

Influence of Exposure Concentration or Dose on the Distribution of Particulate Material in Rat and Human Lungs

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Differences among species in the anatomic sites of particle retention could influence responses to inhaled particles. In this study, we used morphometric techniques to examine the influence of exposure concentration on particle retention in histologic sections from rats and humans. The rats had been exposed for 24 months to diesel exhaust at 0.35, 3.5, or 7.0 mg soot/m³. The human subjects were nonsmokers who did not work as miners, nonsmoking coal miners who worked under the current standard of ≤ 2 mg dust/m³ for 10–20 years (mean = 14 years), and nonsmoking coal miners who worked under the former standard of < 10 mg dust/m³ for 33–50 years (mean = 40 years). The distribution of retained particles within the lung compartments was markedly different between species. In all three groups of rats, 82–85% of the retained particulate material was located in the alveolar and alveolar duct lumens, primarily in macrophages. In humans, 57, 68, and 91% of the retained particulate material was located in the interstitium of the lung in the nonminers, coal miners under the current standard, and coal miners under the former standard, respectively. These results show that chronically inhaled diesel soot is retained predominately in the airspaces of rats over a wide range of exposures, whereas in humans, chronically inhaled particulate material is retained primarily in the interstitium. In humans, the percentage of particles in the interstitium is increased with increased dose (exposure concentration, years of exposure, and/or lung burden). This difference in distribution may bring different lung cells into contact with the retained particles or particle-containing macrophages in rats and humans and may account for differences in species response to inhaled particles. **Key words** coal dust, diesel soot, lung, particles. *Environ Health Perspect* 109:311–318 (2001). [Online 7 March 2001] <http://ehpnet1.niehs.nih.gov/docs/2001/109p311-318nikula/abstract.html>

The scientific and regulatory communities are concerned about potential neoplastic and non-neoplastic health effects of inhaled particles in humans. To date, much of the experimental data on chronic health effects of inhaled particles come from rats. However, an interspecies difference in response, with rats but not mice showing an increased incidence of lung tumors, has been shown for a number of relatively insoluble particles [reviewed by Nikula (1)] including talc (2), carbon black (3), titanium dioxide (3), silica (4), nickel subsulfide (5), cadmium sulfide (6,7), cadmium sulfide (6,7), and cadmium chloride (6,7). Several chronic inhalation bioassays of diesel exhaust in rats, Syrian hamsters, and mice have been conducted [reviewed by Nikula (1)]. These bioassays have consistently shown that diesel exhaust, inhaled chronically at high concentrations, causes increased incidences of lung tumors in rats. Exposures resulting in an increased incidence of lung tumors in rats also caused progressive soot accumulation, primarily within alveolar macrophages, alveolar epithelial hyperplasia, chronic-active inflammation, focal fibrosis, and epithelial metaplasia. None of the studies using Syrian hamsters has demonstrated a diesel exhaust-related

increase in lung tumors, although soot accumulated in hamster lungs. Mild bronchiolar-alveolar hyperplasia, which was much less severe than in comparably exposed rats, has been observed in hamsters that chronically inhaled diesel exhaust (8).

Inconsistent results have been obtained in studies using mice. Incidences of lung tumors increased in some female groups of strain A and Sencar mice, strains sensitive to chemical carcinogenesis, but results have been generally negative in other strains. Lung tumors were not increased in male and female CD-1 mice exposed to diesel exhaust under conditions identical to those that were carcinogenic to rats (9). These mice progressively accumulated soot, primarily in alveolar macrophages, and septal fibrosis and bronchiolization of alveolar ducts were observed in areas of soot accumulation. However, the amounts of inflammation and epithelial hyperplasia were less in mice than in rats (9,10). These results from studies using diesel exhaust and other particles have raised concerns that rats may be more prone than other species to develop persistent pulmonary epithelial hyperplasia, metaplasia, and tumors in response to inhaled particles.

Considerable effort has been expended in measuring and modeling pulmonary deposition of inhaled particles in rodents and other species, and several excellent reviews have been written [e.g., Miller (11)]. However, chronic lung tissue responses to inhaled particles, especially those that are not highly reactive or toxic, may depend more on anatomic sites of particle retention than on sites of initial deposition. Anatomical differences between rats and primates (humans and nonhumans) could affect sites of long-term particle retention. The functional unit of the lung, the acinus, is composed of the terminal bronchiole and the air spaces it supplies (12). Because rats lack respiratory bronchioles, they have simple acini. Species with branching respiratory bronchioles between the terminal bronchiole and alveolar ducts have more complex acini. Macaque monkeys and humans have similar numbers of generations of respiratory bronchioles between the terminal bronchiole and alveolar ducts (13,14), and they have larger alveoli and alveolar ducts than rats (15). Therefore, monkeys and humans have more complex, larger acini than rats.

The amount of interstitial connective tissue in the lung also differs, with rats having less and primates more. A greater portion of the pulmonary parenchyma (composed of air in alveoli and ducts, capillary blood, and septal tissue) is composed of septal tissue in rhesus monkeys and humans than in rats (16–19). In primates, lymphatic vessels exist at the alveolar level adjacent to the respiratory bronchioles (20,21). Accumulation of dust-containing macrophages in the loose connective tissue surrounding these lymphatics may be an event in the formation of coal macules

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in coal workers' lungs (22). In rats, which lack respiratory bronchioles, lymphatic vessels are located adjacent to the terminal bronchioles, but not at the alveolar level. Finally, rats have thin pleura, relatively few pleural lymphatics, and no interlobular connective tissue, whereas humans have thick pleura, relatively abundant pleural lymphatics, and abundant interlobular connective tissue (23–25). Nonhuman primates generally have thicker pleura and more pleural lymphatics than rats; they have scant interlobular connective tissue (23,26). Although rats and primates differ anatomically, the structural differences may not be the sole explanation for differences in particle retention. Differences in clearance kinetics will also influence sites of long-term particle retention (11). However, it is likely that many differences in clearance kinetics among species are related to differences in respiratory tract anatomy (27).

Rats and monkeys differ in anatomic patterns of particle retention in the lungs after identical exposures to diesel exhaust or coal dust (CD) at a single exposure concentration (2 mg/m³) for 2 years (28). However, within each species, the pattern of particle retention does not differ by particle type (diesel soot or CD) (28). At this single exposure concentration, F344 rats retain particles of diesel soot and CD primarily within macrophages located within the lumens of alveolar ducts and alveoli. Identically exposed cynomolgus monkeys retain the diesel soot and CD particles primarily within macrophages located in the pulmonary interstitium. The interstitial sites with the greatest particle accumulation, in descending order of amount, in the monkeys are the alveolar septa, pleura, interstitium of respiratory bronchioles, and perivascular interstitium.

