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Dust Overloading of Lungs: Investigations of Various Materials, Species Differences, and Irreversibility of Effects

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ABSTRACT

In separate inhalation investigations, rodents (Wistar rats, Fischer-344 rats, Syrian golden hamsters, NMRI and C57BL mice) were exposed to various dusts such as test toner (polymer pigmented with carbon black), polyvinyl chloride, carbon black, diesel exhaust and two crystalline forms of titanium dioxide (anatase and rutile). The animals inhaled various concentrations (0.8 to 64 mg/m^3) of these particles for up to 2 years.
Alveolar clearance retardation was detectable above a retained pulmonary burden of 0.5 mg per rat lung, and a substantial decrease in the clearance rate (about a factor of 6) was observed following heavy dust loading, exceeding 10 mg dust per rat lung. Above a threshold lung burden, signs of lung overloading persisted 15 months after cessation of exposure in F-344 rats. Retardation of alveolar clearance was also observed in hamsters, commencing at higher lung burdens (normalized to lung weight) than in rats. At high dust exposure levels, persistent pulmonary inflammation was present in both species. In rats the concentration of lavagable cells remained constant, with decreased macrophages and increased polymorphonuclear neutrophils (PMN) noted, while in hamsters, the cell count increased substantially in both macrophages and PMN's. A retarded particle clearance was also observed in mice at a lung burden above 1 mg/lung.
These results, accompanied by published accounts, indicate that the lung overloading phenomenon is noted among a variety of species and materials. It is generally observed upon exceeding a threshold lung burden with particles of low solubility and low acute toxicity for considerable periods of time.

INTRODUCTION

The Fraunhofer Institute of Toxicology and Aerosol Research has investigated the inhalation toxicity of insoluble particles for more than 10 years. The studies were performed for various reasons; therefore different species, materials and exposure conditions were selected. Since several of these investigations were conducted under Good Laboratory Practice (GLP) guidelines according the Organisation for Economic Co-Operation and Development (OECD) , standard measurements such as body weight, food consumption, biochemical and

Key words: Lung, retention, alveolar clearance, insoluble particles, dust overloading, inflammation, fibrosis

hematologic parameters, organ weight, histopathologic examinations etc. were performed. In addition, in most of these investigations we measured particle deposition, clearance and retention, a number of biochemical and cytological parameters and pulmonary function.
The characteristic findings at the highest exposure level in most of these rodent inhalation studies included: increased lung weight, disproportionate retention of test material, i.e. the ratio of the retained mass to the aerosol concentration increased, decreased or obliterated alveolar clearance, impaired pulmonary function and persistent inflammatory responses. These changes were reflected by a variety of histopathological alterations which are only partly covered within this paper and are reported separately. The pulmonary changes were ascribed to "lung overloading", a generic response of the lung to the long-term presence of large quantities of insoluble particles. Dust overloading of the lungs was reported in various studies (Ferin and Feldstein 1979; Vostal et al. 1982; Muhle et al. 1984 and 1988; Vincent et al. 1985; Wolff et al. 1987). This paper will focus on some of the common outcome characteristics of various studies conducted in our laboratory.

MATERIALS AND METHODS

The properties of the various particles evaluated are shown in Table 1. The test toner used in copy machines was composed of about 90 % styrene/1-butylmethacrylate, a high molecular weight random copolymer (CAS 25213-39-2) and 10 % of a medium color, high purity furnace type carbon black (CAS 1336-86-4). A 9000-type xerographic toner material (Xerox Corp. Rochester, USA) was specially prepared for this study and was enriched about 10-fold in respirable sized particles, relative to the commercial toner, such that it was about 35 % respirable according to the American Conference of Governmental Industrial Hygienists (ACGIH) criteria.
Polyvinyl chloride powder (PVC, Chem. Werke Hüls, FRG) and two types of titanium dioxide (TiO_2, rutile, type "Bayer Titan T", Bayer AG, FRG and anatase, type "P 25", Degussa, FRG) were used. A characteristic difference between the two types of TiO_2 was the diameter of the primary particles (Table 1).

TABLE 1

Properties of the Particles

Particle type	Diameter of primary particles [µm]	Mass median aerodynamic diameter [µm]	Geometric standard deviation	Density [g/cm^3]
Test toner (carbon black pigmented polymer)	~4	4	1.5	1.15
Polyvinyl chloride powder (PVC)	~1.3	1.3	2.07	1.3
Titanium dioxide (rutile)	0.2-0.7	1.1	1.6	4.26
Titanium dioxide (anatase)	0.02-0.04	0.80	1.8	3.84
Carbon black (Printex 90)	~ 0.014	0.64	2.1	~ 2
Diesel exhaust particles	~ 0.05	0.25	2.9	~2

High purity carbon black (type "Printex 90", Degussa, FRG) was taken as a reference material to diesel exhaust. The fraction extractable by toluene was < 0.1 %.

A 40 kilowatt 1.6 l diesel engine served as a source for the exhaust emissions. The engine was mounted on a computer-controlled test bench and was operated continuously according to the US 72 test driving cycle. For further details see Heinrich et al. (1986).

A dry aerosol dispersion technique was used for the first 5 types of particles listed in Table 1 (Koch et al. 1986). Aerosols of carbon black and anatase consisted of aggregates of primary particles.

Animals were exposed in horizontal flow type whole body inhalation chambers (Heinrich et al. 1985) at aerosol concentrations listed in Tables 2 and 3. Animals inhaled one of these test materials for up to two years. Results shown in these tables originate from female rats; pooled data of males and females are given only in Study A. In Study E, Syrian golden hamsters were used. After serial sacrifices during the course of the studies, the retained mass in lungs was analyzed (5-8 lungs per investigation). After digestion of the lung tissue, toner, PVC, carbon black and diesel soot were determined by light absorption spectroscopy. Titanium dioxide was analyzed by atomic absorption spectroscopy.

Alveolar clearance was determined at various times within the studies using short-term nose only exposure to radioactive labelled tracer particles (^{85}Sr-polystyrene with an MMAD of about 3.5 μm). The decrease of the γ-activity in the thoracic area was measured over a 75-100 days period (Bellmann et al. 1989) .

For calculation of the clearance rate coefficients or half-times, an exponential curve fit was performed on these data excluding measurements before day 15 to omit faster clearance processes of the upper respiratory tract and to allow a simplified and concise description of the clearance kinetics.

The total dust concentration and the duration of exposure of the various studies, the retained mass, the retained volume and the half-time of the alveolar clearance are presented in Tables 2 and 3. With the exception of Study A the retained mass reflects the value at the middle of the period in which clearance of labelled particles was measured, which enables documentation of the relationship between both parameters. In cases in which the retention was not determined at this date, the values were interpolated from adjacent measurements.

Bronchoalveolar lavagate was obtained by a twofold lavage with 4 ml saline. The lavagate was analyzed for cytological and biochemical parameters (Henderson et al. 1987).

