial clearance has been reported, with dogs generally having faster clearance rates than guinea pigs, rats, or rabbits (Felicetti et al., 1981). The half-time ($t_{1/2}$) values for tracheobronchial clearance of relatively insoluble particles are usually on the order of hours, as compared to alveolar clearance, which is on the order of hundreds of days in humans and dogs. The clearance of particulate matter from the tracheobronchial region is generally recognized as being biphasic or multiphasic (Raabe, 1982). Some studies have shown that particles are cleared from large, intermediate, and small airways with $t_{1/2}$ of 0.5, 2.5, and 5 h, respectively. However, reports have indicated that clearance from airways is biphasic and that the long-term component for humans may take much longer for a significant fraction of particles deposited in this region, and may not be complete within 24 h as generally believed (Stahlhofen et al., 1990; ICRP, 1994).

Although most of the particulate matter will be cleared from the tracheobronchial region towards the larynx and ultimately swallowed, the contribution of this fraction relative to carcinogenic potential is unclear. With the exception of conditions of impaired bronchial clearance, the desorption $t_{1/2}$ for particle-associated organics is generally longer than the tracheobronchial clearance times, thereby making uncertain the importance of this fraction relative to toxicity in the respiratory tract (Pepelko, 1987). However, Gerde et al. (1991a) showed that for low-dose exposures, particle-associated PAHs were released rapidly at the site of deposition. The relationship between the early clearance of poorly soluble particles of 4 μm aerodynamic diameter from the tracheobronchial regions and their longer-term clearance from the alveolar region is illustrated in Figure 3-2.

Cuddihy and Yeh (1986) reviewed respiratory tract clearance of particles inhaled by humans. Depending on the type of particle (ferric oxide, Teflon discs, or albumin microspheres), the technique employed, and the anatomic region (midtrachea, trachea, or main bronchi), particle velocity (moved by mucociliary transport) ranged from 2.4 to 21.5 mm/min. The highest velocities were recorded for midtracheal transport, and the lowest were for main bronchi. In one study, an age difference was noted for tracheal mucociliary transport velocity (5.8 mm/min for individuals less than 30 years of age and 10.1 mm/min for individuals over 55 years of age).

Cuddihy and Yeh (1986) described salient points to be considered when estimating particle clearance velocities from tracheobronchial regions: these include respiratory tract airway dimensions, calculated inhaled particle deposition fractions for individual airways, and thoracic (A + TB) clearance measurements. Predicted clearance velocities for the trachea and main bronchi were found to be similar to those experimentally determined for inhaled radiolabeled particles, but not those for intratracheally instilled particles. The velocities observed for inhalation studies were generally lower than those of instillation studies. Figure 3-3 illustrates a comparison of the short-term clearance of inhaled particles by human subjects and the model predictions for this clearance. However, tracheobronchial clearance via the mucociliary escalator is of limited importance for long-term clearance.

Exposure of F344 rats to whole DPM at concentrations of 0.35, 3.5, or 7.1 mg/m^3 for up to 24 mo did not significantly alter tracheal mucociliary clearance as assessed by clearance of ^{99m}Tc-macroaggregated albumin instilled into the trachea (Wolff et al., 1987). The authors stated that measuring retention would yield estimates of clearance efficiency comparable to measuring the velocity for transport of the markers in the trachea. The results of this study were in agreement with similar findings of unaltered tracheal mucociliary clearance in rats exposed to DPM (0.21, 1.0, or 4.4 mg/m^3) for up to 4 mo (Wolff and Gray, 1980). However, the 1980 study by Wolff and Gray, as well as an earlier study by Battigelli et al. (1966), showed that acute exposure to high concentrations of diesel exhaust soot (1.0 and 4.4 mg/m^3 in the study by Wolff and Gray [1980] and 8 to 17 mg/m^3 in the study by Battigelli et al. [1966]) produced transient reductions in tracheal mucociliary clearance. Battigelli et al. (1966) also noted that the compromised tracheal clearance was not observed following cessation of exhaust exposure.

That tracheal clearance does not appear to be significantly impaired or is impaired only transiently following exposure to high concentrations of DPM is consistent with the absence of pathological effects in the tracheobronchial region of the respiratory tract in experimental animals exposed to DPM. The apparent retention of a fraction of the deposited dose in the airways could be cause for some concern regarding possible effects in this region, especially in light of the results from simulation studies by Gerde et al. (1991b) suggesting that release of PAHs from particles may occur within minutes and therefore at the site of initial deposition.

However, the absence of effects in the TB areas in long-term DPM studies and experimental evidence that particle-associated PAHs are released at the site of particle deposition together suggest that these PAHs and other organics may be of lesser importance in tumorigenic responses of rats than originally suspected. On the other hand, however, a larger fraction of particles are translocated to the interstitium of the respiratory tract in primates (and therefore presumably in humans) than in rats, including the interstitium of the respiratory bronchioles, an anatomical site absent in rats (Section 3.6) (Nikula et al., 1997a,b). Moreover, eluted PAHs in the TB region are retained longer than those in the alveoli (Gerde et al., 1999), allowing time for activation. Thus PAHs may have a role in human response to diesel exhaust that cannot be evaluated with the rat model.

Also, impairment of mucociliary clearance function as a result of exposure to occupational or environmental respiratory tract toxicants or to cigarette smoke may significantly enhance the retention of particles in the TB region. For example, Vastag et al. (1986) demonstrated that not only smokers with clinical symptoms of bronchitis but also symptom-free smokers have significantly reduced mucociliary clearance rates. Although impaired tracheobronchial clearance could conceivably have an impact on the effects of deposited DPM in the conducting airways, it does not appear to be relevant to the epigenetic mechanism likely responsible for diesel exhaust-induced rat pulmonary tumors.

Poorly soluble particles such as DPM that are deposited within the TB region are cleared predominantly by mucociliary transport towards the oropharynx, followed by swallowing. Poorly soluble particles may also be cleared by traversing the epithelium by endocytotic processes, and enter the peribronchial region. Clearance may occur following phagocytosis by airway macrophages, located on or beneath the mucous lining throughout the bronchial tree, or via macrophages that enter the airway lumen from the bronchial or bronchiolar mucosa (Robertson, 1980).

3.3.2.3. *A Region*

A number of investigators have reported on the alveolar clearance kinetics of human subjects. Bohning et al. (1980) examined alveolar clearance in eight humans who had inhaled <0.4 mg of ^{85}Sr-labeled polystyrene particles (3.6 ± 1.6 μm diam.). A double-exponential model best described the clearance of the particles and provided $t_{1/2}$ values of 29 ± 19 days and 298 ± 114 days for short-term and long-term phases, respectively. It was noted that of the particles deposited in the alveolar region, 75% ± 13% were cleared via the long-term phase. Alveolar retention $t_{1/2}$ values of 330 and 420 days were reported for humans who had inhaled aluminosilicate particles of MMAD 1.9 and 6.1 μm (Bailey et al., 1982). In a comprehensive study Bailey et al. (1985) followed the long-term retention of inhaled particles in a human

respiratory tract. The retention of 1 and 4 μm fused aluminosilicate particles labeled with strontium-85 and yttrium-88, respectively, was followed in male volunteers for about 533 days. Approximately 7% of the initial lung deposit of 1 μm particles and 40% of the 4 μm particles were associated with a rapid clearance phase corresponding to the calculated tracheobronchial deposits. Retention of the remaining material followed a two-component exponential function, with phases having half-times of the order of tens of days and several hundred days, respectively.

Quantitative data on clearance rates in humans having large lung burdens of particulate matter are lacking. Bohning et al. (1982) and Cohen et al. (1979), however, did provide evidence for slower clearance in smokers, and Freedman and Robinson (1988) reported slower clearance rates in coal miners who had mild pneumoconiosis with presumably high lung burdens of coal dust. Although information on particle burden and particle overload relationships in humans is much more limited than in experimental animal models, inhibition of clearance does seem to occur. Stöber et al. (1967) estimated a clearance $t_{1/2}$ of 4.9 years in coal miners with nil or slight silicosis, based on postmortem lung burdens. The lung burdens and estimated exposure histories ranged from 2 to 50 mg/g of lung or more, well above the value at which clearance impairment is observed in the rat. Furthermore, impaired clearance resulting from smoking or exposure to other respiratory toxicants may increase the possibility of an enhanced particle accumulation effect resulting from exposure to other particle sources such as DPM.

Normal alveolar clearance rates in laboratory animals exposed to DPM have been reported by a number of investigators (Table 3-2). Because the rat is, historically, the species for which experimentally induced lung cancer data are available and for which most clearance data exist, it is the species most often used for assessing human risk, and reviews of alveolar clearance studies have been generally limited to this species.

Chan et al. (1981) subjected 24 male F344 rats to nose-only inhalation of DPM (6 mg/m^3) labeled with ^{131}Ba or ^{14}C for 40 to 45 min and assessed total lung deposition, retention, and elimination. Based on radiolabel inventory, the deposition efficiency in the respiratory tract was 15% to 17%. Measurement of ^{131}Ba label in the feces during the first 4 days following exposure indicated that 40% of the deposited DPM was eliminated via mucociliary clearance. Clearance of the particles from the lower respiratory tract followed a two-phase elimination process consisting of a rapid ($t_{½}$ of 1 day) elimination by mucociliary transport and a slower ($t_{½}$ of 62 days) macrophage-mediated alveolar clearance. This study provided data for normal alveolar clearance rates of DPM not affected by prolonged exposure or particle overloading.

Several studies have investigated the effects of exposure concentration on the alveolar clearance of DPM by laboratory animals. Wolff et al. (1986, 1987) provided clearance data ($t_{½}$) and lung burden values for F344 rats exposed to diesel exhaust for 7 h/day, 5 days/week for 24 mo. Exposure concentrations of 0.35, 3.5, and 7.1 mg of DPM/m^3 were employed in this whole

body-inhalation exposure experiment. Intermediate (hours-days) clearance of $^{67}Ga_2O_3$ particles (30 min, nose-only inhalation) was assessed after 6, 12, 18, and 24 mo of exposure at all of the DPM concentrations. A two-component function described the clearance of the administered radiolabel:

$$F_{(t)} = A\exp(-0.693\ t/t_1) + B\exp(-0.693\ t/t_2), \qquad (3\text{-}1)$$

where $F_{(t)}$ was the percentage retained throughout the respiratory tract, *A* and *B* were the magnitudes of the two components (component *A* included nasal, lung, and gastrointestinal clearance, while component *B* represented intermediate lung clearance) and τ_1 and τ_2 were the half-times for the *A* and *B* components, respectively. The early clearance half-times (τ_1), were similar for rats in all exposure groups at all time points except in the high-exposure (7.1 mg/m^3) group following 24 mo of exposure, which was faster than the controls. Significantly longer *B* component retention half-times, representing intermediate clearance probably from nonciliated structures such as alveolar ducts and alveoli, were noted after as little as 6 mo exposure to DPM at 7.1 mg/m^3 and 18 mo exposure to 3.5 mg/m^3.

Nose-only exposures to ^{134}Cs fused aluminosilicate particles (FAP) were used to assess long-term (weeks-months) clearance. Following 24-mo exposure to DPM, long-term clearance of ^{134}Cs-FAP was significantly ($p<0.01$) altered in the 3.5 (cumulative exposure [C × T] of 11,760 mg·h/m^3) and 7.1 mg/m^3 C × T = 23,520 mg·h/m^3) exposure groups ($t_{½}$ of 264 and 240 days, respectively) relative to the 0.35 mg/m^3 and control groups ($t_{½}$ of 81 and 79 days, respectively). Long-term clearance represents the slow component of particle removal from the alveoli. The decreased clearance correlated with the greater particle burden in the lungs of the 3.5 and 7.1 mg/m^3 exposure groups. Based on these findings, the cumulative exposure of > 11,760 mg·h/m^3 (or 3.5 mg/m^3 for a lifetime exposure) represented a particle overload condition resulting in compromised alveolar clearance mechanisms; the clearance rate at the lowest concentration (0.35 mg/m^3; cumulative exposure of 118 mg·h/m^3) was not different from control rates (Figure 3-4).

Heinrich et al. (1986) exposed rats 19 h/day, 5 days/week for 2.5 years to DPM at a particle concentration of about 4 mg/m^3, equal to a C × T of 53,200 mg·h/m^3. The deposition in the alveolar region was estimated to equal 60 mg. The lung particle burden was apparently sufficient to result in a "particle overload" condition (Section 3.4). With respect to the organic matter adsorbed onto the particles, the authors estimated that over the 2.5-year period, 6-15 mg of particle-bound organic matter had been deposited and was potentially available for biological

effects. This estimation was based on the analysis of the diesel exhaust used in the experiments, values for rat ventilatory functions, and estimates of deposition and clearance.

Accumulated burden of DPM in the lungs following an 18-mo, 7 h/day, 5 days/week exposure to diesel exhaust was reported by Griffis et al. (1983). Male and female F344 rats exposed to 0.15, 0.94, or 4.1 mg DPM/m^3 were sacrificed at 1 day and 1, 5, 15, 33, and 52 weeks after exposure, and DPM was extracted from lung tissue dissolved in tetramethylammonium hydroxide. Following centrifugation and washing of the supernatant, DPM content of the tissue was quantitated using spectrophotometric techniques. The analytical procedure was verified by comparing results to recovery studies using known amounts of DPM with lungs of unexposed rats. Lung burdens were 0.035, 0.220, and 1.890 mg/g lung tissue, respectively, in rats exposed to 0.15, 0.94, and 4.1 mg DPM/m^3. Long-term retention for the 0.15 and 0.94 mg/m^3 groups had estimated half-times of 87 ± 28 and 99 ± 8 days, respectively. The retention $t_{1/2}$ for the 4.1-mg/m^3 exposure group was 165 ± 8 days, which was significantly (p<0.0001) greater than those of the lower exposure groups. The 18-mo exposures to 0.15 or 0.96 mg/m^3 levels of DPM C × T equivalent of 378 and 2,368 mg·h/m^3, respectively) did not affect clearance rates, whereas the exposure to the 4.1 mg/m^3 concentration C × T = 10,332 mg·h/m^3) resulted in impaired clearance.

Lee et al. (1983) described the clearance of DPM (7 mg/m^3 for 45 min or 2 mg/m^3 for 140 min) by F344 rats (24 per group) and Hartley guinea pigs exposed by nose-only inhalation with no apparent particle overload in the lungs as being in three distinct phases. The exposure protocols provided comparable total doses based on a ^{14}C radiolabel. $^{14}CO_2$ resulting from combustion of ^{14}C-labeled diesel fuel was removed by a diffusion scrubber to avoid erroneous assessment of ^{14}C intake by the animals. Retention of the radiolabeled particles was determined up to 335 days after exposure and resulted in a three-phase clearance with retention $t_{1/2}$ values of 1, 6, and 80 days. The three clearance phases are taken to represent removal of tracheobronchial deposits by the mucociliary escalator, removal of particles deposited in the respiratory bronchioles, and alveolar clearance, respectively. Species variability in clearance of DPM was also demonstrated because the Hartley guinea pigs exhibited negligible alveolar clearance from day 10 to day 432 following a 45-min exposure to a DPM concentration of 7 mg/m^3. Initial deposition efficiency (20% ± 2%) and short-term clearance were, however, similar to those for rats.

Lung clearance in male F344 rats preexposed to DPM at 0.25 or 6 mg/m^3 20 h/day, 7 days/week for periods lasting from 7 to 112 days was studied by Chan et al. (1984). Following this preexposure protocol, rats were subjected to 45-min nose-only exposure to ^{14}C-DE, and alveolar clearance of radiolabel was monitored for up to 1 year. Two models were proposed: a normal biphasic clearance model and a modified lung retention model that included a slow-clearing residual component to account for sequestered aggregates of macrophages. The first model described a first-order clearance for two compartments: $R(t) = Ae^{-u1t} + Be^{-u2t}$. This yielded

clearance $t_{1/2}$ values of 166 and 562 days for rats preexposed to 6.0 mg/m^3 for 7 and 62 days, respectively. These values were significantly (p<0.05) greater than the retention $t_{1/2}$ of 77 ± 17 days for control rats. The same retention values for rats of the 0.25 mg/m^3 groups were 90 ± 14 and 92 ± 15 days, respectively, for 52- and 112-day exposures and were not significantly different from controls. The two-compartment model represents overall clearance of the tracer particles, even if some of the particles were sequestered in particle-laden macrophages with substantially slower clearance rates. For the second model, which excluded transport of the residual fractions in sequestered macrophage aggregates, slower clearance was observed in the group with a lung burden of 6.5 mg (exposed to 6.0 mg/m^3 for 62 days), and no clearance was observed in the 11.8 mg group (exposed to 6.0 mg/m^3 for 112 days). Clearance was shown to be dependent on the initial burden of particles, and therefore the clearance $t_{1/2}$ would increase in higher exposure scenarios. This study emphasizes the importance of particle overloading of the lung and the ramifications on clearance of particles; the significant increases in half-times indicate an increasing impairment of the alveolar macrophage mobility and subsequent transition into an overload condition as is discussed further in Section 3.4.

Long-term alveolar clearance rates of particles in various laboratory animals and humans have been reviewed by Pepelko (1987). Although retention $t_{1/2}$ varies both among and within species and is also dependent on the physicochemical properties of the inhaled particles, the retention $t_{1/2}$ for humans is much longer (>8 mo) than the average retention $t_{1/2}$ of 60 days for rats.

