CHAPTER 3

NOTE TO READERS

Because Chapter 3 recently has merged with Chapter 2, time did not permit renumbering of the remaining chapters for this draft. Chapters 4 through 12 will be renumbered in the next version.

4. DOSIMETRIC FACTORS

4.1. INTRODUCTION

Diesel engine emissions consist of a complex mixture of gases, vapors, and particles made up of a carbon core with a great variety of organic agents adsorbed to the surface. The tumorigenic response observed in rats exposed to diesel exhaust is the result of pulmonary overloading with diesel soot particles. Data from studies in rats have shown that exposure duration and particle surface area and/or particle mass are relevant dosimetric factors. These same studies have shown that particle-adsorbed organics may not be instrumental in the tumorigenic response in rats. Whether similar tumorigenic mechanisms in humans exposed at low doses are critical dosimetric factors in estimating human risk to diesel exhaust is uncertain. Although assessment of dose-response relationships may permit more advanced extrapolations from high experimental exposure concentrations to ambient levels and from animal test species to humans, the question of mechanistic similarities in a tumorigenic response between rats and humans remains unanswered.

A review of animal carcinogenicity studies (Chapter 7) revealed that the gaseous phase alone failed to induce increases in lung tumors in any of the long-term studies in rats or mice. Because of the very limited positive data for this fraction and because the potential carcinogens likely to be present in this fraction (formaldehyde and acetaldehyde) induce upper respiratory tract tumors, which were not seen in the whole-exhaust studies, the gaseous phase is not considered separately in determining carcinogenic risk of diesel exhaust. Noncancer endpoints examined in these studies (Chapter 5) also were more affected by the whole exhaust compared to the gas phase of the exhaust. Because the tumorigenic effects observed in rats are likely to be a function of particle overload, the dosimetry of particles will be emphasized in this chapter. The dosimetry of particle-adsorbed organics, however, will be included because they may be of greater importance for humans exposed at low concentrations.

With a single exception (Iwai et al., 1986), the tumors reported in the diesel exhaust inhalation studies reviewed in Chapter 7 all occurred in the lungs. However, these findings are questionable because of the relatively small number of animals used and the fact that the lymphomas reported by Iwai et al. (1986) were not observed in other studies where rats were similarly exposed. Because particle overload carcinogenesis occurs under pulmonary overload conditions, dosimetric considerations are limited to the lung.

The insoluble carbon core of the exhaust particle and its persistence in the lung are now recognized as critical factors in the particle overload phenomenon that apparently leads to the tumorigenic response observed in rats. Furthermore, total particle surface area appears to be an important determinant in the particle overload phenomenon. The contribution of extractable

organics has been shown to be inconsequential in this response (this subject is discussed in more detail in Chapter 10). It is, however, important to note that dose determination for inhaled compounds remains problematic among toxicologists (Dahl et al., 1991).

The dosimetric aspects considered will include deposition in the conducting airways and alveolar regions, normal particle clearance mechanisms and rates in both regions, clearance rates during lung overload, elution of organics from the particles, particle transport to extraalveolar sites, and the interrelationships of these factors in determining the target organ dose.

4.2. REGIONAL DEPOSITION OF INHALED PARTICLES

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, and density) 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 diesel exhaust particulate matter 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. Where appropriate, 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).

4.2.1. Physical Processes, Physiological/Anatomical Considerations, and Particle Characteristics

Deposition of particles may occur through several processes or combinations thereof, including diffusion, sedimentation (gravitational settling), interception, electrostatic precipitation, and impaction. 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 resulting from these processes 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 nasopharyngeal, tracheobronchial, and alveolar regions of the respiratory tract.

Upon inhalation of particulate matter such as diesel exhaust, deposition will occur throughout the respiratory tract. Because of high airflow velocities and abrupt directional

changes in the nasopharyngeal and tracheobronchial regions, inertial impaction is a primary deposition mechanism (especially for particles ≤ 2.5 µm mass median diameter [MMD]). 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 diesel exhaust particles, with an MMD ≤ 1 µm and small aspect ratio.

Because their MMD is generally ≤ 1 µm, diesel exhaust particles are subject to deposition in the alveolar region. Based on animal data regarding the site of origin of diesel exhaust-induced tumors, particle deposition in the alveolar region may be of greatest concern relative to the carcinogenic potential of diesel particulate matter and/or the adsorbed organics. However, such data for humans are not available. For such small particles, diffusion would be especially prevalent in this region, whereas sedimentation would become less significant, especially for particles of MMD <0.5 µm.

Respiratory tract anatomy and ventilatory characteristics are crucial in determining regional deposition patterns and are also responsible for interspecies and interindividual variability in both deposition of particulate matter and inhaled dose. The variability in size and branching configurations of conducting airways is an important determinant of interspecies variability in deposited dose. Because of the anatomical complexity and variability in ventilatory patterns, a precise categorization of airflow dynamics for a given species or for a specific portion of the respiratory tract is difficult. For more extensive discussions of deposition processes, refer to reviews by Morrow (1966), Raabe (1982), U.S. Environmental Protection Agency (1982), Phalen and Oldham (1983), Lippmann and Schlesinger (1984), Raabe et al. (1988), and Stöber et al. (1993).

Exposure to whole diesel exhaust will also result in inhalation of gas-phase components such as formaldehyde, acrolein, and sulfur dioxide, all of which have been demonstrated to be sensory irritants. It is also known that these irritants affect respiratory rates (Kane and Alarie, 1978, 1979). The sensory irritant-induced reduction of respiratory rate is mediated through stimulation of free nerve endings of the afferent trigeminal nerve (Ulrich et al., 1972). This physiologic reflex response has been shown to be a concentration-dependent response (Alarie, 1966, 1973; Kane and Alarie, 1978). Several studies have also shown that mice appear to be responsive to sensory irritants relative to alteration of respiratory patterns (Alarie, 1973; Kane and Alarie, 1978, 1979). However, only very low levels of these irritants are present in diesel engine exhaust; consequently, their significance in affecting delivered dose through changes in respiration may be small.

4.2.2. Species Variability in Regional Dose

The variability in the anatomy of conducting airways among species results in interspecies variability of inhaled dose. Because different species breathing the same aerosol will not receive the same dose to the respiratory tract, it is generally accepted that exposure concentration is not an accurate description of respiratory tract dose (Brain and Mensah, 1983).

The deposition of inhaled diesel particles in the respiratory tract of humans and mammalian species has been reviewed by Schlesinger (1985). He showed that physiological differences in the breathing mode for humans (nasal or oronasal breathers) and experimental animals (obligatory nose breathers), combined with different airway geometries, resulted in significant differences in lower respiratory tract deposition for larger particles (>1 µm). 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 diesel exhaust particles in rats, hamsters, and humans on the basis of calculations of the models of Schum and Yeh (1980) and Weibel (1963). In Figure 4-1, relative deposition patterns in the lower respiratory tract (trachea = generation 1; alveoli = generation 23) are very similar among hamsters, rats, and humans. Variations in alveolar deposition of diesel exhaust particles 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.