The purpose of the present study was to extend our morphometric examinations of particle retention in rats and monkeys to humans. We also examined the influence of exposure concentration or lung burden on the distribution of retained particles in rat and human lungs.

Materials and Methods

Rat lung tissues and exposures. The histologic sections of rat lung examined for the present study were from an interim sacrifice that was part of the Mauderly et al. (29) bioassay of F344 rats exposed 7 hr/day, 5 days/week for up to 30 months to diluted, whole diesel exhaust at soot concentrations of 0.35, 3.5, or 7.0 mg soot/m³. These groups are referred to as low, medium, and high exposure, respectively. Details concerning the animals, care, and exposures were described by Mauderly et al. (29). The lungs (eight per group; four of each sex) examined for the present study were from rats exposed

for 24 months and sacrificed the day after the last exposure. These lungs had been fixed via intratracheal instillation of 10% neutral buffered formalin at a constant hydrostatic pressure of 20 cm for 24 hr. These tissues were chosen because they provided a large range of particle exposures and lung burdens. There were no lung tumors in the low-exposure group in the bioassay. In the medium-exposure group, there was an increase in benign lung tumors. In the high-exposure group, there was a significantly increased incidence of malignant lung tumors in the bioassay. At the medium- and high-exposure concentrations, the rats accumulated lung burdens of soot greater than those that would be predicted from results in the low-exposure concentration, suggesting a decrease in particle clearance (29).

Human cases. The Institutional Review Board of the Lovelace Respiratory Research Institute certified that the project was exempt from the requirements of Title 45, CFR Part 46, Protection of Human Subjects. The three groups of human cases were referred to as controls, low-dose coal miners, and high-dose coal miners. These groups represented a range of exposures to dusts from urban ambient levels to high occupational levels. The urban ambient control group (five cases) was composed of male, life-long nonsmokers who were residents of Calgary, Alberta, Canada. Calgary has a population of 850,000, and jobs are predominantly white-collar or in light industry. All five cases in the control group were young, with ages ranging from 21 to 33 years (27.6 ± 5.0, mean ± SD). Three had worked in offices, one as a security guard, and one as a waiter. Only the latter would have been exposed to significant second-hand cigarette smoke. Three individuals died from natural causes involving the central nervous system, one in an accident, and one from a self-inflicted gunshot wound to the head. The subjects were randomly selected from the never-smoking male cases in the control group for an epidemiologic study concerning risk factors for sudden death from asthma in the prairie provinces (30). Left lungs were received fresh at autopsy and inflated with 2.5% glutaraldehyde at 25 cm of water pressure. Representative samples were obtained from left upper and lower lobes for histologic analysis.

The selection of miner cases was based on a database search of materials in the National Coal Worker's Autopsy Study. The low-dose miners (11 cases) were male underground coal miners who were employed as miners at the time of death and had died suddenly as a result of accidents. They worked under the current dust exposure standard of ≤ 2 mg dust/m³ for 10–20 years and had an average underground mining exposure of 14.1 ± 3.1

years (mean ± SD). The mean age for these 11 miners was 35.5 ± 4.8 years. The high-dose miners (5 cases) were male underground coal miners who were employed as miners at the time of death (2 cases) or who had retired from mining within 1–2 years of death (3 cases). These individuals died from natural causes involving the cardiovascular system or neoplasia. They worked under the former dust exposure standard of < 10 mg dust/m³ for 33–50 years and had an average underground mining exposure of 40.0 ± 6.2 years. The mean age for the high-dose miners was 63.4 ± 7.1 years. The miners in both groups were never smokers. The terms “low-dose” and “high-dose” miners are used to signify the two groups of miners with different total exposures to CD. In this classification, “dose” refers to the combined interactions of working under different exposure concentration standards and for different lengths of times. The lungs from miners had been fixed at autopsy by intra-airway instillation of 10% neutral buffered formalin, and representative samples of upper and lower lobes were obtained.

Before conducting the morphometric study, 29 cases from miners that fit the criteria above were assembled. We examined the histologic sections of lung, and cases were excluded if the lungs exhibited characteristics or lesions that had been established *a priori* as criteria for exclusion. These exclusion criteria were selected because they were thought to possibly alter particle location or interfere with accurate anatomic determination of particle location. The exclusion criteria consisted of inadequate fixation (poor inflation or autolysis such that accurate anatomic localization of particles would be difficult), marked pulmonary edema, hemorrhage, pneumonia, evidence of primary or metastatic lung tumors, and lesions consistent with silicosis. We excluded 13 cases based on these criteria.

Morphometry. The lung sections examined had been cut at 5 μm and stained with hematoxylin and eosin. There were five sections of lung (one from each lobe) for each rat and two to four sections of human lung. We used the point-counting method of planimetry (31) to estimate the volume density of particulate material in the lung sections and the volume percentage of the total particulate material in defined anatomic compartments of the lung. These compartments are listed and defined in Table 1. Ten compartments were used for the human lungs, and the corresponding eight compartments were used for the rat lungs. These anatomic lung compartments do not differentiate between intracellular and extracellular location of particles, which is not always possible to ascertain in paraffin sections of lung tissue examined by light microscopy. For example, particulate material within alveolar

macrophages in the alveolar lumen or extracellular in the alveolar lumen was counted as particulate material in the alveolar lumen.

A systematic, random sampling scheme was used to capture digitized images of the lung using a 3-chip CCD camera (Optronix Engineering, Coleta, CA, USA) interfaced to an Olympus BH2-RFCA microscope (Olympus America, Inc., Melville, NY, USA) and a Macintosh Quadra 950 computer (Apple Computer, Cupertino, CA, USA). The images were captured using a 40× microscope objective and projected onto the computer monitor screen at a final magnification of 1280×. A sampling grid (Stereology Toolbox, Davis, CA, USA) consisting of 64 points (low-exposure rats and human controls) or 42 points (medium- and high-exposure rats and low- and high-dose miners) was superimposed over each image. We recorded the number of points hitting particulate material, the location of each point, and the number of points hitting the lung section but not hitting particulate material. The small amount of particulate material in the low-exposure group rats and the human controls necessitated the greater sampling intensity (more points per grid). The number of test points for each rat or human case was defined as the total number of test points in the lung (i.e., the sum of the points hitting particulate material in the lung plus the points in the lung not hitting particulate material).