For statistical analysis, data were examined by analysis of variance (ANOVA), followed by Dunnett's test to compare various treatment groups with controls.

RESULTS

Subchronic Inhalation Study of Toner in Rats (study A)

Fischer-344 rats were exposed for 90 days to 1, 4, 16 and 64 mg/m^3 of toner. The retained quantities of toner at the end of the exposure period are shown in Table 2. The greater than proportional increase of retained test material at 16 and 64 mg/m^3 compared to 4 mg/m^3 can be seen in Figure 1, where retention is normalized by the product of aerosol exposure concentration and minute volume. The minute volume correction was applied for pooling the results for male and female animals, which had slightly different minute volumes. The latter was calculated from the body weight using the procedure of Stahl (1967). In this investigation, alveolar toner clearance was determined using the retained quantity of toner in excised rat lungs. The results of both sexes are combined only in this study because this direct determination of the retention kinetics led to higher statistical fluctuations compared to the measurements with labelled particles which were used in the other studies. Animals were sacrificed at days 1, 25, 50 and 75, after removal from exposure. Retention

TABLE 2

Mean and Standard Deviation of Exposure Atmospheres, Retained Mass and Particle Volume in Lungs and Half-times of Alveolar Clearance (with 95% Confidence Limits) in Four Different Inhalation Studies in Rats

Material	Total dust concentration [mg/m^3]	Retained mass [mg/lung]	Retained volume [µl/lung]	Half-time of alveolar clearance [days]	
				^{85}Sr PS Mean (95% CL)	Test material[a] Mean (95% CL)
Study A. Exposure: 30 hr/week for 3 months (Muhle et al., 1990a)					
Toner	1.0 ± 0.2	0.085 ± 0.032	0.074		79 (34-∞)
Toner	4.0 ± 0.6	0.275 ± 0.059	0.239		86 (52-245)
Toner	16.1 ± 1.4	1.86 ± 0.28	1.62		186 (107-636)
Toner	63.2 ± 5.3	11.5 ± 0.8	10.0		>1000 (276-∞)
Study B. Exposure: 30 hr/week for 22.5 months (Muhle et al., 1990b, Bellmann et al. 1990)					
Control air	0	0	0	65 (57-77)	
Toner	1.0 ± 0.1	0.19 ± 0.04	0.169	* 84 (74-98)	
Toner	4.1 ± 0.1	1.36 ± 0.36	1.18	** 187 (122-402)	
Toner	16.0 ± 0.9	12.2 ± 1.3	10.6	** 307 (150-∞)	
TiO_2 (Rutile)	5.0 ± 0.7	2.21 ± 0.37	0.519	** 93 (78-117)	
Study C. Exposure: 25 hr/week for 8 months (Bellmann et al., 1986)					
Control air	0	0	0	57 (51-63)	
PVC	3.3 ± 0.5	0.56 ± 0.16	0.43	71 (64-80)	85 (65-123)
PVC	8.3 ± 0.9	2.09 ± 0.29	1.61	** 122 (95-164)	120 (92-169)
PVC	20.2 ± 1.8	7.24 ± 1.10	5.57	** 184 (108-630)	277 (164-884)
Study D. Exposure: 95 hr/week for 4.5 months (Creutzenberg et al., 1990)					
Control air	0	0	0	61 (52-76)	
Diesel exhaust	0.8 ± 0.5	0.95 ± 0.25	0.47	** 109 (72-224)	
Diesel exhaust	2.4 ± 1.2	4.67 ± 1.19	2.3	** 292 (211-475)	
Diesel exhaust	6.8 ± 1.6	14.4 ± 2.3	7.2	**>1000 (902-∞)	
Carbon black	7.4 ± 1.5 [b]	13.7 ± 2.0	6.9	** 472 (283-1420)	
TiO_2 (Anatase)	7.2 ± 1.2 [b]	14.2 ± 2.2	3.7	**>1000 (548-∞)	

- Levels of significance (Dunnett's test of corresponding clearance rate coefficients)
  * $P < 0.05$ ** $P < 0.01$

- [a] Clearance measurement of toner or PVC particles after cessation of exposure

- [b] Exposure concentrations during the first 4 months:
  7.4 ± 1.5 and 7.2 ± 1.2 mg/m^3 for carbon black and TiO_2, respectively.
  Corresponding values for the 4 following months: 12.0 ± 1.5 and 14.8 ± 3.2 mg/m^3 and after 8 months 12.3 ± 1.9 and 9.4 ± 3.2 mg/m^3, respectively.

TABLE 3

Mean and Standard Deviation of Exposure Atmospheres, Retained Mass and Particle Volume in Lungs and Half-times of Alveolar Clearance (with 95% Confidence Limits) in Male Hamster in Study (E)

Material	Total dust concentration [mg/m³]	Retained mass [mg/lung]	Retained volume [µl/lung]	Half-time of alveolar clearance (^{85}Sr PS) Mean (95% CL) [days]
Study E. Exposure: 30 hr/week for 10.5 months				
Control air	0	0	0	109 (91-135)
Toner	4.0 ± 0.3 [a]	0.11 ± 0.03	0.10	* 72 (56-102)
Toner	16.0 ± 0.3 [a]	0.43 ± 0.06	0.37	103 (86-127)
Toner	63.7 ± 1.9 [a]	3.03 ± 0.66	2.63	** 246 (150-672)
TiO_2 (Rutile)	30.6 ± 2.1 [a]	16.7 ± 1.24	3.88	** 641 (279-∞)

- Levels of significance (Dunnett's test of corresponding clearance rate coefficients)
  * $P < 0.05$ ** $P < 0.01$

- [a] Exposure concentrations during the first 5 months:
  1.5 ± 0.2, 6.1 ± 0.4, 24.5 ± 1.6, 39.3 ± 7.3 mg/m³

data of days 25–75 were fitted by an exponential curve $M = M_0e^{-kt}$. The clearance rate coefficient k with the standard error range was obtained for each group by linear regression.
The calculated alveolar clearance half-times are shown in Table 2. At the aerosol concentrations of 1 and 4 mg/m^3, appreciable test material clearance, with a retention half-time around 80 days, was obtained. Some retardation of clearance was observed at 16 mg/m^3.

