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Lung Particle Overload: Implications for Occupational Exposures to Particles

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Chronic pulmonary inflammation, pulmonary fibrosis, and even lung tumors developed in a number of chronic inhalation studies in rats with highly insoluble nonfibrous particles of low cytotoxicity. Concerns were expressed that these responses are due to excessive particulate lung burdens, and the term "particle overload" was coined to characterize these conditions. The hallmark of the particle-overloaded lung is an impairment of alveolar macrophage (AM)-mediated lung clearance which has been demonstrated in all species tested so far and which eventually leads to accumulation of excessive lung burdens. Experimental evidence suggests that the volume of the particles phagocytized by AM is most critical for causing their impaired clearance function, and that the condition of lung overload is reached once the retained lung particle burden reaches a level equivalent to a volume of approximately 1 μl/g of lung. Cytotoxic particles also cause impaired AM clearance function, yet at a much lower lung burden which does not qualify as particle overload. Significant species differences exist with respect to the induction of adverse chronic effects in response to lung overload; i.e., mice and hamsters are less prone to developing chronic inflammation and pulmonary fibrosis, and lung tumors have been observed only in rat studies. Lung tumors or fibrosis in the rats were seen only at lung burdens having caused impaired particle clearance, and a threshold dose for the adverse chronic effects can be postulated which is defined by a lung particle burden not causing impairment of clearance. Thus, the lung tumors observed in chronic rat studies at very high particulate exposure concentrations may not be relevant for human extrapolation to low-exposure concentrations. Evidence in humans suggests that particle-overloaded lungs, e.g., in coal workers, respond with fibrosis, but no increased incidence of lung tumors has been found in this group. However, it cannot be excluded that other types of chronically inhaled particles may have a carcinogenic potential in the human lung if accumulating to very high lung burdens. More research is needed for a detailed understanding of the basic mechanism leading to nonfibrous particle-induced tumorigenesis in the lungs of different species. Altered particle accumulation and retention kinetics and chronic inflammation in the overloaded lung indicate that the maximum tolerated dose (MTD) has been exceeded. No specific guidelines for inhalation studies defining the MTD have been established; general guidelines are not necessarily applicable for chronic inhalation studies. One suggestion is to define the MTD in chronic particle inhalation studies as a maximum functionally tolerated dose (MFTD) based on a functional parameter of lung particle clearance. However, other endpoints based on an evaluation of alveolar inflammation and lung histology should be included as well. For the estimation of the MTD or MFTD a range-finding study of sufficient length, preferably 3 months, would be required. It is important to realize, however, that with increasing chronicity of exposure and increasing age of the animals, a shift in the dose–response relationship may occur. One important conclusion from our understanding of lung particle overload is that occupational exposure limits should be set to prevent impaired AM-mediated lung clearance which will avert the accumulation of high pulmonary dust burdens; conceivably, this will also prevent the subsequent induction of adverse chronic effects including inflammation, fibrosis, and tumors since these particle-induced tumors are likely secondary to continued inflammation, tissue damage, and cell proliferation. Extrapolation from rat studies of such exposure limits predicts that present occupational standards for highly insoluble particles of low cytotoxicity may not prevent lung overload in humans and should be reevaluated.

INTRODUCTION

Understanding the effect of very high lung particle burden is of critical importance for the interpretation of results from experimental chronic inhalation studies with highly insoluble particles of low cytotoxicity. Results of these studies in rodents imply that rather benign particles can induce serious adverse pulmonary effects if inhaled chronically at high concentrations. These parti-

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cles were formerly categorized as "nuisance" dusts under occupational exposure conditions, but are now categorized as particles not otherwise classified (PNOC; ACGIH, 1994). Chronic inhalation studies in rats involving such types of particles have resulted in pulmonary fibrosis and even lung tumors at high exposure concentrations which also affected lung clearance mechanisms, and evidence has been accumulating that these effects are toxicological implications of a condition of lung "particle overload" (Morrow, 1988). This concept is now widely accepted. However, not every tumorigenic response observed in a chronic particle inhalation study in rats should *a priori* be labeled as being a consequence of particle overload without having a thorough understanding of this concept. A careful analysis of all available data is required for an interpretation of results from such studies which is most important with respect to human extrapolation.

Thus, before a decision is made that the results of a chronic particle inhalation study fit the category of particle overload, a critical review of the study data is imperative. This paper will discuss the concept of particle overload, including pulmonary particle retention kinetics, overload-associated effects, and the existence of a threshold. Important species differences between rats, hamsters, and mice will be pointed out, the necessity of defining a maximum tolerated dose for chronic inhalation studies in rodents will be stressed, and extrapolation of results from experimental particle inhalation studies to humans will be discussed.

THE PARTICLE OVERLOAD CONCEPT

A number of chronic inhalation studies with highly insoluble particles of relatively low toxicity have been published over the past decade. Particle types that have been investigated include TiO_2, toner particles, PVC particles, carbon black, diesel soot, talc, coal dust, petroleum coke, oil shale, and volcanic fly ash (Martin *et al.*, 1977; MacFarland *et al.*, 1982; Lee *et al.*, 1985; Wehner *et al.*, 1986; Heinrich *et al.*, 1986, 1992; Mauderly *et al.*, 1987; Klonne *et al.*, 1987; Muhle *et al.*, 1990a; NTP, 1993; Mauderly, 1994). In most studies rats were used, but also mice and hamsters were exposed in several of these studies. Additionally, singular studies were performed in guinea pigs, monkeys, cats, rabbits, and ponies. Despite this large variety of species, there are only a limited number of studies directly comparing the responses of the inhaled particulate compounds between different species, and these comparative studies are limited to rats, mice, and hamsters. Typically, exposure concentrations in these studies ranged anywhere from ~1 to ~30 mg/m^3, exceptionally even up to a concentration of 250 mg/m^3 in a two-year TiO_2 inhalation study (Lee *et al.*, 1985). As a result, the retained particulate lung burdens were quite high, reaching several milligrams per gram of lung tissue. Resulting adverse effects have best been studied in rats. These effects involve a sequence of inflammatory responses, altered particle kinetics, altered morphology, and finally chronic disease states including fibrosis and even the induction of benign and malignant lung tumors (Heinrich *et al.*, 1986; Henderson *et al.*, 1988; Muhle *et al.*, 1990a; Mauderly, 1994) (Table 1). A characteristic event in addition to the dose-dependent increase in pulmonary inflammatory cells is a significant depression of the alveolar macrophage (AM)-mediated clearance of particles which in turn leads to increased retention and accumulation of particles in the lung accompanied by an increased access of these particles into the pulmonary interstitium. A typical finding is also the occurrence of aggregated AM in focal areas of the alveoli and the occurrence of cell proliferative responses of epithelial and interstitial cells. The latter may be causally related to the induction of focal fibrotic lesions and development of lung tumors in the rats.