The volume density of particulate material in the lung, which is a volume ratio of particulate material to lung, was calculated for each rat or human case from the number of points hitting particulate material divided

by the total number of test points × 100%. These data are estimates of the relative amount of retained particulate material in the lung and can be thought of as a “lung burden” in histologic sections.

The volume percentage of the total particulate material in a defined anatomic compartment is a volume ratio of particulate material in a compartment to total particulate material and was calculated for each rat or human case from the number of points hitting particulate material in a defined compartment divided by the total number of points hitting particulate material × 100%. These data are estimates of the anatomic distribution of the retained particulate material. Although particulate material was categorized as being in 1 of 8 (rats) or 10 (humans) specific anatomic compartments, the compartments were grouped into broader categories for statistical analyses. One broader anatomic compartment was referred to as parenchymal lumens and consisted of lumens of alveolar ducts and alveoli in rats and lumens of respiratory bronchioles, alveolar ducts, and alveoli in humans. The other broad anatomic compartment was referred to as interstitium and consisted of all the interstitial compartments (i.e., the remaining compartments other than parenchymal lumens and lumens of conducting airways).

To determine if the amount of material in parenchymal lumens or the interstitium was simply related to the size of the corresponding luminal or interstitial compartment, we calculated the relative compartmental retention of particulate material in rat and human lungs. The relative compartmental retention was

calculated as the percentage of the particulate material in parenchymal lumens or the interstitium divided by the percentage of the lung composed of parenchymal lumens or interstitium. The expected ratio would be 1 if particles were uniformly and randomly scattered throughout the lung. A ratio < 1 would mean that there were few particles relative to the size of the compartment, and a ratio > 1 would mean that particles were more densely packed into that compartment compared to other parts of the lung.

Because the lungs from miners were not fixed at a constant hydrostatic pressure, differences in degree of inflation could have affected the results for the relative volume densities of particulate material in the lungs (i.e., volume of particulate material/volume of lung). Thus, these data must be viewed as only crude estimates of the amount of particulate material in the histologic sections. However, differences in inflation would not have affected the location of the particulate material (i.e., percentage of total particulate material in defined anatomic compartments); thus, the differences would not have affected the comparisons of particle location across species (rat versus human) or within species across exposure scenarios.

We determined the volume fraction of the lung occupied by the various compartments, as represented in these lung sections, so that values obtained from these lung sections could be compared with normal values to assess the lung inflation and sampling. Using a systematic, random sampling scheme and the same imaging system described above, images were captured using a 10× objective and projected onto the screen at a final magnification of 320×. A 42-point grid was superimposed over each image, and the number of points hitting each anatomic compartment of the lung section was recorded. We calculated the volume fraction of the lung occupied by specific compartments for each rat or human case from the number of points hitting the compartment divided by the total number of test points.

Because of the anatomic differences between rat and human lungs, it was not possible to evaluate the sections while blinded to species. However, because a randomly placed test system of grid points was used to generate the morphometric data, the data are free from selection bias by the morphometrist.

Statistical analyses. We used one-way analysis of variance (ANOVA) to determine if there were significant differences among exposure (rats) or dose (humans) groups in the volume density of particulate material in the lungs. When the ANOVAs indicated that the differences were significant, then the Student-Newman-Keuls multiple comparison method was used to isolate the group or

Table 1. Morphometric compartments for particle counts.

Point hitting particle in (1–10)
1. Pleura Including associated connective tissue and lymphatics
2. Organized lymphoid tissue, e.g., intrapulmonary lymph nodes
3. Bronchovascular interstitium of conducting airways Including airway walls, peribronchovascular connective tissue, and associated vessel walls. Includes lumens of lymphatics
4. Lumen of conducting airway
5. Interstitium of respiratory bronchiole (human) Including respiratory bronchiolar wall, peribronchiolar and perivascular connective tissue, and associated vessel walls. Includes lumens of lymphatics Not including septa of alveolar outpocketings
6. Lumen of respiratory bronchiole (human)
7. Interlobular and intersegmental connective tissue and perivascular interstitium Includes interlobular and intersegmental connective tissue and lumens of lymphatics. Also includes perivascular adventitia and lymphatic capillaries surrounding pulmonary arterioles, veins, and venules not associated with conducting airways or respiratory bronchioles
8. Septum of alveolar duct or alveolus Including septa of alveolar outpocketings of respiratory bronchioles in humans
9. Lumen of alveolar duct or alveolus
10. Scar An interstitial compartment with fibroplasia (collagen) and distortion of the pulmonary architecture such that the normal underlying associated structure could not be identified
11. Point hitting lung section but not hitting a particle

For each field, points hitting the lung section (test points) were counted in one of 11 categories. Thus, total points hitting the lung section, total points hitting particles, and points hitting particles in each defined lung compartment could be calculated.

groups that differed from the others. To determine any differences among exposure or dose groups in the distribution of particulate material in the parenchymal lumens versus the interstitium, we compared the difference between the portion in the lumen and interstitium across groups by ANOVA. If the ANOVAs indicated significant differences, then the Student-Newman-Keuls multiple comparison method was used to isolate the group or groups that differed from the others. To determine any significant differences between the portion of particulate material in the parenchymal lumens or interstitium, we used paired *t*-tests. Similarly, to determine if exposure or dose groups differed in the relative compartment retention of particulate material in the parenchymal lumens versus the interstitium, the difference between relative compartment retention of particulate material in the lumen and interstitium was compared across groups by

ANOVA. The ANOVAs did not detect significant differences across groups, so the groups were pooled, and paired *t*-tests were used to determine if any differences between the relative compartment retention of particulate material in the parenchymal lumens or interstitium were significant. The significance level was set at $p < 0.05$ for all statistical tests.

Results

Qualitative assessment of particle location.

Histopathologic findings in rats exposed to diesel exhaust at these concentrations have been described (29). Qualitative light microscopic examination of the rat lungs to assess the distribution of the retained particulate material showed that the soot particles were mostly located in alveolar macrophages within alveolar and alveolar duct lumens (Figure 1) in all three exposure groups. Some of the particulate material in alveolar and

alveolar duct lumens was extracellular. Compared to the amount of the total particulate material that was in lumens, less of the particulate material was in the interstitium. It was not always possible to discern the intracellular versus extracellular location of the interstitial soot based on the light microscopic examination of the 5- μ m sections. However, much of this material appeared to be within macrophages.

Histopathologic findings in lungs of coal miners have been described in detail (32). Qualitative assessment of CD location in the lung sections showed that most of the retained particulate material was located in the interstitium (Figure 2) in miners from both CD exposure groups. The degree of interstitialization of particulate material was most pronounced in the high-dose miners. Similar to the findings in rats, the particulate material that was in alveolar and airway lumens was mostly within macrophages.