Clearance from the A region occurs via a number of mechanisms and pathways, but the relative importance of each is not always certain and may vary between species. Particle removal by macrophages comprises the main nonabsorptive clearance process in this region. Alveolar macrophages reside on the epithelium, where they phagocytize and transport deposited material, which they contact by random motion or via directed migration under the influence of local chemotactic factors (Warheit et al., 1988).

Particle-laden macrophages may be cleared from the A region along a number of pathways (U.S. EPA, 1996). Uningested particles or macrophages in the interstitium may traverse the alveolar-capillary endothelium, directly entering the blood (Raabe, 1982; Holt, 1981); endocytosis by endothelial cells followed by exocytosis into the vessel lumen seems, however, to be restricted to particles <0.1 μm diameter, and may increase with increasing lung burden (Lee et al., 1985; Oberdörster, 1988). Once in the systemic circulation, transmigrated macrophages, as well as uningested particles, can travel to extrapulmonary organs.

Alveolar macrophages constitute an important first-line cellular defense mechanism against inhaled particles that deposit in the alveolar region of the lung. It is well established that a host of diverse materials, including DPM, are phagocytized by AMs shortly after deposition (White and Garg, 1981; Lehnert and Morrow, 1985) and that such cell-contained particles are generally

rapidly sequestered from both the extracellular fluid lining in the alveolar region and the potentially sensitive alveolar epithelial cells. In addition to this role in compartmentalizing particles from other lung constituents, AMs are prominently involved in mediating the clearance of relatively insoluble particles from the air spaces (Lehnert and Morrow, 1985). Although the details of the actual process have not been delineated, AMs with their particle burdens gain access and become coupled to the mucociliary escalator and are subsequently transported from the lung via the conducting airways. Although circumstantial, numerous lines of evidence indicate that such AM-mediated particle clearance is the predominant mechanism by which relatively insoluble particles are removed from the alveolar region of the lungs (Gibb and Morrow, 1962; Ferin, 1982; Harmsen et al., 1985; Lehnert and Morrow, 1985; Powdrill et al., 1989).

The removal characteristics for particles deposited in the alveolar region of the lung have been descriptively represented by numerous investigators as a multicompartment or multicomponent process in which each component follows simple first-order kinetics (Snipes and Clem, 1981; Snipes et al., 1988; Lee et al., 1983). Although the various compartments can be described mathematically, the actual physiological mechanisms determining these differing clearance rates have not been well characterized.

Lehnert et al. (1988, 1989) performed studies using laboratory rats to examine particle-AM relationships over the course of alveolar clearance of low to high lung burdens of noncytotoxic microspheres (2.13 μm diam.) to obtain information on potential AM-related mechanisms that form the underlying bases for kinetic patterns of alveolar clearance as a function of particle lung burdens. The intratracheally instilled lung burdens varied from 1.6×10^7 particles (about 85 μg) for the low lung burden to 2.0×10^8 particles (about 1.06 mg) for the mid-dose and 6.8×10^8 particles (about 3.6 mg) for the highest lung burden. The lungs were lavaged at various times postexposure and the numbers of spheres in each macrophage counted. Although such experiments provide information regarding the response of the lung to particulate matter, intratracheal instillation is not likely to result in the same depositional characteristics as inhalation of particles. Therefore, it is unlikely that the response of alveolar macrophages to these different depositional characteristics will be quantitatively similar.

The $t_{1/2}$ values of both the early and later components of clearance were virtually identical following deposition of the low and medium lung burdens. For the highest lung burden, significant prolongations were found in both the early, more rapid, as well as the slower component of alveolar clearance. The percentages of the particle burden associated with the earlier and later components, however, were similar to those of the lesser lung burdens. On the basis of the data, the authors concluded that translocation of AMs from alveolar spaces by way of the conducting airways is fundamentally influenced by the particle burden of the cells so translocated. In the case of particle overload that occurred at the highest lung burden, the

translocation of AMs with the heaviest cellular burdens of particles (i.e., greater than about 100 microspheres per AM) was definitely compromised.

On the other hand, analysis of the disappearance of AMs with various numbers of particles indicates that the particles may not exclusively reflect the translocation of AMs from the lung. The observations are also consistent with a gradual redistribution of retained particles among the AMs in the lung concurrent with the removal of particle-containing AMs via the conducting airways. Experimental support suggestive of potential processes for such particle redistribution comes from a variety of investigations involving AMs and other endocytic cells (Heppleston and Young, 1973; Evans et al., 1986; Aronson, 1963; Sandusky et al., 1977; Heppleston, 1961; Riley and Dean, 1978).

3.3.3. Translocations of Particles to Extra-Alveolar Macrophage Compartment Sites

Although the phagocytosis of particles by cells free within the lung and the mucociliary clearance of the cells with their particulate matter burdens represent the most prominent mechanisms that govern the fate of particles deposited in the alveolar region, other mechanisms exist that can affect both the retention characteristics of relatively insoluble particles in the lung and the lung clearance pathways for the particles. One mechanism is endocytosis of particles by alveolar lining (Type I) cells (Sorokin and Brain, 1975; Adamson and Bowden, 1978, 1981) that normally provide >90% of the cell surface of the alveoli in the lungs of a variety of mammalian species (Crapo et al., 1983). This process may be related to the size of the particles that deposit in the lungs and the numbers of particles that are deposited. Adamson and Bowden (1981) found that with increasing loads of carbon particles (0.03 μm diam.) instilled in the lungs of mice, more free particles were observed in the alveoli within a few days. The relative abundance of particles endocytosed by Type I cells also increased with increasing lung burdens of the particles, but instillation of large particles (1.0 μm) rarely resulted in their undergoing endocytosis. A 4 mg burden of 0.1 μm diameter latex particles is equivalent to 8×10^{12} particles, whereas a 4 mg burden of 1.0 μm particles is composed of 8×10^{9} particles. Regardless, DPM with volume median diameters between 0.05 and 0.3 μm (Frey and Corn, 1967; Kittleson et al., 1978) would be expected to be within the size range for engulfment by Type I cells should suitable encounters occur. Indeed, it has been demonstrated that DPM is endocytosed by Type I cells in vivo (White and Garg, 1981).

Unfortunately, information on the kinetics of particle engulfment (endocytosis) by Type I cells relative to that by AMs is scanty. Even when relatively low burdens of particulate matter are deposited in the lungs, some fraction of the particles usually appears in the regional lymph nodes (Ferin and Fieldstein, 1978; Lehnert, 1989). As will be discussed, endocytosis of particles by Type I cells is an initial, early step in the passage of particles to the lymph nodes. Assuming

particle phagocytosis is not sufficiently rapid or perfectly efficient, increasing numbers of particles would be expected to gain entry into the Type I epithelial cell compartment during chronic aerosol exposures. Additionally, if particles are released on a continual basis by AMs that initially sequestered them after lung deposition, some fraction of the "free" particles so released could also undergo passage from the alveolar space into Type I cells.

The endocytosis of particles by Type I cells represents only the initial stage of a process that can lead to the accumulation of particles in the lung's interstitial compartment and the subsequent translocation of particles to the regional lymph nodes. As shown by Adamson and Bowden (1981), a vesicular transport mechanism in the Type I cell can transfer particles from the air surface of the alveolar epithelium into the lung's interstitium, where particles may be phagocytized by interstitial macrophages or remain in a "free" state for a poorly defined period that may be dependent on the physicochemical characteristics of the particle. The lung's interstitial compartment accordingly represents an anatomical site for the retention of particles in the lung, especially so for primates. Whether or not AMs, and perhaps polymorphonuclear neutrophils (PMNs) that have gained access to the alveolar space compartment and phagocytize particles there, also contribute to the particle translocation process into the lung's interstitium remains a controversial issue.

Translocation of particulate matter to the various interstitial spaces within the lung is a prominent phenomenon occurring at least at high (occupational) exposures that has been examined extensively for both DPM and coal dust in a species comparison between rats and primates (Nikula et al., 1997a,b). Detailed pulmonary morphometry conducted on F344 rats and cynomolgus monkeys that had been exposed for 24 months to occupational levels of DPM (1.95 mg/m^3; see Lewis et al., 1989) showed major differences in the pulmonary sites of particulate deposition. In rats about 73% of DPM was present in the alveolar ducts/alveoli and 27% in interstitial compartments; for monkeys the corresponding figures were markedly different at 43% and 57%. The corresponding pulmonary histopathology confirmed that both species were affected, although rats are more sensitive, as incidence and severity scores for alveolar effects ranged from 15 of 15 with severity scores from 1-4 (minimal to moderate), whereas for monkeys the corresponding values were only 4 of 15 at a range of 0-2 (not observed to minimal). Similarly, both species exhibited histopathology at the interstitial sites of deposition but with effects in monkeys being slightly more severe (1 of 15 graded as slight, 14 of 15 graded as minimal) than those in rats (14 of 15 graded as slight, 1 of 15 graded as minimal). The basis for this interspecies difference may be due to any number of clear contrasts that exist between rat and primate lungs, including anatomical (primates and humans have respiratory bronchioles whereas rats do not), kinetic (primates and human clearance processes allow more residence time of particles in the lung than do those in rats), or morphological (primates and humans have more

interstitial tissue, more and thicker pleura, and wider interstitial spaces than do rats). The analysis of Kuempel (2000) using human occupational data clearly showed that models require an interstitialization process to provide adequate fits to the empirical human (miners') lung deposition data discussed in that study. Hypotheses about possible mechanisms for the interstitialization process are scant, although Harmsen et al. (1985) provided some evidence in dogs that migration of AMs may contribute to the passage of particles to the interstitial compartment and also may be involved in the subsequent translocation of particles to draining lymph nodes. Translocation to the extrapulmonary regional lymph nodes apparently can involve the passage of free particles as well as particle-containing cells via lymphatic channels in the lungs (Harmsen et al., 1985; Ferin and Fieldstein, 1978; Lee et al., 1985). Further, it has been noted that particles accumulate both more rapidly and more abundantly in lymph nodes that receive lymphatic drainage from the lung (Ferin and Feldstein, 1978; Lee et al., 1985). As a final point, it should be stressed that further investigation is required to confirm the character and even existence of the interstitialization process in the lungs of humans with exposures to particles at lower environmental concentrations, or to submicrometer particles such as DPM.

3.3.3.1. *Clearance Kinetics*

The clearance kinetics of PM have been reviewed in the PM CD (U.S. EPA, 1996) and by Schlesinger et al. (1997), the results of which indicate that clearance kinetics may be profoundly influenced by several factors. The influence of time, for example, is definitively showed by the work of Bailey et al. (1985; discussed above), who showed that the rate of clearance from the pulmonary region to the GI tract decreased nearly fourfold from initial values to those noted at 200 days and beyond after particle inhalation.

3.3.3.2. *Interspecies Patterns of Clearance*

The inability to study the retention of certain materials in humans for direct risk assessment requires the use of laboratory animals. Adequate toxicological assessment necessitates that interspecies comparisons consider aspects of dosimetry including knowledge of clearance rates and routes. The basic mechanisms and overall patterns of clearance from the respiratory tract are similar in humans and most other mammals. Regional clearance rates, however, can show substantial variation between species, even for similar particles deposited under comparable exposure conditions (U.S. EPA, 1996; Schlesinger et al., 1997; Snipes et al., 1989).

In general, there are species-dependent rate constants for various clearance pathways. Differences in regional and total clearance rates between some species are a reflection of differences in mechanical clearance processes. For consideration in assessing particle dosimetry,

the end result of interspecies differences in clearance is that the retained doses in the lower respiratory tract can differ between species, which may result in differences in response to similar particulate exposures.

3.3.3.3. *Clearance Modifying Factors and Susceptible Populations*

A number of host and environmental factors may modify clearance kinetics and may consequently make individuals exhibiting or afflicted with these factors particularly susceptible to the effects resulting from exposure to DPM. These include age, gender, physical activity, respiratory tract disease, and inhalation of irritants (U.S. EPA, 1996, Section 10.4.2.5). Respiratory tract clearance appears to be prolonged in a number of pathophysical conditions in humans, including chronic sinusitis, chronic bronchitis, ashthma, chronic obstructive lung disease, and various acute respiratory infections.

3.3.3.4. *Respiratory Tract Disease*

Earlier studies reviewed in the PM CD (U.S. EPA, 1996) noted that various respiratory tract diseases are associated with alterations in overall clearance and clearance rates. Prolonged nasal mucociliary clearance in humans is associated with chronic sinusitis or rhinitis, and cystic fibrosis. Bronchial mucus transport may be impaired in people with bronchial carcinoma, chronic bronchitis, asthma, and various acute infections. In certain of these cases, coughing may enhance mucus clearance, but it generally is effective only if excess secretions are present.

The rates of A region particle clearance are reduced in humans with chronic obstructive lung disease and in laboratory animals with viral infections, whereas the viability and functional activity of macrophages are impaired in human asthmatics and in animals with viral-induced lung infections (U.S. EPA, 1996). However, any modification of functional properties of macrophages appears to be injury specific, reflecting the nature and anatomic pattern of disease.

3.4. PARTICLE OVERLOAD

3.4.1. Introduction

Some experimental studies using laboratory rodents employed high exposure concentrations of relatively nontoxic, poorly soluble particles. These particle loads interfered with normal clearance mechanisms, producing clearance rates different from those that would occur at lower exposure levels. Prolonged exposure to high particle concentrations is associated with what is termed particle overload. This is defined as the overwhelming of macrophage-mediated clearance by the deposition of particles at a rate exceeding the capacity of that clearance pathway. Aspects and occurrence of this phenomenon have already been alluded to in earlier portions of this chapter on alveolar clearance (Section 3.3.2.3). The relevance of this

phenomenon for human risk assessment has long been the object of scientific inquiry. A monograph on this matter and many others relevant to DPM has appeared (ILSI, 2000), and the results, opinions, and judgments put forth therein are used extensively in this chapter and in this assessment.

Wolff et al. (1987) used ^{134}Cs-labeled fused aluminosilicate particles to measure alveolar clearance in rats following 24-mo exposure to low, medium, and high concentrations of diesel exhaust (targeted concentrations of DPM of 0.35, 3.5 and 7.1 mg/m^3). The short-term component of the multicomponent clearance curves was similar for all groups, but long-term clearance was retarded in the medium and high exposure groups (Figure 3-4). The half times of the long-term clearance curves were 79, 81, 264, and 240 days, respectively, for the control, low-, medium-, and high-exposure groups. Clearance was overloaded at the high and medium but not at the low exposure level. Lung burdens of DPM were measured after 6, 12, 18, and 24 mo of exposure. The results (Figure 3-5) indicate that the lung burden of freshly deposited particles was appreciably increased in the two highest exposures post 6 mo., whereas the lung burden at the low-exposure level remained the same throughout all time periods examined.

Morrow (1988) has proposed that the condition of particle overloading in the lungs is caused by a loss in the mobility of particle-engorged AMs and that such an impediment is related to the cumulative volumetric load of particles in the AMs. Morrow (1988) has further estimated that the clearance function of an AM may be completely impaired when the particle burden in the AM is of a volumetric size equivalent to about 60% of the normal volume of the AM. Morrow's hypothesis was the initial basis for the physiology-oriented multicompartmental kinetic (POCK) model derived by Stöber et al. (1989) for estimating alveolar clearance and retention of relatively insoluble, respirable particles in rats.

A revised version of this model refines the characterization of the macrophage pool by including both the mobile and immobilized macrophages (Stöber et al., 1994). Application of the revised version of the model to experimental data suggested that lung overload does not cause a dramatic increase in the total burden of the macrophage pool but results in a great increase in the particle burden of the interstitial space, a compartment that is not available for macrophage-mediated clearance. The revised version of the POCK model is discussed in greater detail in the context of other dosimetry models below.

Oberdörster and co-workers (1992) assessed the alveolar clearance of smaller (3.3 μm diam.) and larger (10.3 μm diam.) polystyrene particles, the latter of which are volumetrically equivalent to about 60% of the average normal volume of a rat AM, after intratracheal instillation into the lungs of rats. Even though both sizes of particles were found to be phagocytized by AMs within a day after deposition, and the smaller particles were cleared at a normal rate, only minimal

lung clearance of the larger particles was observed over an approximately 200-day postinstillation period, thus supporting the volumetric AM overload hypothesis.

It has been hypothesized that when the retained lung burden approaches 1 mg particles/g lung tissue, overloading will begin in the rat (Morrow, 1988); at 10 mg particles/g lung tissue macrophage-mediated clearance of particles would effectively cease. Overloading appears to be a nonspecific effect noted in experimental studies, generally in rats, using many different kinds of poorly soluble particles (including TiO_2, volcanic ash, DPM, carbon black, and fly ash) and results in A region clearance slowing or stasis, with an associated inflammation and aggregation of macrophages in the lungs and increased translocation of particles into the interstitium (Muhle et al., 1990; Lehnert, 1990; Morrow, 1994). Following overloading, the subsequent retardation of lung clearance, accumulation of particles, chronic inflammation, and the interaction of inflammatory mediators with cell proliferative processes and DNA may lead to the development of fibrosis, epithelial cell mutations, and fibrosis in rats (Mauderly, 1996). The phenomenon of overload has been discussed in greater detail in the previous PM CD (U.S. EPA, 1996).