However, 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 4-1 compares the predicted deposited doses of diesel exhaust particles 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 4-1, the absolute deposited dose 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 absolute deposited dose in relevant target areas are important and have to be considered when extrapolating the results from diesel exhaust exposure studies in animals to humans. The differences are less on a surface area basis than on a lung

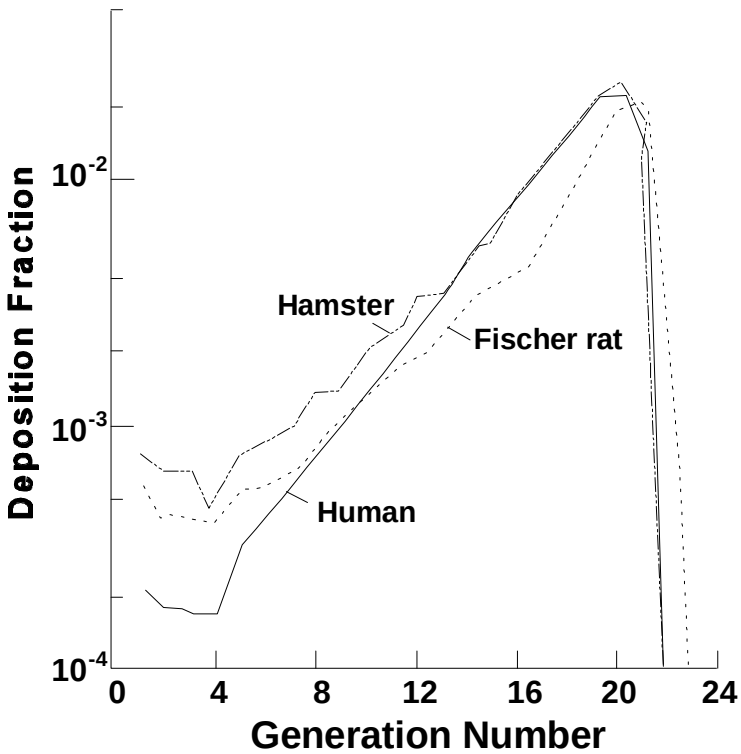

Figure 4-1. Deposition distribution patterns of inhaled diesel exhaust particles in the airways of different species.

Source: Xu and Yu (1987).

Table 4-1. Predicted doses of inhaled diesel exhaust particles 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/mi/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 and M_1 = mass of particles deposited in total lung.
M_2 = mass of particles deposited in the alveolar region only.
Based on the following conditions: (1) MMAD = 0.2 µm, σ = 1.9, ϕ = 0.3, and ρ = 1.5 g/cm^3; (2) particle concentration = 1 mg/m^3; and (3) nose-breathing.

Source: Xu and Yu (1987).

volume basis (Table 4-1). This is due to larger alveolar diameters in humans and concomitantly lower surface area per unit of lung volume.

The alternative, perhaps more accurate physiologically, is to consider deposition rate relative to exposure concentration; the deposition rate 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.

4.3. RESPIRATORY TRACT CLEARANCE RATES

4.3.1. Tracheobronchial Clearance

The dynamic relationship between deposition and clearance is responsible for determining lung burden at any point in time. Clearance of highly insoluble particles from the tracheobronchial region is mediated primarily by mucociliary transport and is 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; those for alveolar clearance may be 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 conducting 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).

Although most of the particulate matter cleared from the tracheobronchial region will ultimately be 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 carcinogenesis in the respiratory tract

(Pepelko, 1987). Gerde et al. (1991a) showed that for low-dose exposures, particle-associated PAHs were rapidly released, thereby suggesting retained particle-associated PAHs may be of lesser importance in tumorigenic responses than originally believed. The relationship between the early clearance of insoluble particles (4 µm aerodynamic diameter) from the tracheobronchial regions and their longer-term clearance from the alveolar region is illustrated in Figure 4-2.

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: respiratory tract airway dimensions, calculated inhaled particle deposition fractions for individual airways, and thoracic 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 for intratracheally instilled particles. The velocities observed for inhalation studies were generally lower than those of instillation studies. Figure 4-3 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 retention.

Exposure of F344 rats to whole DPM at concentrations of 0.35, 3.5, or 7.0 mg/m^3 for up to 24 mo did not significantly alter tracheal mucociliary clearance of ^{98m}Tc-macroaggregated albumin instilled into the trachea (Wolff et al., 1987). The assessment of tracheal clearance was determined by measuring the amount of material retained 1 h after instillation. 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

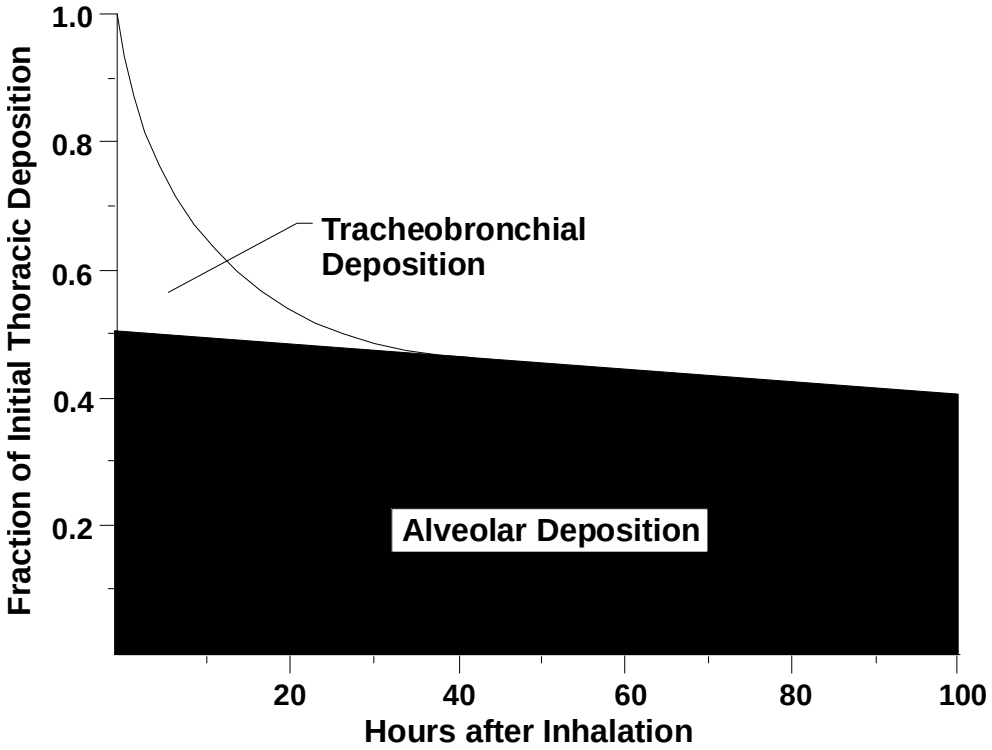

Figure 4-2. Clearance of insoluble particles deposited in tracheobronchial and alveolar regions.