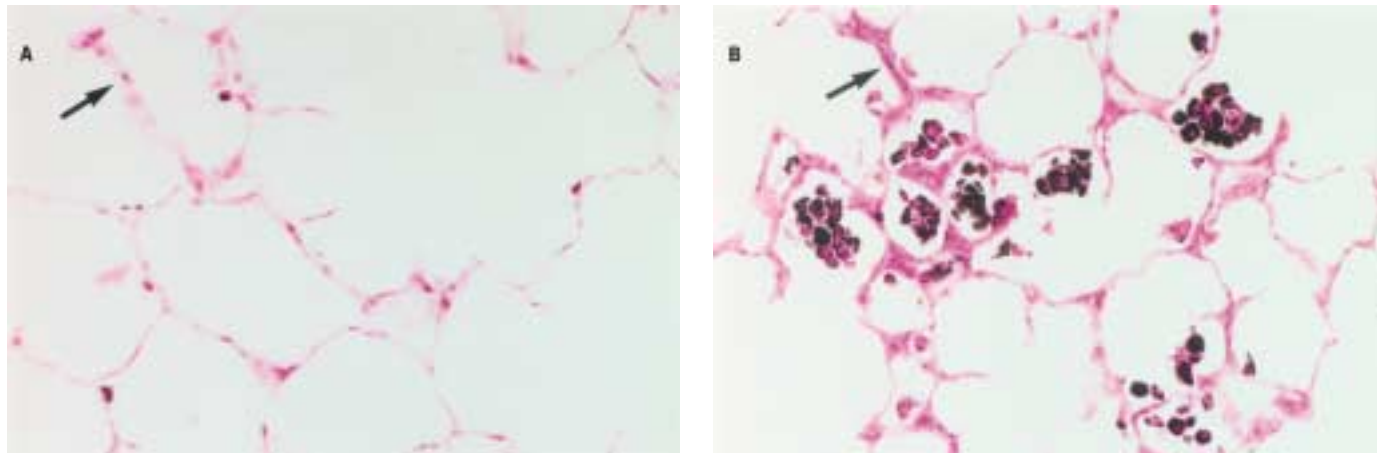


Figure 1. Lung sections from rats exposed to diesel exhaust. (A) rats exposed to low concentrations of diesel exhaust. (B) Rats exposed to high concentrations of diesel exhaust. Regardless of exposure concentration, most of the particulate material is located in macrophages in the lumens of alveoli and alveolar ducts. Arrows point to particulate material in the interstitium.

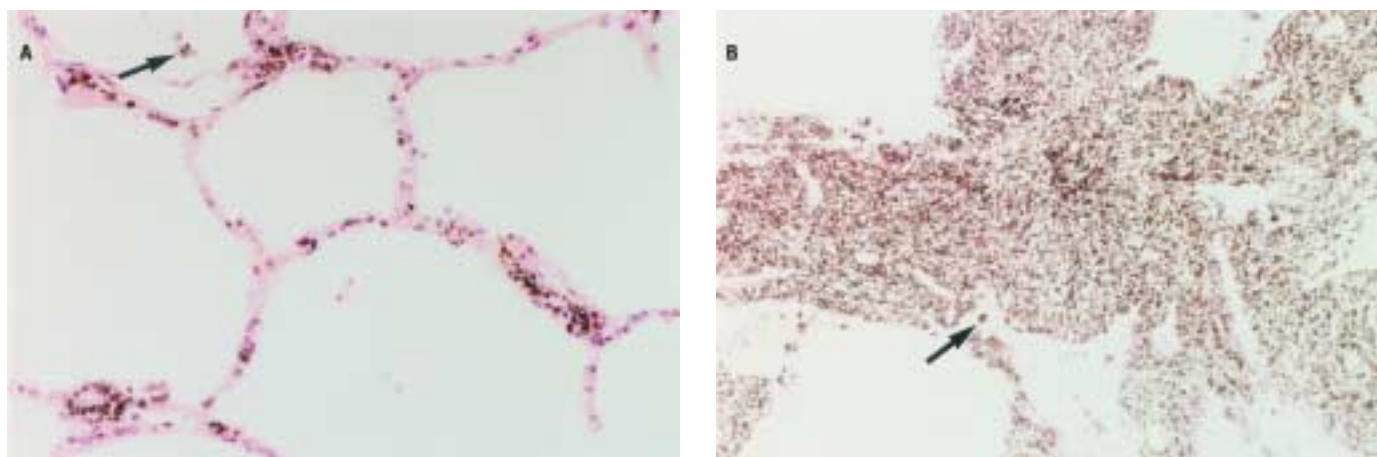


Figure 2. Lung sections from coal miners. Most of the particulate material is located in the interstitium in (A) low-dose and (B) high-dose miners. Arrows point to material in lumens.

Again, the intracellular versus extracellular location of the interstitial soot was frequently difficult to discern based on the light microscopic examination of the 5- μm sections. Much of the material was clearly located within macrophages, but other CD particles seemed to be extracellular.

Morphometry. To determine if exposure had caused significant changes in the proportion of the lung composed of the major compartments used in the analyses in this study, the difference between the percentage of the lung composed of parenchymal lumens and the percentage composed of interstitium was compared across exposure groups of rats using ANOVA. The three exposure groups did not differ significantly. To assess the sampling of lung tissue (i.e., parenchymal tissue versus nonparenchymal tissue), we compared data from these rats with published data. For the rats in this study, the mean volume fraction of alveolar parenchyma (alveolar air and alveolar septa and parenchymal scars) was 88% of the total lung. This value is similar to that reported for normal F344 rats of 82% for the volume fraction of alveolar parenchyma in the lung (16).

To determine if exposure had caused significant changes in the proportion of the lung composed of the major compartments, the difference between the percentage of the lung composed of parenchymal lumens and the percentage composed of interstitium was compared across the three human dose groups using ANOVA. The three dose groups were not significantly different, although there was a tendency for an increasing amount of the lung to be composed of interstitial tissue with increasing dose. The interstitial compartment composed 32, 37, and 44% of the lung in the controls, low-dose, and high-dose miners, respectively. There was a corresponding tendency for a decreasing proportion of the lung to be comprised of parenchymal lumens as the dose

increased. Again, we compared parenchymal versus nonparenchymal tissue to assess the inflation and sampling of the lungs. In the human lung sections, the mean volume fraction of alveolar parenchyma was 85% of the total lung and was not different across groups. The volume fraction of alveolar parenchyma is 85–90% of the total human lung fixed under physiologic conditions (33). These results suggest that the sampling was representative of the human lung and the degree of inflation was similar across groups. Overall, these results suggest that the values for volume density of particulate material in the lungs can be accepted as reasonable estimates.