3.4.2. Relevance to Humans

The relevance of lung overload to humans, and even to species other than laboratory rats and mice, is not clear. Although likely to be of little relevance for most "real world" ambient exposures of humans, this phenomenon is of concern in interpreting some long-term experimental exposure data and perhaps for human occupational exposure. In addition, relevance to humans is clouded by the suggestion that macrophage-mediated clearance is normally slower and perhaps less important in humans than in rats (Morrow, 1994), and that there can be significant differences in macrophage loading between species. Particle overload appears to be an important factor in the pulmonary carcinogenicity observed in rats exposed to DPM. Studies described in this section provide additional data showing a particle overload effect. A study by Griffis et al. (1983) demonstrated that exposure (7 h/day, 5 days/week) of rats to DPM at concentrations of 0.15, 0.94, or 4.1 mg/m^3 for 18 mo resulted in lung burdens of 0.035, 0.220, and 1.89 mg/g of lung tissue, respectively. The alveolar clearance of those rats with the highest lung burden (1.89 mg/g of lung) was impaired, as determined by a significantly greater (p<0.0001) retention $t_{1/2}$ for DPM. Impaired clearance was reflected in the greater lung burden/exposure concentration ratio at the highest exposure level. Similarly, in the study by Chan et al. (1984), rats exposed for 20 h/day, 7 days/week to DPM (6 mg/m^3) for 112 days had an extraordinarily high lung particle burden of 11.8 mg, with no alveolar particle clearance being detected over 1 year.

Muhle et al. (1990) indicated that overloading of rat lungs occurred when lung particle burdens reached 0.5 to 1.5 mg/g of lung tissue and that clearance mechanisms were totally

compromised at lung particle burdens ≥10 mg/g for particles with a specific density close to 1, observations that are concordant with those of Morrow (1988).

Pritchard (1989), utilizing data from a number of diesel exhaust exposure studies, examined alveolar clearance in rats as a function of cumulative exposure. The resulting analysis noted a significant increase in retention $t_{1/2}$ values at exposures above 10 mg/m^3·h/day and also showed that normal lung clearance mechanisms appeared to be compromised as the lung DPM burden approached 0.5 mg/g of lung.

Animal studies have revealed that impairment of alveolar clearance can occur following chronic exposure to DPM (Griffis et al., 1983; Wolff et al., 1987; Vostal et al., 1982; Lee et al., 1983) or a variety of other diverse poorly soluble particles of low toxicity (Lee et al., 1986, 1988; Ferin and Feldstein, 1978; Muhle et al., 1990). Because high lung burdens of relatively insoluble, biochemically inert particles result in diminution of normal lung clearance kinetics or in what is now called particle overloading, this effect appears to be more related to the mass and/or volume of particles in the lung than to the nature of the particles per se. Particle overload relates only to poorly soluble particles of low toxicity. It must be noted, however, that some types of particles may be cytotoxic and impair clearance at lower lung burdens (e.g., crystalline silica may impair clearance at much lower lung burdens than DPM). Regardless, as pointed out by Morrow (1988), particle overloading in the lung modifies the dosimetry for particles in the lung and thereby can alter toxicologic responses.

Although quantitative data are limited regarding lung overload associated with impaired alveolar clearance in humans, impairment of clearance mechanisms appears to occur, and at a lung burden generally in the range reported to impair clearance in rats, i.e., approximately 1 mg/g lung tissue. Stöber et al. (1967), in their study of coal miners, reported lung particle burdens of 2 to 50 mg/g lung tissue, for which estimated clearance $t_{1/2}$ values were very long (4.9 years). Freedman and Robinson (1988) also reported slower alveolar clearance rates in coal miners, some of whom had a mild degree of pneumoconiosis. It must be noted, however, that no lung cancer was reported even among those miners with apparent particle overload.

3.4.3. Potential Mechanisms for an AM Sequestration Compartment for Particles During Particle Overload

Several factors may be involved in the particle-load-dependent retardations in the rate of particle removal from the lung and the corresponding functional appearance of an abnormally slow clearing or particle sequestration compartment. As previously mentioned, one potential site for particle sequestration is the containment of particles in the Type I cells. Information on the retention kinetics for particles in the Type I cells is not currently available. Also, no

morphometric analyses have been performed to date to estimate what fraction of a retained lung burden may be contained in the Type I cell population of the lung during lung overloading.

Another anatomical region in the lung that may be a slow clearing site is the interstitial compartment (Kuempel, 2000). Little is known about the kinetics of removal of free particles or particle-containing macrophages from the interstitial spaces, or what fraction of a retained burden of particles is contained in the lung's interstitium during particle overload. The gradual accumulation of particles in the regional lymph nodes and the appearance of particles and cells with associated particles in lymphatic channels and in the peribronchial and perivascular lymphoid tissue (Lee et al., 1985; White and Garg, 1981) suggest that the mobilization of particles from interstitial sites via local lymphatics is a continual process.

Indeed, it is clear from histologic observations of the lungs of animals chronically exposed to DPM that Type I cells, the interstitium, the lymphatic channels, and pulmonary lymphoid tissues could collectively comprise subcompartments of a more generalized slow clearing compartment.

Although these sites must be considered potential contributors to the increased retention of particles during particle overload, a disturbance in particle-associated AM-mediated clearance is undoubtedly the predominant cause, inasmuch as, at least in animals, the AMs are the primary reservoirs of deposited particles. The factors responsible for a failure of AMs to translocate from the alveolar space compartment in lungs with high particulate matter burdens remain uncertain, although a hypothesis concerning the process involving volumetric AM burden has been offered (Morrow, 1988).

Other processes also may be involved in preventing particle-laden AMs from leaving the alveolar compartment under conditions of particle overload in the lung. Clusters or aggregates of particle-laden AMs in the alveoli are typically found in the lungs of laboratory animals that have received large lung burdens of a variety of types of particles (Lee et al., 1985), including DPM (White and Garg, 1981; McClellan et al., 1982). The aggregation of AMs may explain, in part, the reduced clearance of particle-laden AM during particle overload. The definitive mechanism(s) responsible for this clustering of AMs has not been elucidated to date. Whatever the underlying mechanism(s) for the AM aggregation response, it is noteworthy that AMs lavaged from the lungs of diesel exhaust-exposed animals continue to demonstrate a propensity to aggregate (Strom, 1984). This observation suggests that the surface characteristics of AMs are fundamentally altered in a manner that promotes their adherence to one another in the alveolar region, and that AM aggregation may not simply be directly caused by their abundant accumulation as a result of immobilization by large particle loads. Furthermore, even though overloaded macrophages may redistribute particle burden to other AMs, clearance may remain inhibited (Lehnert, 1988). This

may, in part, be because attractants from the overloaded AMs cause aggregation of those that are not carrying a particle burden.

3.5. BIOAVAILABILITY OF ORGANIC CONSTITUENTS PRESENT ON DIESEL EXHAUST PARTICLES

Because it has been shown that DPM extract is not only mutagenic but also contains known carcinogens, the organic fraction was originally considered to be the primary source of carcinogenicity in animal studies. Since then evidence has been presented that carbon black, lacking an organic component, is capable of inducing lung cancer at exposure concentrations sufficient to induce lung particle overload. This suggested that the relatively insoluble carbon core of the particle may be of greater importance for the pathogenic and carcinogenic processes observed in the rat inhalation studies conducted at high exposure concentrations. (See Chapter 7 for a discussion of this issue.) However, lung cancer reported in epidemiology studies was associated with diesel exposure levels far below those inducing particle overload in lifetime studies in rats. It is therefore reasoned that compounds in the organic fraction of DPM may have some role in the etiology of human lung cancers.

The bioavailability of toxic organic compounds adsorbed to DPM can be influenced by a variety of factors. Although the agent may be active while present on the particle, most particles are taken up by AMs, a cell type not generally considered to be a target site. In order to reach the target site, elution from the particle surface is necessary followed by diffusion and uptake by the target cell. Metabolism to an active form by either the phagocytes or the target cells is also required for activity of many of the compounds present.

3.5.1. In Vivo Studies

3.5.1.1. *Laboratory Investigations*

Several studies reported on the retention of particle-adsorbed organics following administration to various rodent species. In studies reported by Sun et al. (1982, 1984) and Bond et al. (1986), labeled organics were deposited on DPM following heating to vaporize away the organics originally present. Sun et al. (1982) compared the disposition of either pure or diesel particle-adsorbed benzo[a]pyrene (BaP) following nose-only inhalation by F344 rats. About 50% of particle-adsorbed BaP was cleared with a half-time of 1 h, predominantly by mucociliary clearance. The long-term retention of particle-adsorbed ^{3}H-BaP of 18 days was approximately 230-fold greater than that for pure ^{3}H-BaP (Sun et al., 1982). At the end of exposure, about 15% of the ^{3}H label was found in blood, liver, and kidney. Similar results were reported in a companion study by Bond et al. (1986), and by Sun et al. (1984) with another PAH, 1-nitropyrene, except the retention half-time was 36 days.

Ball and King (1985) studied the disposition and metabolism of intratracheally instilled ^{14}C-labeled 1-NP (>99.9% purity) coated onto DPM. About 50% of the ^{14}C was excreted within the first 24 h; 20% to 30% of this appeared in the urine, and 40% to 60% was excreted in the feces. Traces of radiolabel were detected in the trachea and esophagus. Five percent to 12% of the radiolabel in the lung co-purified with the protein fraction, indicating some protein binding. The corresponding DNA fraction contained no ^{14}C above background levels.

Bevan and Ruggio (1991) assessed the bioavailability of BaP adsorbed to DPM from a 5.7-L Oldsmobile diesel engine. In this study, exhaust particles containing 1.03 μg BaP/g particles were supplemented with exogenous ^{3}H-BaP to provide 2.62 μg BaP/g of exhaust particles. In vitro analysis indicated that the supplemented BaP eluted from the particles at the same rate as the original BaP. Twenty-four hours after intratracheal instillation in Sprague-Dawley rats, 68.5% of the radiolabel remained in the lungs. This is approximately a 3.5-fold greater proportion than that reported by Sun et al. (1984), possibly because smaller amounts of BaP adsorbed on the particles resulted in stronger binding or possibly because of differences between inhalation exposure and intratracheal exposure. At 3 days following administration, more than 50% of the radioactivity remained in the lungs, nearly 30% had been excreted into the feces, and the remainder was distributed throughout the body. Experiments using rats with cannulated bile ducts showed that approximately 10% of the administered radioactivity appeared in the bile over a 10-h period and that less than 5% of the radioactivity entered the feces via mucociliary transport. Results of these studies showed that when organics are adsorbed to DPM the retention of organics in the lungs is increased considerably. Because retention time is very short following exposure to pure compounds not bound to particles, it can be concluded that the increased retention time is primarily the result of continued binding to DPM. The detection of labeled compounds in blood, systemic organs, urine, and bile as well as the trachea, however, provides evidence that at least some of the organics are eluted from the particles following deposition in the lungs and would not be available as a carcinogenic dose to the lung. As discussed in Section 3.6.3, most of the organics eluted from particles deposited in the alveolar region, especially PAHs, are predicted to rapidly enter the bloodstream and thus not to contribute to potential induction of lung cancer.

3.5.1.2. *Studies in Occupationally Exposed Humans*

DNA adducts in the lungs of experimental animals exposed to diesel exhaust have been measured in a number of animal experiments (World Health Organization, 1996). Such studies, however, provide limited information regarding bioavailability of organics, as positive results may well have been related to factors associated with lung particle overload, a circumstance reported by Bond et al. (1990), who found carbon black, a substance virtually devoid of organics, to induce

DNA adducts in rats at lung overload doses. These authors showed that levels of DNA adducts present in pulmonary type II cells from the lungs of rats (n=15) exposed to equivalent conditions of either carbon black or diesel exhaust (each at 6.2 mg/m^3) were nearly the same and 4- to 5-fold more than air-exposed controls. This similarity was noted despite a difference of nearly three orders of magnitude in solvent-extractable organic content between diesel exhaust (30%) and carbon black (0.04%). None of the diesel exhaust or carbon black adducts comigrated with BPDE (BaP diol epoxide).

On the other hand, DNA adduct formation and/or mutations in blood cells following exposure to DPM, especially at levels insufficient to induce lung overload, can be presumed to be the result of organics diffusing into the blood. Hemminki et al. (1994) reported increased levels of DNA adducts in lymphocytes of bus maintenance and truck terminal workers. Österholm et al. (1995) studied mutations at the hprt-locus of T-lymphocytes in bus maintenance workers. Although they were unable to identify clear-cut exposure-related differences in types of mutations, adduct formation was significantly increased in the exposed workers. Nielsen et al. (1996) reported significantly increased levels of lymphocyte DNA adducts, hydroxyvaline adducts in hemoglobin, and 1-hydroxypyrene in urine of garage workers exposed to diesel exhaust.

3.5.2. In Vitro Studies

3.5.2.1. *Extraction of Diesel Particle-Associated Organics by Biological Fluids*

In vitro extraction of mutagenic organics by biological fluids can be estimated by measurement of mutagenic activity in the particular fluid. Using this approach, Brooks et al. (1981) reported extraction efficiencies of only 3% to 10% that of dichloromethane following DPM incubation in lavage fluid, serum, saline, albumin, or dipalmitoyl lecithin. Moreover, extraction efficiency did not increase with incubation time up to 120 h. Similar findings were reported by King et al. (1981), who also reported that lung lavage fluid and lung cytosol fluid extracts of DPM were not mutagenic. Serum extracts of DPM did exhibit some mutagenic activity, but considerably less than that of organic solvent extracts. Furthermore, the mutagenic activity of the solvent extract was significantly reduced when combined with serum or lung cytosol fluid, suggesting protein binding or biotransformation of the mutagenic components. Siak et al. (1980) assessed the mutagenicity of material extracted from DPM by bovine serum albumin in solution, simulated lung surfactant, fetal calf serum (FCS), and physiological saline. Only FCS was found to extract some mutagenic activity from the DPM. Keane et al. (1991), however, reported positive effects for mutagenicity in *Salmonella* and sister chromatid exchange in V79 cells exclusively in the supernatant fraction of DPM dispersed in aqueous mixtures of dipalmitoyl phosphatidyl choline, a major component of pulmonary surfactant, indicating that pulmonary surfactant components can extract active components of DPM and result in bioavailability.

The ability of biological fluids to extract organics in vitro and their effectiveness in vivo remains equivocal because of the character of the particular fluid. For example, extracellular lung fluid is a complex mixture of constituents that undoubtedly have a broad range of hydrophobicity (George and Hook, 1984; Wright and Clements, 1987), which is fundamentally different from serum in terms of chemical composition (Gurley et al., 1988). Moreover, assessments of the ability of lavage fluids, which actually represent substantially diluted extracellular lung fluid, to extract mutagenic activity from DPM clearly do not reflect the in vivo condition. Finally, except under very high exposure concentrations, few particles escape phagocytosis and possible intracellular extraction. In this respect, Hiura et al. (1999) have shown that whole DPM, but not carbon black or diesel particles devoid of organics, induces apoptosis, apparently through generation of oxygen radicals. This study implicates organic compounds present on DPM. It also indicates the bioavailability of organics for generation of radicals from reaction with particle-associated organics or following elution from DPM.

3.5.2.2. *Extraction of DPM-Associated Organics by Lung Cells and Cellular Components*

A more likely means by which organics may be extracted from DPM and metabolized in the lung is either through particle dissolution or extraction of organics from the particle surface within the phagolysosomes of AMs and other lung cells. This mechanism presupposes that the particles are internalized. Specific details about the physicochemical conditions of the intraphagolysosomal environment, where particle dissolution in AMs presumably occurs in vivo, have not been well characterized. It is known that phagolysosomes constitute an acidic (pH 4 to 5) compartment in macrophages (Nilsen et al., 1988; Ohkuma and Poole, 1978). The relatively low pH in the phagolysosomes has been associated with the dissolution of some types of inorganic particles (some metals) by macrophages (Marafante et al., 1987; Lundborg et al., 1984), but few studies provide quantitative information concerning how organics from DPM may be extracted in the phagolysosomes (Bond et al., 1983). Whatever the mechanism, assuming elution occurs, the end result is a prolonged exposure of the respiratory epithelium to DPM organics, which include low concentrations of carcinogenic agents such as PAH.

Early studies by King et al. (1981) found that when pulmonary alveolar macrophages were incubated with DPM, amounts of organic compounds and mutagenic activity decreased measurably from the amount originally associated with the particles, suggesting that organics were removed from the phagocytized particles. Leung et al. (1988) studied the ability of rat lung and liver microsomes to facilitate transfer and metabolism of BaP from diesel particles. ^{14}C-BaP coated diesel particles, previously extracted to remove the original organics, were incubated directly with liver or lung microsomes. About 3% of the particle-adsorbed BaP was transferred to the lung microsomes within 2 h. Of this amount about 1.5% was metabolized, for a total of about

0.05% of the BaP originally adsorbed to the DPM. Although transformation is slow, the long retention of particles, including DPM, in humans may cause the fraction eluted and metabolized to be considerably higher than this figure.

