TABLE 1
Measured and Normalized Lung Talc Burden of Rats at the End of the 4-Week Inhalation Study of Talc

	Exposure concentrations, mg/m³			
	0 Control	2 (target) 2.3 (actual)	6 (target) 4.3 (actual)	18 (target) 17 (actual)
Male				
n	5	5	5	5
μg talc/lung (from NTP report)	4.3 ± 1.6	81.6 ± 2.1	186.0 ± 9.3	846.0 ± 5.5
μg talc/lung per mg/m³ exposure concn	—	35.5	43.3	49.8
(% increase over low concn)			(+22%)	(+40%)
Female				
n	5	4	5	5
μg talc/lung	0.58 ± 0.24	56.5 ± 1.6	127.2 ± 9.3	546.0 ± 35.2
μg talc/lung per mg/m³ exposure concn	—	24.6	29.6	32.1
(% increase over low concn)			(+20%)	(+30%)

clearance mechanisms, and evidence has been accumulating that these effects are toxicological implications of a condition of lung "particle overload" (Morrow, 1988). Since this concept is now widely accepted and since it has implications for human extrapolation, results from chronic particle inhalation studies in rodents need to be carefully analyzed. The purpose of this article is a critical evaluation of the chronic NTP talc study with respect to the overload concept.

THE NTP TALC STUDY

Four-Week Talc Inhalation Study

A subchronic 4-week inhalation study using concentrations of 6 and 18 mg talc/m³ was performed prior to initiating the chronic study (NTP, 1993). The purpose of this study apparently was to select appropriate exposure concentrations for the long-term study by avoiding effects associated with excessive particle loading of the lung, or, in other words, avoiding that a maximum tolerated dose (MTD) is exceeded. It has been suggested that in chronic carcinogenicity rodent bioassays the highest dose should be at the MTD level with subsequent lower doses at one-half and one-fourth of the MTD (Haseman, 1985). With respect to inhalation studies with particles, participants at a recent NTP-sponsored workshop on Maximal Aerosol Exposure Concentrations in Inhalation Studies (Lewis *et al.*, 1989) recommended the following: 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

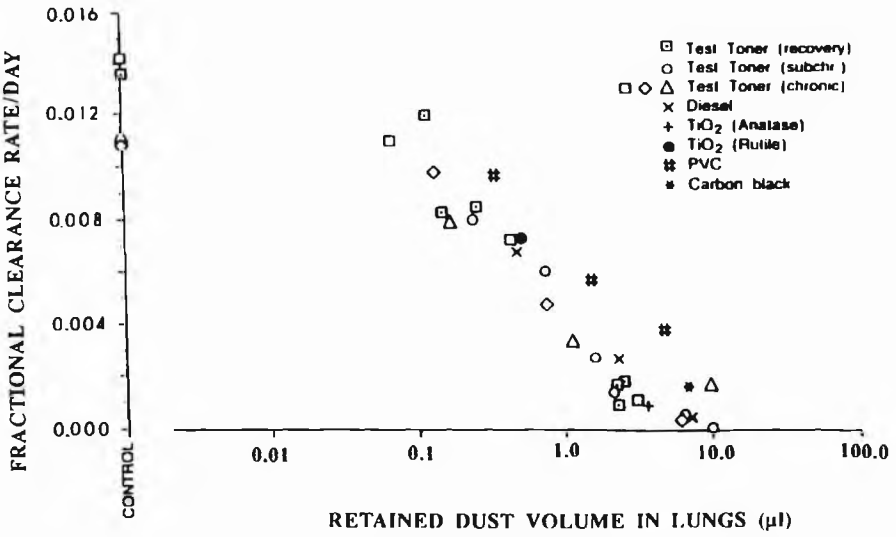

FIG. 1. Pulmonary particle clearance rates and volumetric lung burdens of different retained particles in rats (adapted from Morrow *et al.*, 1991).

TABLE 2

Average Daily Deposited Doses of Inhaled Talc in Rats and Mice Estimated by Using Reported Deposition and Breathing Parameters for Normal Animals and Talc Exposure Parameters Given in the NTP Report

Species	Exposure concn; mg/m^3	Average daily deposition μg
Rat		
Males	6	28.1
	18	84.3
Females	6	21.4
	18	63.4
Mouse		
Males	6	0.98
	18	2.94
Females	6	0.91
	18	2.74

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. Additionally, Muhle *et al.* (1990b) suggested to define a maximum functionally tolerated dose (MFTD) for chronic inhalation studies with particles which they arbitrarily defined as a particulate lung burden causing a two- to fourfold prolongation of lung particle clearance.