Since the altered particle-retention kinetics caused by a depressed AM clearance function is regularly seen at particulate burdens above ~1–3 mg in the rat lung, the term lung "particle overload" was coined to indicate primarily a significant prolongation of AM-mediated particle clearance due to high pulmonary particle load. It should be emphasized that this concept of particle overload specifically applies to highly insoluble particles of low cytotoxicity. Other more cytotoxic particles affect AM-mediated particle clearance as well, but at much lower lung burdens, e.g., crystalline SiO_2, metal compounds like CdO and NiO, ultrafine particles in the nanometer size range, and natural and man-made fibers which either are too long to be phagocytized by AM or are more cytotoxic. Thus, it should be cautioned not to view every impairment of AM-mediated particle clearance as a condition of particle overload. Interestingly, even PNOCs can elicit a highly toxic pulmonary response if they consist of ultrafine particles. For example, inhaled ultrafine TiO_2 (diameter of primary particles, 20 nm) has significantly greater pulmonary toxicity (Oberdörster *et al.*, 1992a) and also induces lung tumors in rats at much lower lung burdens (Heinrich, 1994) than submicronic TiO_2 of 200- to 300-nm-diameter particle size. Aspects of particle deaggregation upon deposition in the lung, the high specific surface area, and increased interstitial translocation of these ultrafine particles all appear to be important for their effects. However, this will not be further discussed in this article.

With respect to low toxicity particles in the overloaded lung, the high-particulate lung burden seems to overwhelm the capacity of AM to clear the deposited particles efficiently from the alveolar region. If exposure continues, the retained lung burden increases more than would be predicted with normal clearance kinetics; consequently, the high lung burden increases the potential to cause irreversible chronic effects such as fibrosis and even lung tumors which were found in rats exposed to high concentrations of TiO_2, diesel soot, carbon black,

TABLE 1
Particle-Induced Pulmonary Effects in Rats

EXPOSURE DURATION / DOSE INCREASE

inflammatory cells ↑ (AM; PMN)	*inflammation*
biochemical lavage parameters ↑	
alveolar epithelial leakiness	
lung weight ↑	
AM clearance function ↓	*particle kinetics*
particle retention ↑	
AM aggregates	
interstitial access of particles (LN)	
cell proliferation ↑ *type II cells* *fibroblasts*	*morphology*
"bronchiolization"	
collagen ↑	
fibrotic foci	*chronic disease*
emphysematous lesions	
benign and malignant tumors	

and talc (Lee *et al.*, 1985; Mauderly *et al.*, 1987; Heinrich *et al.*, 1992; NTP, 1993).

Particle Retention Kinetics during Lung Overload

Highly insoluble particles deposited in the lower respiratory tract are eliminated mainly by two important mechanisms: The mucociliary escalator efficiently removes deposited particles in the conducting airways due to ciliary beating within hours; in the alveolar region the main removal mechanisms for deposited particles are AM which can be attracted to the site of particle deposition (Warheit *et al.*, 1988) and phagocytize those particles for transport toward the mucociliary escalator (Schlesinger, 1985; Oberdörster, 1988; Snipes, 1989). AM-mediated particle clearance from the alveolar space is a slow process which can be approximated by a mono-exponential decay function with a clearance rate of ~1% per day for the rat, equivalent to an alveolar retention halftime of ~70 days. Up to 10-fold longer alveolar retention halftimes have been indirectly determined for particles in humans (Bailey *et al.*, 1985). If the deposition rate of chronically inhaled particles in the alveolar region is less than or at the most equal to the clearance rate, it can be predicted that the resulting lung burden will reach an equilibrium in the alveolar region after approximately 5 retention halftimes have elapsed. Lung clearance mechanisms under these conditions function properly and no excessive accumulation of particles is to be expected. Upon cessation of exposure a normally functioning lung eliminates the alveolar particle burden according to the species-specific retention halftimes. This scenario under normal low-exposure conditions for the rat is shown schematically in Fig. 1, lower curve, assuming a $1\frac{1}{2}$-year chronic exposure to a low particle concentration and subsequent clearance after cessation of exposure.

On the other hand, this normal behavior of pulmonary accumulation and clearance of highly insoluble particles during chronic exposure changes significantly when exposure concentrations are so high that the deposition rate in the alveolar region exceeds the AM-mediated clearance rate, resulting in a much higher particulate

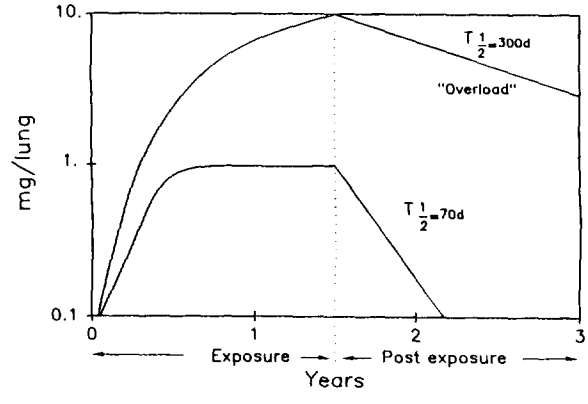

FIG. 1. Pulmonary accumulation and clearance of highly insoluble low-toxicity particles in rats during and after inhalation exposure to low and high inhaled concentrations.

lung burden. This occurs despite the fact that there is an adaptive response of increased AM numbers in the lung: As shown in the schematic representation of this overload scenario in Fig. 1, upper curve, lung particle burden continues to increase until exposure ceases. Upon termination of exposure, particles are cleared with a significantly slower rate corresponding to a significantly increased alveolar retention halftime compared to the normal condition. It has been observed in rat studies with very high lung burdens that the prolongation of the alveolar retention halftime can be irreversible (Bellmann *et al.*, 1991). Associated with the excessive accumulation of particles in the rat lungs and their slowed elimination is an increased translocation of particles into the pulmonary interstitium which will significantly contribute to enhanced adverse interstitial effects, e.g., the induction of pulmonary fibrosis (Adamson *et al.*, 1989; Bowden *et al.*, 1989).