In analyzing phagolysosomal dissolution of various ions from particles in the lungs of Syrian golden hamsters, however, Godleski et al. (1988) demonstrated that solubilization did not necessarily result in clearance of the ions (and therefore general bioavailability) in that binding of the solubilized components to cellular and extracellular structures occurred. It is reasonable to assume that phagocytized DPM particles may be subject to similar processes and that these processes would be important in determining the rate of bioavailability of the particle-bound constituents of DPM.

Alveolar macrophages or macrophage cell lines that were exposed to high concentrations of DPM in vitro were observed to undergo apoptosis, which was attributed to the generation of reactive oxygen radicals (ROR) (Hiura et al. 1999). Further experimentation showed that DPM with the organic constituents extracted was no longer able to induce apoptosis or generate ROR. The organic extracts alone, however, were able to induce apoptosis as well as the formation of stress-activated protein kinases that play definitive roles in cellular apoptotic pathways. The injurious effects of nonextracted DPM or of DPM extracts were observed to be reversible by the antioxidant radical scavenger N-acetyl cysteine. These data suggest strongly that, at least at high concentrations of DPM, the organic constituents contained on DPM play a central role in cellular toxicity and that this toxicity may be attributable to the generation of ROR.

3.5.3. Modeling Studies

Gerde et al. (1991a,b) described a model simulating the effect of particle aggregation and PAH content on the rate of PAH release in the lung. According to this model, particle aggregation will occur with high exposure concentrations, resulting in a slow release of PAHs and prolonged exposure to surrounding tissues. However, large aggregates of particles are unlikely to form at doses typical of human exposures. Inhaled particles, at low concentrations, are more likely to deposit and react with surrounding lung medium without interference from other particles. The model predicts that under low-dose exposure conditions, more typical in humans, particle-associated organics will be released more rapidly from the particles because they are not aggregated. Output from this model suggests strongly that sustained exposure of target tissues to PAHs will result from repeated exposures, not from increased retention due to association of PAHs with carrier particles. This distinction is important because at low doses PAH exposure and lung tumor formation would be predicted to occur at sites of deposition rather than retention, as occurs with high doses.

The site of release of PAHs influences effective dose to the lungs because, as noted previously, at least some free organic compounds deposited in the lungs are rapidly absorbed into the bloodstream. Gerde et al. (1991b) predicted PAHs would be retained in the alveoli less than 1 min, whereas they may be retained in the conducting airways for hours. These predictions were based on an average diffusion distance to capillaries of only about 0.5 μm in the alveoli, as compared to possibly greater than 50 μm in the conducting airways such as the bronchi. An experimental study by Gerde et al. (1999) provided support for this prediction. Beagle dogs were exposed to ^{3}H-BaP adsorbed on the carbonaceous core of DPM at a concentration of 15 μg BaP/gm particles. A rapidly eluting fraction from DPM deposited in the alveoli was adsorbed into the bloodstream and metabolized in the liver, whereas the rapidly eluting fraction from DPM deposited in the conducting airways was to a large extent retained and metabolized in situ in the airway epithelium. Thus, organics eluting from DPM depositing in the conducting airways (i.e., the TB region) would have a basis for a longer residence time in the tissues (and for consequent biological activity) than would organics eluting from DPM depositing in the pulmonary parenchyma. And, given the same overall deposited dose of DPM to the total pulmonary system, a deposited dose with a higher proportion in the TB region would incur a higher probability of tissue interactions with any eluted organics. This may be the case when comparing regional doses of DPM to humans as compared to rats for two reasons. First, one deposition model (Freijer et al., 1999) projects that for air concentrations of DPM at either 0.1 or 1.0 mg/m^{3}, a higher proportion of the total DPM dose to the pulmonary system would be deposited in the TB area for humans at 31% (TB/Total; 0.098 / 0.318) than for rats at only 16% (0.04 / 0.205). Second, comparative morphometry data of DPM from chronically exposed rats and primates showed higher levels of DPM adjacent to conducting airways in primates (i.e., the interstitium of the respiratory bronchioles) than were present in parallel regions in the rat (interstitium of the alveolar ducts) (Nikula et al., 1997a,b). The focal nature of this deposition could give rise to localized high concentrations of any organics eluted.

Overall, the results of studies presented in Section 3.6 provide evidence that at least some of the organic matter adsorbed to DPM deposited in the respiratory tract is eluted. The percentage taken up and metabolized to an active form by target cells is, however, uncertain. Organics eluted from particles deposited in alveoli are likely to rapidly enter the bloodstream via translocation across endothelial cells, where they may undergo metabolism by enzymes such as cytochromes P-450 that are capable of producing reactive species. Organics eluted from particles deposited in the conducting airways (the bronchioles, bronchi, and trachea) may also undergo metabolism in other cell types such as the Clara cells with constituent or inducible cytochrome P-450 species. Risk of harmful effects for particles deposited in the conducting airways is predicted to be greater because solubilized organic compounds will be retained in the thicker tissue longer,

allowing for metabolism by epithelial cells lining the airways. Furthermore, since some deposition conducting airways occurs primarily at bifurcations, localized higher concentrations may occur. At present, unfortunately, the available data are insufficient to accurately model the effective dose of organics in the respiratory tract of humans or animals exposed to DPM.

3.5.4. Bioavailability/Deposition of Organics

Using the data presented by Xu and Yu (1987), it is possible to calculate the total mass of DPM, as well as the total organic mass and specific carcinogenic PAHs deposited in the lungs of an individual exposed to DPM. For example, the annual deposition of DPM in the lungs of an individual exposed continuously to 1 $\mu g/m^3$ DPM can be estimated to be about 420 μg based on total lung volume (see Table 3-1). About 0.7% of particle mass consists of PAHs (see Section 2.2.6.2, Chapter 2) for a total of 2.94 μg. Of this amount, the deposited mass of nitro-polycyclic aromatic compounds, based on data by Campbell and Lee (1984), would equal 37 ng, while the deposited mass of 7 PAHs that tested positive in cancer bioassays (U.S. EPA, 1993), and measured by Tong and Karasek (1984), would range from 0.16 to 0.35 μg. Exercises similar to this have been carried out by others, e.g., Valberg and Watson (1999). However, the possibility that high concentrations of DPM may result in localized areas of deposition (such as the conducting airways), the fact that human exposures may be considerably greater than those presupposed in the exercise (e.g., 1 $\mu g/m^3$), the nature of the assays (i.e., in vitro in Chapter 4 vs. actual inhalation exposures), and the findings that DNA adducts may result from other known noncarcinogens such as carbon black (Bond et al., 1990) make the interpretation of such exercises problematic and their meaning unclear.

3.6. MODELING THE DEPOSITION AND CLEARANCE OF PARTICLES IN THE RESPIRATORY TRACT

3.6.1. Introduction

The biological effects of inhaled particles are a function of their disposition, i.e., their deposition and clearance. This, in turn, depends on their patterns of deposition (i.e., the sites within which particles initially come into contact with airway epithelial surfaces and the amount removed from the inhaled air at these sites) and clearance (i.e., the rates and routes by which deposited materials are removed from the respiratory tract). Removal of deposited materials involves the competing processes of macrophage-mediated clearance and dissolution-absorption. Over the years, mathematical models for predicting deposition, clearance and, ultimately, retention of particles in the respiratory tract have been developed. Such models help interpret experimental data and can be used to make predictions of deposition for cases where data are not

available. A review of various mathematical deposition models was given by Morrow and Yu (1993) and in U.S. EPA (1996).

Currently available data for long-term inhalation exposures to poorly soluble particles (e.g., TiO_2, carbon black, and DPM) show that pulmonary retention and clearance of these particles are not adequately described by simple first-order kinetics and a single compartment representing the alveolar macrophage particle burden. Several investigators have developed models for deposition, transport, and clearance of poorly soluble particulate matter in the lungs. All of these models identify various compartments and associated transport rates, but empirically derived data are not available to substantiate many of the assumptions made in these models.

3.6.2. Dosimetry Models for DPM

3.6.2.1. *Introduction*

The extrapolation of toxicological results from laboratory animals to humans, the goal of this chapter, requires the use of dosimetry models for both species that include, first, the deposition of DPM in various regions of the respiratory tract, and second, the transport and clearance of the particles, including adsorbed constituents, from their deposited sites. Therefore the ideal model structure would incorporate both deposition and clearance in animals and humans.

Deposition of particles in the respiratory tract, as described above, can be by impaction, sedimentation, interception, and diffusion, with the contribution from each mechanism a function of particle size, lung structure, and size and breathing parameters. Because of the size of diesel particles, under normal breathing conditions most of this deposition takes place by diffusion, and the fraction of the inhaled mass that is deposited in the thoracic region (i.e., TB plus A regions) is substantially similar for rats and humans.

Among deposition models that include aspects of lung structure and breathing dynamics, the most widely used have been typical-path or single-path models (Yu, 1978; Yu and Diu, 1983). The single-path models are based on an idealized symmetric geometry of the lung, assuming regular dichotomous branching of the airways and alveolar ducts (Weibel, 1963). They lead to modeling the deposition in an average regional sense for a given lung depth. Although the lower airways of the lung may be reasonably characterized by such a symmetric representation, there are major asymmetries in the upper airways of the tracheobronchial tree that in turn lead to different apportionment of airflow and particulate burden to the different lung lobes. The rat lung structure is highly asymmetric because of its monopodial nature, leading to significant errors in a single-path description. This is rectified in the multiple-path model of the lung, which incorporates asymmetry and heterogeneity in lung branching structure and calculates deposition at the individual airway level. This model has been developed for the rat lung (Anjilvel and Asgharian, 1995; Freijer et al., 1999) and, in a limited fashion because of insufficient morphometric data, for

the human lung (Subramaniam et al., 1998; Yeh and Schum, 1980). Such models are particularly relevant for fine and ultrafine particles such as occur in DPM. However, models for clearance have not yet been implemented in conjunction with the use of the multiple-path model.

Clearance of particles in the respiratory tract takes place (1) by mechanical processes: mucociliary transport in the ciliated conducting airways and macrophage phagocytosis and migration in the nonciliated airways, and (2) by dissolution. The removal of material such as the carbonaceous core of DPM is largely by mechanical clearance, whereas the clearance of the organics adsorbed onto the carbon core is principally by dissolution.

Several clearance models currently exist, some specifically for humans and others specific for laboratory animals. They differ significantly in the level of physiological detail that is captured in the model and in the uncertainties associated with the values of the parameters used. All of these models identify various compartments and associated transport rates, but empirically derived data are not available to validate many of the assumptions made in the models. A review of the principal human and animal deposition/clearance models, including candidate models for use in animal-to-human extrapolation in this assessment, are considered below.

3.6.2.2. *Human Models*

The International Commission on Radiological Protection (ICRP) recommends specific mathematical dosimetry models as a means to calculate the mass deposition and retention by different parts of the human respiratory tract and, if needed, tissues beyond the respiratory tract. The latest ICRP-recommended model, ICRP66 (1994), considers the human respiratory tract as four general anatomical regions: the ET region, which is divided into two subregions; the TB region, which is also subdivided into two regions; and the gas-exchange tissues, which are further defined as the alveolar-interstitial (AI) region but are exactly comparable to the pulmonary or A region. The fourth region is the lymph nodes. Deposition in the four regions is given as a function of particle size with two different types of particle size parameters: activity median thermodynamic diameter (AMTD) for deposition of particles ranging in size from 0.0005 to 1.0 μm and the activity median aerodynamic diameter (AMAD) for deposition of particles from 0.1 to 100μm. Reference values of regional deposition are provided and guidance is given for extrapolating to specific individuals and populations under different levels of activity. This model also includes consideration of particle inhalability, a measure of the degree to which particles can enter the respiratory tract and be available for deposition. After deposition occurs in a given region, two different intrinsic clearance processes act competitively on the deposited particles: particle transport, including mucociliary clearance from the respiratory tract and physical clearance of particles to the regional lymph nodes; and absorption, including movement of material to blood and both dissolution-absorption and transport of ultrafine particles. Rates of

particle clearance derived from studies with human subjects are assumed to be the same for all types of particles. The ICRP model provides average concentration or average number values on a regional basis, i.e., mass or number deposited or retained in the ET, TB, or A regions. Additionally, while the ICRP66 model was developed primarily for use with airborne radioactive particles and gases in humans, its use for describing the dosimetry of inhaled mass of nonradioactive substances in humans is also appropriate.

An alternative new human respiratory tract dosimetry model that developed concurrently with the new ICRP model is being proposed by the National Council on Radiation Protection (NCRP). This model was described in outline by Phalen et al. (1991). As with the 1994 ICRP66 model (ICRP66, 1994), the proposed NCRP model addresses (1) inhalability of particles, (2) new subregions of the respiratory tract, (3) dissolution-absorption as an important aspect of the model, and (4) body size (and age). The proposed NCRP model defines the respiratory tract in terms of a naso-oro-pharyngo-laryngeal (NOPL) region, a TB region, a pulmonary (P) region, and the lung-associated lymph nodes (LN). The rates of dissolution-absorption of particles and their constituents are derived from clearance data from humans and laboratory animals. The effect of body growth on particle deposition is also considered in the model, but particle clearance rates are assumed to be independent of age. The NCRP model does not consider the fate of inhaled materials after they leave the respiratory tract. Although the proposed NCRP model describes respiratory tract deposition, clearance, and dosimetry for radioactive substances inhaled by humans, the model can also be used for evaluating inhalation exposures to all types of particles. Graphical outputs of regional deposition fractions from both the ICRP66 (1994) and draft NCRP models presented in U.S. EPA (1996) indicate approximately 15% would be deposited in the alveolar region at the MMAD of DPM, 0.2 μm.

3.6.2.3. *Animal Models*

Strom et al. (1988) developed a multicompartmental model for particle retention that partitioned the alveolar region into two compartments on the basis of the physiology of clearance. The alveolar region has a separate compartment for sequestered macrophages, corresponding to phagocytic macrophages that are heavily laden with particles and clustered, and consequently have significantly lowered mobility. The model has the following compartments: (1) tracheobronchial tree, (2) free particulate on the alveolar surface, (3) mobile phagocytic alveolar macrophages, (4) sequestered particle-laden alveolar macrophages, (5) regional lymph nodes, and (6) gastrointestinal tract. The model is based on mass-dependent clearance (the rate coefficients reflect this relationship), which dictates sequestration of particles and their eventual transfer to the lymph nodes. The transport rates between various compartments were obtained by fitting the calculated results to lung and lymph node burden experimental data for both exposure

and postexposure periods. Because the number of fitted parameters was large, the model is not likely to provide unique solutions that would simulate experimental data from various sources and for different exposure scenarios. For the same reason, it is not readily possible to use this model for extrapolating to humans.

Stöber and co-workers have worked extensively in developing models for estimating retention and clearance of relatively insoluble respirable particles (as DPM) in the lung. Their most recent work (1994), a revised version of the POCK model, is a rigorous attempt to incorporate most of the physiologically known aspects of alveolar clearance and retention of inhaled relatively insoluble particles. Their multicompartmental kinetics model has five subcompartments. The transfer of particles between any of the compartments within the alveolar region is macrophage mediated. There are two compartments that receive particles cleared from the alveolar regions: the TB tract and the lymphatic system. The macrophage pool includes both mobile and particle-laden immobilized macrophages. The model assumes a constant maximum volume capacity of the macrophages for particle uptake and a material-dependent critical macrophage load that results in total loss of macrophage mobility. Sequestration of those macrophages heavily loaded with a particle burden close to a volume load capacity is treated in a sophisticated manner by approximating the particle load distribution in the macrophages. The macrophage pool is compartmentalized in terms of numbers of macrophages that are subject to discrete particle load intervals. Upon macrophage death, the phagocytized particle is released back to the alveolar surface; thus phagocytic particle collection competes to some extent with this release back to the alveolar surface. This recycled particle load is also divided into particle clusters of size intervals defining a cluster size distribution on the alveolar surface. The model yields a time-dependent frequency distribution of loaded macrophages that is sensitive to both exposure and recovery periods in inhalation studies.

The POCK model also emphasizes the importance of interstitial burden in the particle overload phenomenon and indicates that particle overload is a function of a massive increase in particle burden of the interstitial space rather than total burden of the macrophage pool. The relevance of the increased particle burden in the interstitial space lies with the fact that this compartmental burden is not available for macrophage-mediated clearance and, therefore, persists even after cessation of exposure.

Although the POCK model is the most sophisticated in the physiological complexity it introduces, it suffers from a major disadvantage. Experimental retention studies provide data only on total alveolar and lymph node mass burdens of the particles as a function of time. The relative fraction of the deposition between the alveolar subcompartments in the Stöber model therefore cannot be obtained experimentally; the model thus uses a large number of parameters that are simultaneously fit to experimental data. Although the model predictions are tenable, experimental

data are not currently available to substantiate the proposed compartmental burdens or the transfer rates associated with these compartments. Thus, overparameterization in the model leads to the possibility that the model may not provide a unique solution that may be used for a variety of exposure scenarios, and for the same reason, cannot be used for extrapolation to humans. Stöber et al. have not developed an equivalent model for humans; therefore the use of their model in our risk assessment for diesel is not attempted.

