PARTICLE OVERLOAD IN THE RAT LUNG AND LUNG CANCER
Implications for Human Risk Assessment

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CURRENT INFORMATION ON LUNG OVERLOAD IN NONRODENT MAMMALS: CONTRAST WITH RATS

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Because the concept of lung overload is based primarily on the results of chronic inhalation studies in which rats inhaled large amounts of relatively nontoxic, poorly soluble dusts, the extent to which biological manifestations of lung overload in rats can be extrapolated to other mammalian species, especially humans, is being questioned. Rats exhibit relatively fast pulmonary clearance of dust and appear to retain pulmonary burdens of dust predominantly in macrophages within alveoli. In contrast, larger animals and humans exhibit slower pulmonary clearance of dust and appear to retain pulmonary burdens of dust predominantly in the pulmonary interstitium. Chronic inhalation of dust by the larger species is therefore predicted to result in different dust accumulation patterns in the lungs than seen in rats. These patterns may dictate the extent to which species other than rats exhibit manifestations of lung overload. Results of several studies of coal miners, hard rock miners, and asbestos workers demonstrated that large pulmonary burdens of dust can accumulate in humans. An important unanswered question is the extent to which pulmonary clearance might have been altered during the chronic exposures of larger species and humans. It is possible for significant burdens of dust to accumulate in lungs with normal clearance. Published results of a UO_2 chronic inhalation study with rats, monkeys, and dogs suggest that pulmonary clearance was not altered in monkeys and dogs under chronic exposure conditions that resulted in altered pulmonary clearance in the rats. These results strongly support the concept that relationships among species relative to lung overload may be attributable more to differences in patterns of dust accumulation in the lung than to the amounts of accumulated dust. In summary, the available data from monkeys, dogs, and humans suggest that lung overload in rats may not be directly relevant to larger mammals and humans. However, the issue of lung overload in species other than rodents is still important and requires additional attention to determine the extent to which the various manifestations of lung overload in rats can be applied to other species, including humans.

Chronic inhalation of poorly soluble dusts can result in substantial pulmonary burdens of the dust that may produce adverse biological effects. Throughout this article, "pulmonary" refers to the alveolar-interstitial region of the respiratory tract. The kinds of dust encountered in chronic inhalation exposures can be compact particles, which have aspect ratios (length/diameter) <3, or fibers, which have aspect ratios ≥3. Differences exist between compact particles and fibers relative to deposition, retention, and clearance patterns in the respiratory tract, particularly for fibers >15 μm in length. This article dis-

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cusses general principles that are believed to be common to compact particles and fibers having lengths <15 μm. Therefore, for discussion purposes, compact particles and fibers are not differentiated in this article, and the term "dust" is used to describe all types of respirable, poorly soluble materials.

Chronic inhalation of poorly soluble dust can cause lung overload, which results when macrophage-mediated dust clearance is overwhelmed by the amount of dust deposited in the lung (Mauderly, 1996). Lung overload has been described primarily on the basis of observations in rodent species, and rats appear to be exceptionally susceptible to the development of lung overload (Mauderly, 1996). Other rodent species have not been studied as extensively, but available data suggest that mice and Syrian hamsters are not as susceptible as rats to lung overload.

The extent to which lung overload in rats can be extrapolated to other species, especially larger mammals and humans, has not been determined. A few studies have been reported in which monkeys, dogs, and cats were chronically exposed to respirable dusts. Rats were included in most of those studies, thereby allowing comparisons to be made between rats and one or both of these larger animal species. The human database relevant to chronic inhalation of respirable dusts consists primarily of coal miners, hard rock miners, and asbestos workers. Therefore, although some direct comparisons are possible between rats, monkeys, and dogs, it is very difficult to make such direct comparisons between humans and any other species. However, dogs, monkeys, and humans appear to have similar deposition, retention, and clearance patterns for inhaled dusts (Snipes, 1989), so some of the results from chronic inhalation studies with these species may be predictive for humans.

This article focuses on rats, monkeys, dogs, cats, and humans. A brief interspecies comparison of pulmonary clearance of dust is presented first. Next, lung overload in rats is summarized, followed by a broader discussion of published data relevant to the concept of lung overload in monkeys, dogs, and humans. Finally, the data for rats, monkeys, and dogs are compared and contrasted, yielding some tentative conclusions about lung overload in larger mammalian species.

PULMONARY CLEARANCE OF DUST

When small amounts of dust deposit in the pulmonary airspaces, the dust is efficiently phagocytized by pulmonary alveolar macrophages (PAMs). Some of the dust becomes incorporated into the pulmonary interstitium, and the rest remains in the pulmonary airspaces in macrophages. The pulmonary deposit of dust is subjected to the competing processes of macrophage-mediated clearance and dissolution–absorption to remove it from the lung. These competing processes clear most of the deposited dust with an effective half-time of about 2 mo for most rodents and about 2 yr for large mammalian species, including humans (Snipes, 1989). Figure 1 presents examples of