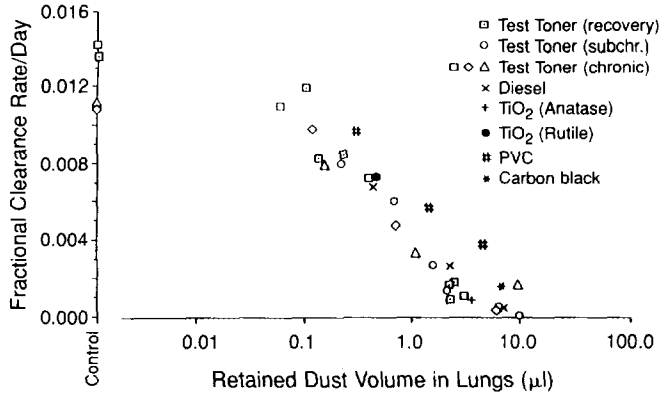

FIG. 2. Pulmonary particle clearance rates and volumetric lung burdens of different retained particles in rats (adapted from Morrow *et al.*, 1991).

Particles in the pulmonary interstitium form a sequestration compartment from which clearance is even less efficient than that from the alveolar spaces. Other sequestration sites may include aggregates of particle-laden macrophages and particle-containing epithelial cells as suggested by Lehnert (1990). While the existence of such sequestration compartments is an important secondary component to explain and model prolonged particle clearance (Stöber *et al.*, 1993), the questions are why and when AM loaded with particles initially are adversely affected in their clearance functions. Lehnert *et al.*, (1990) found that both the random and the chemotaxin-directed migration of AM are depressed *in vitro* when macrophages have phagocytized high particle burdens *in vivo*. A similar loss in AM motility after ingestion of high numbers of particles has been described for dog and rat macrophages by Mueller *et al.* (1990), and Brown *et al.*, (1992) found that the chemotactic responses of bronchoalveolar leukocytes from rats inhaling coal mine dust and quartz were reduced and remained so after a 30-day recovery period.

Conceivably, a mechanical impediment of AM burdened with phagocytized particles may cause this decreased response to chemotactic stimuli as well as the decrease in random migration. Morrow (1988) introduced this idea and hypothesized that it is the volumetric loading of the AM that eventually will affect their clearance function. Based on lung burden and lung clearance data from a number of published investigations, he proposed a volumetric overload hypothesis which states that phagocytized particulate lung burdens which on average amount to 6% of the normal AM volume may be critical for initiating the impaired AM clearance behavior. Morrow (1988) suggested further that the AM clearance function is fully depressed once the phagocytized volume reaches ~60% of the normal AM volume. Supporting these volumetric estimates is a study by Dorries and Valberg (1992), who concluded from their results that possible effects on the cytoskeleton of AM occur when ~7–8% of the AM volume is filled by phagocytized particles; and Lehnert (1990) found that AM with particulate volume loads equal to or in excess of 60% of their normal volumes are the main sequestrating compartments to which diminished rates of lung clearance can be virtually totally attributed. In another *in vivo* study designed to test the volumetric overload hypothesis we confirmed that rat AM with a phagocytized particle load equivalent to 60% of their normal volume do not eliminate these particles from the lung (Oberdörster *et al.*, 1992a). Morrow's volumetric hypothesis implies that the appropriate particle parameter to describe an overload-induced prolongation of particle clearance should be the retained pulmonary particle volume rather than the retained particle mass; i.e., a density correction must be applied when the lung dose is given in terms of retained mass. The 6% AM volume suggested by Morrow (1988) to signal the beginning of AM overload can be translated into a volumetric lung burden of ~1 μl of particles/g of lung.

Morrow *et al.* (1991) reevaluated this volumetric overload concept and compared alveolar clearance rates observed in different rat studies with different types of particles on the basis of the retained volumetric lung burden. Figure 2 represents their findings of a very good correlation between increasing retained volumetric lung burdens of very different types of particles and diminishing lung clearance rates. Diminished clearance rates occurred for both the chronically inhaled different particle types and the additionally administered trace amounts of labeled microspheres for measuring lung clearance. Inasmuch as the particle load is not evenly distributed among AM (Lehnert *et al.*, 1992), large numbers of AM do not contain particles and yet are apparently not able to efficiently clear small doses of newly deposited particles. Bellmann *et al.* (1991) suggested the existence of strong chemotactic signals generated by particle-containing AM which in turn will affect the clearance function of AM with no or very low burden, and Morrow (1992) in addressing this problem pointed out some experimental evidence that AM phagocytic ac-

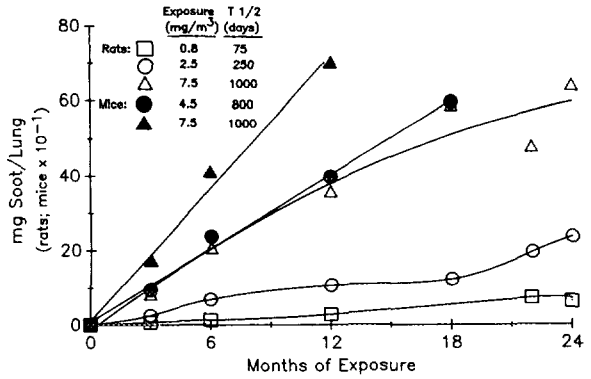

FIG. 3. Accumulation of diesel soot particles in lungs of rats and mice with estimated pulmonary retention halftimes during chronic exposure (after data from Heinrich *et al.*, 1992).

tivity and AM mobility can be affected by factors generated in the alveolar space. Clearly, basic mechanistic events have yet to be understood to explain all facets of the overload condition; however, the volumetric overload concept at present provides a plausible answer to explain prolonged particle clearance rates in the overloaded lung.

Since the particulate volume–clearance rate correlations shown in Fig. 2 are based on findings from rat inhalation studies, the question arises whether other species show similar effects in high-exposure inhalation studies with particles. Among the few studies providing sufficient information to allow such species comparison are the chronic diesel soot inhalation studies in rats and mice and the chronic toner inhalation studies in rats and hamsters at the Fraunhofer Institute in Germany (Heinrich *et al.*, 1992; Muhle *et al.*, 1990a). Figures 3. and 4 show results of these studies with respect to particle accumulation during the chronic exposure. Alveolar retention halftimes estimated from these data show the characteristic increase in retention halftime with increasing exposure concentration and lung dust burden, respectively, in the diesel study in rats; the high-exposure groups did not show equilibrium lung burdens. With respect to mice it is evident from Fig. 3 that pulmonary lung burdens in the mice also did not reach an equilibrium at the 12- or 18-month exposure time points. Based on a pulmonary retention halftime of about 55 days for low particle burdens in mice (Kreyling, 1990), equilibrium lung burdens are expected to be reached after about 300 days of exposure. Likewise, hamsters exposed to high concentrations of toner particles showed a continuous increase in lung burden reflecting a decreasing alveolar clearance rate for toner with increasing lung burdens rather than achieving an equilibrium (Fig. 4).