Thus, using results of a subchronic talc inhalation study for establishing a MTD for the chronic study is most appropriate. Results of the 4-week talc inhalation study in rats showed indeed that "the ratio of lung burden to exposure concentration was somewhat higher at the 6 and 18 mg/m^3 exposure levels" (NTP, 1993, Appendix F). Also, the NTP report continues: "The increase in talc burden with exposure concentration may have been because the maximum ability of the respiratory tract to clear particles was exceeded at the 6 and 18 mg/m^3 exposure levels."

Despite this conclusion from the range-finding study, the subsequent chronic study was performed at the two concentrations for which impaired lung clearance was detected in the 4-week study, i.e., the MTD may have been exceeded at both exposure concentrations. Thus, the purpose of the 4-week talc inhalation study remains unclear since the results apparently were not applied to selecting levels for the chronic study in rats. Table 1 shows these results after recalculation from the original data supplied in Appendix F of the NTP report (1993). Recalculation of the data was necessary because calculation of the normalized lung burden was done incorrectly in the report, although the right conclusions were drawn about impaired lung clearance.

Table 1 shows that talc lung burdens in rats normalized to the exposure concentration increased by 20 to 40% at the different exposure levels (the actual exposure concentration in the 4-week study was recalculated based on the information in the NTP report). If lung clearance mechanisms for particles are not impaired, then lung particle accumulation should be proportional to the exposure concentration and the lung burdens from different exposure concentrations normalized by the exposure concentration should not be significantly different. Table 1 shows that these normalized lung burdens were different, between 20 and 40% more than predicted. To achieve the measured 20% greater than expected normalized lung burden after only 4 weeks of exposure the impairment of lung clearance must have been very high, and this result of a 4-week study might have been very alarming with respect to selecting appropriate exposure concentrations for the long-term study.

However, using an exposure duration of only 4 weeks as a range-finding study for a subchronic study makes it very difficult to get an accurate prediction of the accumulation kinetics. A longer 13-week study, although more expensive, would be highly preferable since it allows for a far better evaluation and prediction of the long-term pulmonary accumulation kinetics of particles. Nevertheless, according to the report, the 4-week talc study identified the 6 and 18 mg/m^3 exposure levels as causing effects on lung clearance mechanisms in rats. The results in mice from the 4-week study were less clear, only female mice exhibited a more than 20% increase in normalized lung talc burden at both the 6 and 18 mg/m^3 levels.

Lung clearance will also be impaired from inhalation of more toxic particles such as crystalline silica, but in this case impairment will occur at much lower lung burdens than those of low-toxicity particles, e.g., TiO_2 or toner. The question to be answered, therefore, is: Does

TABLE 3

Actual and Predicted Lung Talc Burden in Rats in the Chronic NTP Study (mg/lung)

	Months			
(mg/m^3)	6	12	18	24
	Males			
6				
Found	3.15	5.38	12.36	18.45
Predicted	2.36	2.76	2.82	2.84
18				
Found	12.95	25.74	46.62	42.65
Predicted	7.08	8.29	8.47	8.51
	Females			
6				
Found	2.44	4.52	8.66	9.23
Predicted	1.78	2.08	2.13	2.14
18				
Found	8.39	13.58	27.49	29.81
Predicted	5.99	6.23	6.37	6.39

talc deposited in the lung behave like a cytotoxic particle or like a more benign particle, i.e., does the lung overload condition develop with talc particles? Results of the pulmonary talc accumulation of the chronic talc study should provide an answer to this question.

For an understanding of these results it will be useful to briefly review findings from other chronic inhalation studies in rats with highly insoluble particles of low cytotoxicity. Morrow *et al.* (1991) evaluated respective studies with different particles on the basis of their retained volumetric lung burden and their observed lung clearance rates. The use of the correlation between these two parameters is based on the volumetric lung overload concept (Morrow, 1988) which states that alveolar macrophages (AM) become impaired in their particle clearance function if a certain fraction of their cell volume is filled by phagocytized particles. That means, the volumetric rather than the gravimetric particulate lung burden is of greater relevance to correlate with lung clearance for a range of different particle types. Figure 1 illustrates this correlation between increasing retained volumetric lung burdens of different particle types and decreasing lung clearance rates.