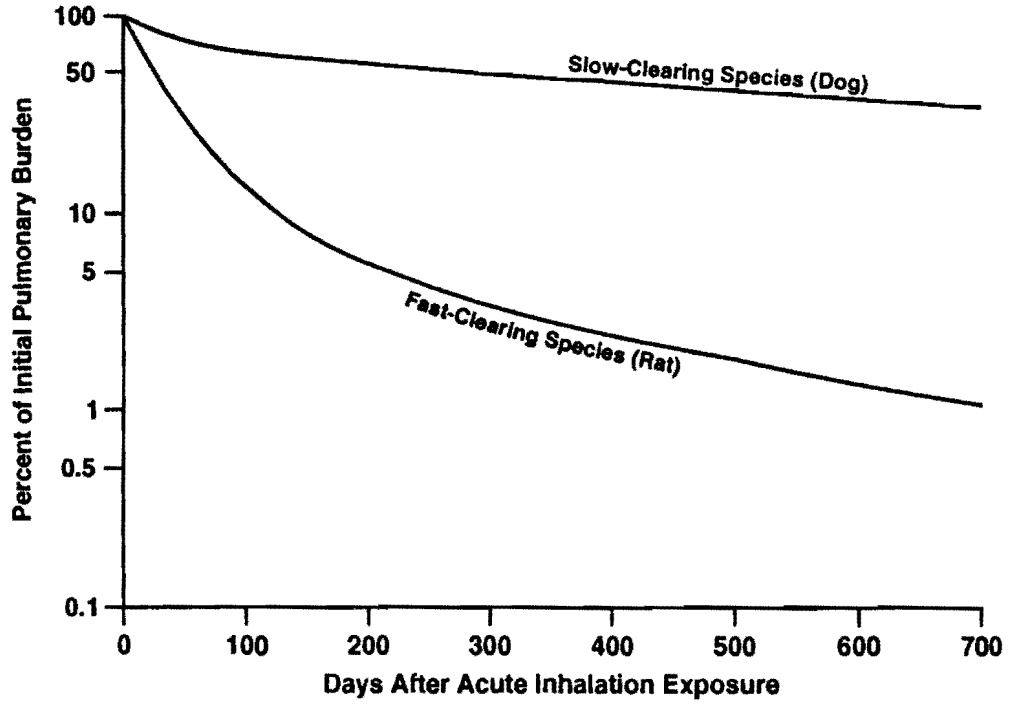

FIGURE 1. Example of pulmonary clearance for poorly soluble dust inhaled acutely by a slow-clearing mammalian species (dog) and fast-clearing species (rat).

pulmonary clearance patterns typical for rats (fast-clearing species) and dogs (slow-clearing species). The reasons for these differences in pulmonary clearance rates have not been fully determined but may be related to dust retention sites within the lung.

During chronic inhalation exposures, PAMs phagocytize dust and appear to function normally in spite of the fact that the lung is accumulating the dust. Pulmonary clearance may proceed at a normal rate, and an equilibrium pulmonary burden of dust may eventually be reached when the pulmonary deposition rate equals the effective pulmonary clearance rate. Figure 2 presents simulated patterns for the pulmonary accumulation of dust predicted for a fast-clearing species (rat) and slow-clearing species (dog) during a 1-yr chronic inhalation exposure to an aerosol of dust, followed by patterns for clearance during a 1-yr recovery period. The model used to make these predictions for cumulative pulmonary burdens of dust was described by Pritsker (1974) and uses a Fortran-based numerical integration of differential equations. The model output includes an estimate of the pulmonary burden of dust for every day of interest following an inhalation exposure. Chronic exposures are simulated by defining the exposure days for the study (Monday through Friday, for example) and summing the amounts of dust retained in the lung from each daily inhalation exposure throughout the defined chronic exposure period. For the example shown in Figure 2, the dust was 2 μm mass median aerodynamic diameter at a concentration of 1 mg dust/m^3, with

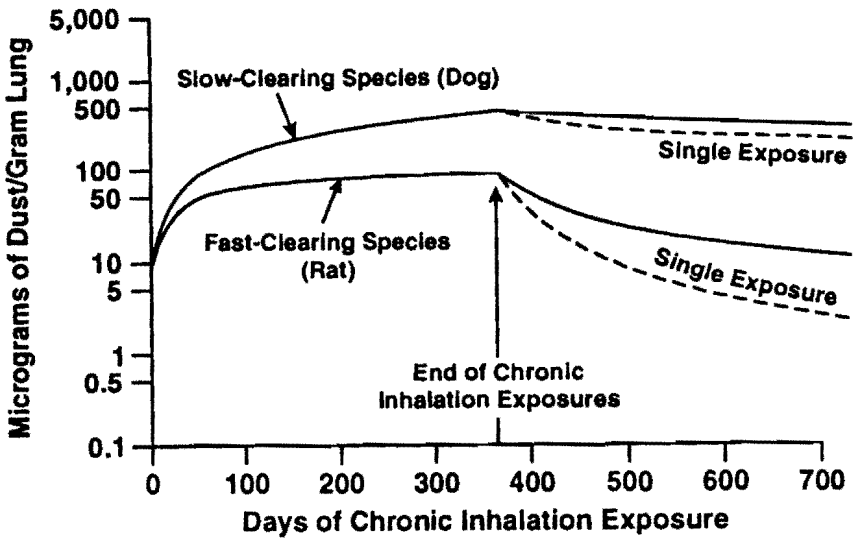

FIGURE 2. Simulated patterns for the pulmonary accumulation of dust predicted for a fast-clearing species (rat) and slow-clearing species (dog) during a 1-yr chronic inhalation exposure to an aerosol of dust, followed by patterns for clearance during a 1-yr recovery period (solid lines). The simulated chronic exposures were 8 h/day, 5 days/wk to a hypothetical 2-μm mass median aerodynamic diameter aerosol containing 1 mg dust/m^3, with a dissolution–absorption half-time of 1000 days. Dashed lines represent clearance patterns for the dust deposited on day 365, with the intercepts adjusted for ease of visual comparison with the clearance curves for the recovery period. Deposition and clearance parameters are explained in text.

an assumed dissolution–absorption half-time of 1000 days. The simulated exposures were 8 h/day, Monday through Friday. Pulmonary deposition was assumed to be 0.07 for rats and 0.23 for dogs (Snipes, 1989) for this hypothetical aerosol. Minute ventilation (L/min • g lung) was assumed to be 0.078 for rats and 0.022 for dogs (Table 1). The calculated daily deposition of dust per gram of lung was 2.6 μg for rats and 2.4 μg for dogs. Physical clearance parameters are presented in Table 2.

TABLE 1. Physiological parameters for rats, monkeys, and dogs

	Body weight (kg)	Lung weight (g)	Minute ventilation (L/min)	Minute ventilation per g lung (L/min • g lung)
Rat	0.25[a]	1.6[a]	0.13[b]	0.078
Monkey	2.45[a]	22.3[a]	0.78[b]	0.035
Dog	10.0[c]	110[c]	2.39[b]	0.022

[a]From Phalen (1984).

[b]Calculated using power law prediction parameters (Stahl, 1967); minute ventilation = 379 • (kg body wt)$^{0.8}$.

[c]Cumulative data from the Inhalation Toxicology Research Institute normalized to a 10.0-kg dog.

TABLE 2. Physical clearance parameters used for modeling pulmonary clearance of dust inhaled by rats, monkeys, and dogs

Species	Clearance via mucociliary transport pathway	Clearance to thoracic lymph nodes
Rat	$0.028\ \exp^{-0.01t} + 0.0018$	$0.0007\ \exp^{-0.5t}$
Monkey and dog	$0.008\ \exp^{-0.022t} + 0.0001$	0.0002

Note. Parameters are given in terms of fraction of existing pulmonary burden physically cleared per day. Adapted from Snipes (1989). Physical clearance parameters were based on data collected in dogs and were assumed to be the same for monkeys.