3.6.2.4. *Combined Models (for Interspecies Extrapolation)*

Currently available data for long-term inhalation exposures to poorly soluble particles (e.g., TiO_2, carbon black, and DPM) show that pulmonary retention and clearance of these particles are not adequately described by simple first-order kinetics and a single compartment representing the alveolar macrophage particle burden. A two-compartment lung model that could be applied to both humans and animals was developed by Smith (1985) and includes alveolar and interstitial compartments. For uptake and clearance of particles by alveolar surface macrophages and interstitial encapsulation of particles (i.e., quartz dust), available experimental data show that the rate-controlling functions followed Michaelis-Menton type kinetics, whereas other processes affecting particle transfer are assumed to be linear. The model was used in an attempt to estimate interstitial dust and fibrosis levels among a group of 171 silicon carbide workers; the levels were then compared with evidence of fibrosis from chest radiographs. A significant correlation was found between estimated fibrosis and profusion of opacities on the radiographs. This model provides as many as seven different rate constants derived by various estimations and under various conditions from both animal and human sources. The model was intended for estimation of generalized dust described only as respirable without any other regard to sizing for establishing the various particle-related rate constants. As most of the described functions could not be validated with experimental data, the applicability of this model, especially for particulates in the size range of DPM, was unclear.

Yu et al. (1991; also reported as Yu and Yoon, 1990) have developed a three-compartment lung model that consists of tracheobronchial (T), alveolar (A), and lymph node (L) compartments (Appendix A, Figure A-1) and, in addition, considered filtration by a nasopharyngeal or head (H) compartment. Absorption by the blood (B) and gastrointestinal (G) compartments was also considered. Although the treatment of alveolar clearance is physiologically less sophisticated than that of the Stöber et al. model, the Yu model provides a more comprehensive treatment of clearance by including systemic compartments and the head, and including the clearance of the organic components of DPM in addition to the relatively insoluble carbon core.

The tracheobronchial compartment is important for short-term considerations, whereas long-term clearance takes place via the alveolar compartment. In contrast to the Stöber and Strom approaches, the macrophage compartment in the Yu model contains all of the phagocytized particles; that is, there is no separate (and hypothetical) sequestered macrophage subcompartment. Instead, in order to progress beyond the classical human ICRP66 retention model, Yu has addressed the impairment of long-term clearance (the overload effect) by using a set of variable transport rates for clearance from the alveolar region as a function of the mass of DPM in the alveolar compartment. A functional relationship for this was derived mathematically (Yu et al., 1989) based upon Morrow's hypothesis for the macrophage overload effect discussed earlier in the section on pulmonary overload. The extent of the impairment depends on the initial particle burden, with greater particulate concentration leading to slower clearance.

Within this model DPM is treated as being composed of three material components: a relatively insoluble carbonaceous core, slowly cleared organics (10% particle mass), and fast-cleared organics (10% particle mass). Such a partitioning of organics was based on observations that the retention of particle-associated organics in lungs shows a biphasic decay curve (Sun et al., 1984; Bond et al., 1986). For any compartment, each of these components has a different transport rate. The total alveolar clearance rate of each material component is the sum of clearance rates of that material from the alveolar to the tracheobronchial, lymph, and blood compartments. In the Strom and Stöber models discussed above, the clearance kinetics of DPM were assumed to be entirely dictated by those of the relatively insoluble carbonaceous core. For those organic compounds that get dissociated from the carbon core, clearance rates are likely to be very different, and some of these compounds may be metabolized in the pulmonary tissue or be absorbed by blood.

The transport rates for the three components were derived from experimental data for rats using several approximations. The transport rates for the carbonaceous core and the two organic components were derived by fitting to data from separate experiments. Lung and lymph node burdens from the experiment of Strom et al. (1988) were used to determine the transport rate of the carbonaceous core. The Yu model incorporates the impairment of clearance by including a mass dependency in the transport rate. This mass dependency is easily extracted because the animals in the experiment were killed over varying periods following the end of exposure.

It was assumed that the transport rates from the alveolar and lymph compartments to the blood were equal and independent of the particulate mass in the alveolar region. The clearance rates of particle-associated organics for rats were derived from the retention data of Sun et al. (1984) for benzo[a]pyrene and the data of Bond et al. (1986) for nitropyrene adsorbed on diesel particles.

In their model Yu et al. (1991) make two important assumptions to carry out the extrapolation in consideration of inadequate human data. First, the transport rates of organics in the DPM do not change across species. This is based upon lung clearance data of inhaled lipophilic compounds (Schanker et al., 1986), where the clearance was seen to be dependent on the lipid/water partition coefficient. In contrast, the transport rate of the carbonaceous core is considered to be significantly species dependent (Bailey et al., 1982). DPM clearance rate is determined by two terms in the model (see Equation A-82 in Appendix A). The first, corresponding to macrophage-mediated clearance, is a function of the lung burden and is assumed to vary significantly across species. The second term, a constant, corresponding to clearance by dissolution, is assumed to be species independent. The mass-dependent term for humans is assumed to vary in the same proportion as in rats under the same unit surface particulate dose. The extrapolation is then achieved by using the data of Bailey et al. (1982) for the low lung burden limit of the clearance rate. This value of 0.0017/day was lower than the rat value by a factor of 7.6. This is elaborated further in Appendix A. Other transport rates that have lung burden dependence are extrapolated in the same manner.

The Bailey et al. (1982) experiment, however, used fused monodisperse aluminosilicate particles of 1.9 and 6.1 μm aerodynamic diameters. Yu and co-workers have used the longer of the half-times obtained in this experiment; in using such data for DPM 0.2 μm in diameter, they have assumed the clearance of relatively insoluble particles to be independent of size over this range. This appears to be a reasonable assumption because the linear dimensions of an alveolar macrophage are significantly larger, roughly 10 μm (Yu et al., 1996). However, Snipes (1979) has reported a clearance rate (converted here from half-time values) of 0.0022/day for 1 and 2 μm particles but a higher value of 0.0039/day for 0.4 μm particles. In the absence of reliable data for 0.2 μm particles, clearance rate pertaining to this much larger particle size is being used. Although such a choice may underestimate the correct clearance rate for DPM, the resulting error in the output (i.e., a human equivalent concentration) is likely to be only more protective of human health. Long-term clearance rates for particle sizes more comparable to DPM are available, e.g., iron oxide and polystyrene spheres (Waite and Ramsden, 1971; Jammet et al., 1978), but these data show a large range in the values obtained for half-lives or are based upon a very small number of trials, and therefore compare unfavorably with the quality of data from the Bailey experiment.

The deposition fractions of particulate matter in the pulmonary and tracheobronchial regions of the human lung remain relatively unchanged over the particle size range between 0.2 and 1.0 μm, on the basis of the analysis done with the ICRP66 (1994) model as documented in the PMCD (U.S. EPA, 1996). As the clearance of relatively insoluble particles is also likely to remain the same over this range, the dosimetry results in this report for the carbonaceous core

component of DPM could also be extended to other particles in this size range within the $PM_{2.5}$. For respirable particles with diameters larger than this range, e.g., between 1.0 and 3.5 μm, the extent of the fraction deposited in the pulmonary region is unclear. Results from the ICRP66 (1994) model predict little change in human deposition for this diameter range, whereas the earlier model of Yu and Diu (1983) predicts a significant increase. It is therefore unclear if either model would be applicable for particles in this range without changing the value for the deposition fractions. As mentioned above, however, regional deposition fractions from both the ICRP66 (1994) and draft NCRP models presented in U.S. EPA (1996) indicate approximately 15% would be deposited in the alveolar region at the MMAD of DPM, 0.2 µm. These values compare favorably with the human alveolar deposition in humans specific for DPM, which has been extimated with the Yu model to be 7% to 13% (Yu and Xu, 1986).

Although there was good agreement between experimental and modeled results, this agreement follows a circular logic (as adequately pointed out by Yu and Yoon [1990]) because the same experimental data that figured into the derivation of transport rates were used in the model. Nevertheless, even though this agreement is not a validation, it provides an important consistency check on the model. Further experimental data and policy definitions on what constitutes validation would be necessary for a more formal validation.

The model showed that at low lung burdens, alveolar clearance is dominated by mucociliary transport to the tracheobronchial region, and at high lung burdens, clearance is dominated by transport to the lymphatic system. The head and tracheobronchial compartments showed quick clearance of DPM by mucociliary transport and dissolution. Lung burdens of both the carbonaceous core and organics were found to be greater in humans than in rats for similar periods of exposure.

The Yu and Yoon (1990) version of the model provides a parametric study of the dosimetry model, examining variation over a range of exposure concentrations, breathing scenarios, and ventilation parameters; particle mass median aerodynamic diameters; and geometric standard deviations of the aerosol size distribution. It examines how lung burden varies with age for exposure over a lifespan, provides dosimetry extrapolations to children, and examines changes in lung burden with lung volume. The results showed that children would exhibit more diminished alveolar clearance of DPM at high lung burden than adults when exposed to equal concentrations of DPM. These features make the model easy to use in risk assessment studies. The reader is referred to Appendix A for further details on the model and for analyses of the sensitivity of the model to change in parameter values.

The Yu model presents some uncertainties in addition to those discussed earlier in the context of particle size dependence of clearance rate. The reports of Yu and Yoon (1990) as well as Yu et al. (1991) underwent extensive peer review; we list below the most important among the

model uncertainties discussed by the review panel. The experimental data used by the Yu model for adsorbed organics used passively adsorbed radiolabeled compounds as surrogates for combustion-derived organics. These compounds may adhere differently to the carbon core than do those formed during combustion. Yu has estimated that slowly cleared organics represent 10% of the total particle mass; the actual figure could be substantially less; the reviewers estimate that the amount of tightly bound organics is probably only 0.1% to 0.25% of the particle mass.

The model was based upon the experimental data of Strom et al. (1988), where Fischer-344 rats were exposed to DPM at a concentration of 6.0 mg/m^3 for 20 h/day and 7 days/week for periods ranging from 3 to 84 days. Such exposures lead to particle overload effects in rats, whereas human exposure patterns are usually to much lower levels at which overload will not occur. Parameters obtained by fitting to data under the conditions of the experimental scenario for rats may not be optimal for the human exposure and concentration of interest.

The extrapolation of retained dose from rats to humans assumed that the macrophage-mediated mechanical clearance of the DPM varies with the specific particulate dose to the alveolar surface in the same proportion in humans and in rats, whereas clearance rates by dissolution were assumed to be invariant across species. This assumption has not been validated.

It should also be noted that the Yu et al. (1991) model does not possess an interstitial compartment. The work of Nikula et al. (1997a,b) and of Kuempel (2000) provide compelling information on the significance of an extensive interstitilization process in primates and in humans. Kuempel (2000) developed a lung dosimetry model to describe the kinetics of particle clearance and retention in coal miners' lungs. Models with overloading of lung clearance, as observed in rodent studies, were found to be inadequate to describe the end-of-life lung dust burdens in those miners. The model that provided the best fit to the human data included a sequestration process representing the transfer of particles to the interstitium. These findings are consistent with a study showing reduced lung clearance of particles in retired coal miners (Freedman and Robinson, 1988) and with studies showing increased retention of particles in the lung interstitium of humans and nonhuman primates compared to rodents exposed to coal dust and/or diesel exhaust (Nikula et al., 1997a,b). Because the Yu model has not been validated on human data and does not include an interstitial compartment, it is acknowledged that this model may therefore underpredict the lung dust burdens in humans exposed to occupational levels of dust. However, it is also not known whether the model based on coal miner data (Kuempel, 2000) would also describe the clearance and retention processes in the lungs of humans with exposures to particles at lower environmental concentrations, or to submicrometer particles such as diesel exhaust particulate. Further investigation of these issues is needed.

3.6.2.5. *Use of the Yu et al. (1991) Model for Interspecies Extrapolation*

In addressing the objectives of this chapter, i.e., consideration of what is known and applicable to DPM concerning particle disposition and the bioavailability of adsorbed organics on DPM, it is apparent that the database is considerable for both the processes involved in particle dosimetry and for DPM. This information makes the goal of predicting a human internal dose from animal data through a model utilizing this database both feasible and appropriate.

In their charge to EPA through "Science and Judgment in Risk Assessment" (NRC, 1995), the National Research Council opines that EPA should have principles for judging when and how to depart from default options. The extensive data presented in this chapter (including the model of Yu), their scientific validity, and the limitations of the current default procedures provide a basis for departing from the default options currently identified by the Agency for extrapolating from animals to humans. The default option of assuming external concentrations of DPM in animal studies as being representative of a human concentration (and an equivalent internal dose) is clearly not adequate given the vast differences in the basic processes of deposition and clearance between animals and humans documented by these data. Use of an alternate default option, the Agency's dosimetric adjustment procedures for inhaled particles in animal-to-human scenarios (described in U.S. EPA, 1994), is also inadequate as only deposition is predicted and then only down to an MMAD of 0.5 μm, whereas the MMAD of DPM is typically 0.2 μm or smaller. Models have been described in this section that consider both deposition and retention specifically for DPM in both laboratory animals and in humans. These points provide justification for moving away from default options and utilizing the best scientific information available (i.e., that integrated into deposition/clearance models) in performing the animal-to-human extrapolation.

Of the models evaluated in this chapter, that of Yu et al. (1991) is uniquely equipped to perform animal-to-human extrapolation for DPM. The model structure is parsimonious, with three lung compartments (tracheobroncial, pulmonary, lymph node). Design of the model incorporated both human and animal information, utilizing empirical clearance data from both rats and humans. In addition to DPM, this model considers deposition and clearance of two classes of organics adsorbed onto DPM. The model does have limitations, such as a lack of definitive information on variability of the results and absence of a lung compartment (interstitial) that could well be of importance to humans. It is, however, considered that the attributes considerably outweigh the detractions in choosing this model as a means to perform animal-to-human extrapolation for DPM.

3.7. SUMMARY

The most consistent historical measure of exposure for diesel exhaust is DPM in units of μg or mg particles/m^3, with the underlying assumption that all components of diesel emissions

(e.g., organics in the form of volatilized liquids or gases) are present in proportion to the DPM mass. DPM is used as the basic dosimeter for effects from various scenarios such as chronic and acute exposures as well as for different endpoints such as irritation, fibrosis, or even cancer. There is, however, little evidence currently available to prove or refute DPM as being the most appropriate dosimeter.

DPM dose to the tissue is related to the extent of the deposition and clearance of DPM. DPM may deposit throughout the respiratory tract via sedimentation or diffusion, with the latter being prevalent in the alveolar region. Particles that deposit upon airway surfaces may be cleared from the respiratory tract completely or may be translocated to other sites by regionally distinct processes that can be categorized as either absorptive (i.e., dissolution) or nonabsorptive (i.e., transport of intact particles via mucociliary transport). With poorly soluble particles such as DPM, clearance by dissolution is insignificant compared to the rate of clearance as an intact particle. Other mechanisms that can affect retention of DPM include endocytosis by alveolar lining cells and interstitialization, which lead to the accumulation of DPM in the interstitial compartment of the lung and subsequent translocation of DPM to lymph nodes; interstitialization of poorly soluble particles is prominent in primates and humans as compared to rodents. For poorly soluble particles such as DPM, species-dependent rate constants exist for the various clearance pathways that can be modified by factors such as respiratory tract disease.

In rats, prolonged exposure to high concentrations of particles may be associated with particle overload, a condition that is defined as the overwhelming of macrophage-mediated clearance by the deposition of particles at a rate exceeding the capacity of that clearance pathway. This condition seems to begin to occur in rats when the pulmonary dust burden exceeds about 1 mg particles/g lung tissue. On the other hand, there is no clear evidence for particle overload in humans. Macrophage-mediated clearance appears to be slower and perhaps less important in humans than in rats, and interstitialization of poorly soluble particulate matter may be of greater consequence in humans than in rats.

The degree of bioavailability of the organic fraction of DPM is still somewhat uncertain. However, reports of DNA alterations in occupationally exposed workers, as well as results of animal studies using radiolabeled organics deposited on DPM, indicate that at least a fraction of the organics present are eluted prior to particle clearance. Carcinogenic organics eluted in regions where diffusion may be a relatively long process, such as in the conducting airways vs the alveolar region, may remain in the lung long enough to be metabolized to an active form or to interact directly with vital cellular components. The current information suggests that DPM-associated organics could be involved in a carcinogenic process, although the quantitative data are far from adequate to make any firm conclusions.

Use of laboratory animal data in an assessment meant to be applied to humans obligates some form of interspecies extrapolation. Review and evaluation of the considerable, specific database in humans and animals on disposition of DPM, its adsorbed organics, and other poorly soluble particles led to the judgment that default options available for interspecies dosimetry adjustment could be set aside for more scientifically valid, DPM-specific processes. Refinement of this evaluation led to the identification and choice of the Yu et al. (1991) model to conduct interspecies extrapolation. This model has a three-compartment lung consisting of tracheobronchial, alveolar, and lymph node compartments. It treats DPM as being composed of the insoluble carbonaceous core, slowly cleared organics, and fast-cleared organics, and considers in an integrative manner the simultaneous processes of both deposition and clearance through empirical data derived from both laboratory animals and humans. Also, the model has some limited consideration of model variability in its outputs describing dose to the lung. Major assumptions made in this model include that transport rates of organics in DPM do not change across species and that the transport rate of the carbonaceous core is species dependent, with the clearance rate varying with the dose to the alveolar surface in the same proportion in humans as in rats. Limitations of the model include the lack of definitive information on variability and the lack of a biological compartment (the interstitium) that may be of consequence in humans. The basis of this model is to derive an internal dose from an external DPM concentration by utilizing species-specific physiological and pharmacokinetic parameters and, as such, is considered to have addressed the pharmacokinetic aspects of interspecies dosimetry. This aspect of the model addresses some of the critical data needs for the quantitative analysis of noncancer effects from DPM, the subject of Chapter 6.