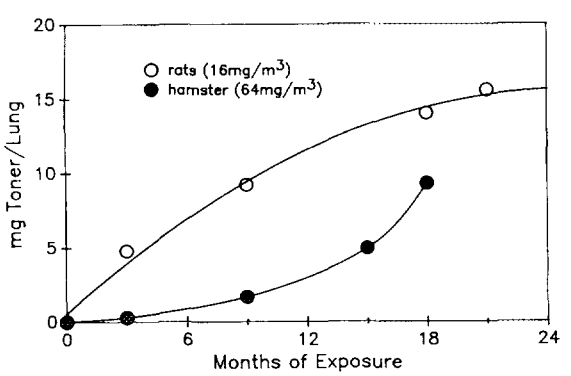

FIG. 4. Pulmonary accumulation of toner particles in lungs of rats and hamsters during chronic exposure (after data from Muhle *et al.*, 1990a).

TABLE 2
Increase in Retention Halftime with Volumetric Lung Burden of Particles

Lung burden (μl/g lung)	Rat	Mouse	Hamster
1	1.5	1.3	1.8
5	4.4	4.3	7.3
10	6.9	8.0	12.5
20	12.6	22.8	(22.5)

Note. Relative $T_{1/2}$ for trace amount of particles = 1.0

Table 2 compares the relative increase in alveolar retention halftime with increasing lung burden in rats, mice, and hamsters estimated from this database. The alveolar retention halftimes in mice and hamsters, like those in rats, increase significantly with increasing volumetric lung burden, and the conclusion is justified that the phenomenon of impaired lung clearance due to high particulate lung burdens occurs not only in rats but also in other mammalian species. Is this also true for humans? An obvious group in which to examine such effects would be coal workers, who are known to have been exposed to high concentrations of airborne particles resulting in large lung burdens of coal dust accumulating over time. Indeed, Stöber *et al.* (1965) determined coal dust burdens in miners' lungs ranging from <5 to >40 grams/lung (average ~15 g/lung). Translating these human pulmonary dust burdens to the rat lung indicates that a lung overload condition had clearly been reached, i.e., on average ~15 mg/g lung. Stöber *et al.* (1965) estimated also the retention kinetics of coal mine dust in the miners' lungs based on their exposure history and concluded that the alveolar retention halftime of this dust was ~5 years. This is considerably longer than the normal retention halftime for particles (trace doses) determined in the well-designed study in human volunteers by Bailey *et al.* (1985). They calculated pulmonary retention halftimes of ~400 days (ranging from ~180 to 700 days). Likewise, Freedman and Robinson (1988) determined by a magnetopneumographic method that the long-term alveolar retention halftime in coal miners was substantially longer than the retention halftime in

TABLE 3
Pulmonary Effects in Different Species Related to High Particle Load

	Rat	Mouse	Hamster	Evidence in coal workers[a]
Prolonged particle clearance	++	++	++	[X]
Inflammation	++	+	(+)	X
Cell proliferation	++	+	(+)	X
Fibrotic foci	++	+/−	(+)	X
Localized emphysema	+	−	(−)	X
Tumors	++	−	−	−

Note. Overall response to highly insoluble low-toxicity particles: rats > mice > hamsters; rats > primates (?).

[a] The evidence in coal workers (X) is not meant as a quantitative comparison to the three rodent species but merely indicates that a given adverse response has been observed in these workers. As detailed in the text, indirect evidence ([X]) in coal workers for prolonged lung clearance comes from studies by Friedman and Robinson (1988), Friedman *et al.* (1988), and Stöber *et al.* (1965).

nonminers measured by them with the same method in a different study (Freedman *et al.*, 1988). Thus, these findings in a human population with high dust exposures are consistent with the concept of high particulate lung burden causing prolongation of dust clearance, quite similar to what has been found in experimental animals.

Overload-Associated Effects: Responses beyond a Threshold?

As described in the previous section, adverse effects observed in chronic rat inhalation studies include inflammation, cell proliferation, focal fibrosis, localized emphysema, and benign and malignant lung tumors. Although prolonged particle clearance occurred also in mice and hamsters (Table 2), subsequent chronic effects (e.g., fibrosis) have been seen in mice to a much lesser degree, and hamsters were even less responsive than mice in this respect. In fact, increased incidences of lung tumors have not been observed in either mice or hamsters in chronic nonfibrous particle inhalation studies. Table 3 compares the different rodent species in this regard based on studies recently summarized by Muhle *et al.*, (1990a), Morrow *et al.* (1991, 1992), Dungworth (1994), and Mauderly (1994). The typical focal accumulation of particle-laden AM observed in particle overloaded lungs of rats does not occur to the same degree in mice in which this response remains more diffuse (Henderson *et al.*, 1988). This might be an important difference contributing to the difference in chronic events between the two species. Furthermore, antioxidant (glutathione) levels in lung tissue during particle exposure were found to increase in mice but not in rats (Henderson *et al.*, 1988), which conceivably also significantly influences the development of chronic lung injury if it is based on oxidative damage (see below). Clearly, the rat is the more sensitive species among the three to show lung overload-associated chronic adverse effects, and the hamster appears to be the least sensitive. Which might be the more appropriate species to use for extrapolating such effects to humans?

Evidence in coal workers (Table 3) shows that there is some degree of chronic inflammatory and cell proliferative responses, and the occurrence of fibrosis and emphysema is well established in these workers; however, no epidemiological study so far has shown evidence of increased lung tumor incidences in these workers; on the contrary, even lower lung tumor incidences than expected have been reported in some studies (Merchant *et al.*, 1986; Parkes, 1982). Epidemiological studies on the carcinogenicity of other particle types which had induced lung tumors in the overloaded rat lung, i.e., diesel soot and carbon black, either showed no increased pulmonary carcinogenicity (carbon black; Robertson and Ingalls, 1989) or that there was limited evidence for increased carcinogenicity at low exposure concentrations which predictably will not lead to lung particle overload (diesel exhaust; Garshick *et al.*, 1987, 1988). Possibly, carcinogenic organic compounds adsorbed on diesel particles play a significant role even at low exposure levels in lungs of diesel-exposed workers, whereas they are apparently unimportant for lung tumor induction in the particle-overloaded rat lung (Oberdörster and Yu, 1990). It appears, therefore, that the lung tumors associated with a high pulmonary particle load in the rat studies are a very species-specific response to low-toxicity nonfibrous particles, do not occur in any of the other laboratory species, and may also not occur in humans.