Predicted accumulation and clearance patterns for the chronically inhaled dust are indicated by the solid lines in Figure 2. The predicted cumulative burdens of dust after 1 yr of chronic exposure were 89 μg/g lung for the rats and 440 μg/g lung for the dogs. The dashed lines are included in the figure to illustrate what the predicted clearance patterns are for the dust deposited in the lungs of these 2 species on day 365, with the starting positions of the clearance curves moved to intercept the curves for chronic exposures for visual effect. The slower clearance of the pulmonary burdens of dust that were accumulated under chronic exposure conditions is due to the fact that the accumulated pulmonary burdens of dust are mainly comprised of the slow-clearing portions of dust that deposited during the chronic exposure period.

The predicted pulmonary burdens of dust in Figure 2 apply to normal pulmonary clearance. As discussed later, pulmonary accumulation and clearance patterns change substantially under chronic exposure conditions that cause altered pulmonary clearance and lung overload.

LUNG OVERLOAD IN RATS

A rapid response to chronic inhalation of large amounts of dust is recruitment of additional PAMs into the pulmonary airspaces (Brain, 1971; Adamson & Bowden, 1978; Brain, 1985). Bowden (1987) noted that the recruitment of macrophages into the lung is closely associated with the burden of dust reaching the pulmonary airspaces and that the system can be overloaded by either too much dust or an inadequate cellular response. Continued inhalation of large amounts of dust overwhelms the functional abilities of PAMs, adversely affects macrophage-mediated clearance, and causes an inflammatory response. Large numbers of polymorphonuclear leukocytes (PMNs) enter the pulmonary airspaces (Larsen et al., 1983; Lehnert et al., 1985) as part of the inflammatory response. The PMNs represent the most prominent migratory cells present in most inflammatory conditions and are considered to have important roles in many kinds of lung disease. With continued inhalation of large amounts of dust, the accumula-

tion of dust in the lungs proceeds at an accelerated rate. Long-term chronic inhalation exposures of rats to high concentrations of poorly soluble dust that result in altered pulmonary clearance can generally be expected to produce pulmonary fibrosis and possibly neoplasia.

An example from Wolff et al. (1987) of pulmonary burdens of diesel soot in rats exposed chronically to large amounts of diesel exhaust is shown in Figure 3. At selected times during the study, groups of 4 or 16 rats were sacrificed, and pulmonary burdens of diesel soot were measured. The lines in Figure 3 are the result of simulation modeling using the presumption of normal dust deposition and pulmonary clearance throughout the study. Predicted and measured pulmonary burdens agreed reasonably well for rats exposed to 0.35 mg soot/m^3, at least during the first year of the study. Measured and predicted pulmonary burdens of diesel soot did not agree either early or late in the study for rats exposed to 3.5 or 7.0 mg soot/m^3; pulmonary burdens of diesel soot were lower than predicted early in the study and were about 5 times higher after 24 mo of chronic exposure. These latter two cases are typical of patterns observed during chronic exposures to dust that result in lung overload in rats.

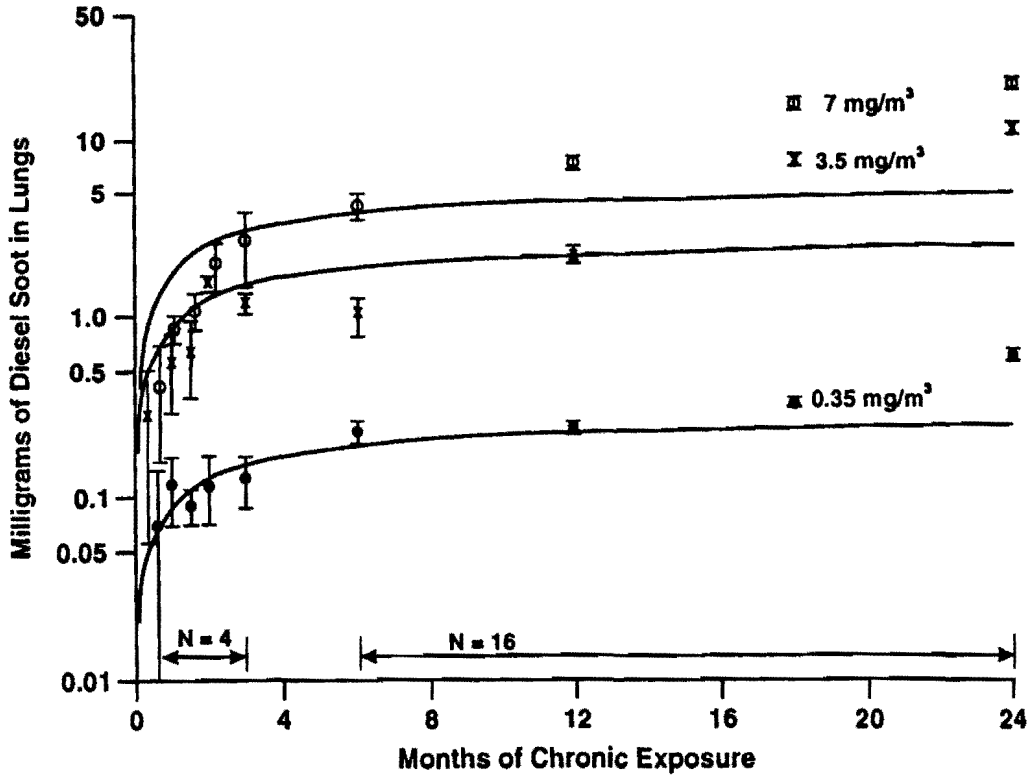

FIGURE 3. Measured and predicted pulmonary burdens of diesel soot in rats exposed 7 h/day, 5 days/wk for 2 yr to diesel exhaust at concentrations of 0.35, 3.5, or 7.0 mg soot/m^3. Values are mean ± SE for 4 or 16 rats per time point at which lung burdens were measured. Data adapted from Wolff et al. (1987).

COMPARATIVE DATA FROM CHRONIC DUST INHALATION STUDIES

Table 3 summarizes all of the chronic dust inhalation experiments known to this author that included nonrodent mammals. The studies included several types of dust, and most or all of the exposure protocols indicated in Table 3 appear to be adequate to cause lung overload in rats. The majority of these studies emphasized histopathological evaluations of the respiratory tract, generally for animals sacrificed after the end of the chronic exposures or after a postexposure period. The histopathology findings were not substantially different among the studies, with the exception of the study with UO_2, in which radiation damage to lung was probably a confounding factor. Pulmonary burdens of the test materials were not measured in most of these studies, so it is not possible to compare and contrast pulmonary accumulation patterns for the chronically inhaled dusts and relate those patterns to histopathological findings. Fortunately, Leach et al. (1970, 1973) reported pulmonary burdens of U for rats, monkeys, and dogs chronically exposed to UO_2, and the data are adequate for making comparisons among these three species relative to lung overload; these studies are discussed in detail later.