Source: Chuddihy and Yeh, 1986.

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.

The fact 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. However, the apparent retention of a fraction of the deposited dose in the airways is cause for some concern regarding possible carcinogenic effects in this region, especially in light of the results from simulation studies by Gerde et al. (1991b) that suggested that release of polycyclic aromatic hydrocarbons (PAHs) from particles may occur within minutes and at the site of initial deposition. Moreover, impairment of mucociliary clearance function as a result of exposure to either occupational or environmental respiratory tract toxicants

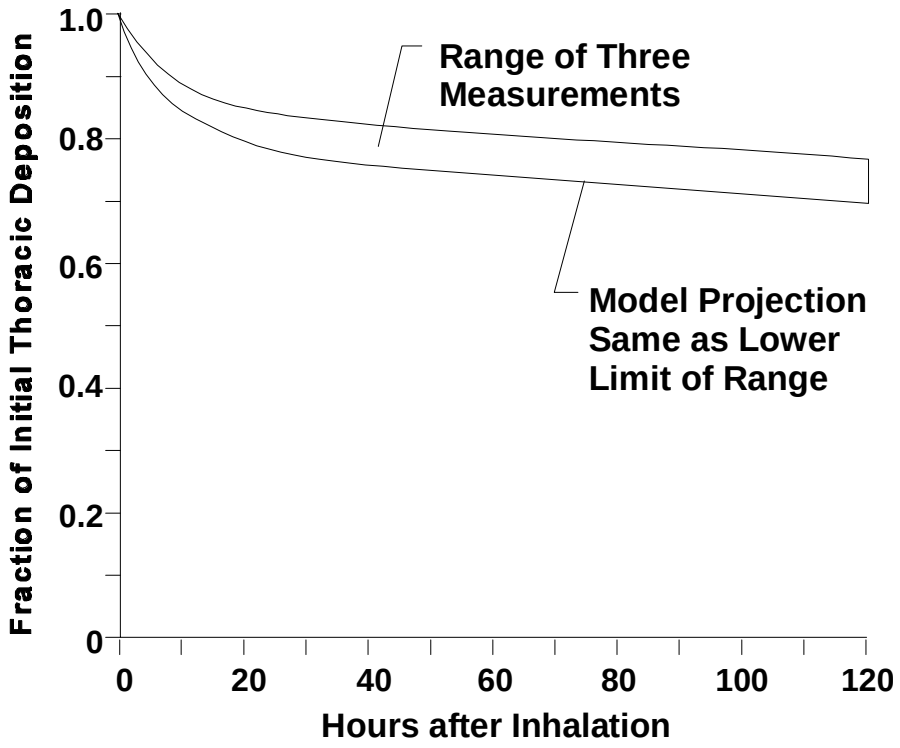

Figure 4-3. Short-term thoracic clearance of inhaled particles as determined by model prediction and experimental measurement.

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

or to cigarette smoke will significantly enhance the retention of particles in this 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 in diesel exhaust-induced rat pulmonary tumors.

4.3.2. Clearance From the Alveolar Region

4.3.2.1. *Alveolar Clearance in Humans*

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 (Bailey et al., 1982).

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 individuals who had mild pneumoconiosis. Although information on particle burden and particle overload relationships in humans is much more limited than for 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 ranged from 2 to 50 mg/g of lung or more, well above the value for which sequestration 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 diesel exhaust.

4.3.2.2. *Alveolar Clearance in Animals*

Normal alveolar clearance rates in animals have been reported by a number of investigators. Because the rat is 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 7.0 mg of soot/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:

$$F_{(t)} = A \exp(-0.693\ t/\tau_1) + B \exp(-0.693\ t/\tau_2),$$

where $F_{(t)}$ was the percentage retained throughout the respiratory tract, *A* and *B* were the magnitudes of the two components (component *A* representing the amount cleared from nasal, lung, and gastrointestinal compartments and component *B* representing intermediate clearance from the lung compartment), and τ_1 and τ_2 were the half-times for the *A* and *B* compartments, respectively. The early retention half-times (τ_1), representing clearance from primary, ciliated conducting airways, were similar for rats in all exposure groups at all time points except for those in the high exposure (7.0 mg/m^3) group following 24 mo of exposure, whose clearance rate was faster than that of the controls. Significantly longer *B* compartment retention half-times, representing the early clearance from nonciliated passages such as alveolar ducts and alveoli, were noted after as few as 6 months of exposure to DPM at 7.0 mg/m^3 and 18 months of 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-month 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 7.0 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 7.0 mg/m^3 exposure groups. Based on these findings, the cumulative exposure of 11,760 mg·h/m^3 represented a particle overload condition resulting in compromised alveolar clearance mechanisms.

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. This is 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 sufficient to result in a particle overload condition. With respect to the organic matter adsorbed onto the particles, the authors estimated that over the 2.5-year period, 60 to 150 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. 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 significant lung DPM burdens and impaired clearance.

In a subsequent study (Lee et al., 1983), a three-phase model was used to describe 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) exposed by nose-only inhalation with no apparent particle overload in the lungs. 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 the derivation of a three-phase clearance of the particles. The resulting retention $t_{1/2}$ values for the three phases were 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 by the fact that 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-diesel exhaust 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, and no clearance was observed in the 11.8 mg group. 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. Based on these data, a particle overload effect was demonstrated for both the high and low exposure levels (equivalent to C × T dose of 840 [transitional overload] and 7,440 $mg \cdot h/m^3$).

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 generally much longer (>8 mo) than the average retention $t_{1/2}$ of 60 days for rats.

4.3.2.3. *Lung Burden and Pulmonary Overload Resulting in Impaired Clearance*

Particle overload appears to be an important factor in the diesel emission-induced pulmonary carcinogenicity observed in rats. 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 35, 220, and 1,890 µg/g of lung tissue, respectively. The alveolar clearance of those rats with the highest lung burden (1,890 µg/g of lung) was impaired, as determined by a significantly greater ($p<0.0001$) retention $t_{1/2}$ for DPM. This is 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 a total 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.

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 $mg/m^3 \cdot h/day$ and also

showed that normal lung clearance mechanisms appeared to be compromised as the lung DPM burden approached 0.5 mg/g of lung.