The Chronic Talc Inhalation Study

The kinetics of talc accumulation in lungs of both rats and mice during the chronic study deserve special attention. As discussed above, results of a number of studies have shown that chronic exposure of rats to inhaled low-toxicity particles at high concentrations will exceed the capacity of lung clearance mechanisms to effectively eliminate these particles from the alveolar spaces (e.g., Morrow, 1988; Muhle *et al.*, 1990a,b). This overload phenomenon occurs at an average volumetric lung burden of retained particles approaching ~1 μl/rat lung (see Fig. 1). In the following paragraphs this value will be used to evaluate the results of the chronic NTP talc study for retarded particle clearance, possibly indicating lung overload. An evaluation of the lung dosimetry needs to be performed for this purpose, including deposition and retention behavior of the inhaled talc particles.

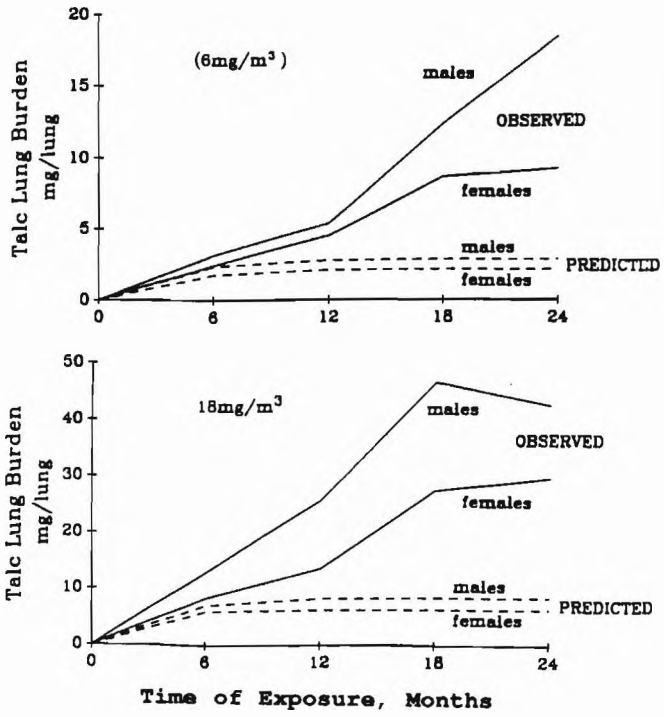

FIG. 2. Observed and predicted talc accumulation in lungs of male and female rats during 2 years of exposure to 6 mg/m³ (top) and 18 mg/m³ (bottom).

TABLE 4

Average Pulmonary Retention Halftimes and Average Clearance Rates for Talc in Rats Estimated from Measured Pulmonary Talc Burdens in the Chronic NTP Study

	(mg/m³)	$T_{1/2}$, days	Clearance rate/day
Males	6	300	2.31×10^{-3}
	18	300	2.31×10^{-3}
Females	6	250	2.77×10^{-3}
	18	280	2.48×10^{-3}

Deposition of particles of different aerodynamic diameters in rodent lungs has been studied by Raabe *et al.* (1977, 1988), and the results from their studies indicate that the particle sizes used in the NTP chronic talc study (2.7–3.6 μm) have a deposition efficiency in the rat lung of ~10% of the inhaled dose and in mice lungs of ~2.5% of the inhaled dose. Using these values and assuming an average body weight during the study for male and female rats of ~400 and ~280 g, respectively, and for mice of 34 g (male) and 31 g (female), the daily deposited dose for the two inhaled concentrations (6 and 18 mg/m³) can be estimated using rat and mouse specific breathing parameters. Results of these estimates are given in Table 2.