The published results for the studies identified in Table 3 contained varying amounts of detail describing exposure conditions, dust distribution patterns in the lungs, and pulmonary pathology. The following information relevant to dust retention patterns in the lungs and pathology that can be compared with what is typically seen in rats subjected to lung overload was extracted from the studies. The intent was to extract generalizations that would be useful in discussing lung overload, rather than attempt to make direct comparisons among these studies.

Gross and Nau (1967) chronically exposed rats and monkeys to lignite dust for 12 mo and evaluated lungs of the animals between 13 and 24 mo. The authors noted accumulations of lignite dust in alveoli of rats and monkeys and indicated that the most striking lesion in the rat lungs (not found in monkeys) was "a lipid pneumonia characterized by foci of expanded alveoli containing large lipophages and lipophagic debris, as well as finely dispersed black dust." No dust was noted in perivascular or peribronchial interstitial tissues in the rats. Pulmonary lesions were not reported for the monkeys. The report by Klonne et al. (1987) included similar findings in rats and monkeys that chronically inhaled coke dust; in addition to phagocytosis of the coke dust by PAMs of both species, the rats had "chronic inflammation and focal areas of fibrosis, bronchiolization, schlerosis, squamous alveolar metaplasia, and keratin cyst formation." Again, lesions were not noted in the lungs of the monkeys.

Leach et al. (1970, 1973) reported that UO_2 in the lungs of monkeys and dogs occurred chiefly as interstitial aggregates, which did not appear to increase in size or number after exposures longer than 2–3 yr. In rats, however, the pigment was located almost exclusively within macrophages lying in alveoli and bronchi, with less interstitialization. Monkeys developed pul-

TABLE 3. Chronic inhalation studies conducted with monkeys, dogs, and cats; rats were included in most of the studies, as indicated

Species	Type of dust	Particle size[a] (polydisperse)	Exposure concentration (mg/m^3)	Exposure frequency	Exposure duration	Evaluation times	References
Rat, monkey	Lignite	~3.8 μm MMAD	0, 7.7	7 h/day; 5 days/wk	12 mo	Between ~13 and 24 mo	Gross and Nau (1967)
Rat, monkey	Coke	3.1 μm MMAD	0, 10.2, 30.7	6 h/day; 5 days/wk	24 mo	3, 6, 12, 18, and 24 mo for rats; 24 mo for monkeys	Klonne et al. (1987)
Rat, monkey, dog	Uranium oxide	1.03 μm MMD	0, 5	6 h/day; 5 days/wk	Up to 60 mo	Various times during study[b]	Leach et al. (1970, 1973)
Rat, monkey	Diesel exhaust	~0.3 μm MMD	0, 2	7 h/day; 5 days/wk	24 mo	3, 6, 12, and 24 mo for rats; 24 mo for monkeys	Lewis et al. (1989)[c]
Rat, monkey	Coal	<7 μm MMAD	0, 2	7 h/day; 5 days/wk	24 mo	3, 6, 12, and 24 mo for rats; 24 mo for monkeys	Lewis et al. (1989)[c]
Rat, monkey	Shale	4–5 μm MMAD	0, 10, 30	6 h/day; 5 days/wk	24 mo	24 mo	MacFarland et al. (1982)
Monkey	Fly ash	~2.6 μm MMD	0, 0.16, 0.46	~23.6 h/day	18 mo	18 mo	MacFarland et al. (1971)
Monkey	Coal	N.I.[d]	0, 2	6.5 h/day; 24 mo	24 mo	24 mo	Moorman et al. (1975)
Rat, monkey	Fiberglass	See reference for details	0, 5, 15	7 h/day; 5 days/wk	21 or 18 mo[e]	21 or 18 mo, or 80% mortality	Moorman et al. (1988)
Monkey	Carbon black	N.I.	0, 1.6, 2.4	7 h/day; 5 days/wk	Some for 7.1 yr	Insufficient information in the reference	Nau et al. (1962)
Monkey	Carbon black	N.I.	Not clear	6 h/day; 6 days/wk	About 3.1 yr	After about 3.1 yr of chronic exposure	Nau et al. (1976)
Cat	Diesel exhaust	90% <1 μm; 50% <0.3 μm	0, 6, or 12[f]	8 h/day, 7 days/wk	24 mo	After 1 and 2 yr	Pepelko and Peirano (1983); Hyde et al. (1985)
Monkey	Manganese dioxide	N.I.	0, 0.7, 3	22 h/day; 7 days/wk	10 mo	10 mo	Suzuki et al. (1978)

[a]MMD, mass median diameter; MMAD, mass median aerodynamic diameter.

[b]Rats were exposed for 12 mo and observed for an additional 12 mo; dogs and monkeys were exposed for 5 yr and observed for an additional 5 yr. Lung burden data were collected throughout the study.

[c]Rats and monkeys were also exposed to a combination of 1 mg diesel exhaust/m^3 + 1 mg coal dust/m^3.

[d]N.I., not indicated or not clear.

[e]Rats were exposed for 21 mo; monkeys were exposed for 18 mo.

[f]Exposed to 6 mg soot/m^3 during the first year, and to 12 mg soot/m^3 during the second year of the study.

monary fibrosis that was minimal at 3.6 yr and progressively more notable after longer exposure periods and during the postexposure period. Pulmonary fibrosis was also noted in dogs at extended times after stopping the chronic exposures to UO_2. The lungs of 19 dogs were evaluated from 6 to 11 yr after starting the chronic exposures. Bronchiolar metaplasia was noted in six lungs, adenoma was noted in two lungs, and adenocarcinomas were noted in two lungs. These lesions were not found in lungs of control dogs, or in exposed rats and monkeys. The authors concluded that the pathology produced in lungs of the monkeys and dogs was due to alpha radiation produced by the U.