Suggested mechanisms underlying overload-associated effects in the rat lungs are schematically depicted in Fig. 5. Focal points in this scheme are inflammatory processes with activation of AM and neutrophils (PMN) upon particle uptake with concomitant release of a number of mediators which include inflammatory cytokines and growth factors of important biological activities with respect to cell–cell interactions (Driscoll *et al.*, 1990; Driscoll and Maurer, 1991) and oxidants and proteases. Some of these mediators in turn can lead to epithelial damage and increased epithelial cell proliferation as well as interstitial cell proliferation, the latter of which is amplified by the increasing access of particles into the pulmonary interstitium where they can directly interact with interstitial cells (Adamson *et al.*, 1989; Bowden *et al.*, 1989). These interstitial events in conjunction with the previously mentioned focal accumulation of AM in the alveolar space (i.e., high focal levels of released mediators) then could initiate the formation of fibrotic foci. In addition, the interstitial access of particles conceivably creates chemotactic stimuli leading to a shift in the chemotactic gradient from the alveolar space to the interstitium (Oberdörster *et al.*, 1992b) which could contribute to the inhibition of AM-mediated clearance of particles toward the mucociliary escalator. Fur-

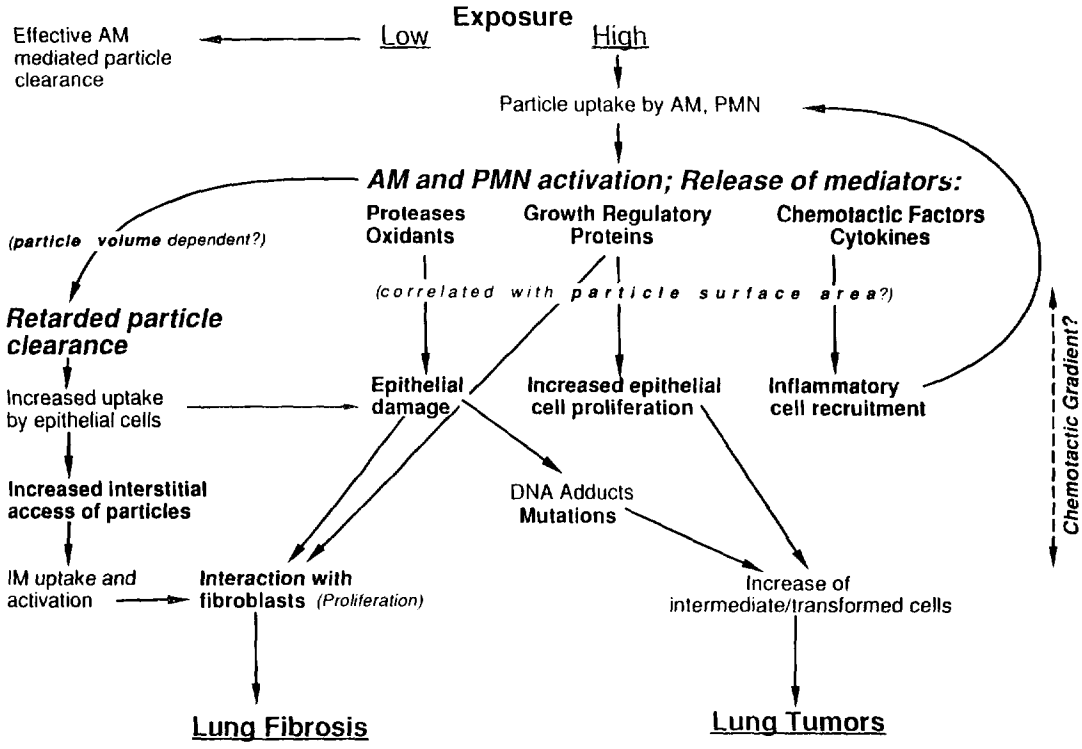

FIG. 5. Suggested mechanisms to explain particle overload-associated adverse effects in rat lungs during chronic particle exposure.

thermore, formation of DNA adducts can occur as described after exposure of rats to diesel soot and carbon black particles (Bond *et al.*, 1990; Wolff *et al.*, 1990), possibly due to the action of oxygen-derived radicals; resulting increased mutational rates may eventually lead to lung tumor formation. Thus, there appears to be a sequence of pulmonary events during high particle exposure in rats involving AM activation → acute inflammation and impaired clearance → further particle accumulation → chronic inflammation and focal fibrosis → epithelial cell proliferation → mutations → metaplasia → lung tumors.

Since low lung burdens of particles can be effectively eliminated from the lung by AM-mediated particle clearance, it is likely and biologically plausible that adverse responses seen in the rat lung at higher lung burdens reflect a nonlinear response, and it is tempting to predict that below a certain threshold of lung particle burdens none of these effects would occur. What is, therefore, the evidence for the existence of a threshold?

As discussed in the first section of this article, prolongation of AM-mediated particle clearance, the hallmark of lung overload, occurs only when a certain particulate lung burden equivalent to a specific AM load is achieved and exceeded during a chronic inhalation study (see Fig. 2). This impairment of clearance develops when the deposition rate of inhaled particles is greater than their alveolar clearance rate, and we can safely propose that a threshold dose exists in the lung for this specific effect. Evidence of a threshold for the other adverse effects is only conjectural, i.e., for chronic inflammation, fibrosis, cell proliferation, mutation, and lung tumors. If all of these are a consequence of the impaired particle clearance and the subsequent accumulation of particles in the lung, there may be a likely mechanistic sequence from chronic inflammatory events to cell proliferation to mutation and to lung tumors as discussed above (Fig. 5). Indeed, as reported recently by Driscoll *et al.* (1994), *in vitro* exposure of rat lung epithelial cells to inflammatory cells lavaged from the lungs of quartz-exposed rats resulted in a significantly increased mutation frequency of the *hprt* gene, indicating that mediators (e.g., oxidants) released from inflammatory cells are causally involved in mutational events. Interim results from our laboratory of a subchronic inhalation study with different concentrations of carbon black particles in rats show that at the end of a 13-week exposure period inflammatory lung lavage parameters, in particular influx of PMN, are not increased at the lowest exposure concentration (Fig. 6). Mutational events in the alveolar epithelial cells of the carbon black-exposed rats seem to parallel the cellular inflammatory responses, consistent with the results of Driscoll *et al.* (1994). The ongoing evaluation of the cell proliferative response and mutation frequencies of the epithelial cells in this subchronic carbon black study will show whether the hypothesized correlation exists between these endpoints and the observed cell-ular inflammatory response. The present result would be consistent with the