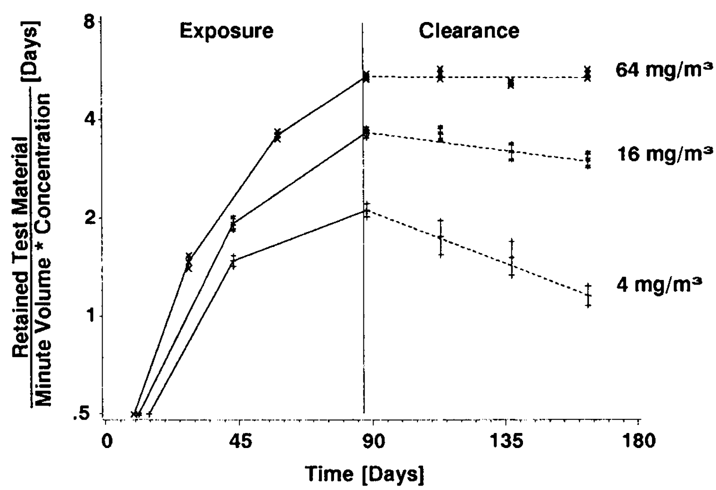

FIGURE 1. Lung Burden of Test Toner during the 90-Day Inhalation Study of Toner in Rats and 90-Day Post-Exposure Period, Normalized to the Minute Volume of the Rats and Exposure Concentration. Pooled Data for Male and Female Animals.

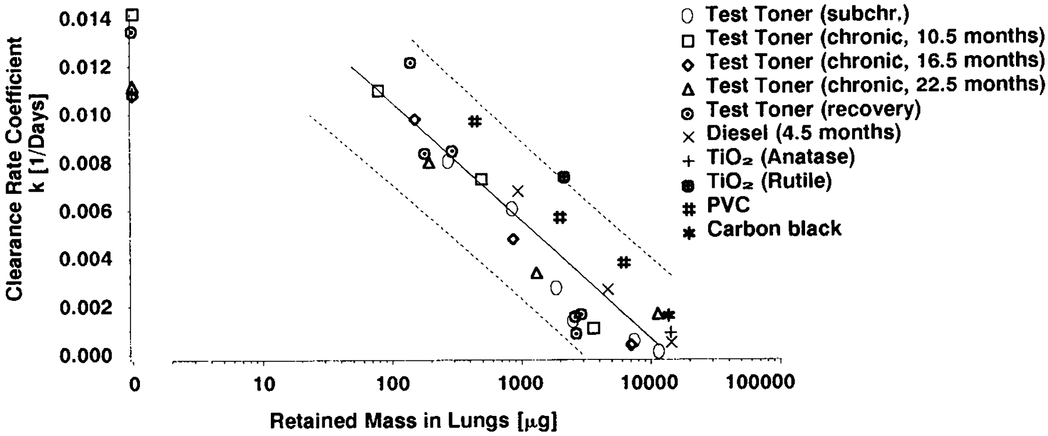

FIGURE 2. Clearance Rate Coefficient of Labelled Particles (^{85}Sr-Polystyrene) or Toner Particles as a Function of the Retained Mass of the Various Test Materials. Compilation of the Various Studies performed in Rats. Lines: Regression Curve with 95% Confidence Interval

At the highest exposure concentration (64 mg/m^3), toner particle clearance had practically ceased. An illustration of the various clearance rates at the respective pulmonary loads (and exposure concentrations) is shown in Figure 2, indicated as test toner (subchr.).
For comparison of effects of particles differing in density, the retained volume of particles is a more useful parameter than the retained mass, as alveolar macrophages show an upper volumetric uptake limit (Bowden 1987, Morrow 1988). Results presented in Figure 3 demonstrate the decrease of the clearance rate coefficient as a function of the retained dust volume in the lungs. This graph also contains results of further studies which will be introduced later. For further details of this study see Muhle et al. (1990a).

Chronic Inhalation Study of Toner and TiO_2 (Rutile) in Rats (Study B)

The quantity of test toner and TiO_2 retained in the lungs of female Fischer-344 rats at 22.5 months of exposure is summarized in Table 2, Study B.

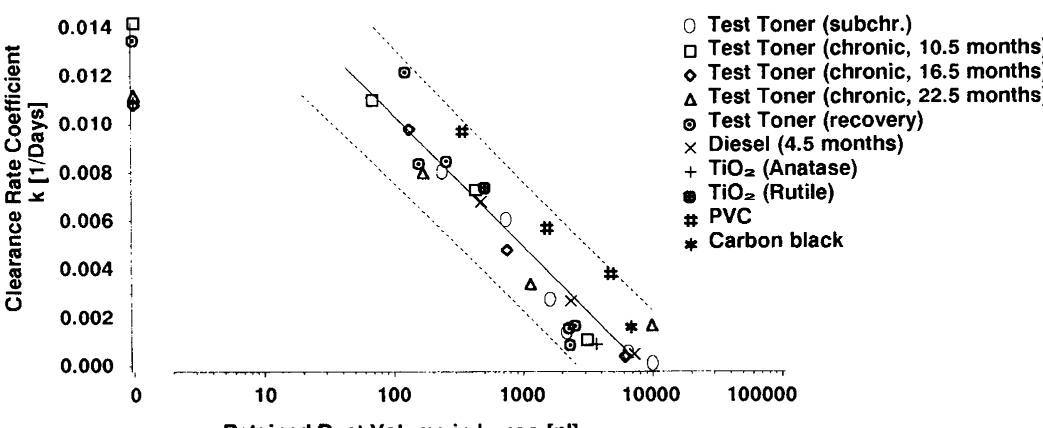

FIGURE 3. Clearance Rate Coefficient of Labelled Particles (^{85}Sr-Polystyrene) or Toner Particles as a Function of the Retained Dust Volume of the Various Test Materials. Compilation of the Various Studies performed in Rats. Lines: Regression Curve with 95% Confidence Interval

The lung burden in males was about 40 % higher than in females, which is influenced by the higher body weight and minute volume of the males. For better comparison with other rat studies, only the data of females are shown in Table 2, Studies B to D. The lung weight in the toner high exposure group was increased by 40 % at 22.5 months of the study.
In Table 2, the clearance half-time values with 95 % confidence limit for the tracer aerosols are presented. Half-times were 65 days for controls. Retardation of alveolar clearance started when the retained mass in lungs reached a level of about 0.5 mg per rat lung and amounted to about 300 days after heavy dust overloading (>10 mg per rat lung). This is also documented by Figure 2 in which these results are shown under test toner (chronic). Also included in this figure are clearance and retention data after 10.5 and 16.5 months of the study, which are not listed in Table 2.
Figure 4 shows the retained material in lungs normalized to the aerosol concentration. In the absence of lung overloading, the three lines for toner should be superimposable. This Figure demonstrates the overproportional increase of the toner lung burden in the 4 and 16 mg/m^3 exposure groups compared to the 1 mg/m^3 exposure group. This illustrates the slight overloading of lung clearance in the medium and substantial overloading in the high toner exposure groups.
A semi-empirical model was developed for calculation of retained masses in rat lungs. This model takes into account the relationship between the retained mass (m) and clearance rate coefficient (k) as documented by Figure 2. Further, the deposited mass per time (D) can be calculated from the deposition fraction, the aerosol concentration and the inhaled volume per time. The time dependence of the retained lung burden m is described by the relationship

$$dm/dt = D - km$$

Through an iterative process the lung burden at day (t+1) can be calculated from the previous day's lung burden (m_t).

$$m_{(t+1)} = D + (1-k)\ m_{(t)}$$

The lines in Figure 4 are calculated by this model.