As parallels have been drawn between DPM and $PM_{2.5}$ in other chapters, it is perhaps appropriate to compare them also from the aspect of dosimetry. Obvious comparisons include the nature of the particle distribution, defined artificially for $PM_{2.5}$ as compared with the thorough characterization of DPM for both MMAD (which, at around 0.2 µm, is typically more than an order of magnitude less than the $PM_{2.5}$ cutoff) and geometric standard deviation. It is clear that a larger portion of $PM_{2.5}$ particles than DPM would be above the aerodynamic equivalent diameter (d_{ae}) of 0.5 µm, which is often considered as a boundary between diffusion and aerodynamic mechanisms of deposition. This would imply that a somewhat larger portion of DPM may pass on to the lower respiratory tract than would $PM_{2.5}$. Alveolar depostion in humans specific for DPM has been estimated with the Yu model to be 7%-13% (Yu and Xu, 1986). This fractional deposition may be compared to one calculated for $PM_{2.5}$ and reported in U.S. EPA (1996a); assuming a MMAD of 2.25 µm and a geometric standard deviation of 2.4, a fractional alveolar deposition of 10.2% was reported. This value is within the range and quite comparable to that

obtained by Yu and Xu (1986), indicating that little difference may exist in alveolar deposition between DPM and $PM_{2.5}$, at least for this assumed geometric standard deviation.

Table 3-1. Predicted doses of inhaled DPM per minute based on total lung volume (M), total airway surface area (M_1), or surface area in alveolar region (M_2)

Species	M (10^{-3} μg/min/cm^3)	M_1 (10^{-6} μg/min/cm^2)	M_2 (10^{-6} μg/min/cm^2)
Hamster	3.548	3.088	2.382
Fischer rat	3.434	3.463	2.608
Human	0.249	1.237	0.775

$M = \frac{\text{mass DPM deposited in lung per minute}}{\text{total lung volume}}$

$M_1 = \frac{\text{mass DPM deposited in lung per minute}}{\text{total airway surface area}}$

$M_2 = \frac{\text{mass DPM deposited on the unciliated airways per minute}}{\text{surface area of the unciliated airways}}$

Based on the following conditions: (1) mass median aerodynamic diameter (MMAD) = 0.2 μm; geometric standard deviation (σ_g) = 1.9; packing density (ϕ) = 0.3; and particle mass density (ρ) = 1.5 g/cm^3; (2) particle concentration = 1 mg/m^3; and (3) nose-breathing. For humans, total lung volume = 3200 cm^3, total airway surface area = 633,000 cm^3, surface area of the unciliated airways = 627,000 cm^3.

Source: Xu and Yu, 1987.

Table 3-2. Alveolar clearance in laboratory animals exposed to DPM

Species/sex	Exposure technique	Exposure duration	Particles mg/m^3	Observed effects	Reference
Rats, F-344, M	Nose only; Radiolabeled DPM	40-45 min	6	Four days after exposure, 40% of DPM eliminated by mucociliary clearance. Clearance from lower RT was in 2 phases. Rapid mucociliary ($t_{1/2}$ = 1 day); slower macrophage-mediated ($t_{1/2}$ = 62 days).	Chan et al. (1981)
Rats, F-344	Whole body; assessed effect on clearance of $^{67}Ga_2O_3$ particles	7 h/day 5 days/week 24 mo	0.35 3.5 7.1	τ_1 significantly higher with exposure to 7.1 mg/m^3 for 24 mo; τ_2 significantly longer after exposure to 7.1 mg/m^3 for 6 mo and to 3.5 mg/m^3 for 18 mo.	Wolff et al. (1986, 1987)
Rats	Whole body	19 h/day 5 days/week 2.5 years	4	Estimated alveolar deposition = 60 mg; particle burden caused lung overload. Estimated 6-15 mg particle-bound organics deposited.	Heinrich et al. (1986)
Rats, F-344, MF	Whole body	7 h/day 5 days/week 18 mo	0.15 0.94 4.1	Long-term clearance was 87 ± 28 and 99 ± 8 days for 0.15 and 0.94 mg/m^3 groups, respectively; $t_{1/2}$ = 165 days for 4.1 mg/m^3 group.	Griffis et al. (1983)
Rats, F-344;	Nose-only; Radiolabeled ^{14}C	45 min 140 min	7 2	Rats demonstrated 3 phases of clearance with $t_{1/2}$ = 1, 6, and 80 days, representing tracheobronchial, respiratory bronchioles, and alveolar clearance, respectively. Guinea pigs demonstrated negligible alveolar clearance from day 10 to 432.	Lee et al. (1983)
Guinea pigs, Hartley		45 min	7		
Rats, F-344		20 h/day 7 days/week 7-112 days	0.25 6	Monitored rats for a year. Proposed two clearance models. Clearance depends on initial particle burden; $t_{1/2}$ increases with higher exposure. Increases in $t_{1/2}$ indicate increasing impairment of AM mobility and transition into overload condition.	Chan et al. (1984)

RT = respiratory tract.
AM = alveolar macrophage.
τ_1 = clearance from primary, ciliated airways.
τ_2 = clearance from nonciliated passages.

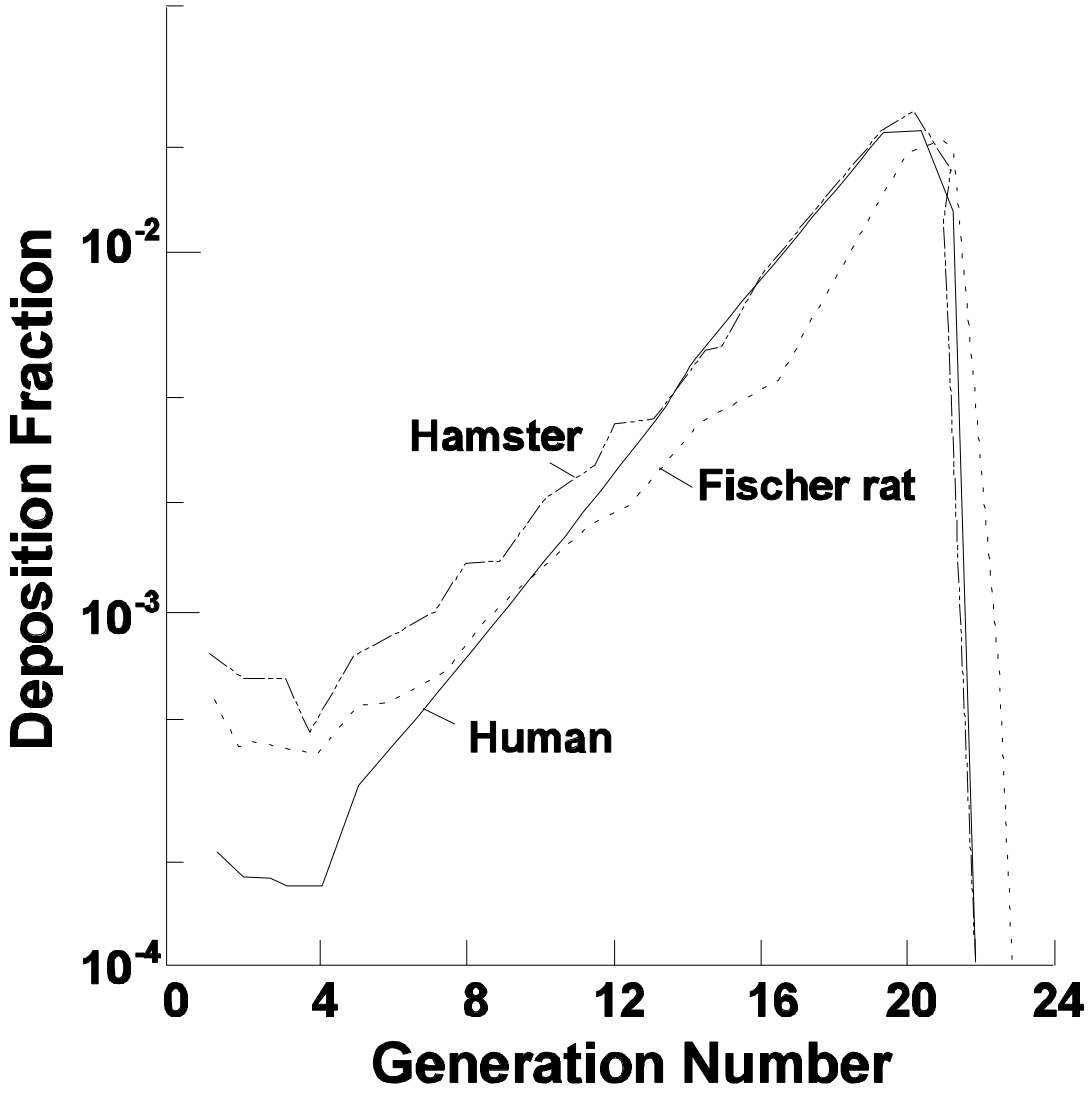

Figure 3-1. Modeled deposition distribution patterns of inhaled diesel exhaust particles in the airways of different species. Generation 1-18 are TB; >18 are A.

Source: Xu and Yu, 1987.

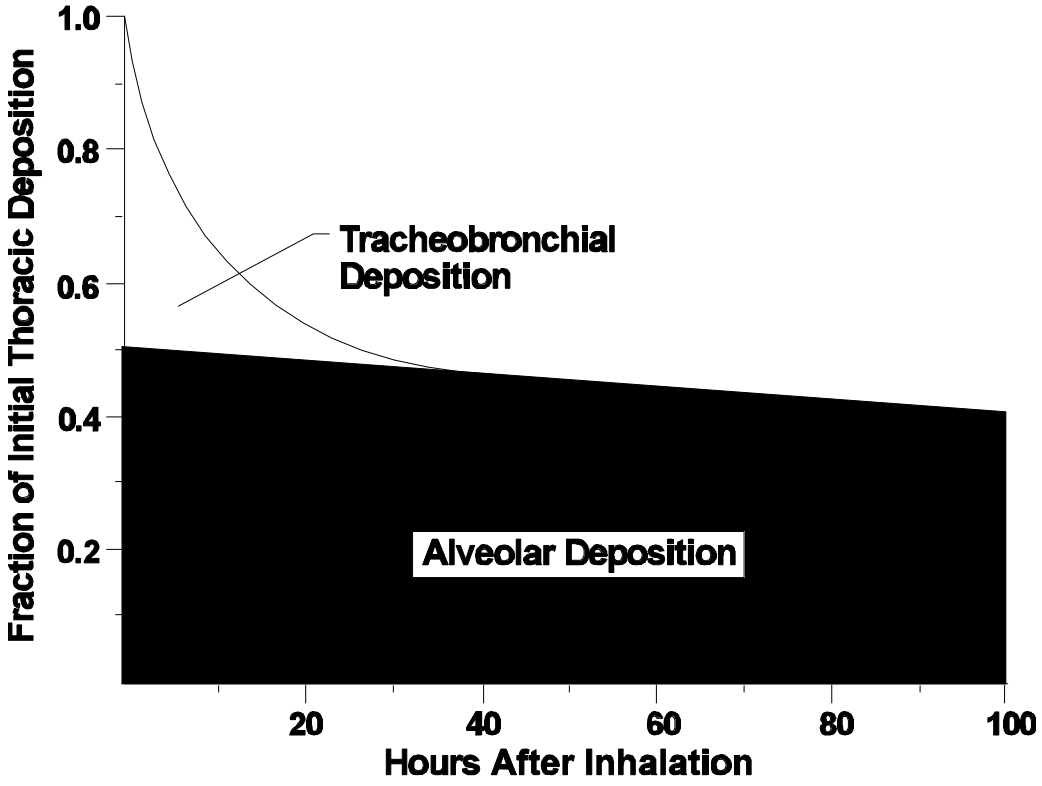

Figure 3-2. Modeled clearance of poorly soluble 4-μm particles deposited in tracheobronchial and alveolar regions in humans.

Source: Cuddihy and Yeh, 1986.

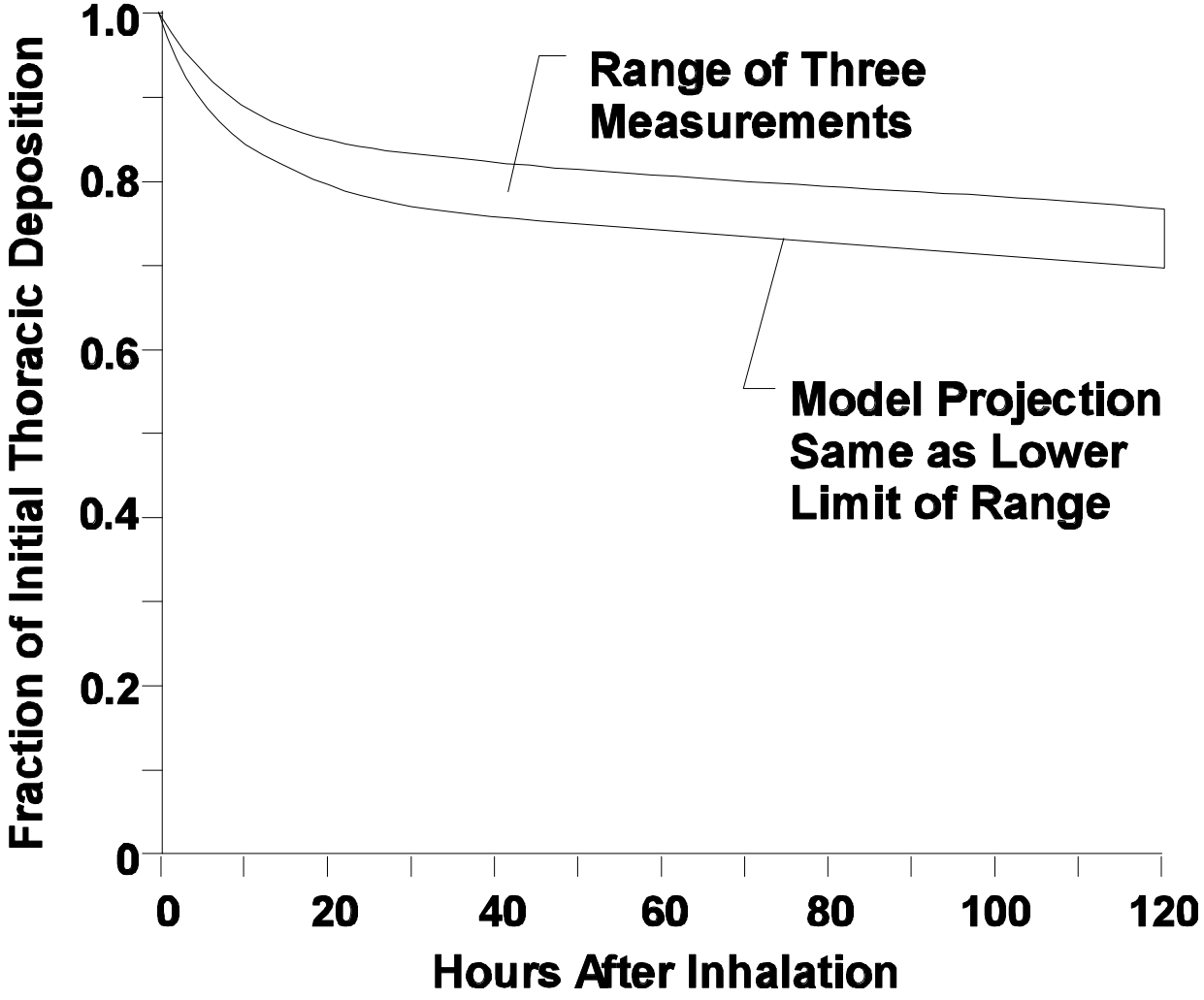

Figure 3-3. Short-term thoracic clearance of inhaled particles as determined by model prediction and experimental measurement.

Source: Cuddihy and Yeh, 1986 (from Stahlhofen et al., 1980).