Volume densities of particulate material.

The volume densities of particulate material in the lungs of rats exposed to the low, medium, and high concentrations of diesel exhaust are shown in Figure 3. The volume density of particulate material in the lung increased significantly with each exposure concentration. The volume densities of particulate material in the human lungs at different exposure concentrations of CD are shown in Figure 4. The volume density of particulate material increased with dose and was significantly greater in the high-dose than in the low-dose or control groups. These data illustrate that for both species, the groups examined represent not only a range of exposure concentrations from low to high, but that the amount of material retained in the lung also differs significantly across exposure groups. Thus, examination of these cases was appropriate to the goal of determining the influence of exposure concentration or lung burden on the anatomic location of retained particles in the lungs of rats and humans.

Distribution of particulate material. In the three groups of rats, approximately 81–85% of the particulate material was located in alveolar and alveolar duct lumens either within macrophages or extracellularly.

Seven to 12% of the particulate material was located in alveolar and alveolar duct septa, and the remaining material was scattered among the interstitial compartments, with no single compartment containing more than 3% of the particulate material. The lumens of the conducting airway contained no more than 0.3% of the particulate material.

Figure 5 shows the effect of exposure concentration on the distribution of particulate material in the rat lungs. There were no significant differences among exposure groups in the distribution of particulate material in parenchymal lumens and interstitium. In all three groups of rats, the portion of the particulate material in the parenchymal lumens was significantly greater than the portion in the interstitium. Thus, the tendency of the rat to retain particles primarily in macrophages located in alveolar and alveolar duct lumens is not only seen in low-exposure groups but also when macrophage-mediated clearance is overloaded.

In the human lungs, approximately 57–91% of the particulate material was located in the interstitium. In the controls, the interstitial compartments with the greatest amount of particulate material were the alveolar and alveolar duct septa; the interlobular, intersegmental, and perivascular connective tissue; and the pleura. These compartments contained 27, 16, and 6% of the retained particulate material, respectively. In the low-dose miners, the interstitial compartments with the greatest amount of particulate material were the interlobular, intersegmental, and perivascular connective tissue; the alveolar and alveolar duct septa; the interstitium of respiratory bronchioles; and the pleura. These compartments contained 26, 22, 8, and 6% of the retained particulate material, respectively. In the high-dose miners, the interstitial compartments with the greatest amount of particulate material were the interlobular, intersegmental,

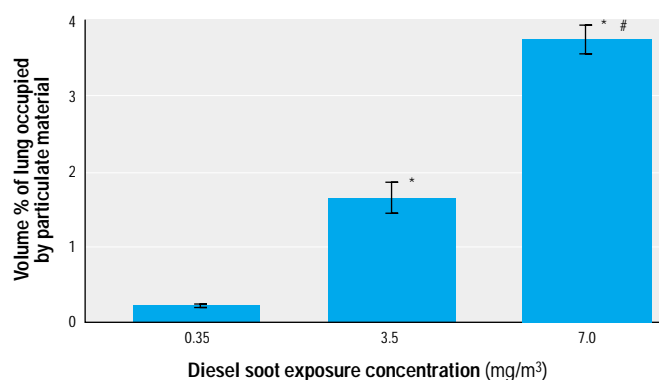


Figure 3. The volume density (mean \pm SE) of particulate material in rat lungs at different diesel soot exposure concentrations. The volume density of particulate material increased significantly with each dose.

*Significantly different from low exposure group. #Significantly different from medium exposure group.

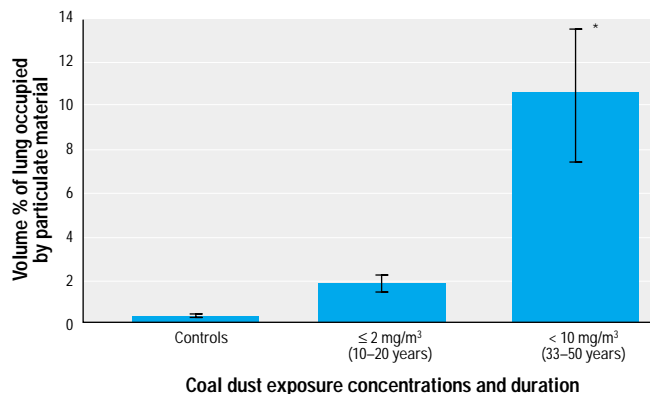


Figure 4. The volume density (mean \pm SE) of particulate material in human lung sections at different particle exposure concentrations.

*The volume density of particulate material was significantly greater in the high-dose than in the control group.

and perivascular connective tissue; scars; the alveolar and alveolar duct septa; the pleura; bronchovascular interstitium of conducting airways; and the interstitium of respiratory bronchioles. These compartments contained 27, 26, 17, 10, 6, and 6% of the retained particulate material, respectively. Nine to 43% of the particulate material was in the parenchymal lumens in the human lungs, and almost all of this material was in the lumens of alveoli and alveolar ducts. No more than 2% of the retained material was in the lumens of respiratory bronchioles and conducting airways (combined).

Two of the high-dose miners were employed as miners at the time of death, and three were recently retired. Eighty-six and 94% of the particulate material was located in the interstitium of the two active high-dose miners, and 88, 93, and 97% of the particulate material was located in the interstitium of the retired high-dose miners. Because of the similarities between the active and recently retired high-dose miners, they were considered together in the following analyses.

Figure 6 shows the effect of exposure concentration or lung burden on the distribution of particulate material in the human lung sections. A significantly greater portion of the particulate material was distributed in the interstitium versus the parenchymal lumens in the high-dose than the low-dose miners or controls. The portion of the particulate material in the interstitium was significantly greater than the portion in the parenchymal lumens in the low-dose and high-dose miners. Although there was a tendency for more particles to be in the interstitium in the controls, the difference was not significant.

Relative compartmental retention. We calculated the relative compartmental retention to normalize the amount of material in each compartment to the size of the compartment. The relative compartmental retention

of particulate material in rat lungs was similar across groups in rats (Figure 7). Across all three groups, the amount of particulate material in lumens relative to the size of the luminal compartment was significantly greater than the comparable ratio for the interstitium. These data suggest that retained particles or retained particles within macrophages are not randomly distributed within the lung such that the amount in a given compartment simply reflects the size of that compartment. Instead, particles are retained in parenchymal lumens to a greater extent than in the interstitium, even when the fact that more of the lung is composed of parenchymal lumens is considered.