An exposure-related decrease in the fraction of lavagable macrophages and an increase in the fraction of lavagable polymorphonuclear neutrophils (PMN) and

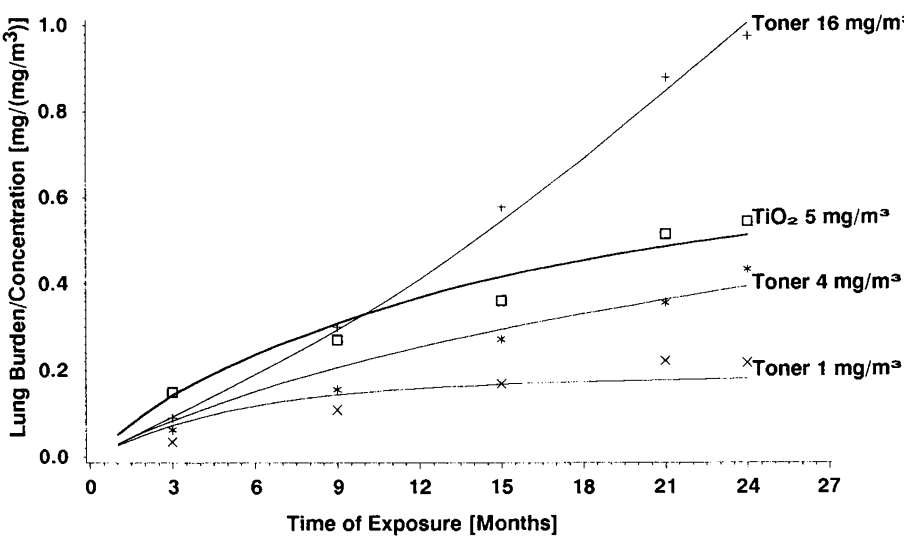

FIGURE 4. Lung Burden of Test Materials Pooled for Male and Female Rats, Normalized to the Exposure Concentration. Chronic Inhalation Study of Toner and TiO_2 (Study B). Lines: Model Calculation

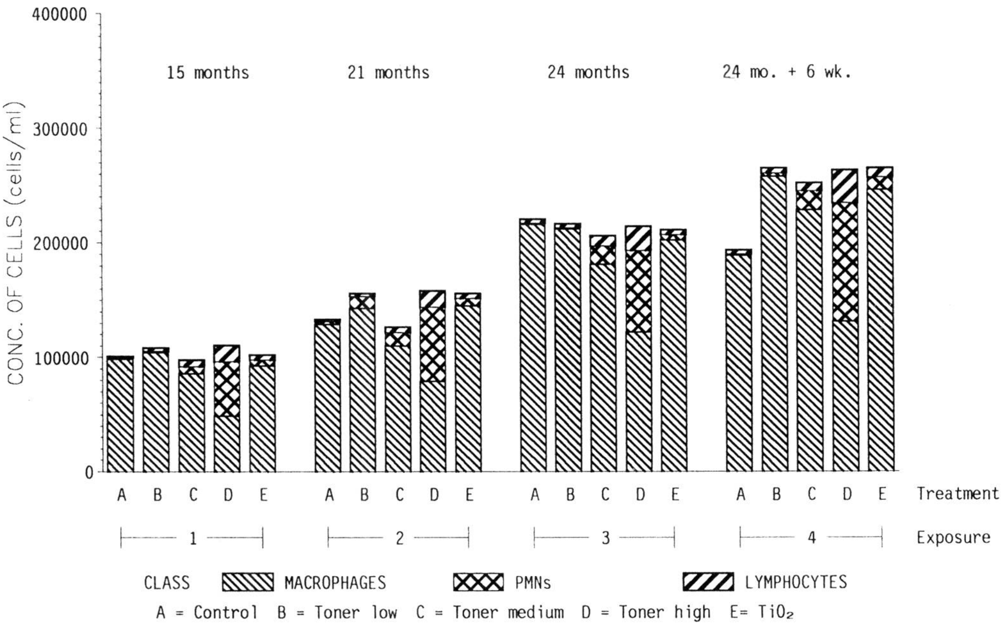

FIGURE 5. Differential Cell Count in Bronchoalveolar Lavagate up to 24 Months of Exposure to Toner and TiO_2 in Rats. Aerosol Concentration as Listed in Table 2, Study B.

lymphocytes were observed at the toner middle and high exposure level in the bronchoalveolar lavagate, indicating persistent inflammatory responses. This cytologic pattern persisted throughout the study as the results at 15, 21, 24 and 25.5 months were quite similar (see Figure 5). The results from the toner low and TiO_2-exposed groups were comparable to control.

An important lesion observed was lung fibrosis, the extent of which was found to increase with both toner exposure level and duration. In those animals which showed a survival time of more than 21 months, a slight to moderate degree of fibrosis in 92 % of the rats at the toner high and a very slight degree of fibrosis in 22 % at the toner middle exposure were observed. There was no fibrosis reported in the toner low exposure group at any time. Detailed data are presented by Muhle et al. (1990b) and Bellmann et al. (1990).

Recovery Study after Subchronic Inhalation of Toner in Rats

The reversibility or permanence of lung overloading was investigated in a follow-up study using Fischer-344 rats and the same test toner.

Exposure conditions were selected to achieve dust overloading conditions in the high exposure group (40 mg/m^3) but not in the low exposure group (10 mg/m^3) after 3 months of exposure. A 15-month post-exposure period followed with periodic measurements.

The quantity of test toner retained in the lungs was determined at 3, 6, 9, 12 and 18 months of the study. The values of retained masses after 3 months of exposure were 0.4 and 3.0 mg/lung at the low and high toner exposure levels, respectively (see Table 4). The corresponding values after 15 months of clearance were 0.12 and 2.6 mg/lung, respectively.

The alveolar toner clearance half-times were calculated from the toner retention measurements during the post-exposure period. For the high dose group a half-time of about 2800 days and for the low dose group a half-time of 277 days were calculated. If one assumes that the quantities of toner present at 15 months post exposure are sequestered in the lungs into compartments without clearance, the corresponding half-times after subtraction of this amount are 51 and 321 days, respectively.

TABLE 4

Retention of Toner in Lungs after Three Months of Exposure and a Subsequent Observation Period of 15 Months.

Total dust concen-tration [mg/m³]	Retained mass [mg/lung]					Calculated clearance half-time of toner [days]	
	Exposure + observation period [months]						
	3	3 + 3	3 + 6	3 + 9	3 + 15		Assuming sequestration
10.1±0.2	0.40±0.05	0.20±0.06	0.16±0.04	0.12±0.03	0.12±0.05	277	51
40.9±1.3	3.01±0.40	2.86±0.21	2.51±0.35	2.77±0.26	2.65±0.33	2845	321

Labelled particles, polystyrene latex (^{85}Sr, MMAD-3.5 µm) were periodically inhaled by the nose-only route and were used to measure alveolar clearance. The alveolar clearance of the tracer aerosol was retarded at both toner exposure levels (Table 5). At the low exposure level, the degree of clearance retardation was slight and a partial recovery of the clearance behavior was noted after six months post-exposure. In contrast, at the toner high exposure level, alveolar clearance was substantially impaired and no indication of a reversal in this response was apparent during the 15-month observation period.