Applying the normal pulmonary retention halftime for highly insoluble particles in rat lungs of ~70 days and assuming that no impaired clearance of the deposited particles occurred, the build-up of the talc particles over the exposure period of 24 months can be predicted from the daily deposited dose. The respective results are given in Table 3 and Fig. 2, together with the actual measured talc burdens in the rats during the chronic study. Under the assumption that no impaired particle clearance occurred, the lung burdens of talc particles are predicted to achieve an equilibrium after about 12 months of exposure in both male and female rats. In contrast to this prediction, the actual accumulation of inhaled talc was quite different in the chronic study exceeding the predicted values beyond 6 months of exposure and either approaching an equilibrium much later or not at all. This

is indicative of a prolonged clearance of the deposited talc particles in the rat lungs compared to normal conditions. Estimates of the pulmonary retention halftimes and the respective average pulmonary clearance rates for the talc particles which would best describe the actual observed talc burdens are presented in Table 4. These estimated pulmonary retention halftimes of the retained talc particles range between 250 and 300 days, which is markedly longer than the normal retention halftime for highly insoluble particles in rat lungs of ~70 days.

It can be concluded from this analysis that at both the high- and low-exposure concentrations the pulmonary retention halftime for talc particles compared to that of other particles was increased, which may be indicative of lung overload as discussed earlier. It may be surprising that the estimated retention halftimes for both exposure levels are quite similar, although the talc lung burdens between the two dose groups are quite different. However, it must be kept in mind that the data in Table 8 are estimates only of the retention halftimes and, unlike data reported for other studies, they are not based on actual measurements of pulmonary particle clearance.

Prolonged particle clearance in the lung is not necessarily an indication of particle overload. For example, cytotoxic particles like SiO_2 will significantly retard particle clearance in the lung at much lower lung burdens than those seen with more benign particles like TiO_2. To analyze this aspect further, the actual lung gravimetric burdens found in the chronic NTP talc study in the lungs of the rats (Table 3) were converted into volumetric lung burdens of talc (specific density of talc: 2.8 g/cm^3). The results for the rat are given in Table 5 for the two different exposure concentrations and sexes. Table 5 shows that already at 6 months of exposure even in the low-dose groups the volumetric lung burden of the retained talc particles approaches a value of 1 μl per rat lung, suggested by Morrow (1988) to indicate the beginning of lung particle overload. By the end of the exposure, all of the exposed groups are clearly in the volumetric overload range.

TABLE 5
Volumetric Lung Burden of Talc in Rats Found in the Chronic NTP Study (μl/lung)

		Months			
	(mg/m^3)	6	12	18	24
Males	6	1.13	1.92	4.41	6.59
	18	4.63	9.19	16.65	15.23
Females	6	0.87	1.61	3.09	2.23
	18	3.00	4.85	9.82	10.65

It is helpful to view this finding in the context of other reported results. Figure 3 shows the results of several long-term particle inhalation studies summarized by Morrow *et al.* (1991) which were discussed earlier in this paper (Fig. 1). The data of Tables 4 and 5, associated talc clearance rates and retained volumetric talc lung burdens, and respective results from a crystalline SiO_2 study (Oberdörster *et al.*, 1994) are added to this Figure. The following conclusions can be drawn: One is that the datapoint for highly cytotoxic SiO_2 lies clearly outside the reported correlation curve for low-cytotoxicity particles. The other is that the values obtained from the present NTP talc study fit quite nicely into the datapoints of Morrow *et al.* (1991) indicating that, indeed, these talc particles behave much like the other particles of low cytotoxicity with respect to volumetric impairment of lung clearance.