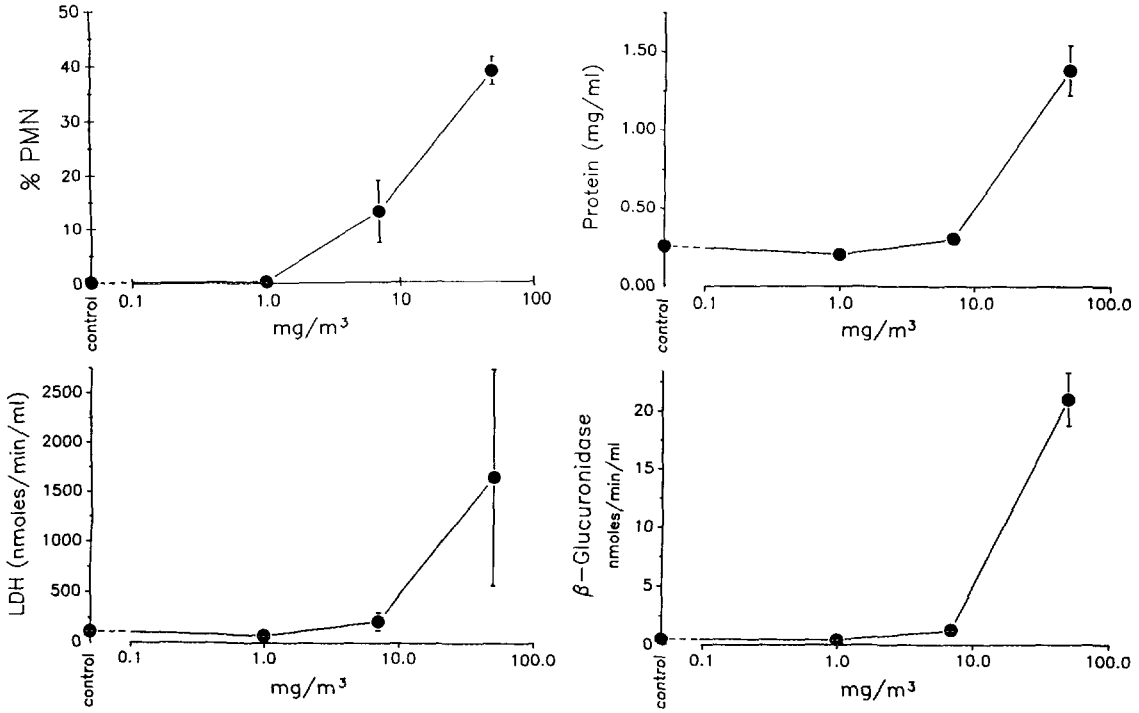

FIG. 6. *Cellular and noncellular parameters in lavage fluid of extensively lavaged lungs of rats after 13 weeks of exposure for 6 hr/day, 5 days/* week to 1.0, 7.0, and 50 mg/m^3 of carbon black. The neutrophil (PMN) response seems to be the most sensitive endpoint and is expressed as percentage cells of total lavaged cells.

concept of a threshold for the inflammatory response associated with the overload phenomenon.

With respect to fibrogenic and tumorigenic effects in long-term rat inhalation studies with particles of low cytotoxicity, there is good evidence that these effects occurred only when pulmonary clearance was disturbed. The scheme in Fig. 7 displays these observations: The normalized lung burden (normalized to the exposure concentration) in the rat during a chronic inhalation study with particles not inducing impaired clearance is represented by a common accumulation curve exhibiting an equilibrium since the alveolar deposition rate of inhaled particles is less or at the most equal to their alveolar clearance rate. This curve reflects the fact that under normal conditions the equilibrium lung burden is linearly correlated with the exposure concentration. However, in cases where the deposition rate exceeds the clearance rate the normalized lung burdens will accumulate more than predicted, fibrosis is likely to occur in the rat lung, and lung tumors could be—but do not necessarily have to be—induced at a later stage. For example, chronically inhaled particle concentrations of TiO_2 at 50 mg/m^3 or of toner at 16 mg/m^3 clearly lead to overload-induced prolonged pulmonary particle clearance and fibrosis in rats but did not induce lung tumors, whereas 250 mg/m^3 TiO_2 additionally resulted in the induction of lung tumors (Lee *et al.*, 1985; Muhle *et al.*, 1991). These nonlinear exposure–dose–response relationships support the concept of a threshold phenomenon for lung tumors observed in the chronically particle-overloaded rat lung. However, more data are needed to better understand the basic mechanisms of particle-induced lung tumors, e.g., the importance of inflammatory cytokines and of reactive oxygen species for proliferative and mutational events.

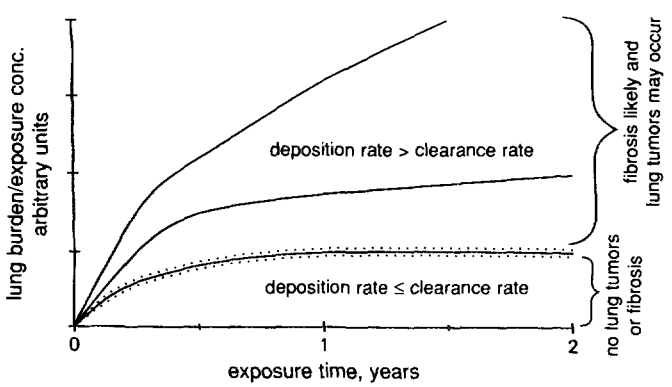

FIG. 7. Pulmonary accumulation of particles normalized by the exposure concentration and the correlation with chronic adverse effects during a chronic rat inhalation study indicating the existence of a threshold below which no fibrogenic or tumorigenic pulmonary responses occur. Dotted lines indicate some variation around normal curve.

Although the existence of a threshold for particle

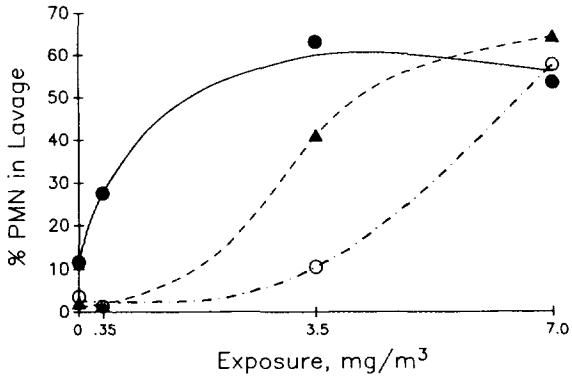

FIG. 8. Exposure–response relationships for alveolar inflammation as determined by neutrophils in lung lavage of rats exposed for different time periods to diesel exhaust, 7 hr/day, 5 days/week (Henderson *et al.*, 1988). The response is expressed as percentage neutrophils of total nucleated cells in lung lavage. The shape of the response curve changes with increasing exposure duration and age of the animals. Exposure duration: ○, 6 months; ▲, 12 months; ●, 24 months.