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 AM. 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 biologically insoluble, respirable particles. The model provides for physiology-oriented multicompartmental considerations and is based on the dominant role of alveolar macrophages.

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). A diagram of the POCK model is shown in Figure 4-4. For characterizing these macrophage tools, 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. Application 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 of the particle burden of the interstitial space, a compartment that is not available for macrophage-mediated clearance. The model predictions, however, regarding the subcompartmental concentrations of particles require an experimental database at a reasonably wide range of external dose rates.

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 sizes of particles were found to be phagocytized by AM 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 overload hypothesis.

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 aerosols (Lee et al., 1986; Lee et al., 1988; Ferin and

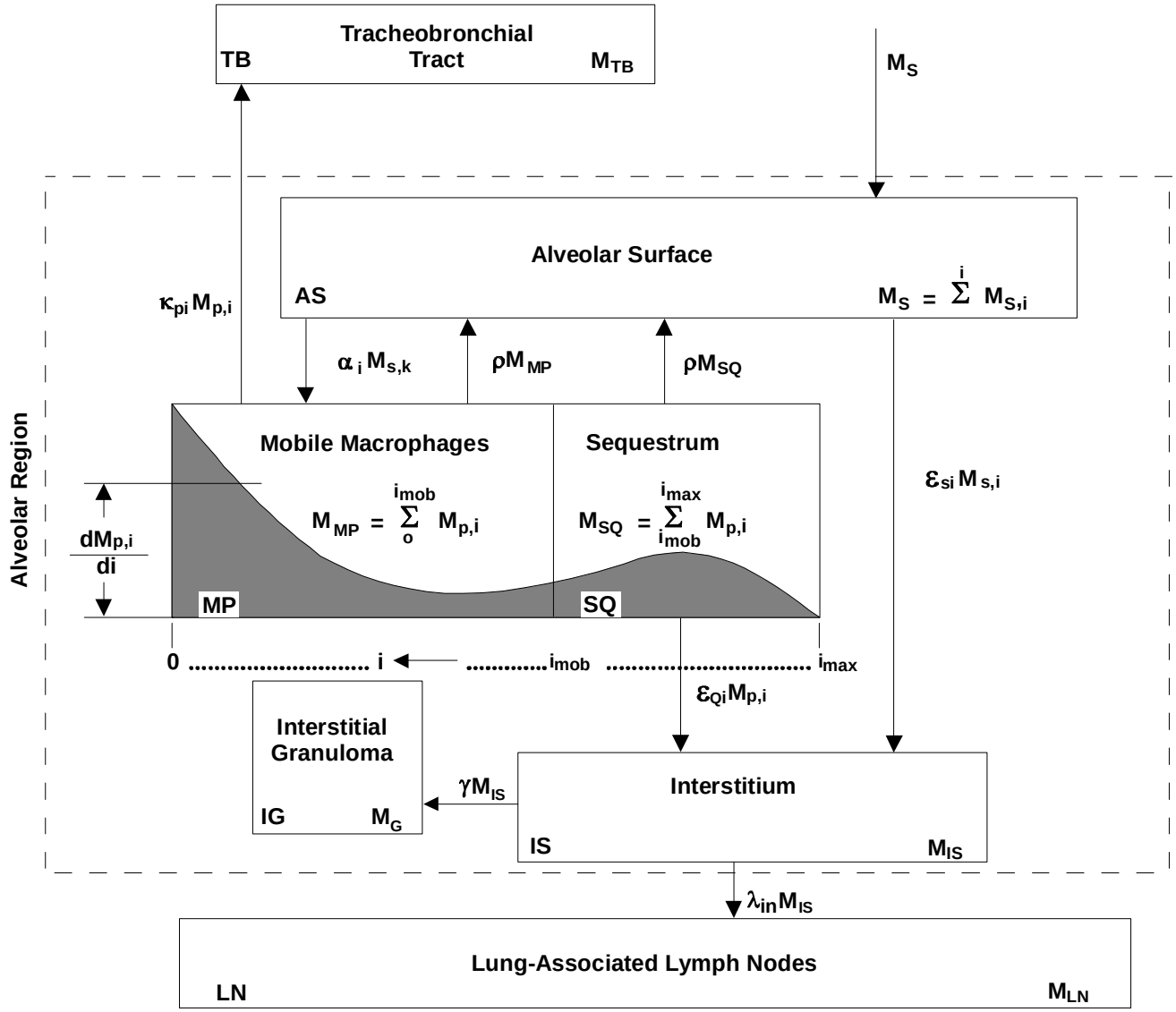

Figure 4-4. Physiology-oriented compartmental kinetics model of retention of inhaled insoluble particles in rats.

Source: Stöber et al. (1994).

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Feldstein, 1978; Muhle et al. 1990). Because high lung burdens of 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. It must be noted, however, that some types of particles may impair clearance at lower lung burdens (e.g., silica may impair clearance at much lower lung burdens than DPM). This could be due to toxic agents adsorbed to the particle surface, or in the case of silica, surface-associated organics. 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. 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 for those miners with apparent particle overload.

4.3.3. Role of AMs in the Clearance of Particulate Matter

4.3.3.1. *AM-Mediated Clearance of Particulate Matter*

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 the 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 in nature, numerous lines of evidence indicate that such AM-mediated particle clearance is normally the predominant mechanism by which relatively insoluble particles are removed from 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 lung's alveolar region 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 physiologic mechanisms determining these differing clearance rates have not been well characterized.

Lehnert et al. (1988, 1989) performed a study 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 studied 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 would. 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 they may not exclusively reflect the translocation of AM from the lung. The observations are also consistent with a gradual redistribution of retained particles among the lung's AMs concurrent with the removal of particle-containing AMs via the conducting airways per se. Experimental support suggestive of potential processes for such particle redistribution comes from a variety of investigations involving AM and other endocyte cell types (Heppleston and Young, 1974; Evans et al., 1986; Aronson, 1963; Sandusky et al., 1977; Heppleston, 1961; Riley and Dean, 1978).

4.3.3.2. *Translocations of Particles to Extraalveolar Macrophage Compartment Sites*

Although the phagocytosis of particles by lung-free cells 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 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 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. Whether or not AMs, and perhaps polymorphonuclear lymphocytes (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. Evidence that such migration of AMs may contribute significantly to the passage of particles to the interstitial compartment and also may be involved in the subsequent translocation of particles to draining lymph nodes has been obtained with the dog model (Harmsen et al., 1985).

The fate of particles once they enter the lung's interstitial spaces remains unclear. Some particles, as previously indicated, are phagocytized by interstitial macrophages, whereas others apparently remain in a free state in the interstitium for some time without being engulfed by interstitial macrophages. It is unknown if interstitial macrophages subsequently enter the alveoli with their engulfed burdens of particles and thereby contribute to the size of the resident AM population over the course of lung clearance. Moreover, no investigations have been conducted to date to assess the influence that the burden of particles with an interstitial macrophage may have on the AM's ability to migrate into the alveolar space compartment.