TABLE 5

Alveolar Clearance Half-time of Labelled Particles after Three Months of Exposure to Toner and a Subsequent Observation Period of 15 Months

Group	Alveolar clearance half-time with 95% confidence interval [days]			
Dust con-centration	Exposure + observation period [months]			
	3	3 + 3	3 + 6	3 + 12
[mg/m³]	Mean (C.L.)	Mean (C.L.)	Mean (C.L.)	Mean (C.L.)
Control	45 (39-54)	45 (42-49)	39 (35-45)	75 (58-105)
Toner 10	66 (55-84)	81 (71-96)	55 (49-61)	86 (68-117)
Toner 40	229 (170-352)	635 (378-1991)	329 (217-678)	308 (209-579)

Bronchoalveolar lavage was performed periodically. The results of the differential cell count are presented in Figure 6. A large influx of PMNs and a moderate increase in lymphocytes as well as an increase in lavaged cell concentration were observed in the toner high group. The increase in elevated PMN and lymphocyte responses persisted throughout the observation period of 15 months.

Histopathological investigations after the 90-day exposure period showed a multifocal intra-alveolar accumulation of particle-laden macrophages to a moderate degree in the 40 mg/m^3 exposure group and minimal changes in the 10 mg/m^3 group. No fibrosis was diagnosed at this stage. After a 15-month observation period, a treatment-related interstitial fibrosis of a mild focal nature was observed in the subpleural region in two out of five rats in the toner high exposure group, with no fibrosis in the low exposure group. This delayed appearance of fibrosis is interpreted as a result of the persistent inflammatory reaction in the lungs and is a consequence of dust overloading. For further results see Bellmann et al. (1989) and Creutzenberg et al. (1989).

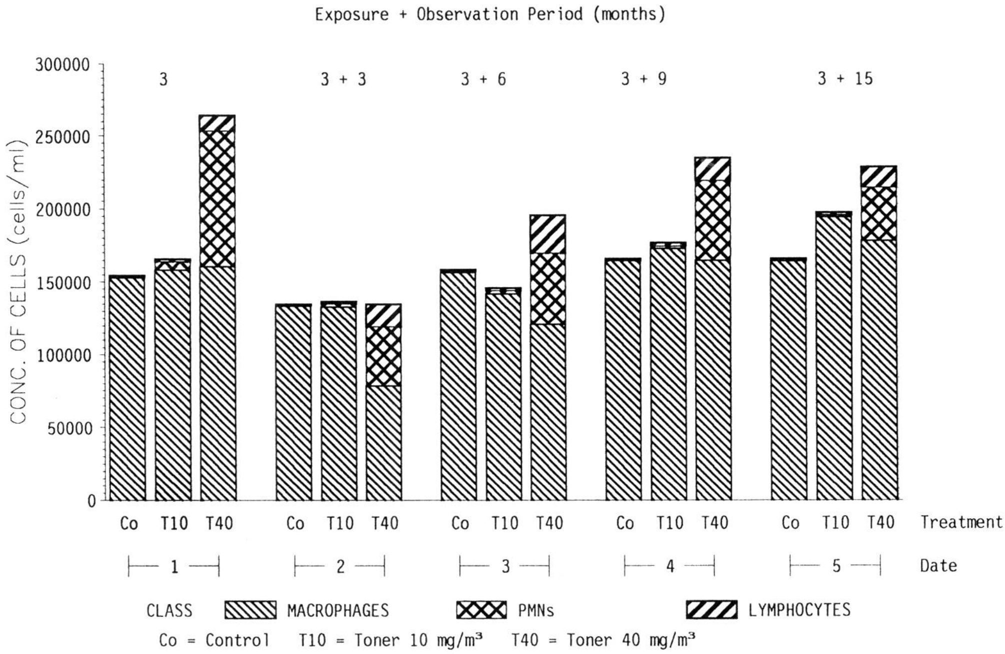

FIGURE 6. Differential Cell Count in Bronchoalveolar Lavagate Showing Persistence of PMN Presence During a 15-Month Recovery Period.

Inhalation Study with Polyviny Chloride (PVC) Powder in Fischer-344 Rats (Study C)

The exposure conditions and the retained mass after a seven-month exposure period are listed in Table 2, Study C. The retention of the inhaled PVC powder was determined at 1, 3 and 5 months after cessation of exposure. After exposure to high PVC concentrations the alveolar clearance of the inhaled material was slower by a factor of 3.3 compared to the exposure to the low concentration. Similar effects were found for the clearance of a superimposed spike of ^{85}Sr-polystyrene which showed a retardation factor of 3.2, compared to the control group (see Table 2). At the medium PVC exposure concentration, there was a moderate or slight retardation of lung clearance. Obviously the ^{85}Sr-polystyrene particles served as good surrogate particles to follow alveolar clearance. A dose-dependent increase of PMN was detected in the bronchoalveolar lavage after 8 months inhalation of PVC powder.

Chronic Inhalation Study of Toner and TiO_2 (Rutile) in Hamsters (Study E)

The aerosol concentrations in this study were changed after 5 months as indicated in the footnote of Table 3. For the three toner groups the aerosol concentration was increased by a factor of 2.7 whereas for TiO_2 the concentration was lowered by one quarter. The reason for this change was that interim retention measurements had shown that the desired similar volumetric lung burden in the toner high and TiO_2 exposure group would not have been reached by the original values.
The quantity of test toner and TiO_2 retained in the lungs of male hamsters at 16.5 months of exposure is shown in Table 3.
The lung weight was increased by 36 % and 77 % in the toner high and TiO_2 exposure groups, respectively, at 18 months in the study. The retained mass in lungs normalized by the aerosol concentration is shown in Figure 7. The graph shows an overproportional increase of the lung burden in the toner high exposure group.
The alveolar clearance half-times for male hamsters after an exposure period of 10.5 months are shown in Table 3, documenting the phenomenon of "dust