The relative compartmental retention of particulate material in the human lung sections was similar across dose groups (Figure 8). The amount of particulate material in the interstitium relative to the size of the interstitial compartment was significantly greater than the comparable ratio for the lumens in all three human dose groups. These data show that the particulate material is more densely packed in the interstitium than in the lumens of humans. In addition, these data show that although a significantly greater portion of the particulate material was distributed in the interstitium in the high-dose than the low-dose miners or controls (Figure 6), the tendency for a greater amount of interstitial tissue in the high-dose miners resulted in a similar relative compartmental retention across dose groups. In other words, the interstitial compartment expanded in proportion to the increased interstitial particles.

Discussion

Ideally, one would like to compare particle location across a range of exposures using the same particulate material in rats and humans and with data for exposure concentrations and lung burdens in the humans as well as in

the rats. Materials that would allow such an experimental design were not available. However, we have shown that for retained diesel soot and CD in rats and monkeys, the anatomic pattern of particle retention is characteristic of the species and does not differ between diesel soot and CD (28). Therefore, these exposures and tissues were chosen as the best available to allow the influence of particle “dose” on the pattern of particle retention to be examined in rat and human lungs. Because exposure and lung burden data were not available for the human cases, we used urban ambient controls and miners who worked under different exposure standards and for differing amounts of time underground to establish three dose groups. The available data for the rats and human cases and the volume densities of particulate material in the lung sections show that this study design did allow comparisons of particle retention across a wide range of exposure concentrations and resultant lung burdens.

Both the qualitative and quantitative examination of histologic sections of rat lungs show that chronically inhaled diesel soot is retained predominantly in alveolar macrophages in parenchymal lumens of rats over a wide range of exposure concentrations and resultant lung burdens. Over the range of exposure concentrations examined, 0.35–7.0 mg/m³, the exposure concentration did not significantly influence the anatomic sites of particle retention.

Interstitialization of soot particles in diesel soot-exposed rats has often been noted as duration of exposure increases and at high exposure concentrations (9,34,35). Accumulation of particles in the interstitium has been called a key feature of the lung overload phenomenon (36). The present study shows that interstitialization of particles is not unique to exposures associated with lung clearance overload in rats.

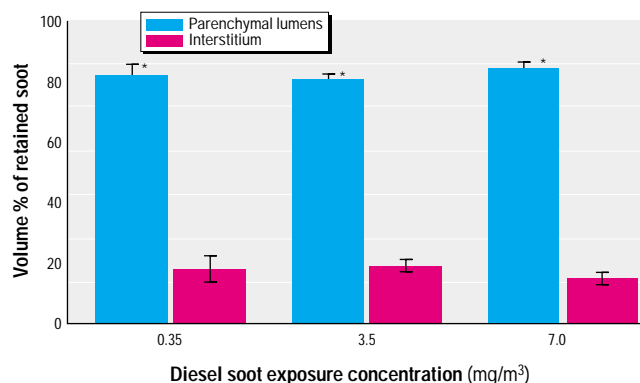


Figure 5. The distribution of particulate material (mean ± SE) in lungs of rats exposed to diesel soot at different concentrations.

*The portion of the particulate material that was located in parenchymal lumens was significantly greater than the portion in the interstitium in all three dose groups and did not vary with exposure concentration or resultant lung burden.

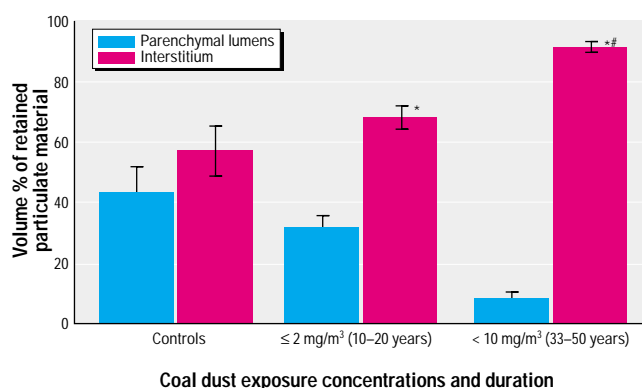


Figure 6. The distribution of particulate material (mean ± SE) in human lung sections at different doses.

*The portion of the particulate material in the interstitium was significantly different than the portion in the parenchymal lumens in the low-dose and high-dose miners. #The distribution of particulate material in the interstitium versus the lumens was significantly greater in the high-dose miners than in the low-dose miners and controls.

The anatomic pattern of particle retention in rats does not switch to a new pattern as the lung burden switches from one not associated with slowing of clearance to one associated with clearance overload. The data show that, as the amount of retained material increases, the amount retained in the parenchymal lumens and in the interstitium both increase and in approximately the same proportion.

At low lung burdens, the amount of particulate material in the interstitium (approximately 20% of a small total) is easily overlooked in qualitative observations. At high lung burdens, this 20% is readily noticed and described by pathologists. This may have led to the impression that interstitialization is a new phenomenon that is especially significant to lung clearance overload. The present study is a reminder that in lung clearance overload in the rat, the total lung burden progressively increases as the amount in the parenchymal lumens also progressively increases. The importance of this large amount of retained material should not be discounted merely because it is not a newly observed phenomenon.

The present study does not address the mechanism of interstitialization in either species. Direct penetration of particles into the interstitium, movement of particle-containing macrophages into the interstitium, and incorporation into the interstitium as a consequence of alveolar collapse and fibrosis (32,34,35,37) are all possibilities. Indeed, the predominant mechanism may change as the lung burden and particle-associated pathology changes.

In nonminers and coal miners, chronically inhaled particulate material is retained

primarily in the interstitium. The portion of the retained material in the interstitium is related to dose expressed as exposure concentration, years of exposure, and/or lung burden. As the amount of retained material in the interstitium increases, the size of the interstitial compartment increases and corresponds to the diagnosis of pulmonary fibrosis. This fibrosis is a frequently described component of coal workers' pneumoconiosis.

The high-dose miners had a significantly greater volume density of particulate material and a greater portion of the retained material in the interstitium than the low-dose miners and controls. In this study, "dose" or the amount of particulate material in the lung was a consequence of both the exposure concentration and years worked underground. The study design does not allow the contributions of exposure concentration versus time to the greater interstitialization in the high-dose miners to be differentiated. In addition, the study design does not allow the contributions of other confounding factors such as geographical differences or cause of death to the greater interstitialization of particles in the high-dose miners to be determined.