At least some particles that gain entry into the interstitial compartment can further translocate to the extrapulmonary regional lymph nodes. This process 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). It is conceivable that the mobility of the interstitial macrophages could be particle-burden limited, and under conditions of high cellular burdens a greater fraction of particles that accumulate in the lymph may reach these sites as free particles. Whatever the process, existing evidence indicates that when lung burdens of particles result in a particle-overload condition, particles accumulate both more rapidly and abundantly in lymph nodes that receive lymphatic drainage from the lung (Ferin and Feldstein, 1978; Lee et al., 1985).

4.3.3.3. *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 lung's Type I cell population during lung overloading.

Another anatomical region in the lung that may be a slow clearing site is the interstitial compartment. Little is known about either 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 are sites that could represent subcompartments of a more generalized slow clearing compartment.

Although these sites must be considered to be 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 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 has been offered involving volumetric AM burden (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 agglutinating 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 due to attractants from the overloaded AMs causing aggregation of those that are not carrying a particle burden.

4.3.3.4. *Physiologically Based Models for Pulmonary Retention and Clearance of Insoluble Particles*

Currently available data for long-term inhalation exposures to insoluble 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 insoluble particulate matter in the lungs. 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 these models.

A two-compartment model was developed by Smith (1985) that 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, while other processes affecting particle transfer are assumed to be linear. Although this model provides rate constants as functions that vary depending on the conditions within the various compartments, most of the described functions could not be validated with experimental data.

Strom et al. (1988) developed a multicompartmental model for particle retention that includes 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 will dictate sequestration of particles and their eventual transfer to the lymph nodes.

Yu et al. (1989) considered a three-compartment model that includes a macrophage compartment containing all of the phagocytized particles, an interstitial compartment, and a lymph node compartment. Analyzing the clearance of insoluble particles from the rat lung in terms of alveolar macrophage mobility kinetics indicated the reduced clearance in overload conditions to be a function of reduced macrophage mobility (due to volumetric increase in cell size from phagocytized particles) and an increased deposition of particles in the interstitial space.

The most recent version of the physiology-oriented multicompartmental kinetics model derived by Stöber and co-workers (1994) (see Section 4.3.2.3., Figure 4-4) addresses alveolar clearance and retention of inhaled insoluble particles more rigorously by incorporating five subcompartments representing alveolar particle load and the respective kinetics associated with the transfer of particle load among these compartments. This 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 model predictions are tenable, experimental data are not currently available to validate the proposed compartmental burdens or the transfer rates associated with these compartments. Stöber and co-workers noted that the POCK model should not be generalized beyond its design and cannot readily be used for interspecies extrapolation.

4.4. 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. Evidence presented in more recent studies, however, indicates that the insoluble carbon core of the particle may fully explain the pathogenic and carcinogenic processes observed in the rat inhalation studies. (See Chapter 10 for a discussion of this issue.) Although the organic constituents appear to be of limited importance in rats exposed at high concentrations, their contributory potential in other species cannot be summarily discounted.

A key factor in determining a possible contributory effect is the bioavailability of particle-adsorbed components and whether or not the bioavailable fraction represents an effective dose. An initial step, and possibly the rate-limiting step, in the bioavailability of carcinogenic organics present on diesel particles is their dissociation from the particle surface (Vostal, 1983). This section will therefore focus on studies relating to the dissociation of these organics from the particles. However, the critical uncertainty in these study results is whether the organics added to particles by surface adsorption will exhibit desorption characteristics similar to organics attached to the carbon core during pyrolytic condensation. Data are not available that provide a definitive answer to this question.

4.4.1. Laboratory Animal Studies

Several studies reported on the disposition of particle-adsorbed organics following administration to various rodent species. Most of the experiments used intratracheal instillation, although some exposed the animals by nose-only inhalation.

Sun et al. (1984) compared the disposition of diesel particle-adsorbed benzo[a]pyrene (B[a]P) (0.1% by weight) and pure B[a]P following nose-only inhalation by F344 rats. Long-term retention (percentage retained after 7 days) of particle-adsorbed ^{3}H-B[a]P was approximately 230-fold greater than that for pure ^{3}H-B[a]P. Alveolar clearance of particle-associated ^{3}H was biphasic, with a long-term $t_{1/2}$ of 18 days, the latter representing clearance of 59% of the initially deposited radiolabel. Clearance of pure B[a]P aerosol was >99% within

2 h and was apparently the result of alveolar and tracheobronchial epithelial absorption into the blood, rather than the result of mucociliary clearance and subsequent ingestion (Sun et al., 1982). The data therefore indicate that adsorption to the carbonaceous diesel particle prolongs retention of the organic components.

A companion study (Bond et al., 1986) examined the biological fate of ^{14}C-1-nitropyrene (^{14}C-NP), both in pure form and adsorbed to DPM, following 1-h nose-only inhalation by male F344 rats. Concentrations of ^{14}C-NP ranged from 0.05 to 1.1 mg/m^3 of air, and DPM concentrations, where used, ranged from 3.7 to 6.1 mg/m^3 of air. The results indicated that long-term lung retention of ^{14}C-NP adsorbed onto DPM was 80-fold greater ($t_{1/2}$ = 36 days) than that for pure ^{14}C-NP, demonstrating again that adsorption onto the diesel particles prolongs the release of the PAHs.

Residence time is also prolonged when organics are adsorbed to other types of particles. For example, Creasia et al. (1976) found that when crystalline B[a]P was instilled into the lungs of mice, it was removed from the respiratory tract with a $t_{1/2}$ of ~1.5 h, but when the B[a]P was adsorbed to 0.5 to 1.0 µm carbon particles, its $t_{1/2}$ in the respiratory tract increased to ~36 h. Hence, the adsorption of B[a]P to the carbon particles increased the lung retention of the B[a]P more than 20-fold. Similar results have also been obtained with B[a]P adsorbed to other particle types, including insoluble Ga_2O_3 (Sun et al., 1982) and insoluble ferric oxide (Saffiotti et al., 1964). Consistent with a gradual elution of B[a]P in AMs, Creasia and co-workers (1976) found that the removal of B[a]P when bound to the carbon was faster than the lung clearance of carbon particles only, which had a clearance $t_{1/2}$ of ~7 days.

Ball and King (1985) studied the disposition and metabolism of ^{14}C-labeled 1-NP (>99.9% purity) coated onto DPM. A single dose of ^{14}C-NP (380 µg/g particle) was intratracheally administered (in 0.2-mL buffered saline) at a particle dose of 5 mg per rat. Another group of rats (number not specified) received the labeled ^{14}C-NP in 0.5 mL of buffered saline intragastrically. Additional groups of AGUS strain rats raised conventionally or germ-free received intraperitoneal injections of ^{14}C-NP to determine the role of gastrointestinal flora on the metabolism of 1-NP.