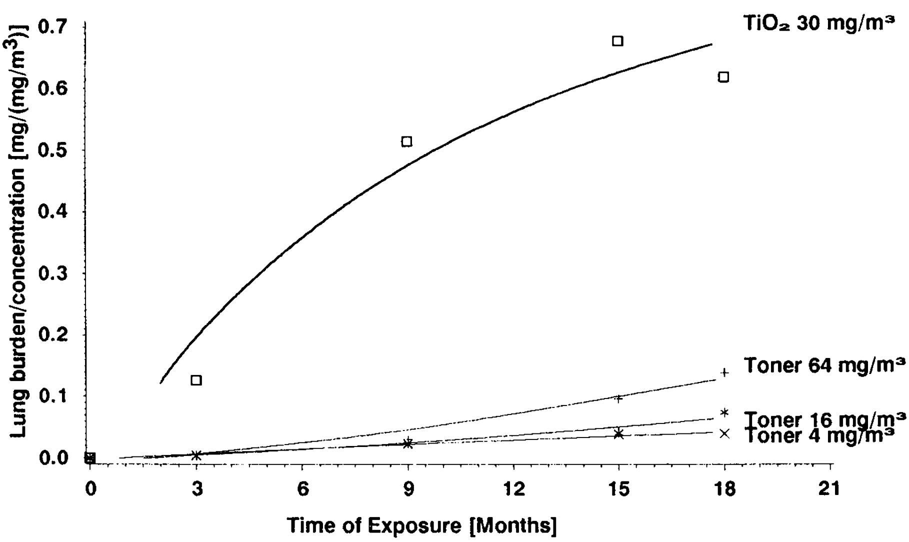

FIGURE 7. Lung Burden of Test Materials for Male Syrian Golden Hamsters, Normalized to the Exposure Concentration. Chronic Inhalation Study of Toner and TiO_2 (Study E)

overloading" also in hamsters. The corresponding values of the clearance rate coefficent are presented in Figure 8. Unlike the results in rat studies, a slight amount of retained dust (0.01 to 0.5 mg per hamster lung) seemed to accelerate the alveolar clearance. It appears that the critical lung burden which leads to a retardation of particle clearance is reached earlier in male than in female hamsters. This interpretation is in accordance with the lower amount of retained toner in the lung of female hamsters. This may be

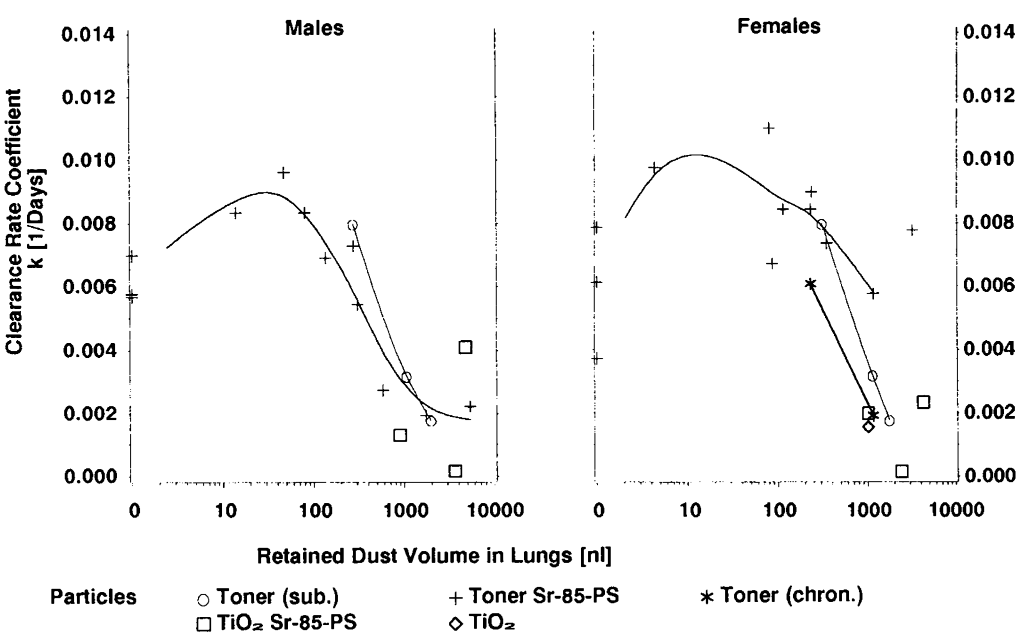

FIGURE 8. Clearance Rate Coefficient of Labelled Particles (^{85}Sr-Polystyrene) or Toner Particles as a Function of the Retained Dust Volume of Toner and TiO_2. Subchronic and Chronic Inhalation Studies in Syrian Golden Hamsters.

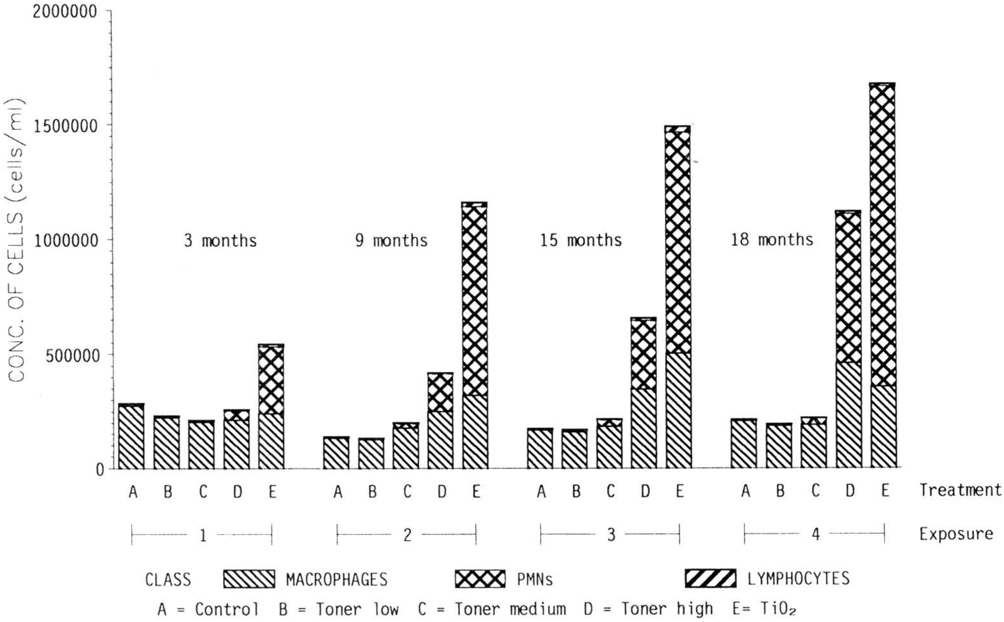

FIGURE 9. Differential Cell Count in Bronchoalveolar Lavagate up to 18 Months of Exposure to Toner and TiO_2 in Syrian Golden Hamsters. Aerosol Concentrations as Listed in Table 3.

influenced by the sex difference in body weight and a resulting difference in the minute volume.
Generally, tracer clearance results in hamsters showed a larger intra-group variability than observed in rats. Similar observations were made on variability of various parameters such as body weight, lung weight and retained mass in lungs. In spite of these statistical fluctuations it is clearly shown that TiO_2 and toner led to a retardation of the alveolar clearance.
The different behavior between hamsters and rats can also be seen in the differential cell count in the bronchoalveolar lavage (compare Figure 9 to Figure 5). Results show an increase of the number of lavagable alveolar macrophages and substantial increase in PMN.
In a recovery experiment after 9 months, female hamsters were removed from the inhalation chambers in the groups exposed to toner medium and high and TiO_2. Animals were kept in filtered air for up to a further 6 months. The retained mass was analyzed after 3 and 6 months. The retained mass and calculated half-time of alveolar clearance are presented in Table 6. For TiO_2 exposure,