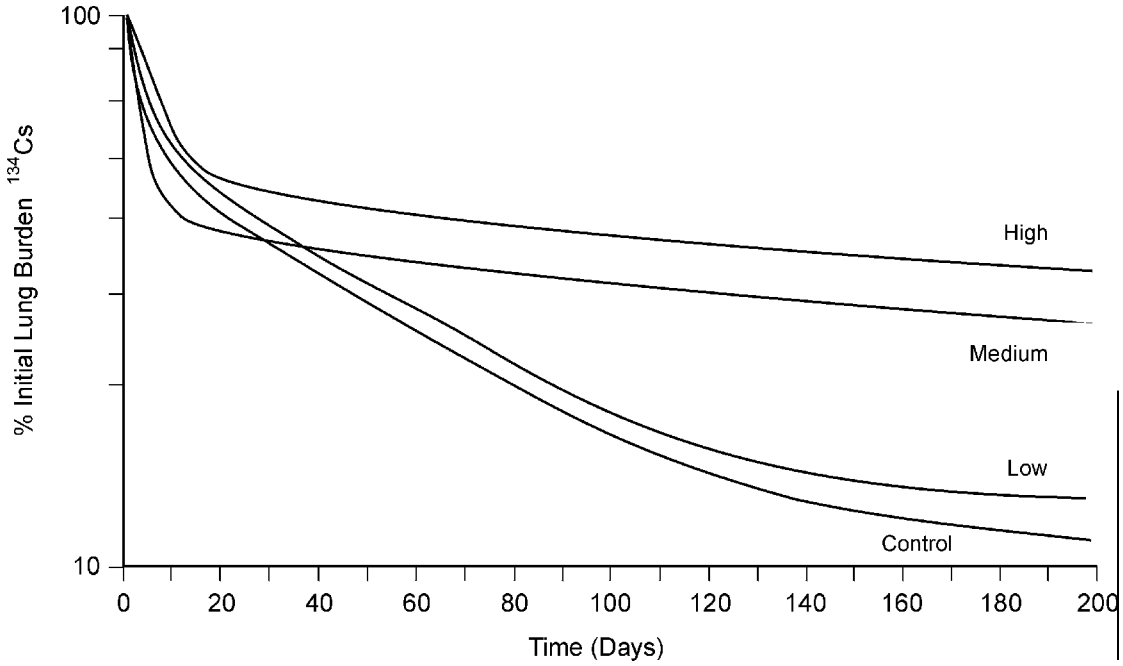

Figure 3-4. Clearance from lungs of rats of ^{134}Cs-FAP fused aluminosilicate tracer particles inhaled after 24 months of diesel exhaust exposure at concentrations of 0 (control), 0.35 (low), 3.5 (medium), and 7.1 (high) mg DPM/m3.

Source: Wolff et al., 1987.

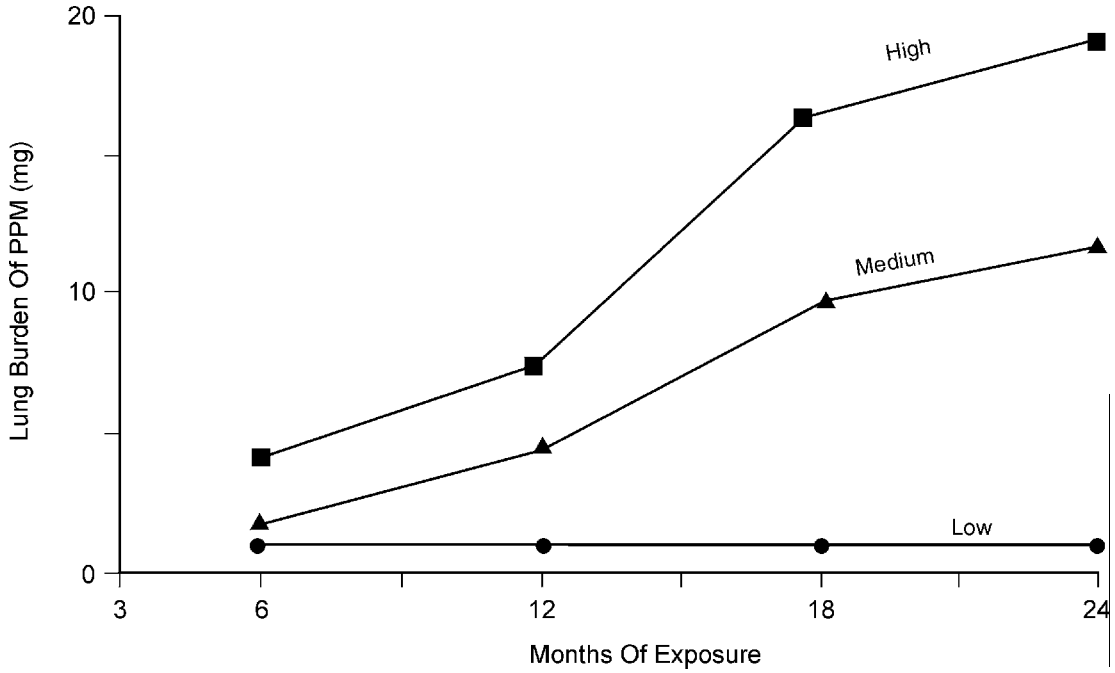

Figure 3-5. Lung burdens of DPM within rats exposed to 0.35 (low) (●), 3.5 (medium) (▲), and 7.1 (high) mg ppm/m^3 (■).

Source: Wolff et al., 1987.

3.8. REFERENCES

Adamson, IYR; Bowden, DH. (1978) Adaptive responses of the pulmonary macrophagic system to carbon: II. Morphologic studies. Lab Invest 38:430-438.

Adamson, IYR; Bowden, DH. (1981) Dose response of the pulmonary macrophagic system to various particulates and its relationship to transepithelial passage of free particles. Exp Lung Res 2:165-175.

Anjilvel, S; Asgharian, B. (1995) A multiple-path model of particle deposition in the rat lung. Fundam Appl Toxicol 28:41-50.

Aronson, M. (1963) Bridge formation and cytoplasmic flow between phagocytic cells. J Exp Med 118:1083-1088.

Bailey, MR; Fry, FA; James, AC. (1982) The long-term clearance kinetics of insoluble particles from the human lung. Ann Occup Hyg 26:273-290.

Bailey, MR; Fry, FA; James, AC. (1985) Long-term retention of particles in the human respiratory tract. J Aerosol Sci 16:295-305.

Ball, LM; King, LC. (1985) Metabolism, mutagenicity, and activation of 1-nitropyrene *in vivo* and *in vitro*. Environ Int 11:355-361.

Battigelli, MC; Hengstenberg, F; Mannela, RJ; et al. (1966) Mucociliary activity. Arch Environ Health 12:460-466.

Bevan, DR; Ruggio, DM. (1991) Bioavailability *in vivo* of benzo[a]pyrene adsorbed to diesel particulate. Toxicol Ind Health 7:125-139.

Bohning, DE; Cohn, SH; Lee, HD; et al. (1980) Two-phase deep-lung clearance in man. In: Pulmonary toxicology of respirable particles: proceedings of the nineteenth annual Hanford life sciences symposium; October 1979; Richland, WA. Sanders, CL; Cross, FT; Dagle, GE; et al., eds. Washington, DC: U.S. Department of Energy; pp. 149-161. Available from: NTIS, Springfield, VA; CONF-791002.

Bohning, DE; Atkins, HL; Cohn, SH. (1982) Long-term particle clearance in man: normal and impaired. In: Inhaled particles V: proceedings of an international symposium; September 1980; Cardiff, Wales. Walton, WH, ed. Ann Occup Hyg 26:259-271.

Bond, JA; Mitchell, CE; Li, AP. (1983) Metabolism and macromolecular covalent binding of benzo[*a*]pyrene in cultured Fischer-344 rat lung type II epithelial cells. Biochem Pharmacol 32:3771-3776.

Bond, JA; Sun, JD; Medinsky, MA; et al. (1986) Deposition, metabolism, and excretion of 1-[^{14}C]nitropyrene and 1-[^{14}C]nitropyrene coated on diesel exhaust particles as influenced by exposure concentration. Toxicol Appl Pharmacol 85:102-117.

Bond, JA; Johnson, NF; Snipes, MB; et al. (1990) DNA adduct formation in rat alveolar type II cells: cells potentially at risk for inhaled diesel exhaust. Environ Mol Mutagen 16:64-69.

Brain, JD; Mensah, GA. (1983) Comparative toxicology of the respiratory tract. Am Rev Respir Dis 128:S87-S90.

Brooks, AL; Wolff, RK; Royer, RE; et al. (1981) Deposition and biological availability of diesel particles and their associated mutagenic chemicals. Environ Int 5:263-267.

Campbell, RM; Lee, ML. (1984) Capillary column gas chromatographic determination of nitro polycyclic aromatic compounds in particulate extracts. Anal Chem 56:1026-1030.

Chan, TL; Lee, PS; Hering, WE. (1981) Deposition and clearance of inhaled diesel exhaust particles in the respiratory tract of Fischer rats. J Appl Toxicol 1:77-82.

Chan, TL; Lee, PS; Hering, WE. (1984) Pulmonary retention of inhaled diesel particles after prolonged exposures to diesel exhaust. Fundam Appl Toxicol 4:624-631.

Cohen, D; Arai, SF; Brain, JD. (1979) Smoking impairs long-term dust clearance from the lung. Science (Washington, DC) 204:514-517.

Cohen, BS; Xiong, JQ; Fang, CP; et al. (1998) Deposition of charged particles on lung airways. Health Phys 74:554-560.

Crapo, JD; Young, SL; Fram, EK; et al. (1983) Morphometric characteristics of cells in the alveolar region of mammalian lungs. Am Rev Respir Dis 128:S42-S46.

Cuddihy, RG; Yeh, HC. (1986) Model analysis of respiratory tract clearance of particles inhaled by people. In: Annual report of the Inhalation Toxicology Research Institute. Muggenburg, BA; Sun, JD, eds. Albuquerque, NM: Lovelace Biomedical and Environmental Research Institute; report no. LMF-115; pp. 140-147.

Evans, MJ; Shami, SG; Martinez, LA. (1986) Enhanced proliferation of pulmonary alveolar macrophages after carbon instillation in mice depleted of blood monocytes by Strontium-89. Lab Invest 54:154-159.

Felicetti, SA; Wolff, RK; Muggenburg, BA. (1981) Comparison of tracheal mucous transport in rats, guinea pigs, rabbits, and dogs. J Appl Physiol: Respir Environ Exercise Physiol 51:1612-1617.

Ferin, J. (1982) Pulmonary alveolar pores and alveolar macrophage-mediated particle clearance. Anat Rec 203:265-272.

Ferin, J; Feldstein, ML. (1978) Pulmonary clearance and hilar lymph node content in rats after particle exposure. Environ Res 16:342-352.

Freedman, AP; Robinson, SE. (1988) Noninvasive magnetopneumographic studies of lung dust retention and clearance in coal miners. In: Respirable dust in the mineral industries: health effects, characterization, and control: proceedings of the international symposium on respirable dust in the mineral industries; October 1986; University Park, PA. Frantz, RL; Ramani, RV, eds. University Park, PA: Pennsylvania State University Press; pp. 181-186.

Freijer, JI; Cassee, FR; Subramaniam, R; et al. (1999) Multiple path particle deposition model developed by the Chemical Industry Institute of Toxicology (CIIT) and the Rijksinstituut Voor Volksgezondheid en Milieu (RIVM), RIVM Publication No. 65001019.

Frey, JW; Corn, M. (1967) Physical and chemical characteristics of particulates in a diesel exhaust. Am Ind Hyg Assoc J 28:468-478.

George, G; Hook, GER. (1984) The pulmonary extracellular lining. Environ Health Perspect 55:227-237.

Gerde, P; Medinsky, MA; Bond, JA. (1991a) Particle-associated polycyclic aromatic hydrocarbons - a reappraisal of their possible role in pulmonary carcinogenesis. Toxicol Appl Pharmacol 108:1-13.

Gerde, P; Medinsky, MA; Bond, JA. (1991b) The retention of polycyclic aromatic hydrocarbons in the bronchial airways and in the alveolar region - a theoretical comparison. Toxicol Appl Pharmacol 107:239-252.

Gerde, P; Muggenberg, BA; Dahl, AR. (1999) Bioavailability, absorption and metabolism of diesel soot-absorbed benzo(a)pyrene after single-breath exposures in dogs. In: Relationships between acute and chronic effects of air pollution: 7th international inhalation symposium; February; Hannover, Federal Republic of Germany; p. 76.

Gibb, FR; Morrow, PE. (1962) Alveolar clearance in dogs after inhalation of an iron 59 oxide aerosol. J Appl Physiol 17:429-432.

Godleski, JJ; Stearns, RC; Katler, MR; et al. (1988) Particle dissolution in alveolar macrophages assessed by electron energy loss analysis using the Zeiss CEM902 electron microscope. J Aerosol Med 1:198-199.

Griffis, LC; Wolff, RK; Henderson, RF; et al. (1983) Clearance of diesel soot particles from rat lung after a subchronic diesel exhaust exposure. Fundam Appl Toxicol 3:99-103.

Gurley, LR; Spall, WD; Valdez, JG; et al. (1988) An HPLC procedure for the analysis of proteins in lung lavage fluid. Anal Biochem 172:465-478.

Harmsen, AG; Muggenburg, BA; Snipes, MB; et al. (1985) The role of macrophages in particle translocation from lungs to lymph nodes. Science (Washington, DC) 230:1277-1280.

Health Effects Institute. (1995) Diesel exhaust: a critical analysis of emissions, exposure, and health effects: a special report of the Institute's Diesel Working Group. Cambridge, MA: Health Effects Institute.

Heinrich, U; Muhle, H; Takenaka, S; et al. (1986) Chronic effects on the respiratory tract of hamsters, mice, and rats after long-term inhalation of high concentrations of filtered and unfiltered diesel engine emissions. J Appl Toxicol 6:383-395.

Hemminki, K; Söderling, J; Ericson, P; et al. (1994) DNA adducts among personnel servicing and loading diesel vehicles. Carcinogenesis 15:767-769.

Heppleston, AG. (1961) Observations on the disposal of inhaled dust by means of the double exposure technique. In: Inhaled particles and vapours: proceedings of an international symposium; March-April 1960; Oxford, United Kingdom. Davies, CN, ed. New York: Pergamon Press; pp. 320-326.

Heppleston, AG; Young, AE. (1973) Uptake of inert particulate matter by alveolar cells: an ultrastructural study. J Pathol 111:159-164.

Heyder, J; Gebhart, J; Rudolf, G; et al. (1986) Deposition of particles in the human respiratory tract in the size range 0.005-15 µm. J Aerosol Sci 17:811-825.

Hiura, TS; Kaxzubowski, MP; Li, N; et al. (1999) Chemicals in diesel exhaust particles generate oxygen radicals and induce apoptosis in macrophages. J Immunol 163:5582-5591.

Holt, PF. (1981) Transport of inhaled dust to extrapulmonary sites. J Pathol 133:123-129.

International Commission on Radiological Protection (ICRP). (1979) Limits for intakes of radionuclides by workers. Oxford, United Kingdom: Pergamon Press; ICRP publication 30, part 1.

ICRP. (1994) Human respiratory tract model for radiological protection: a report of a task group of the International Commission on Radiological Protection. Oxford, United Kingdom: Elsevier Science Ltd. (ICRP publication 66; Annals of the ICRP: v. 24, nos. 1-3).

International Life Sciences Institute (ILSI). (2000) ILSI Risk Science Institute Workshop: the relevance of the rat lung response to particle overload for human risk assessment. Gardner, DE, ed. Inhal Toxicol 12(1-2):1-148.

Jammet, H; Drutel, P; Parrot, R; et al. (1978) Étude de l'épuration pulmonaire chez l'homme après administration d'aérosols de particules radioactives [Study of pulmonary function in man after administration of radioactive particulate aerosols]. Radioprotection 13:143-166.

Keane, MJ; Xing, S-G; Harrison JC; et al. (1991) Genotoxicity of diesel-exhaust particles dispersed in simulated pulmonary surfactant. Mutat Res 260:233-238.

King, LC; Kohan, MJ; Austin, AC; et al. (1981) Evaluation of the release of mutagens from diesel particles in the presence of physiological fluids. Environ Mutagen 3:109-121.

Kittelson, DB; Dolan, DF; Verrant, JA. (1978) Investigation of a diesel exhaust aerosol. Warrendale, PA: Society of Automotive Engineers, Inc.; technical paper series no. 78109.

Kuempel, ED. (2000). Comparison of human and rodent lung dosimetry models for particle clearance and retention. Drug Chem Toxicol 23(1):203-222.

Lee, PS; Chan, TL; Hering, WE. (1983) Long-term clearance of inhaled diesel exhaust particles in rodents. J Toxicol Environ Health 12:801-813.

Lee, KP; Trochimowicz, HJ; Reinhardt, CF. (1985) Transmigration of titanium dioxide (TiO_2) particles in rats after inhalation exposure. Exp Mol Pathol 42:331-343.

Lee, KP; Henry, NW, III; Trochimowicz, HJ.; et al. (1986) Pulmonary response to impaired lung clearance in rats following excessive TiO_2 dust deposition. Environ Res 41:144-167.

Lee, KP; Ulrich, CE; Geil, RG; et al. (1988) Effects of inhaled chromium dioxide dust on rats exposed for two years. Fundam Appl Toxicol 10:125-145.

Lehnert, BE. (1988) Distributions of particles in alveolar macrophages during lung clearance. J Aerosol Med 1:206-207.

Lehnert, BE. (1989) Rates of disappearance of alveolar macrophages during lung clearance as a function of phagocytized particulate burden [abstract]. Am Rev Respir Dis 139(suppl.):A161.

Lehnert, BE. (1990) Alveolar macrophages in a particle “overload” condition. J Aerosol Med 3(suppl. 1):S9-S30.

Lehnert, BE; Morrow, PE. (1985) Association of ^{59}iron oxide with alveolar macrophages during alveolar clearance. Exp Lung Res 9:1-16.

Lehnert, BE; Valdez, YE; Bomalaski, SH. (1988) Analyses of particles in the lung free cell, tracheobronchial lymph nodal, and pleural space compartments following their deposition in the lung as related to lung clearance mechanisms. In: Inhaled particles VI: proceedings of an international symposium and workshop on lung dosimetry; September 1985; Cambridge, United Kingdom. Dodgson, J: McCallum, RI; Bailey, MR; et al., eds. Ann Occup Hyg 32 (suppl. 1):125-140.