Regardless of the route of administration, >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. The ^{14}C excretion pattern for the intratracheally instilled compound was nearly identical to that of the orally administered compound. For animals receiving intratracheally instilled compound, 16% to 38% of the unexcreted dose was in the gastrointestinal tract and 5% to 8% remained in the lungs. 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 protein binding of the 1-NP-derived ^{14}C. However, the corresponding DNA fraction contained no ^{14}C above background levels. The similar excretion kinetics and metabolic profiles for these various routes of

administration indicate that 1-NP becomes bioavailable both in the lungs and the gastrointestinal tract.

Bevan and Ruggio (1991) assessed the bioavailability of B[a]P adsorbed to DPM from a 5.7-L Oldsmobile engine. In this study, exhaust particles were supplemented with exogenous ^{3}H-B[a]P to provide 2.62 µg B[a]P/g of exhaust particles. Distribution of the radioactivity was assessed at 1, 6, 24, or 72 h after intratracheal instillation of these particles into Sprague-Dawley rats (1 mg of DPM suspended in 0.3 mL of 0.15 M NaCl). At 24 h after administration, 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), the difference being attributed to slower pulmonary absorption, less mucociliary transport in intratracheally instilled animals, and differences in administered dose. At 3 days following administration, over 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. The in vitro elution of B[a]P into dimyristoylphosphatidylcholine (DMPC) vesicles was similar for ^{3}H-B[a]P-supplemented DPM and for native DPM (no additional B[a]P added), thereby indicating that the estimation of in vivo bioavailability of B[a]P from the ^{3}H-B[a]P-supplemented DPM was reasonably accurate.

Results of these studies using surface-bound organics showed that the retention of particle-adsorbed organics is prolonged relative to the organic chemicals alone. The studies also indicated that some portion of the adsorbed organics become bioavailable but provided no information regarding the effective dose of these organics or whether experiments using organics adsorbed by pyrolytic condensation would exhibit similar characteristics.

4.4.2. Extraction of Diesel Particle-Associated Organics in Biological Fluids

For mutagenicity testing or biochemical analysis, DPM is usually extracted with organic solvents such as dichloromethane. The efficiency of extraction may be much different, however, from that of the fluids surrounding the particles in the in vivo state. A number of studies have therefore been conducted in which attempts were made to extract DPM with serum or lung lavage fluid. The efficiency of extraction was usually estimated by performing mutagenicity tests on the extracts.

The utility of evaluating extraction by lung fluid or serum may be somewhat limited because particles deposited in the alveoli are normally rapidly ingested by AMs. However, as large lung burdens of DPM are attained, such as during chronic high-concentration exposures to DPM, AMs that become heavily laden may reach their phagocytic capacity, thereby reducing their phagocytic ability. Under these conditions, an increasing fraction of deposited particles could

escape the phagocytic mechanism and be relatively more available over time in the extracellular lung fluid before (1) their removal from the lung by extramacrophagic clearance via the tracheobronchial route, (2) their subsequent engulfment by newly recruited phagocytes, and (3) their engulfment by Type I cells. Even under such conditions, a relatively large mass of the particles will be within AM phagolysosomes, where low pH and enzyme activity would be expected to act on the particles and adsorbed organics.

Particles from a 5.7-L engine operated at idle and from a 2.1-L engine operating on a cycle of varying speed and load were incubated in lavage fluid, serum, saline, albumin, dipalmitoyl lecithin, or dichloromethane (Brooks et al., 1981). The efficiency of extraction by biological fluids was only 3% to 10% that of dichloromethane, based on mutagenicity testing, and did not increase with incubation time up to 120 h. Similar findings were reported by King et al. (1981). In this study, lung lavage fluid and lung cytosol fluid extracts of DPM were not mutagenic. Serum extracts of diesel particles did exhibit some mutagenic activity, but this was considerably less than that for 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 physiologic saline. Only FCS was found to extract some mutagenic activity from the DPM. These investigators concluded that the mutagens in DPM would not be readily available in vivo. This conclusion lacks definitive proof because 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), and it fundamentally differs 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.

Creasia et al. (1976) reported that when B[a]P was adsorbed onto carbon particles larger than would be expected to be easily phagocytized by AMs (15 to 30 µm), the rates of elimination of the B[a]P and the particles from the lung were virtually identical. The data thus indicate little extraction from the particles not phagocytized by AM but only surrounded by epithelial lining fluid.

In summary, because lung fluids appear to be relatively ineffective in the extraction of particle-adsorbed organics and relatively few particles escape phagocytosis, free particles are likely to contribute very little to the acute bioavailability of adsorbed organics. However, during a particle-overload condition, as occurred in the chronic inhalation studies, it can be hypothesized that an increased fraction of the deposited DPM will not be phagocytized and that their adsorbed

organics could be released over a long period with a clearance rate that may be equivalent to that of the particles themselves. In this case, the organics are bioavailable for even longer periods than those from phagocytized particles, from which organics in turn are retained for longer periods than nonadsorbed organics. Data are not available, however, to validate this hypothesis, nor is it known that such extractions would result in effective doses. It is unknown if AMs in particle-overload conditions retain the same capacity to dissolve organics or whether this process is slowed as well, which would also increase the time over which carcinogenic organics are released and available in the overloaded lung. Data of Creasia et al. (1976) showing increased retention of organics adsorbed to large particles could be consistent with this possibility because 15 µm particles may still be phagocytized by AM (Snipes and Clem, 1981; Oberdörster et al., 1991).

4.4.3. Extraction of Diesel Particle-Associated Organics by Alveolar Lung Cells and Other Cell Types

Another more likely mechanism by which organic carcinogens (e.g., PAHs) may be extracted from DPM in the lung is either particle dissolution or extraction of organics from the particle surface within the phagolysosomes of AMs. This mechanism presupposes that the particles are internalized by these phagocytes. Specific details about the physicochemical conditions of the intraphagolysosomal environment, where particle dissolution in AMs presumably occurs in vivo, have not been well characterized. However, it is known that the 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 organic constituents of diesel particles (e.g., B[a]P) may be extracted in the phagolysosomes (Bond et al., 1983). Whatever the mechanism, the end result is a prolonged exposure of the respiratory epithelium to the gradual release of carcinogenic agents.