TABLE 6

Retained Mass at 9 Months of Exposure and Calculated Half-time of Alveolar Clearance (with 95% Confidence Limit) of the Retained Material in a Post-Treatment Observation Period of 6 Months in Female Hamsters in Study E

Nominal aerosol concentration [mg/m^3]		Retained mass [mg/lung]	Half-time of alveolar clearance [days] mean (95% Confidence limit)
6/16	TONER	0.27 ± 0.04	114 (63 - 617)
24/64	TONER	1.34 ± 0.51	359 (129 - ∞)
40/30	TiO_2	10.3 ± 1.67	441 (271 - 1181)

alveolar clearance was retarded, as shown by the half-time of 441 days. Using labelled tracer particles a clearance half-time of 641 days was calculated (Table 3). In the toner high exposure group, the clearance half-time was 359 days compared to 246 days obtained by the tracer method. Although in some cases sex differences in the retention and clearance behavior in hamsters were observed, these data show a considerable congruence.

Chronic Inhalation Study of Diesel Engine Exhaust, Carbon Black and TiO_2 (Anatase) in Wistar Rats and NMRI and C57BL Mice (Study D)

The exposure concentrations in this study for the carbon black and the TiO_2 exposure groups were changed twice, as indicated in the footnote to Table 2, Study D. The reason for these changes was the same as mentioned in the chronic inhalation study of toner in hamsters. Interim retention measurements had shown that the desired similar lung burden in the diesel high, carbon black and TiO_2 exposure groups would not have been reached by the original values (Heinrich et al. 1989).

The quantities of retained masses in lungs after 4.5 months of exposure and the corresponding half-times of the alveolar clearance are presented in Table 2. These values are transferred also to Figure 2, showing the same pattern of impairment of the alveolar clearance with dependence on the retained mass. The time sequence of the retention values is presented in Figure 10.

After 22 months of exposure, the lung weight increased by 1.17, 1.74, 4.37, 5.1 and 4.25 in the three diesel exposure groups, the carbon black and TiO_2 exposure groups, respectively. This substantial elevation of lung weight in the high dose groups led to changes in the mechanical behavior of the lung followed by altered breathing and particle deposition pattern during the course of the study. Mechanical lung function measurement of the rats documented a shallower breathing pattern and a less compliant lung. Clearance measurements using ^{85}Sr-polystyrene particles in the further course of the study also indicated this change in the deposition pattern caused by the considerable increase in lung weight. Therefore, the correlation of the retained dust volume in lungs and the clearance rate of these highly exposed rats did not follow the same pattern as listed in Figure 2. As shown in Figure 11 a high particle load of the lungs accompanied by a considerable increase in the lung weight due to various exposure-related tissue reactions may lead to an altered deposition pattern of the inhaled particles. At this

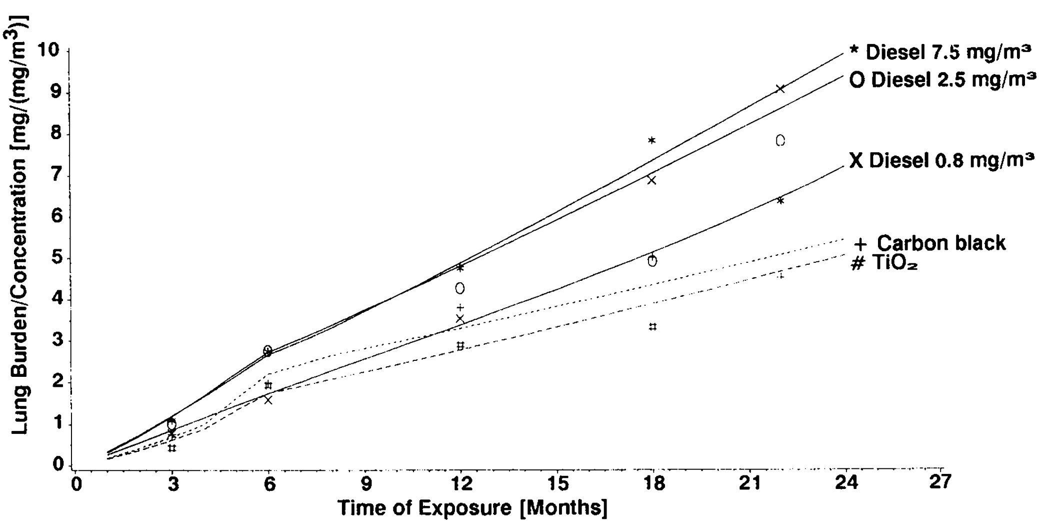

FIGURE 10. Lung Burden of Test Materials of Diesel Soot Particles, Carbon Black and TiO_2 in Female Rats Normalized to the Exposure Concentration (Study D). Lines: Model Calculation

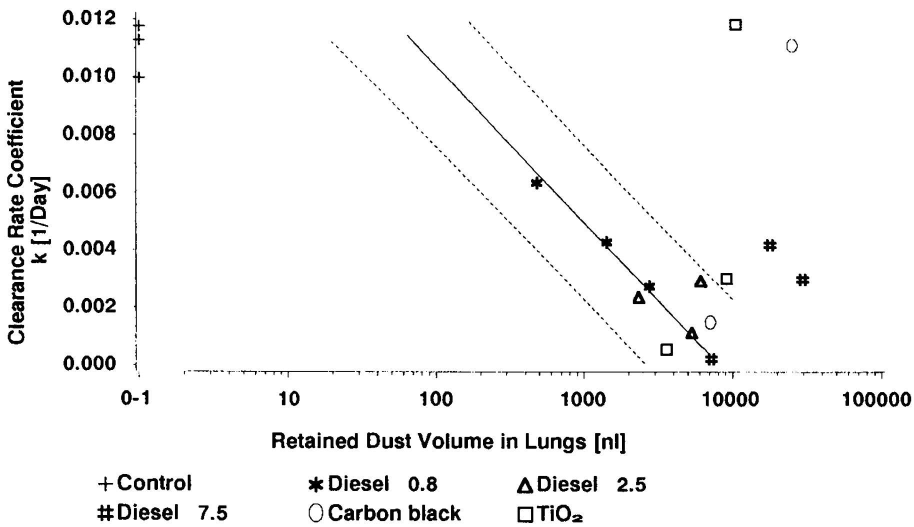

FIGURE 11. Clearance Rate Coefficient of Labelled Particles (^{85}Sr-Polystyrene) as a Function of the Retained Dust Volume Measured after 3, 12, and 18 Months of Exposure. Apparent Normalization of Alveolar Clearance at High Lung Burden (For Detail See Text). Chronic Inhalation of Diesel Exhaust, Carbon Black and TiO_2 in Rats.
Lines: Regression Curve and 95% Confidence Limit from Figure 3.