Lewis et al. (1989) histologically evaluated lungs of rats through 24 mo of exposure to diesel exhaust, coal dust, or a mixture of diesel exhaust and coal dust. Results were similar for exposures to the dusts individually or combined. Collections of macrophages containing black-pigmented dust were observed throughout the lungs of all exposed rats. Most of the macrophages were seen within alveoli; lesser numbers were present in the connective tissues of the bronchopulmonary rays and within bronchial lymphoid tissues. Dust was also observed within the interstitium of the alveolar ducts. Pulmonary responses to the dusts in lungs of rats, especially notable after 24 mo, included accumulations of degenerating foamy macrophages, amorphous granular material, chronic inflammatory cells, and fibrosis.

Monkeys were evaluated by Lewis et al. (1989) after 24 mo of exposure. Aggregates of black dust, which consisted of perivascular, peribronchiolar, and alveolar accumulations, were noted primarily in the distal airways of the monkey lungs. Dust was present within the cytoplasm of macrophages in the alveolar spaces as well as the interstitium. There were no obvious differences in the quantities of dust in the lungs of monkeys from the different exposed groups and no indications of exposure-related fibrosis, focal emphysema, or inflammation.

MacFarland et al. (1982) histologically examined the lungs of rats and monkeys chronically exposed to oil shale dust for 24 mo. Distribution patterns of the dust in lungs of the rats and monkeys were not described. In comparing the pulmonary responses of the rats and monkeys, the authors concluded that nonnodular fibrosis (pleural and/or subpleural), cholesterol clefts with or without microgranulomas, alveolar proteinosis, and proliferative bronchiolitis/alveolitis characterized the inflammatory lung changes in the rats. Mononuclear cell infiltration of the pigment accumulations and/or subacute bronchiolitis and alveolitis were more descriptive of the inflammatory reactions in the lungs of monkeys. In a similar study, monkeys were chronically exposed to fly ash for 18 mo (MacFarland et al., 1971). The lungs of these monkeys contained fly ash in alveolar macrophages, adjacent alveolar walls, and peribronchial lymph nodes. The group of monkeys exposed to 0.46 mg fly ash/m^3 had aggregates of macrophages in small nodules in the alveolar walls, and a minimal fibrotic response was present in a few of the nodules.

Moorman et al. (1975) exposed one group of monkeys to Pennsylvania coal dust, which has been associated with a high incidence of coal workers'

pneumoconiosis, and exposed another group of monkeys to Utah coal dust, associated with a low incidence of coal workers' pneumoconiosis. Both types of coal dust caused similar pulmonary functional impairment (small airway obstruction) after 24 mo of chronic exposure. No histopathological data or lung burdens of coal dust were reported. In a later study (Moorman et al., 1988), rats and monkeys were chronically exposed to fiberglass dust for 21 mo (rats) or 18 mo (monkeys). The only significant effect reported for the monkeys was dust-containing macrophage aggregates, whereas pulmonary responses in the rats included macrophage aggregates and granulomas containing the dust. Grossly visible plaque-like foci occurred in rats because of accumulations of granulomatous foci in pleural and subpleural locations; these pleural plaques were not seen in the monkeys. There were no indications of fiberglass-induced fibrogenic responses in the rats or monkeys.

Nau et al. (1962, 1976) reported the results of studies in which monkeys were chronically exposed to carbon black. Little or no intra-alveolar carbon black was seen, regardless of the length of exposure, and in only rare instances were pigmented macrophages observed. The carbon black was mostly interstitial and characteristically in peribronchial and perivascular locations either free or within macrophages; the alveolar lining cells contained little or no dust. No malignancies were observed in exposed animals. After about 3.1 yr of chronic exposure to carbon black, lungs of monkeys had lesions described by Nau et al. (1976) as comparable to human centrolobular emphysema.

The study by Suzuki et al. (1978) involved only monkeys and emphasized monthly radiographic evaluations during the course of the 10-mo chronic inhalation exposure to manganese dioxide. Evidence of developing pneumoconiosis was noted in radiographs taken as early as 1 mo after initiating chronic inhalation exposures to 3 mg Mn/m^3. Histopathological evaluations were made after the exposures ended. Dust deposits and hyperplasia were noted within pulmonary lymphatic tissues. Some dust was noted in alveoli, but most of the dust was in peribronchiolar and perivascular locations in the pulmonary interstitium. Retention of exudate within the bronchioles, thickening of alveolar walls, pulmonary emphysema, and atelectasis were also noted, but no fibrotic changes were observed.

The results of one major study relevant to dust loading of the lungs of cats have been reported (Pepelko & Peirano, 1983; Hyde et al., 1985). Adult male cats were exposed to diesel exhaust 8 h/day, 7 days/wk for 2 yr. During the first year the exposure concentration was 6 mg $soot/m^3$; during the second year the concentration was 12 mg $soot/m^3$. Pulmonary function tests conducted after the first year did not reveal adverse exposure-related effects. In contrast, pulmonary function tests conducted after the second year revealed an exposure-related restrictive lung disease compatible with a diagnosis of pulmonary fibrosis. Lung weights and lung volumes were decreased in the diesel-exposed cats, which contrasts with a common finding in rats exposed under conditions that produce lung overload; lung

weights are substantially increased in rats with lung overload because of inflammatory responses and fibrosis. Microscopic examination of the cat lungs revealed two major exposure-related lesions in the proximal acinar regions of the lungs: (1) peribronchiolar fibrosis, and (2) bronchiolar epithelial metaplasia. Relative amounts of dust in the pulmonary interstitium and alveoli were not reported.

The cumulative results of these chronic dust inhalation studies suggest that rats accumulated more dust in macrophages in the pulmonary airspaces than in the pulmonary interstitium during chronic inhalation exposures. In contrast, monkeys and dogs had larger accumulations of dust in pulmonary interstitial tissue than in alveolar macrophages. Leach et al. (1970) suggested that uranium may be more rapidly removed from the rat lung because it persisted in alveolar macrophages, presumably providing easier mobilization than uranium in the interstitial deposits found to a greater extent in the lungs of dogs and monkeys. This suggestion may be relevant to most or all other types of dust. Dust retention patterns in the lung may be important factors that influence pulmonary clearance pathways, rates of dust clearance, and pathology resulting from accumulating pulmonary burdens of dust.