Quantitative data on how readily carcinogenic organics may be extracted from DPM in the human lung are not available. As shown by Creasia et al. (1976), B[a]P adsorbed onto 15 to 30 µm carbon particles is removed from the mouse lung at the same rate as the particles. However, B[a]P adsorbed onto 0.5 to 1.0 µm carbon particles was eliminated approximately four times faster than the clearance of the carbon particles. For the rat, Sun and co-investigators (1984) have reported that the $t_{1/2}$ for the lung clearance of B[a]P adsorbed onto DPM over a period consistent with alveolar phase clearance was about 18 days. This latter value is similar to the $t_{1/2}$ for the removal of particles without B[a]P from the rat's lung during the early, more rapid component of alveolar phase clearance (Snipes et al., 1988; Snipes and Clem, 1981; Ferin, 1982; Lehnert et al., 1989; Lehnert, 1989). These findings may suggest that the extraction of organic

components from carrier particles by AMs may differ among species, although differences in the lung burdens administered in the investigations mentioned here may have influenced the outcomes of the studies.

It should be pointed out that studies designed to examine the extraction of organics by AMs have generally focused on B[a]P as a representative procarcinogen associated with diesel particles. Numerous other agents with carcinogenic activity are also associated with diesel particles; these chemical constituents may be extracted from DPM with in vivo kinetics that differ more or less from those of B[a]P. Thus, existing dosimetry models that incorporate desorption of B[a]P from DPM as a representative organic constituent (Yu and Yoon, 1988) may not accurately reflect the actual bioavailability of other procarcinogenic agents on DPM. As discussed in the next section, however, any error in this respect is likely to be minor.

4.4.4. Bioavailability of Adsorbed Compounds as a Function of Particle Clearance Rates and Extraction Rates of Adsorbed Compounds

The bioavailability of toxic organic compounds adsorbed to particles 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. To reach the target site, the agent must first elute from the particle surface. Although elution can be considered a necessary step, it may not always be sufficient. The agent must then diffuse out of the AM into the extracellular fluid and be absorbed by a target cell (e.g., a Type I cell). In analyzing phagolysosomal dissolution of various ions from particles in the lungs of Syrian golden hamsters, Godleski et al. (1988) demonstrated that solubilization did not necessarily result in clearance of the ions and 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. Inability of these constituents to penetrate target cells or to diffuse into the bloodstream is another possible factor limiting bioavailability to lung target cells. Nevertheless, until further research demonstrates otherwise, it is assumed that the rate-limiting factor in the bioavailability of particle-bound organics is the desorption rate from the particle surface.

The long-term clearance $t_{1/2}$ of diesel particles from the lungs of rats in the non-overloaded state was shown to range from about 2 to 3 mo (Chan et al., 1981; Chan et al., 1984; Griffis et al., 1983; Lee et al., 1983). Clearance rate data for DPM in humans are not available. For other types of insoluble particles such as polystyrene, however, $t_{1/2}$ values are close to 1 year (Bohning et al., 1982). The clearance $t_{1/2}$ values for B[a]P and 1-NP from the diesel particle surface, on the other hand, were reported to be only about 18 and 36 days, respectively (Sun et al., 1984; Bond

et al., 1986). The lower $t_{1/2}$ values for clearance of the organics compared with particles themselves indicate that most of the organics are being eluted prior to particle clearance, especially in humans.

For humans, assuming an elution $t_{1/2}$ on the order of 2 to 4 weeks, well over 90% of the organics should desorb from the particles. Even in rats, with more rapid particle clearance rates, most of the organics can be expected to be eluted. Whether particle overload in the lung results in a change in elution rates of the organics is not known. If lung burden of particulate matter is the proper dosimetric factor for induction of pathology or carcinogenesis, target organ dose would be predicted to increase more rapidly than exposure concentration under lung overload conditions.

Gerde et al. (1991a,b) described models simulating the effect of particle aggregation and PAH content on the rate of PAH release in the lung. The investigators used three models, one of which simulated a low-dose situation where only the adsorbed layer of PAH is released from the carrier particle and two of which simulated desorption of PAHs using high PAH levels (with and without an inert carrier dust). Based on the theoretical results obtained, particle retention would be of lesser importance for low-dose situations in which particle-associated PAHs would be rapidly released at the site of particle deposition and not necessarily at the site of particle retention. Frequent low-level exposure, therefore, may result in sustained exposure of target cells and subsequently greater likelihood of tumor formation at the site of initial deposition. For the high-dose situations, as represented by instillation experiments in animals, critical doses to cells are likely to occur at the site of retention because slow release from the particles may increase the dose of metabolites (and increase the risk of tumor formation) before the parent PAH compound can be cleared from the lungs. Generally, the models suggested that the local disposition of PAHs would be more dependent on the behavior of dissolved PAHs in the tissues after their release from the carrier particles than by interactions between the PAHs and the carrier particles. The model predictions were consistent with findings from laboratory animal studies that showed longer PAH retention with higher exposures and longer retention for instillation administration versus inhalation exposure.

Studies by Gerde et al. (1993a,b,c) using beagle dogs provided additional data regarding the dosimetry of inhaled PAH supportive of the previously discussed models. In the Gerde et al. (1993a) study, the dogs were exposed to an aerosolized bolus of PAH crystals (phenanthrene or B[a]P) in a single breath. PAH clearance was measured by monitoring PAH levels in the systemic circulation. Clearance from the alveolar region was dependent on lipophilicity of the PAH; clearance of highly lipophilic PAHs (i.e., B[a]P) was limited by diffusion of the chemical through the alveolar septa, while clearance of moderately lipophilic PAHs (i.e., phenanthrene) was limited by rate of perfusion of the blood. Therefore, bronchi, with their thicker epithelia, would be at

greater risk than alveoli for PAH-induced toxicity at the portal of entry. In the Gerde et al. (1993b) study, small volumes of saline containing either dissolved B[a]P or phenanthrene or a suspension of particulate solvent green or macroaggregated albumin (MAA) were instilled into the mucous lining layer of a primary bronchus or distal tracheas. The highly lipophilic B[a]P was cleared via the mucociliary escalator, some being cleared very rapidly (>90 mm/min). The portion of B[a]P that penetrated the bronchial epithelium exhibited a half-time in the range of 1.4 h, indicating a diffusion-limited uptake of B[a]P by the airways. Although mucociliary clearance is rapid for most of the lipophilic toxicants, the long retention time for the portion that penetrates the epithelium is sufficient for substantial metabolism to occur, resulting in a potential for local toxicity. Gerde et al. (1993c) used the data from the previously described studies to validate models of alveolar clearance (Gerde et al., 1991b), mucous lining penetration (Gerde and Scholander, 1987), and bronchial wall penetration (Gerde et al., 1991b). The analysis provided a reasonable validation of the transport models for these structural regions of the lung. Specifically, alveolar clearance and mucociliary clearance are primarily via molecular diffusion, whereas clearance from bronchial walls involves diffusion, metabolism of a portion of the PAH load, and endocytosis. Such findings suggest that the bronchial epithelium may be especially vulnerable to toxicity induced by diffusion-limited lipophilic substances.