overload-associated effects is highly likely, the question arises whether a given threshold indicated from results of a subchronic study (e.g., alveolar inflammation) will remain the same during the course of a chronic study. For example, does the shape of the curve describing the cellular inflammatory response after a 3-month subchronic inhalation exposure (Fig. 6) persist throughout a chronic 2-year study? Analyses of the inflammatory response after 2 years of exposure to diesel exhaust in rats (Creutzenberg *et al.*, 1990) showed that also at lower exposure concentrations a significant increase in lavageable neutrophils occurred, reflecting a shift compared to the subchronic cellular response to particles shown in Fig. 6. This apparent change in the shape of the exposure–response curve is also demonstrable by results of Henderson *et al.* (1988) shown in Fig. 8. During the course of a chronic exposure to different concentrations of diesel exhaust the shape of the exposure–response for lavage neutrophils changed significantly. This points out that chronic exposure even to low particle concentrations eventually results in an increased neutrophil response although the lung particle burden at 24 months in the low-exposure group was only 0.6 mg diesel soot (Mauderly *et al.*, 1987) and no impaired lung clearance was seen (Wolff *et al.*, 1987). Several mechanistic factors, including animal age-related greater sensitivity, could be involved but will not be discussed here. However, it is of importance to realize that predictions of a specific threshold based on results of a subchronic study may not be valid for the chronic situation. There can be a change in the response with increasing age during continuing exposures commensurate with a significant lowering of a potential threshold. This may have implications also for the selection of a maximum-tolerated dose for chronic exposure studies.

THE MAXIMUM TOLERATED DOSE IN CHRONIC INHALATION STUDIES

One conclusion from the discussions of the particle overload phenomenon is that the rat tumor response is very likely a secondary effect due to the altered lung clearance kinetics resulting in excessively high lung burdens and chronic inflammatory and cell proliferative processes. So far, particle overload-associated lung tumors have not been described in humans; neither have they been observed in hamsters and mice. Obviously, the same retained particle dose in lungs of rats on the one hand and of other species on the other hand does not induce the same response with respect to lung tumors. Is the rat, therefore, a valid model for extrapolation of particle effects to humans? The answer may be no, although we do not know enough about underlying mechanisms of particle-induced lung tumors to fully understand and explain why these significant species differences exist. Until we know more about mechanistic events we may not exclude the possibility that an overloaded lung in humans could also respond with induction of tumors. However, it has to be asked also whether the exposure concentrations used in rat chronic inhalation studies bear any relevancy to encountered lower human exposure conditions, knowing that the tumor response cannot be extrapolated to low concentrations linearly but follows a nonlinear response, very likely exhibiting a threshold. Clearly, the particle-overloaded rat lung responds differently in terms of lung tumor induction than the lungs of other mammalian species tested so far, and we need to decide what the highest exposure level in a rat chronic inhalation study should be. At issue here is the definition of a maximum tolerated dose (MTD) for chronic rodent inhalation assays.

An NCI publication by Sontag *et al.* (1976), "Guidelines for Carcinogen Bioassay in Small Rodents," defines the MTD as follows: "The MTD is defined as the highest dose of a test agent during the chronic study that can be predicted not to alter the animal's longevity from effects other than carcinogenicity." Sontag *et al.* (1976) further suggested that this dose should not produce mortality, clinical signs of toxicity or pathologic lesions, or cause more than a 10% decrement in body weight gain unless the effects are related to a neoplastic response. Subsequently, Haseman (1985) and McConnell (1989) addressed the issue of metabolic overload in the context of selecting an MTD. These authors stress that altered pharmacokinetics due to an extremely high exposure of the animals to the test compound should be carefully evaluated; if it is shown that overload produced the carcinogenic effect in the animal study because the compound does not operate in the range of linear kinetics, this should question the relevancy for human exposure if any observed or anticipated human exposure is well below the level that could trigger a similar carcinogenic response in humans. Recognizing that excessively high

exposures to toxicants can lead to effects which are irrelevant for expected lower human exposures, different agencies recommended similar approaches emphasizing also additional endpoints to be evaluated such as histopathological changes, toxicokinetics, and metabolic parameters for considering metabolic overload at higher doses (IARC, 1980; OSHA, 1980; OECD, 1981; EPA, 1982; NTP, 1984; ILSI, 1984; OSTP, 1985). A suggested standard NCI/NTP design for a chronic carcinogenicity study requires a life-long exposure at three dose levels in rodents, with exposures for the highest dose being at the MTD and subsequent lower doses at one-half MTD and one-quarter MTD (Haseman, 1985).

For chronic inhalation studies with highly insoluble particles of low cytotoxicity the particle overload phenomenon and the MTD question are intimately interrelated. Recommendations of a NTP Workshop on Maximal Aerosol Exposure Concentrations in Inhalation Studies (Lewis *et al.*, 1989) included: The chronic study should not be performed at the highest technologically feasible concentration; three concentrations should be used of which only the highest should show some interference with lung defense mechanisms, i.e., clearance impairment; and the two lower concentrations should show no interference with clearance and particle accumulation. The determination of the highest chronic exposure concentration could be based either on model predictions or on results of a subchronic study in which several exposure levels are tested. Detection of overload could be achieved by administering a labeled test particle probe to the lungs to determine interference with lung clearance. It was emphasized by the workshop participants that it is highly important to determine the kinetics of the test material in the lung as a function of time to recognize altered particle kinetics.

The idea of making use of altered pulmonary particle clearance kinetics to define an MTD was further discussed by Muhle *et al.* (1990b), who suggested defining a maximum functionally tolerated dose (MFTD) for chronic inhalation studies which they arbitrarily defined as a particulate lung burden causing a two- to fourfold prolongation of particle clearance. This appears to be a reasonable suggestion for defining the highest exposure concentration for a chronic particle inhalation study in rodents since it would allow the detection of nonlinear accumulation and retention kinetics in the lung indicative of the particle overload condition (Fig. 7). The prolongation of particle clearance would be determined either through the accumulation kinetics of the particulate compound itself or with labeled test particles in a subchronic study as suggested by Lewis *et al.* (1989).

In addition to this functional parameter it would be very useful to include an evaluation of inflammatory lung lavage parameters, which are very sensitive indicators of pulmonary toxicity, as well as histopathological evaluations (Oberdörster, 1994). How to proceed when selecting the MTD based on either of these different endpoints needs careful consideration. For example, is there a level of pulmonary inflammation determined by cellular lavage parameters that can be defined to reflect a MTD? A possible shift of the exposure–response relationship during a chronic assay (Fig. 8) needs to be considered in this context. Such changes emphasize the difficulty in predicting and selecting the MTD from a subchronic study. A consensus among scientists is urgently needed about the definition and selection criteria of the MTD or MFTD in rodent chronic inhalation assays. In addition, we need to agree on the interpretation of study results derived from exposure levels and related lung doses which substantially exceed the MTD.