point, a much higher fraction of the inhaled material may be deposited in the bronchi resulting in a faster clearance.
This effect had also to be taken into account for the model calculation of particle retention in Figure 10 where a substantial decrease of the deposition rate had to be presumed for carbon black, TiO_2 and for the medium and high diesel exposure groups after 6 months of exposure.
An interstitial fibrosis was observed at serial sacrifices at 12 and 18 months in the carbon black, TiO_2 and diesel high exposure groups.
Retention values of mice principally followed the same pattern as those found in the rat. No steady-state was reached during the exposure period (see Figure 12). Assuming first order kinetics the equation $m = D/k \cdot c \cdot (1-e^{-kt})$ was fitted to the retention values. The deposition rate D and the clearance rate constant k was estimated by a non-linear regression using lung burden m, aerosol concentration c and exposure time t of individual animals. The resulting half-times of diesel retention which were above 500 days are shown in Figure 12. This means that the phenomenon of lung overloading can also be observed in mice.

Effects of particle size

Besides the amount of retained volume there are other factors which also influence the response of the lung. Although the mass median diameters of anatase and rutile were similar, the ratio of retained mass divided by the cumulative exposure dose was considerably higher for anatase. This faster uptake may be partly influenced by a disaggregation of the anatase dust into small primary particles in the lung (Takenaka et al. 1986) which also led to higher inflammatory reactions (Oberdörster et al. 1990).

Effect of animal age

Repeated measurements of the alveolar clearance using ^{85}Sr-labelled polystyrene particles in rats unexposed to dust (control) during their lifespan had consistently shown a slight but statistically significant decrease in alveolar clearance. Typical values at 5 months of age showed a

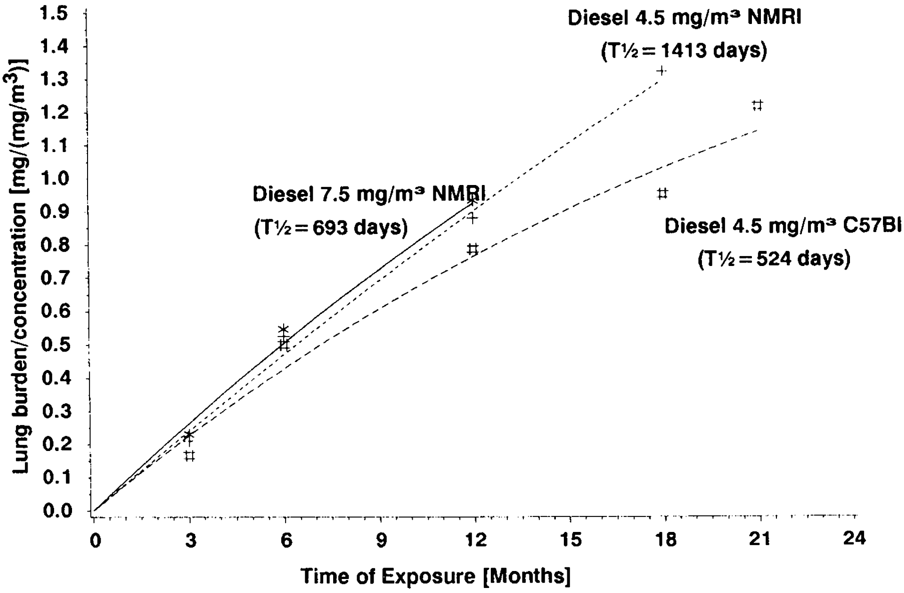

FIGURE 12. Lung Burden of Diesel Soot Particles in Female NMRI and C57BL Mice. Lines According to a First Order Kinetic Model Calculation with Corresponding Half-Times of the Lung Clearance of Diesel Soot Particles.

half-time of 45 days, whereas after 23 months the values increased to 74 days. This is documented by the control values in Figure 2, where the square represents the results from 11-month-old animals and the triangle the results of 23-month-old rats.

DISCUSSION

Accumulation of large quantities of insoluble material in the lungs, clearance impairment and inflammatory response are some of the characteristic signs of lung overloading, a phenomenon which has been discussed by Morrow (1988). In the F-344 rat, lung overloading is generally reached in the range of 0.5 - 1.5 mg or approximately 1 mg of material/g of lung tissue. Since phagocytosis of particles with low solubility and low biological activity depends primarily on their size and not on their density, the most appropriate manner to compare various dusts appears to be the volume of material (Morrow and Mermelstein, 1988).

Similar results of lung overloading were observed upon prolonged exposure of rats to diesel exhaust (Vostal et al. 1982, Chan et al. 1984, Wolff et al. 1986, Heinrich et al. 1986). Vostal (1986) suggested that there is a threshold between cumulative exposure (concentration x time) and particle clearance "overload". As indicated above, it appears to us that a similar threshold relationship should exist between particle clearance "overload" and the onset of fibrosis. The induction of fibrosis after high exposure to TiO_2 was also reported by Lee et al. (1985).

Our results using various test materials and three species indicate that dust overloading of lungs appears to be a generic phenomenon, observed upon over-exposure of the lungs to various particles of low solubility and low acute toxicity.

Characteristic findings of dust overloading of lungs are: a) alveolar clearance retardation of tracer particles, b) increased retention of test materials in the lung, c) increase in lung weight, d) accumulation of dust-laden macrophages, e) persistent inflammation, f) increased epithelial permeability, g) elevated infiltration of neutrophils, h) increased transfer of material to the lung-associated lymph nodes, e) onset of fibrosis after a critical dose (time-integrated concentration) and sufficient time interval. The phenomenon of dust overloading must be taken into account in extrapolating from the highest exposure levels to ambient levels of exposure relevant to man. Effects observed at the high level may be uniquely related to the accumulation of a high burden of insoluble particles. Accordingly the demonstration of "lung overloading" in the various studies indicates that caution should be used in extrapolating the prevalence of lung fibrosis in rats exposed to a high level of dust, to potential fibrogenicity in humans exposed to much lower levels of the same dusts. On the other hand, as dust overloading seems to be a generic phenomenon and taking into account the longer clearance half-time of insoluble particles in humans compared to rodents, it cannot be excluded that a high dust exposure may lead to similar effects in humans as observed in rodents. It should be noted that the effect of dust overloading was also partly observed at exposure concentrations below 10 mg/m^3 respirable dust, i.e. within the range of the occupational dust standards. Assuming that there is a human counterpart to the dust overloading phenomenon at the same lung burden (milligram dust per gram lung), extrapolation modelling can be used to show that the current occupational dust limits do not protect workers sufficiently (Morrow et al. 1991).

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