COMPARISON OF PULMONARY ACCUMULATION AND CLEARANCE PATTERNS FOR UO_2 INHALED BY RATS, MONKEYS, AND DOGS

The chronic inhalation studies with UO_2 reported by Leach et al. (1970, 1973) were unique in that three mammalian species chronically inhaled the same dust for extended periods of time, then were evaluated during postexposure periods equal to or longer than the exposure periods. The rats were chronically exposed for 1 yr, then allowed a postexposure period of 1 yr. The monkeys and dogs were exposed for 5 yr, then allowed a postexposure period of up to 6.5 yr. Animals were sacrificed periodically during the course of the study for histopathology evaluations and to determine pulmonary and thoracic lymph node burdens of uranium. The same evaluations were performed on animals that died for various reasons during the study. The resulting data demonstrate the time course for accumulation of pulmonary burdens of uranium (and thoracic lymph node burdens, not discussed in this article) during the 1- or 5-yr periods of chronic inhalation exposures, followed by the clearance patterns after stopping the chronic exposures. In interpreting the results of the study, one must consider the potential for chemical toxicity of the uranium dust, as well as the fact that radiation exposure and damage to lung tissue could have influenced the results of the study. In spite of those potential confounding factors, the data provide an opportunity to make important comparisons that relate to lung overload among the three species. The data for the three species are plotted in Figures 4–6 as specific pulmonary burden (μg U/g lung) versus time after start of the study.

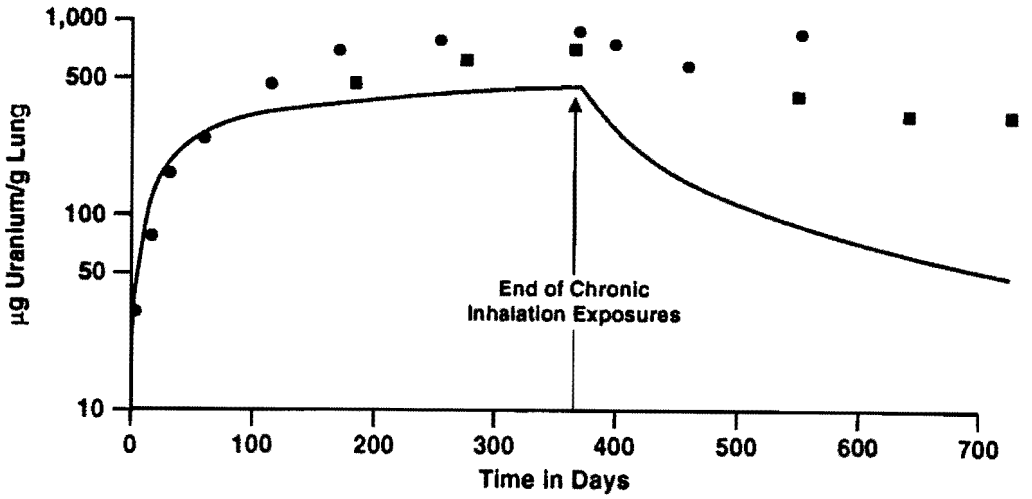

FIGURE 4. Measured and predicted specific pulmonary burdens (µg U/g lung) in rats exposed 6 h/day, 5 days/wk, for 1 yr to 5 mg U/m^3, with a subsequent 1-yr postexposure period. Data adapted from Leach et al. (1970, 1973). Measurements from Study I, ●; measurements from Study II, ■. Each point represents the average value for 2–10 rats.

The same simulation model used to produce Figures 2 and 3 was used to predict pulmonary burdens of dust for the rats, monkeys, and dogs exposed to UO_2. The inhalation input and clearance parameters for U for all three species are summarized in Tables 1, 2, and 4. Table 1 presents physiological parameters used to predict the daily intake of U for the three species. Animal body and lung weights were not presented by Leach et al. (1970, 1973), so the body and lung weights in Table 1 were assumed. Fortunately, accu-

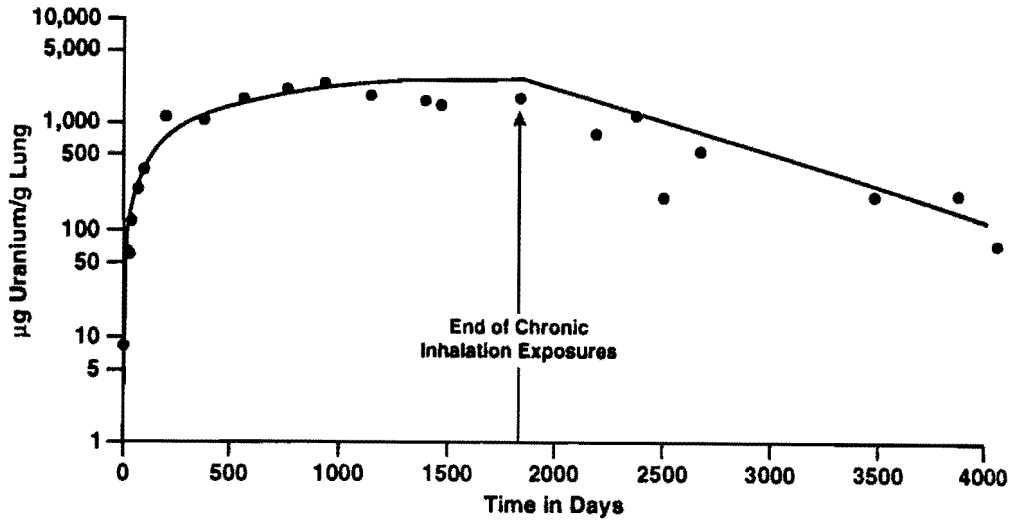

FIGURE 5. Measured and predicted specific pulmonary burdens (µg U/g lung) in dogs exposed 6 h/day, 5 days/wk, for 5 yr to 5 mg U/m^3, with a subsequent 6-yr postexposure period. Data adapted from Leach et al. (1970, 1973). Each point represents the average value for 1–6 dogs.