EXTRAPOLATION TO HUMANS

We can deduce from the foregoing discussion that any highly insoluble particle of low cytotoxicity (e.g., TiO_2, carbon black, diesel soot) will cause lung tumors in rats if accumulating chronically at high enough doses in the lung due to an overload response, i.e., such particles have the potential to cause lung tumors in rats as a secondary response. The emphasis is on the species "rat" and on "high lung doses." However, the existence of a carcinogenic *potential* of inhaled particles in rats is not synonymous with their carcinogenic *potency*. Furthermore, should a particulate compound showing a rat pulmonary carcinogenic potential also be labeled as having a human pulmonary carcinogenic potential?

Some might argue that the types of tumors seen in the rat after particle exposures and their localization are different from tumors found in humans. Tumors diagnosed in rat studies after chronic exposure to carbon black particles and diesel exhaust included benign adenomas, malignant adenocarcinomas, squamous cell carcinomas, adenosquamous carcinomas, and also squamous cysts (Mauderly *et al.*, 1994). Squamous cell carcinomas and adenocarcinomas were found to grow after transplantation into athymic mice, whereas squamous cysts did not (Mauderly *et al.*, 1994), demonstrating that the tumors diagnosed as malignant were indeed neoplasms which would be relevant for humans.

Clearly, however, chronic exposure concentrations as high as 250 mg/m^3 used in a TiO_2 inhalation study (Lee *et al.*, 1985) resulting in lung tumors in rats are completely irrelevant for chronic human exposures, and we should be reasonable in the selection of our experimental exposure concentrations with respect to MTD selection. On the other hand, a particle may be viewed differently if inhaled concentrations of 1 mg/m^3 lead to lung tumor formation in rats as was found in a chronic inhalation study with quartz (Muhle *et al.*, 1989). ACGIH (1993) in its carcinogenicity classification introduced a Category A3, animal carcinogen: "The agent is carcinogenic in experimental animals at a relatively high dose, ... or by mechanisms that are not considered relevant to worker exposure." And it concludes: "Available evidence sug-

gests that the agent is not likely to cause cancer in humans except under uncommon or unlikely routes or levels of exposure." The introduction and definition of a category "animal carcinogen" appears to be more useful and less controversial than classification of a compound as "probably carcinogenic" or "possibly carcinogenic" for humans, terms used by EPA (1986) and IARC (1980) in their classification schemes.

Certainly, the issue of carcinogenicity is extremely important. On the other hand, we should not forget that lung tumors—although attracting most of the attention—were not the only response in rat inhalation studies with highly insoluble low toxicity. Lung fibrotic reactions occurred as well which have also been observed in human populations heavily exposed to different types of particles. The focus on tumorigenicity may, therefore, not be justified, and lung fibrosis has to be considered as well. Both of these responses seem to follow a nonlinear dose–response relationship. Conceptually, preventing lung particle overload-induced impaired lung clearance should also prevent tumorigenic and fibrogenic responses (Fig. 7).

If we accept this concept we can perform some very simple calculations to estimate an occupational exposure level which should provide protection from lung overload. The basis is an equilibrium lung burden which will not cause overload and which is determined by a deposition rate of inhaled particles which is less than or at the most equal to the clearance rate. Assuming for this purpose that a maximum particulate lung burden of 2 mg/g lung should not be exceeded, i.e., this level should be the equilibrium lung burden during a life-long chronic exposure, that would amount to ~2000 mg/human lung at a lung weight of ~1000 g. Three milligrams/day would be cleared applying an average fractional clearance rate of 0.0015 mg/day, which can be applied to the bulk of the material to be cleared (Bailey *et al.*, 1985). The daily deposited amount should at the most be equivalent to this amount cleared per day to avoid overload. Assuming that on average 25% of the inhaled particles will be deposited in the alveolar region, 12 mg of particles can be inhaled daily. If this dose is inhaled during one workshift in a total volume of 10 m^3 air, the inhaled concentration would be 1.2 mg/m^3, which would be a level to avoid lung overload of highly insoluble low-toxicity particles in humans.

A somewhat more refined approach could make use of our understanding of mechanistic aspects involving the volumetric loading of AM with particles suggested by Morrow (1988). As discussed in this article before, prolongation of clearance due to high particle load has been observed in all experimental animals tested so far for this purpose, and overload seems also to occur in humans with heavy dust lung burdens, i.e., coal workers. If we accept, therefore, that a loading of the AM with particles averaging 6% of their volume will signal the beginning stages of an overloaded lung, we can calculate such volumetric lung load of particles using published data about AM numbers and sizes in rat and human lungs. This volumetric particle-lung burden can further be translated to an inhaled particle concentration using well-developed mathematical deposition models of inhaled particles in humans and rats (Schum and Yeh, 1980; Yeh and Schum, 1980). Table 4 shows the result of such calculation using published data on average AM volumes in rats and humans (Crapo *et al.*, 1984; Dethloff and Lehnert, 1988) and assuming a particle density of $\rho = 1$. It turns out (Table 4) that for both rats and humans a 6% AM volume is equivalent to about 1 μl particle volume per gram of lung. If this level is not to be exceeded in the human lung, an occupational exposure limit for a unit density particle of low cytotoxicity should be slightly less than 1 mg/m^3 of respirable particles, indicating that present threshold limit values (TLV) for PNOCs of 10 mg/m^3 are too high. For particles of different density, a density correction needs to be applied, e.g., if we assume that on average PNOCs may have a density of 3 g/cm^3, respective occupational limit values would be 3 mg/m^3.

TABLE 4
Extrapolation of AM Volume Load from Rat to Man

	Average AM volume (μm^3)	AM numbers in lung	6% AM volume (μl/g lung)	Concentration of inhaled particles (d = 3 μm; ρ = 1) to reach 6% AM volume (mg/m^3)[a]
Rat	1000	2.6×10^7	1.04	3.3 (nasal-breathing)
Human	2500	7.0×10^9	1.11	0.8 (oro-nasal-breathing)

[a] During occupational exposure: 8 hr/day; 240 days/year. Man: TV 1250 ml; resp. rate 16 min^{-1}; lung weight 950 g; $T_{1/2}$ = 400 days. Rat: TV 2 ml; resp. rate 73 min^{-1}; lung weight 1.5 g; $T_{1/2}$ = 75 days.

The important conclusion from our present understanding of lung particle overload is that we are dealing with a nonlinear response in terms of associated adverse chronic effects, most likely involving a threshold. It is, therefore, conceivable that these effects can be prevented by avoiding an impairment of the lung particle clearance function, thereby preventing concomitant inflammatory and cell proliferative events in the lung since particle-induced tumors are likely secondary to continued inflammation, tissue damage, and cell proliferation.

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