4.5. CONSIDERATIONS FOR DOSIMETRY MODELING

Although more than one approach is possible in the development of dosimetry models for inhaled DPM, several dosimetry parameters are common to any approach. These include ventilatory rates and volumes, tracheobronchial and alveolar surface area, tracheobronchial and alveolar deposition efficiency, and tracheobronchial and alveolar clearance rates. If the particle-adsorbed organics are considered relevant to the carcinogenic response of the lung, elution $t_{1/2}$ values of these chemicals should be included in the model. The dosimetry models must consider not only species differences but also the effects of extrapolating from high exposure concentrations, which result in an inhibition of particle clearance from the lungs caused by overload. Some models include additional parameters such as transport to lung-associated lymph nodes and clearance of the interstitial compartment, but experimental data are not available to validate many of the assumptions used in these models.

An important consideration is ventilation rate. Numerous estimates of alveolar ventilation rates are available for both humans and rats. Nevertheless, such estimates are still a source of considerable potential error because they are usually resting values. Actual respiration can vary widely with activity or with exposure conditions. Human activity levels are also highly variable. Although it may be necessary to use a single mean value in a model, it would be useful to include

risk estimates for individuals having much greater daily ventilation exchange rates resulting from participation in endurance sports or performance of heavy labor.

Another variable not discussed previously is the adjustment of dose based on metabolic rate. It has been EPA policy to consider that effective dose varies with metabolic rate. Arguments for and against this presumption are beyond the scope of this document. The primary consideration here is the degree of adjustment. EPA has traditionally adjusted for species differences in metabolic rate based on the 2/3 power of body weight as a surrogate for body surface area. This factor is being reappraised within the Agency, and a preliminary proposal to alter this adjustment to the 3/4 power has been made.

A major consideration in the development of dosimetry models is a judgment concerning which fraction of exhaust is responsible for inducing lung cancer. As is discussed in Chapter 10, two approaches are used for quantitative assessment based on animal data. In the first, cancer is assumed to be induced by the organic constituents present on the particle surface, primarily PAHs and nitropyrenes. As discussed herein, in an appropriate model, the effective dose will correlate closely with the deposited dose, with only minor corrections for lung overloading. In the second approach, it is assumed that retained particle burden in the lung fully accounts for the induced lung tumors. A more specific dose parameter for this second approach may be the surface area of the retained particles, as discussed in Chapter 10. In studies with fine and ultrafine TiO_2 particles, Oberdörster et al. (1994) reported that the ultrafine particles resulted in prolonged pulmonary retention, increased translocation to and persistence in the interstitium, increased Type II cell proliferation, presence of fibrotic foci, and impairment of alveolar macrophage function in rats exposed for 12 weeks. Overall, there was a notable correlation between particle surface area and effects. In this case, modeling must account for slowing of clearance during lung overload as well as large differences in normal clearance rates between rats and humans. For this approach, if exposures are at overload levels, low-dose extrapolation may result in a risk estimate considerably different from one based on target organ dose of organics. A third approach could be proposed in which both exhaust components may be operating simultaneously, with the PAHs initiating the carcinogenic process and the particles promoting the process by inducing cell proliferation.

4.6. SUMMARY

Because only very limited evidence exists for diesel exhaust-induced tumors at non-pulmonary sites or for tumor induction by the gaseous fraction alone, dosimetry considerations were limited to either whole exhaust or particulate matter deposited in the lungs. Dosimetric considerations were further limited to the alveolar region of the respiratory tract for several reasons. First of all, most deposited particulate matter is transported by mucociliary transport from the conducting airways in less than 1 day, and exposure to diesel exhaust does not appear to

significantly inhibit this process. However, as previously discussed, there may also be a long-phase retention, especially if tracheobronchial clearance is impaired. In general, the rapid clearance in the conducting airways reduces the time for extraction of organics from the particle surface (although there is evidence for some relatively rapid removal of organics), and some AMs in the mucociliary escalator may contain particles desorbed of organics. Finally, most of the pathologic and carcinogenic effects occur at or distal to the terminal bronchioles in the rat.

Clearance of DPM from the alveolar region varied from about 2 mo in rats to an estimate of 1 year in humans. Under high-exposure regimes, lung overload occurred in rats, leading to slower or near cessation of clearance, thereby increasing lung burdens even further. In addition, with large lung burdens, uptake of particles by Type I cells, passage into the interstitium, and transport to lung-associated lymph nodes were increased. Factors considered to be involved in clearance inhibition included loss of AM mobility with large particle loads and a tendency for AMs to aggregate and become immobilized.

Most biological fluids tested, including lung lavage fluid and serum, were relatively ineffective in the extraction of organic agents adsorbed to the diesel particle surface. Particles deposited in the alveolar region, however, are rapidly phagocytized by AMs, which are more effective in this regard. Although actual elution $t_{1/2}$ values of organics from phagocytosed particles were difficult to obtain, they were generally less than those for the particles themselves, indicating that most organics are released even without inhibition of particle clearance. The gradual elution also prolonged the residence time of organics in the lungs compared with pure organic agents such as B[a]P, possibly avoiding overloading of biological activation systems and thus increasing their effectiveness.

In the development of a dosimetry model to allow both low-dose extrapolation and extrapolation of DPM bioassay data from laboratory animals to humans, several parameters must be accounted for. These include deposition efficiency, particle clearance rates, desorption rates of organics from the particle surface (if other than a particle effect is being considered), lung surface area, and particle surface area. The respiratory rates and volumes are highly variable in both laboratory animals and humans and are also determinants of deposition efficiency. Animal estimates are often based on published values collected under resting conditions. Respiration, however, may be inhibited by the irritant gases present in diesel exhaust, although less so at low dilution ratios. On the other hand, respiration may be either greater or less than estimated resting values, depending on whether exposures were carried out at night when the animals are likely to be awake and active or during the day when they are more likely to be asleep. Human respiratory exchange rates are also quite variable, with the physically active segment of the population at potentially greater risk because of higher doses resulting from higher respiration rates.

Adjustment for particle clearance rate is necessary for two reasons. First of all, many of the laboratory animal experiments were conducted under exposure regimes resulting in an inhibition of clearance caused by an accompanying lung burden overload. If lung burden of particulate matter is considered to be the proper dosimetric variable, then the disproportionately large lung burdens at high levels of exposure must be adjusted for. Second, even under low-exposure regimes, clearance is slower in humans than in rats. If the correct dosimetric variable, on the other hand, is particle-free organic matter, a smaller adjustment for variations in particle clearance rates is required because most of the organics are likely to be eluted from the particles deposited in the alveolar region, even at normal clearance rates. Nevertheless, some adjustment is still necessary, because the organics are seldom all eluted.

4.7. REFERENCES

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