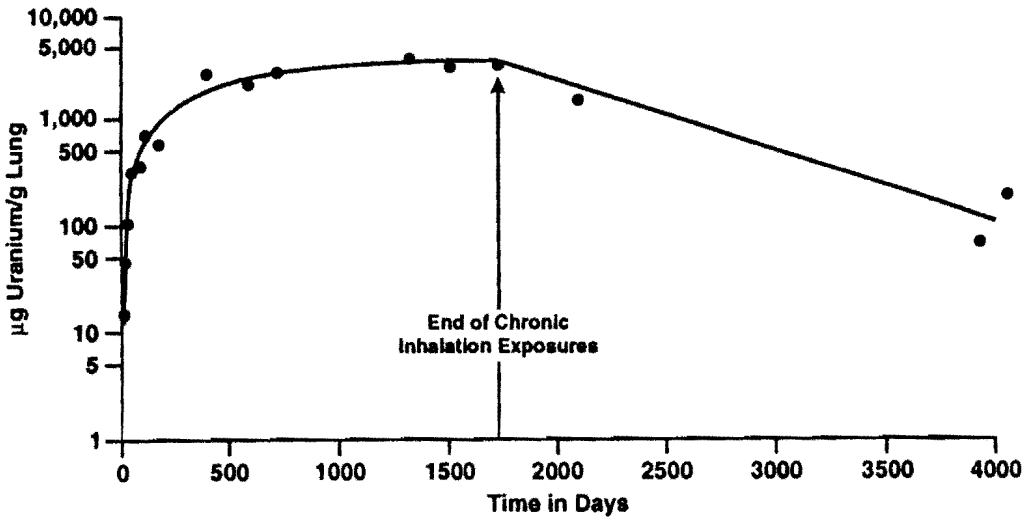

FIGURE 6. Measured and predicted specific pulmonary burdens (µg U/g lung) in monkeys exposed 6 h/day, 5 days/wk, for 5 yr to 5 mg U/m^3, with a subsequent 6-yr postexposure period. Data adapted from Leach et al. (1970, 1973). Each point represents the average value for 1–3 monkeys.

rate body and lung weights were not necessary if the ratio of lung : body weight was the same in the animals as presented in Table 1; if this assumption holds, the minute ventilation per gram lung is essentially independent of body weight for each respective species. The values for minute ventilation per gram lung (Table 1) and deposition fractions (Table 4) were used to calculate daily deposition of U/g lung (Table 4). Each species has its own characteristic physical clearance parameters (Table 2). However, the dissolution–absorption characteristics of the UO_2 were assumed to be the same for all species. The dissolution–absorption rate that best accounted for the U accumulation patterns during the chronic exposures and the clearance patterns after the exposures for all three species was 0.0009/day, which represents a half-time for dissolution–absorption equal to 770 days.

The curve in Figure 4 represents results from the simulation model for rats in which the assumption was made that deposition and clearance were not altered during the course of the study. Results clearly demonstrate that the assumption of normal clearance was not valid. The simulation matches the data reasonably well early in the study, but then it deviates in a pattern typical for lung overload in rats, as shown in Figure 3 for a 2-yr chronic inhalation study with diesel exhaust. The measured pulmonary burdens were substantially higher at later times than predicted by the simulation model. The rate of pulmonary clearance was also notably slower during the postexposure period than predicted by the model. Again, these results are typical of what can be expected for rats chronically exposed under conditions that cause lung overload. The model results for the monkey (Figure 6), based on the assumption that deposition and clearance were normal throughout the study, agree with the measured pulmonary accumulation and clearance data. Data and

TABLE 4. Summary of inhalation exposure parameters used to predict specific pulmonary burdens of U (μg U/g lung) for UO_2 chronically inhaled by rats, monkeys, and dogs

A. Common parameters:	
Exposure atmosphere	5.0 mg U/m^3
Particle mass median aerodynamic diameter	1.03 μm
Particle dissolution–absorption half-time	770 days
Chronic inhalation exposure pattern	6 h/day; 5 days/wk
Duration of chronic exposure:	
Rats	1 yr
Monkeys and dogs	5 yr

B. Calculated daily pulmonary deposition

Species	Deposition fraction[a]	Daily deposition (μg U/g lung)
Rat	0.095	13.9
Monkey	0.22	13.9
Dog	0.22	8.7

[a]Fraction of inhaled UO_2 dust deposited in the pulmonary region of the respiratory tract. Values were adapted from Snipes (1989); the pulmonary deposition fraction was assumed to be the same for monkeys and dogs for this aerosol of UO_2.

modeling results for the dogs agreed well for the first 3 yr of the study; then concentrations of U in lungs were lower than predicted by the model.

Uranium emits alpha radiation, and the lungs of all three species were subjected to alpha radiation damage. The lungs of the rats received about 0.3 Gy of alpha radiation during the 1-yr chronic exposure period, and about 0.3 Gy additional dose during the 1-yr postexposure period. The lungs of the monkeys and dogs received about 8 Gy and 4 Gy, respectively, of alpha radiation dose during the exposure period and about that much additional alpha radiation dose during the postexposure period. The cumulative alpha radiation dose per gram of lung was therefore about a factor of 2 higher in the monkeys than in the dogs.

Leach et al. (1973) concluded that the alpha radiation caused the pulmonary fibrosis noted in monkeys and dogs. Some of the dogs also developed bronchiolar metaplasia, adenomas, or adenocarcinomas. The dogs were apparently more susceptible than monkeys to development of these pulmonary lesions. Weights for lungs were not reported, and the specific pulmonary burdens were reported as micrograms U per gram lung. It is possible that dog lung weights were larger than normal after 3–4 yr of exposure, resulting in low values for specific pulmonary burdens of U. Better agreement might have been obtained between the data and simulations if the results could have been plotted as micrograms U per gram control lung.

Unfortunately, the information needed to make this data conversion was not available.

Neither dogs nor monkeys expressed the pulmonary accumulation and clearance patterns that are distinctive features of lung overload in rats. Pulmonary burdens of U in monkeys and dogs were not higher than predicted, and pulmonary clearance during the postexposure period occurred at the rate predicted by the simulation model. This study suggests that major differences exist between rats and larger species (monkeys and dogs) with respect to lung overload. Table 5 summarizes the results noted by Leach et al. (1970, 1973), expressed in terms of the attributes seen in rats during lung overload. The specific pulmonary burdens in rats were 0.8 mg U/g lung after 1 yr of chronic exposure. Specific pulmonary burdens were about twice that value in monkeys and dogs after 1 yr. The pulmonary burdens in rats were about twice as high as the model predicted assuming normal deposition and clearance. This magnitude of pulmonary burden of dust is less than levels generally associated with lung overload in rats. However, lung overload was apparent in the pattern of accumulation of U in the rat lungs during the 1-yr chronic exposure, as well as during the 1-yr postexposure period. The lungs of monkeys contained about 4 mg U/g lung after 5 yr of chronic exposure; the lungs of dogs contained about 2 mg U/g lung. The projected specific pulmonary burdens for the monkeys were essentially identical to those that were measured; in dogs, the measured burdens were about 2 mg U/g lung after 5 yr, and the projected burdens were about 3 mg U/g lung.