Lehnert, BE; Cline, A; London, JE. (1989) Kinetics of appearance of polymorphonuclear leukocytes and their particle burdens during the alveolar clearance of a high lung burden of particles. Toxicologist 9:77.

Leung, HW; Henderson, RF; Bond, JA; et al. (1988) Studies on the ability of rat lung and liver microsomes to facilitate transfer and metabolism of benzo[a]pyrene from diesel particles. Toxicology 51:1-9.

Lippmann, M; Schlesinger, RB. (1984) Interspecies comparisons of particle deposition and mucociliary clearance in tracheobronchial airways. J Toxicol Environ Health 13:441-469.

Lundborg, M; Lind, B; Camner, P. (1984) Ability of rabbit alveolar macrophages to dissolve metals. Exp Lung Res 7:11-22.

Marafante, E; Lundborg, M; Vahter, M; et al. (1987) Dissolution of two arsenic compounds by rabbit alveolar macrophages *in vitro*. Fundam Appl Toxicol 8:382-388.

Mauderly, JL. (1996) Lung overload: the dilemma and opportunities for resolution. In: Particle overload in the rat lung and lung cancer: implications for human risk assessment. Proceedings of a conference; March 1995; Cambridge, MA. Mauderly, JL; McCunney, RJ; eds. New York: Taylor & Francis.

McClellan, RO; Brooks, AL; Cuddihy, RG; et al. (1982) Inhalation toxicology of diesel exhaust particles. In: Toxicological effects of emissions from diesel engines: proceedings of the EPA diesel emissions symposium; October 1981; Raleigh, NC. Lewtas, J, ed. New York: Elsevier Biomedical; pp. 99-120. (Developments in toxicology and environmental science: v. 10).

Morrow, PE. (1966) International Commission on Radiological Protection (ICRP) task group on lung dynamics, deposition and retention models for internal dosimetry of the human respiratory tract. Health Phys 12:173.

Morrow, PE. (1988) Possible mechanisms to explain dust overloading of the lungs. Fundam Appl Toxicol 10:369-384.

Morrow, PE. (1994) Mechanisms and significance of "particle overload." In: Toxic and carcinogenic effects of solid particles in the respiratory tract: [proceedings of the 4th international inhalation symposium]; March 1993; Hannover, Germany. Mohr, U; Dungworth, DL; Mauderly, JL; et al., eds. Washington, DC: International Life Sciences Institute Press; pp. 17-25.

Morrow, PE; Yu, CP. (1993) Models of aerosol behavior in airways and alveoli. In: Aerosols in medicine: principles, diagnosis and therapy. 2nd rev. ed. Moren, F; Dolovich, MB; Newhouse, MT; et al., eds. Amsterdam: Elsevier; pp. 157-193.

Muhle, H; Bellman, B; Creutzenberg, O; et al. (1990) Dust overloading of lungs after exposure of rats to particles of low solubility: comparative studies. J Aerosol Sci 21:374-377.

National Research Council (NRC). (1995) Science and judgment in risk assessment. Washington, DC: National Research Council.

Nielsen, PS; Andreassen, A; Farmer, PB; et al. (1996) Biomonitoring of diesel exhaust-exposed workers. DNA and hemoglobin adducts and urinary 1-hydroxypyrene as markers of exposure. Toxicol Lett 86:27-37.

Nikula, KJ; Avila, KJ; Griffith, WC; et al. (1997a) Sites of particle retention and lung tissue responses to chronically inhaled diesel exhaust and coal dust in rats and cynomolgus monkeys. In: Proceedings of the sixth international meeting on the toxicology of natural and man-made fibrous and non-fibrous particles; September 1996; Lake Placid, NY. Driscoll, KE; Oberdörster, G, eds. Environ Health Perspect Suppl 105(5):1231-1234.

Nikula, KJ; Avila, KJ; Griffith, WC; et al. (1997b) Lung tissue responses and sites of particle retention differ between rats and cynomolgus monkeys exposed chronically to diesel exhaust and coal dust. Fundam Appl Toxicol 37:37-53.

Nilsen, A; Nyberg, K; Camner, P. (1988) Intraphagosomal pH in alveolar macrophages after phagocytosis *in vivo* and *in vitro* of fluorescein-labeled yeast particles. Exp Lung Res 14:197-207.

Oberdörster, G. (1988) Lung clearance of inhaled insoluble and soluble particles. J Aerosol Med 1:289-330.

Oberdörster, G; Ferin, J; Morrow, PE. (1992) Volumetric loading of alveolar macrophages (AM): a possible basis for diminished AM-mediated particle clearance. Exp Lung Res 18:87-104.

Ohkuma, S; Poole, B. (1978) Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agents. Proc Nat. Acad Sci U S A 75:3327-3331.

Österholm, AM; Fält, S; Lambert, B; et al. (1995) Classification of mutations at the human hprt-locus in T-lymphocytes of bus maintenance workers by multiplex-PCR and reverse transriptase-PCR analysis. Carcinogenesis 16:1909-1995.

Pepelko, WE. (1987) Feasibility of dose adjustment based on differences in long-term clearance rates of inhaled particulate matter in humans and laboratory animals. Regul Toxicol Pharmacol 7:236-252.

Phalen, RF; Oldham, MJ. (1983) Tracheobronchial airway structure as revealed by casting techniques. Am Rev Respir Dis 128:S1-S4.

Powdrill, J; Buckley, C; Valdez, YE; et al. (1989) Airway intra-luminal macrophages: origin and role in lung clearance. Toxicologist 9:77.

Pritchard, JN. (1989) Dust overloading causes impairment of pulmonary clearance: evidence from rats and humans. Exp Pathol 37:39-42.

Raabe, OG. (1982) Deposition and clearance of inhaled aerosols. In: Mechanisms in respiratory toxicology. Witschi, H, ed. Boca Raton, FL: CRC Press; pp. 27-76.

Raabe, OG; Al-Bayati, MA; Teague, SV; et al. (1988) Regional deposition of inhaled monodisperse, coarse, and fine aerosol particles in small laboratory animals. In: Inhaled particles VI: proceedings of an international symposium and workshop on lung dosimetry; September 1985; Cambridge, United Kingdom. Dodgson, J; McCallum, RI; Bailey, MR; et al., eds. Ann Occup Hyg 32(suppl. 1):53-63.

Riley, PA; Dean, RT. (1978) Phagocytosis of latex particles in relation to the cell cycle in 3T3 cells. Exp Cell Biol 46:367-373.

Robertson, B. (1980) Basic morphology of the pulmonary defence system. Eur J Respir Dis 61(suppl. 107):21-40.

Sandusky, CB; Cowden, MW; Schwartz, SL. (1977) Effect of particle size on regurgitative exocytosis by rabbit alveolar macrophages. In: Pulmonary macrophage and epithelial cells: proceedings of the sixteenth annual Hanford biology symposium; September 1976; Richland, WA. Sanders, CL; Schneider, RP; Dagle, GE; et al., eds. Washington, DC: Energy Research and Development Administration; pp. 85-105. (ERDA symposium series: no. 43). Available from: NTIS, Springfield, VA; CONF-760927.

Schanker, LS; Mitchell, EW; Brown, RA, Jr. (1986) Species comparison of drug absorption from the lung after aerosol inhalation or intratracheal injection. Drug Metab Dispos 14:79-88.

Schlesinger, RB. (1985) Comparative deposition of inhaled aerosols in experimental animals and humans: a review. J Toxicol Environ Health 15:197-214.

Schlesinger, RB; Ben-Jebria, A; Dahl, AR; et al. (1997) Disposition of inhaled toxicants. In: Handbook of human toxicology. Massaro, EJ, ed. Boca Raton, FL: CRC Press; pp. 493-550.

Schum, M; Yeh, HC. (1980) Theoretical evaluation of aerosol deposition in anatomical models of mammalian lung airways. Bull Math Biol 42:1-15.

Siak, JS; Chan, TL; Lee, PS. (1980) Diesel particulate extracts in bacterial test systems. In: Health effects of diesel engine emissions: proceedings of an international symposium, v. 1; December 1979; Cincinnati, OH. Pepelko, WE; Danner, RM; Clarke, NA, eds. Cincinnati, OH: U.S. EPA, Health Effects Research Laboratory; pp. 245-262; EPA report no. EPA/600/9-80/057b. Available from: NTIS, Springfield, VA; PB81-173809.

Smith, TJ. (1985) Development and application of a model for estimating alveolar and interstitial dust levels. Ann Occup Hyg 29:495-516.

Snipes, MB. (1979) Long-term retention of monodisperse and polydisperse particles inhaled by beagle dogs, rats and mice. Albuquerque, NM: Lovelace Biomedical and Environmental Research Institute; Inhalation Toxicology Research Institute annual report LF-69; pp. 420-423.

Snipes, MB; Clem, MF. (1981) Retention of microspheres in the rat lung after intratracheal instillation. Environ Res 24:33-41.

Snipes, MB; Olson, TR; Yeh, HC. (1988) Deposition and retention patterns for 3-, 9-, and 15-μm latex microspheres inhaled by rats and guinea pigs. Exp Lung Res 14:37-50.

Snipes, MB; McClellan, RO; Mauderly, JL; et al. (1989) Retention patterns for inhaled particles in the lung: comparisons between laboratory animals and humans for chronic exposures. Health Phys 57(suppl. 1):69-78.

Sorokin, SP; Brain, JD. (1975) Pathways of clearance in mouse lungs exposed to iron oxide aerosols. Anat Rec 181:581-625.

Stahlhofen, W; Gebhart, J; Heyder, J. (1980) Experimental determination of the regional deposition of aerosol particles in the human respiratory tract. Am Ind Hyg Assoc J 41:385-398a.

Stahlhofen, W; Koebrich, R; Rudolf, G; et al. (1990) Short-term and long-term clearance of particles from the upper human respiratory tract as function of particle size. J Aerosol Sci 21(suppl. 1):S407-S410.

Stöber, W; Einbrodt, HJ; Klosterkötter, W. (1967) Quantitative studies of dust retention in animal and human lungs after chronic inhalation. In: Inhaled particles and vapours II: proceedings of an international symposium; September-October 1965; Cambridge, United Kingdom. Davies, CN, ed. Oxford, United Kingdom: Pergamon Press; pp. 409-418.

Stöber, W; Morrow, PE; Hoover, MD. (1989) Compartmental modeling of the long-term retention of insoluble particles deposited in the alveolar region of the lung. Fundam Appl Toxicol 13:823-842.

Stöber, W; McClellan, RO; Morrow, PE. (1993) Approaches to modeling disposition of inhaled particles and fibers in the lung. In: Toxicology of the lung. Gardner, DE; Crapo, JD; McClellan, RO, eds. New York: Raven Press; pp. 527-601.

Stöber, W; Morrow, PE; Koch, W; et al. (1994) Alveolar clearance and retention of inhaled insoluble particles in rats simulated by a model inferring macrophage particle load distributions. J Aerosol Sci 25:975-1002.

Strom, KA. (1984) Response of pulmonary cellular defenses to the inhalation of high concentrations of diesel exhaust. J Toxicol Environ Health 13:919-944.

Strom, KA; Chan, TL; Johnson, JT. (1988) Pulmonary retention of inhaled submicron particles in rats: diesel exhaust exposures and lung retention model. In: Inhaled particles VI: proceedings of an international symposium and workshop on lung dosimetry; September 1985; Cambridge, United Kingdom. Dodgson, J; McCallum, RI; Bailey, MR; et al., eds. Ann Occup Hyg 32(suppl. 1):645-657.

Subramaniam, RP; Miller, FJ; Asgarian, B. (1998) Numerical predictions of regional deposition of fine particles in the human lung using a multiple-path model. Proceedings of the American Association of Aerosol Research, annual meeting; Denver, CO.

Sun, JD; Wolff, RK; Kanapilly, GM. (1982) Deposition, retention, and biological fate of inhaled benzo(*a*)pyrene adsorbed onto ultrafine particles and as a pure aerosol. Toxicol Appl Pharmacol 65:231-244.

Sun, JD; Wolff, RK; Kanapilly, GM; et al. (1984) Lung retention and metabolic fate of inhaled benzo(*a*)pyrene associated with diesel exhaust particles. Toxicol Appl Pharmacol 73:48-59.

Tong, HY; Karasek, FW. (1984) Quantitation of polycyclic aromatic hydrocarbons in diesel exhaust particulate matter by high-performance liquid chromatography fractionation and high-resolution gas chromatography. Anal Chem 56:2129-2134.

U.S. Environmental Protection Agency (U.S. EPA). (1982) Air quality criteria for particulate matter and sulfur oxides. Research Triangle Park, NC: Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office; EPA report no. EPA/600/8-82-029aF-cF. 3v. Available from: NTIS, Springfield, VA; PB84-156777.

U.S. EPA. (1993) Provisional guidance for quantitative risk assessment of polycyclic aromatic hydrocarbons. Office of Health and Environmental Assessment; EPA report no. EPA/600/R-93/089.

U.S. EPA. (1996) Air quality criteria for particulate matter. Research Triangle Park, NC: National Center for Environmental Assessment-RTP Office; report nos. EPA/600/P-95/001aF-cF. 3v. Available from: NTIS, Springfield, VA; PB96-168224.

Valberg, PA; Watson, AY. (1999). Comparative mutagenic dose of ambient diesel engine exhaust. Inhal Toxicol 11(3):215-228.

Vastag, E; Matthys, H; Zsamboki, G; et al. (1986) Mucociliary clearance in smokers. Eur J Respir Dis 68:107-113.

Vostal, JJ; Schreck, RM; Lee, PS; et al. (1982) Deposition and clearance of diesel particles from the lung. In: Toxicological effects of emissions from diesel engines: proceedings of the 1981 EPA diesel emissions symposium; October 1981; Raleigh, NC. Lewtas, J, ed. New York: Elsevier Biomedical; pp. 143-159. (Developments in toxicology and environmental science: v. 10).

Waite, DA; Ramsden, D. (1971) The inhalation of insoluble iron oxide particles in the sub-micron range. Part I. Chromium-51 labelled aerosols. Winfrith, Dorchester, Dorset, United Kingdom: Atomic Energy Establishment; report no. AEEW-R740.

Warheit, DB; Overby, LH; George, G; et al. (1988) Pulmonary macrophages are attracted to inhaled particles through complement activation. Exp Lung Res 14:51-66.

Weibel, ER. (1963) Morphometry of the human lung. New York: Academic Press, Inc.

White, HJ; Garg, BD. (1981) Early pulmonary response of the rat lung to inhalation of high concentration of diesel particles. J Appl Toxicol 1:104-110.

Wolff, RK; Gray, RL. (1980) Tracheal clearance of particles. In: Inhalation Toxicology Research Institute annual report: 1979-1980. Diel, JH; Bice, DE; Martinez, BS, eds. Albuquerque, NM: Lovelace Biomedical and Environmental Research Institute; p. 252; report no. LMF-84.

Wolff, RK; Henderson, RF; Snipes, MB; et al. (1986) Lung retention of diesel soot and associated organic compounds. In: Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Ishinishi, N; Koizumi, A; McClellan, R; et al. eds. Amsterdam: Elsevier Science Publishers BV.; pp. 199-211. (Developments in toxicology and environmental science: v. 13).

Wolff, RK; Henderson, RF; Snipes, MB; et al. (1987) Alterations in particle accumulation and clearance in lungs of rats chronically exposed to diesel exhaust. Fundam Appl Toxicol 9:154-166.

World Health Organization. (1996) Diesel fuel and exhaust emissions. Geneva, Switzerland: World Health Organization, International Programme on Chemical Safety. (Environmental health criteria 171).

Wright, JR; Clements, JA. (1987) Metabolism and turnover of lung surfactant. Am Rev Respir Dis 136:426-444.

Xu, GB; Yu, CP. (1987) Deposition of diesel exhaust particles in mammalian lungs: a comparison between rodents and man. Aerosol Sci Technol 7:117-123.

Yeh, HC; Schum, GM. (1980) Models of human lung airways and their application to inhaled particle deposition. Bull Math Biol 42:461-480.

Yu, CP. (1978) Exact analysis of aerosol deposition during steady breathing. Powder Technol 21:55-62.

Yu, CP; Diu, CK. (1983) Total and regional deposition of inhaled aerosols in humans. J Aerosol Sci 5:599-609.

Yu, CP; Xu, GB. (1986) Predictive models for deposition of diesel exhaust particulates in human and rat lungs. Aerosol Sci Technol 5:337-347.

Yu, CP; Yoon, KJ. (1990) Retention modeling of diesel exhaust particles in rats and humans. Amherst, NY: State University of New York at Buffalo (Health Effects Institute research report no. 40).

Yu, CP; Chen, YK; Morrow, PE. (1989) An analysis of alveolar macrophage mobility kinetics at dust overloading of the lungs. Fundam Appl Toxicol 13:452-459.

Yu, CP; Yoon, KJ; Chen, YK. (1991) Retention modeling of diesel exhaust particles in rats and humans. J Aerosol Med 4(2):79-115.

Yu, CP; Ding, YJ; Zhang, L; et al. (1996) A clearance model of refractory ceramic fibers in the rat lung including fiber dissolution and breakage. J Aerosol Sci 27:151-160.