This morphometric examination of long-term sites of particle retention shows that the anatomic distribution pattern of retained particulate material differs between rat and human lungs. This difference in distribution means that different lung cells are predominantly in contact with the particles or particle-containing macrophages in rats and humans, which suggests that responses may differ between the two species.

The present study suggests that the 80% of the particulate material retained in alveolar

macrophages or extracellularly in parenchymal lumens may be key to the pathologic sequelae of lung clearance overload that are oriented to the luminal surface in rats. Such sequelae to lung clearance overload that are observed with exposure to diesel soot or a number of poorly soluble particles include acute and chronic inflammation, epithelial hyperplasia, alveolar-bronchiolar and squamous metaplasia, and epithelial neoplasia. These epithelial responses could be due to direct contact with particles or to cytokines and growth factors released by particle-laden macrophages. In contrast, in humans with long-term particle retention, the cells in closest contact with particles or cytokines from the majority of the particle-containing macrophages are mesenchymal cells, and fewer are epithelial cells.

Volumetric loading of macrophages, their subsequent inability to move, and their release of proinflammatory cytokines are key to the overload response (38). Monkey and human macrophages have two times and five times the volume of rat macrophages, respectively (39). This suggests that rat macrophages may be more sensitive to overload and more likely to trigger the associated responses than monkey or human macrophages. This difference, combined with the difference in location of particles or macrophages containing particles, may partially account for the tendency of rats to respond to poorly soluble particles with more chronic inflammation and epithelial responses and relatively less fibrosis. In contrast, humans respond to the same or similar particles with more fibrosis and less inflammatory and epithelial response (40,41). Of course,

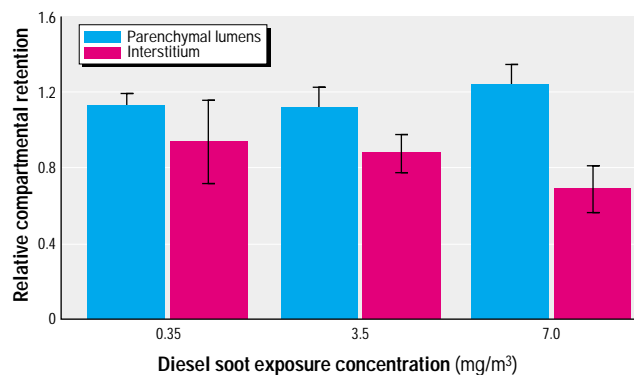


Figure 7. The relative compartmental retention of particulate material (mean \pm SE) in the lungs of rats exposed to diesel soot at different concentrations. The relative compartmental retention was calculated as the percentage of the particulate material in a given compartment divided by the percentage of the lung composed of that compartment. In rats, the relative compartmental retention of particles was similar across exposure groups. The amount of particulate material retained in parenchymal lumens relative to the size of the luminal compartment was significantly greater than the comparable ratio for the interstitium in all groups (combined). These results show that in rat lungs, particles are retained in parenchymal lumens disproportionately to the amount of the lung composed of parenchymal lumens.

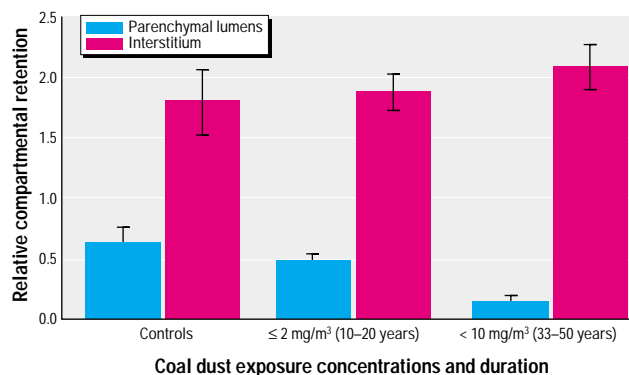


Figure 8. The relative compartmental retention of particulate material (mean \pm SE) in human lung sections. The relative compartmental retention was calculated as the percentage of the particulate material in a given compartment divided by the percentage of the lung composed of that compartment. The relative compartmental retention of particles was similar across dose groups. The amount of particulate material in the interstitium relative to the size of the interstitial compartment was significantly greater than the comparable ratio for the lumens in all groups (combined). These results show that in human lungs, particles are retained in the interstitium disproportionately to the amount of interstitium in the lung.

other factors may contribute to the differences in responses between rats and humans. Such factors might include differences in amounts of various cytokines and growth or chemotactic factors, differences in oxidant generation and antioxidant defenses, and differences in the responsiveness of epithelial cells and fibroblasts to stimulation.