Chronic inhalation of UO_2 apparently did not cause pulmonary inflammation in these three mammalian species. Hyperplasia was not reported for rats or monkeys, but was present in lungs of dogs after about 6 yr. The data strongly suggest that altered pulmonary clearance occurred in the rats, but not

TABLE 5. Summary observations from Leach et al. (1970, 1973) chronic inhalation studies with UO_2

Observation	Rat	Monkey	Dog
Pulmonary burdens (mg U/g fresh lung)			
After 1 yr	0.8[a]	1.5	1.4
After 5 yr	Not applicable	4.0	1.8[b]
Chronic pulmonary inflammation	Not reported	Not reported	Not reported
Pulmonary hyperplasia (macrophages especially)	Not reported	Not reported	After 6–10 yr
Altered macrophage-mediated pulmonary clearance	Yes	No	No
Larger than predicted pulmonary burdens of U	Yes	No	No
Increased interstitial deposits of UO_2	Possibly	Yes	Yes
Increased transport of UO_2 to thoracic lymph nodes	Possibly	Probably	Probably
Interstitial lung disease (fibrosis)	None reported	After 3–6 yr	After 6.5–10 yr
Development of lung tumors	No	No	Yes[c]

[a]Projected to be about 0.8 mg U/g lung after 1 yr.

[b]Projected to be about 3 mg U/g normal lung after 5 yr.

[c]Adenoma, adenocarcinoma (about 4–7 Gy cumulative alpha radiation dose to lung).

in the monkeys or dogs; pulmonary burdens of U were larger than predicted in the rats, but were not larger than predicted in the monkeys or dogs. Increased interstitial deposits were not reported, but could have occurred in all three species. Interstitial lung disease was not reported for rats; it was reported for the monkeys and dogs after 3–4 yr of chronic exposure. The only tumors noted in the studies occurred in lungs of dogs at late times in the study and were probably the result of alpha radiation damage to the lungs.

DATA RELEVANT TO LUNG OVERLOAD IN HUMANS

Only a limited number of human studies have demonstrated a relationship between pulmonary burdens of dust and altered pulmonary function. Lippmann and Timbrell (1990) summarized Timbrell's evaluations of dust loading in lungs of workers who had long-term inhalation exposures to a variety of amphiboles. Timbrell's postmortem analyses of lungs from these individuals led him to conclude (1) that a small percentage of heavily exposed workers had extensive fibrosis and severely altered pulmonary clearance in which there was little or no clearance from parts of the lungs, and (2) that pulmonary clearance of fibers is strongly dependent on the pulmonary burden of fibers and the degree of pulmonary fibrosis. Additionally, the extent of lung fibrosis is described as proportional to the total surface of retained mineral dust for both fibers and compact particles. None of the lungs evaluated by Timbrell had tumors attributable to the accumulated burdens of fibers.

Miners represent another group likely to demonstrate one or more attributes of lung overload if the phenomenon applies to humans. Mauderly (1994) summarized information available from 10 studies published between 1956 and 1986 in which substantial pulmonary burdens of dust were reported in miners; the estimated range for specific pulmonary burdens of accumulated dust in these individuals was about 6–14 mg dust/g wet lung. No increase in lung tumors is thought to be attributable to these specific pulmonary burdens of coal dust in humans.

GENERAL DISCUSSION AND SUMMARY

Several studies with monkeys and dogs, as well as studies with coal miners and hard rock miners, have demonstrated that some attributes of lung overload are seen in these large animal species and humans. Table 6 summarizes lung overload attributes noted in rats, with comparisons to observations to date in monkeys and dogs. Specifically, excessive pulmonary burdens of dust, some accumulations of dust-laden macrophages in pulmonary alveoli, enhanced lymphoid-associated and interstitial deposits of dust, and granulomatous inflammation have been noted.

The signal attribute of lung overload in rats that has not been demonstrated in large animal species or humans is altered clearance. Even though

TABLE 6. Comparison of attributes of lung overload in rats, monkeys, and dogs

Classical attributes and sequelae of lung overload in rats	Rats	Dogs, monkeys, and humans
Chronic pulmonary inflammation	Yes	Not certain
Hyperplasia of macrophages and epithelial cells	Yes	Not certain
Altered pulmonary clearance (overwhelmed macrophage-mediated clearance)	Yes	Probably not
Large pulmonary burdens of particles	Yes	Probably not
Increased interstitialization of deposited particles	Yes	Yes
Increased translocation of particles from lung to thoracic lymph nodes	Probably	Probably
Interstitial lung disease (fibrosis)	Yes	Yes
Production of lung tumors	Yes	No

substantial pulmonary burdens of dust have been reported for some large laboratory animals and humans, altered pulmonary clearance has not been demonstrated. Interestingly, published data described earlier from a chronic inhalation study of UO_2 with rats, monkeys, and dogs conducted at the University of Rochester suggest that altered pulmonary clearance did not occur in monkeys and dogs under chronic exposure conditions that resulted in altered pulmonary clearance in rats (Leach et al., 1970, 1973).

Other important attributes of lung overload in rats that have not been demonstrated in large animal species and humans include widespread accumulations of dust-laden macrophages within alveoli, and development of alveolitis and lung tumors. These findings associated with lung overload in rats suggest a strong correlation between the accumulations of dust-containing macrophages in pulmonary airspaces and the most significant pathology associated with lung overload in rats, lung tumors. The presence of a chronic inflammatory response in the lungs of rats may be a very important consequence of chronic inhalation exposures of rats to substantial amounts of respirable dust. Although accumulations of dust in alveoli of large animal species have been noted in chronic inhalation studies, the relative amounts of dust and numbers of macrophages associated with the dust in alveoli appear to have been less than observed in rats chronically exposed under similar conditions.

In summary, altered pulmonary clearance and pathology associated with lung overload appear to be related to dust retention patterns in the lung. Mammalian species with fast-clearing lungs, rats in particular, retain dust predominantly in macrophages in pulmonary airspaces and appear to be more susceptible to lung overload than are the larger species that clear pulmonary burdens of dust more slowly and retain dust predominantly within the pulmonary interstitium. Results from chronic inhalation studies with monkeys and dogs suggest that slow-clearing species do not develop the most important attributes of lung overload that are elicited in rats under the same exposure conditions, namely, altered pulmonary clearance and lung

tumors. Unfortunately, the species differences noted to date only suggest that the consequences of lung overload in rats may not be relevant to larger mammals such as monkeys, dogs, and humans; additional work, especially lifespan studies with one or more slow-clearing species, may be required to confirm this tentative conclusion.

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