REFERENCES AND NOTES

- Nikula KJ. Rat lung tumors induced by exposure to selected poorly soluble nonfibrous particles. *Inhal Toxicol* 12:97-119 (2000).
- NTP. Toxicology and Carcinogenesis Studies of Talc (CAS No. 14807-96-6) (Non-Asbestiform) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR 421. Research Triangle Park, NC:National Toxicology Program, 1993.
- Heinrich U, Fuhst R, Rittinghausen S, Creutzenberg O, Bellmann B, Koch W, Levens K. Chronic inhalation exposure of Wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide. *Inhal Toxicol* 7:533-556 (1995).
- Saffiotti U. Carcinogenesis by crystalline silica: animal, cellular, and molecular studies. In: *Silica and Silica-Induced Lung Diseases* (Castranova V, Vallyathan V, Wallace WE, eds). Boca Raton, FL:CRC Press, Inc., 1995:345-381.
- NTP. Toxicology and Carcinogenesis Studies of Nickel Subulfide (CAS No. 12035-72-2) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR 453. Research Triangle Park, NC:National Toxicology Program, 1994.
- Heinrich U, Peters L, Ernst H, Rittinghausen S, Dasenbrock C, König H. Investigation on the carcinogenic effects of various cadmium compounds after inhalation exposure in hamsters and mice. *Exp Pathol* 37:253-258 (1989).
- Glaser U, Hochrainer D, Otto FJ, Oldiges H. Carcinogenicity and toxicity of four cadmium compounds inhaled by rats. *Toxicol Environ Chem* 27:153-162 (1990).
- Heinrich U, Muhle H, Takenaka S, Ernst H, Fuhst R, Mohr U, Pott F, Stöber W. 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 (1986).
- Mauderly JL, Banas DA, Griffith WC, Hahn FF, Henderson RF, McClellan RO. Diesel exhaust is not a pulmonary carcinogen in CD-1 mice exposed under conditions carcinogenic to F344 rats. *Fundam Appl Toxicol* 30:233-242 (1996).
- Henderson RF, Pickrell JA, Jones RK, Sun JD, Benson JM, Mauderly JL, McClellan RO. Response of rodents to inhaled diluted diesel exhaust: biochemical and cytological changes in bronchoalveolar lavage fluid and in lung tissue. *Fundam Appl Toxicol* 11:546-567 (1988).
- Miller FJ. Dosimetry of particles in laboratory animals and humans in relationship to issues surrounding lung overload and human health risk assessment: a critical review. *Inhal Toxicol* 12:19-57 (2000).
- Schreider JP, Raabe OG. Structure of the human acinus. *Am J Anat* 162:221-232 (1981).
- Phalen RF, Oldham MJ. Airway structures: tracheo-bronchial airway structure as revealed by casting techniques. *Am Rev Respir Dis* 128:S1-S4 (1983).
- Tyler WS. Small airways and terminal units: comparative subgross anatomy of lungs. *Am Rev Respir Dis* 128:S32-S36 (1983).
- Mercer RR, Crapo JD. Structure of the gas exchange region of the lungs determined by three-dimensional reconstruction. In: *Toxicology of the Lung*. New York:Raven Press, 1988:43-70.
- Pinkerton KE, Barry BE, O'Neil JJ, Raub JA, Pratt PC, Crapo JD. Morphologic changes in the lung during the lifespan of Fischer 344 rats. *Am J Anat* 164:155-174 (1982).
- Kapanci Y, Weibel ER, Kaplan HP, Robinson FR. Pathogenesis and reversibility of the pulmonary lesions of oxygen toxicity in monkeys. II. Ultrastructural and morphometric studies. *Chest* 62:162-169 (1969).
- Kapanci Y, Tosco R, Eggerman J, Gould VE. Oxygen pneumonitis in man: light and electron microscopic morphometric studies. *Chest* 62:162-169 (1972).
- Crapo JD, Barry BE, Gehr P, Bachofen M, Weibel ER. Cell numbers and cell characteristics of the normal human lung. *Am Rev Respir Dis* 126:332-337 (1982).
- Leak LV. Pulmonary lymphatics and their role in the removal of interstitial fluids and particulate matter. In: *Respiratory Defense Mechanisms, Part II* (Brain JD, Proctor DF, Reid LM, eds). New York:Dekker, 1977:631-685.
- Lauweryns JM, Baert JH. The role of the pulmonary lymphatics in the defenses of the diseased lung: morphological and experimental studies of the transport mechanisms of intratracheally instilled particles. *Ann NY Acad Sci* 221:244-275 (1974).
- Heppleston AG. The pathogenesis of simple pneumoconiosis in coal workers. *J Pathol Bacteriol* 67:51-63 (1954).
- McLaughlin RF, Tyler WS, Canada RO. A study of the subgross pulmonary anatomy in various mammals. *Am J Anat* 108:149-165 (1961).
- McLaughlin RF Jr, Tyler WS, Canada RO. Subgross pulmonary anatomy of the rabbit, rat, and guinea pig, with additional notes on the human lung. *Am Rev Respir Dis* 94:380-387 (1966).
- Leak LV, Jamuar MP. Ultrastructure of pulmonary lymphatic vessels. *Am Rev Respir Dis* 128:S59-S65 (1983).
- Tyler WS, Julian MD. Gross and subgross anatomy of lungs, pleura, connective tissue septa, distal airways, and structural units. In: *Comparative Biology of the Normal Lung, Vol I* (Parent RA, ed). Boca Raton, FL:CRC Press, Inc., 1992:37-48.
- Wolff RK. Mucociliary function. In: *Comparative Biology of the Normal Lung, Vol 1* (Parent RA, ed). Boca Raton, FL:CRC Press, 1992:659-680.
- Nikula KJ, Avila KJ, Griffith WC, Mauderly JL. 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 (1997).
- Mauderly JL, Jones RK, Griffith WC, Henderson RF, McClellan RO. Diesel exhaust is a pulmonary carcinogen in rats exposed chronically. *Fundam Appl Toxicol* 9:208-211 (1987).
- Hessel P, Mitchell I, Tough S, Green F, Cockcroft D, Kepron U, Butt J. Risk factors for death from asthma. *Ann Allergy Asthma Immunol* 83:362-368 (1999).
- Elias H, Hyde DM. *A Guide to Practical Sterilology*. New York:Karger, 1983.
- Green FHY, Laqueur WA. Coal workers' pneumoconiosis. *Pathol Annu* 15 (Pt 2):333-410 (1980).
- Weibel ER. *Morphometry of the Human Lung*. New York:Springer Verlag, 1963.
- Ishinishi N, Kuwabara N, Nagase S, Suzuki T, Ishiwata S, Kohono T. Long term inhalation studies on effects of exhaust from heavy and light duty diesel engines on F344 rats. In: *Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust* (Ishinishi N, Koizumi A, McClellan RO, Stöber W, eds). New York:Elsevier Science Publishing Co., 1986:329-348.
- Ishinishi N, Kuwabara N, Takaki Y, Nagase S, Suzuki T, Nakajima T, Maejima K, Kato A, Nakamura M. Long-term inhalation experiments on diesel exhaust. In: *Diesel Exhaust and Health Risk. Results of the HERP Studies*. Tsukuba, Ibaraki, Japan:Japan Automobile Research Institute, Inc., 1988:11-84.
- Morrow PE. Mechanisms and significance of particle overload. In: *Toxic and Carcinogenic Effects of Solid Particles in the Respiratory Tract* (Mohr U, Dungworth DL, Mauderly JL, Oberdörster G, eds). Washington, DC:ILSI Press, 1994:17-25.
- Heppleston AG. Relationship of lipid secretion and particle size to diffuse interstitial change in pneumoconiosis: a pathogenic perspective. *Am J Ind Med* 15:427-439 (1989).
- Morrow PE. Possible mechanisms to explain dust overloading of the lungs. *Fundam Appl Toxicol* 10:369-384 (1988).
- Krombach F, Munzing S, Allmeling AM, Gerlach JT, Behr J, Droger M. Cell size of alveolar macrophages: an interspecies comparison. *Environ Health Perspect* 105(suppl 5):1261-1263 (1997).
- Green FHY. Pulmonary responses to inhaled poorly soluble particulate in the human. *Inhal Toxicol* 12:59-95 (2000).
- Hahn FF, Green FHY, Nikula KJ, Vallyathan V. Comparative pulmonary responses of humans and animals to dust exposures. In: *Advances in the Prevention of Occupational Respiratory Diseases* (Chiyotani K, Hosoda Y, Aizawa Y, eds). Amsterdam:Elsevier Science, 1998:974-978.

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