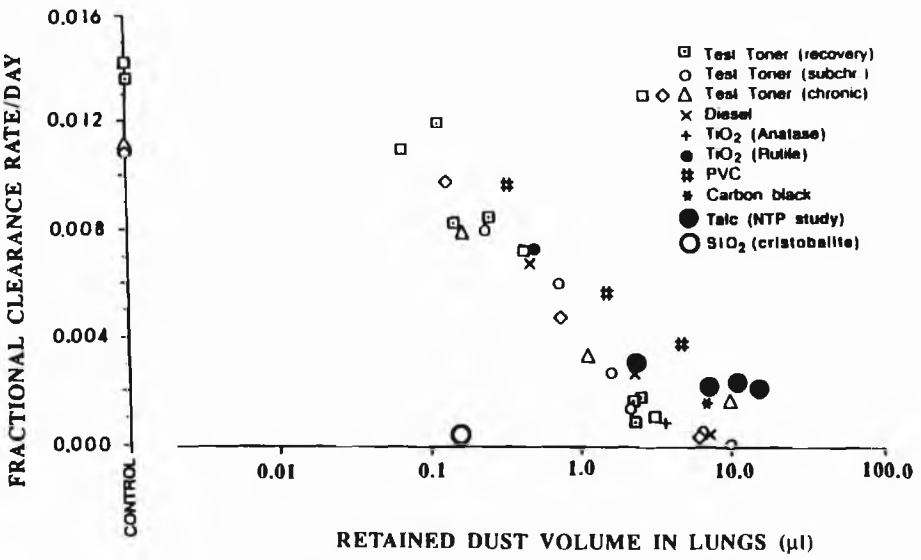

FIG. 3. Pulmonary particle clearance rates and volumetric lung burdens of different retained particles in rats including talc and the highly fibrogenic SiO_2 (see Fig. 2).

TABLE 6
Actual and Predicted Lung Talc Burden in Mice in the Chronic NTP Study (mg/lung)

	Months			
(mg/m^3)	6	12	18	24
	Males			
6				
Actual	0.07	0.17	0.10[a]	0.75
Predicted	0.07	0.08	0.08	0.08
18				
Actual	0.23	1.41	1.91	4.97
Predicted	0.22	0.23	0.23	0.23
	Females			
6				
Actual	0.10	0.11	0.31	0.74
Predicted	0.07	0.07	0.07	0.07
18				
Actual	0.26	0.97	1.75	5.53
Predicted	0.20	0.22	0.22	0.22

[a] Error in the NTP Report?

Similar analyses were applied to the results of the chronic mouse talc study. Normal pulmonary retention data for highly insoluble particles in mouse lungs are documented by Kreyling (1990) who reported respective retention halftimes of about 55 days; this means that an equilibrium lung burden during a chronic particle inhalation study would be reached after about 300 days of exposure. Table 6 shows the lung burden data, measured and predicted values, in lungs of the talc-exposed mice. No equilibrium lung burden is reached during the course of the 2-year exposure, very similar to the rat lungs overloaded with talc particles (Fig. 2), and the measured lung doses are considerably higher than would be predicted if lung clearance would not be prolonged.

Using these data of pulmonary talc accumulation in the mice, average pulmonary retention halftimes and average clearance rates were calculated and the results are given in Table 7. The results clearly show that mice also exhibited a marked increase in pulmonary retention halftime for talc particles with increasing lung burdens, i.e., a severe retardation of normal AM-mediated particle clearance, compared to a normal retention halftime in mice lungs of ~55 days (Kreyling, 1990). It is of interest to note that mice appear to be more sensitive toward overload effects in the talc study as far as impairment of particle clearance is concerned; with respect to fibrotic and tumorigenic effects, the opposite is true, i.e., talc-exposed mice did not show these responses. This is consistent with findings in mice from other studies with different particles (i.e., TiO_2, carbon black, diesel exhaust) in which particle-exposed mice showed a lower pulmonary inflammatory and fibrotic response than rats and mice did not develop lung tumors (Heinrich *et al.*, 1986; Muhle *et al.*, 1990a; Mauderly, 1994).

SIGNIFICANCE OF CARCINOGENICITY OF INHALED TALC OBSERVED IN RATS

The discussion of the chronic NTP study in the preceding paragraphs can be summarized by stating that lung particle clearance in both rats and mice was impaired resulting in altered accumulation kinetics of talc particles chronically inhaled at concentrations of 6 and 18 mg/m^3. However, the finding of greatest concern in these studies is the significantly increased incidence of lung tumors in female rats. An epidemiological study in pottery workers with exposure to talc also showed an increased lung tumor incidence, i.e., increased standardized mortality ratio (SMR) (Thomas and Stewart, 1987; Thomas, 1990). However, these results are very difficult to interpret in terms of their biological plausibility: The SMR for lung tumors was higher in workers exposed to nonfibrous talc as opposed to those exposed to fibrous talc (containing asbestiform fibers), and the latency period after nonfibrous talc exposure may have been as low as 5 years of exposure. In addition, the predominant exposure of the pottery workers was to SiO_2 and other minerals as well as numerous substances including chromium and other metals which were also present in the exposure atmosphere. Smoking status was not evaluated. Thus, no definitive conclusions can be drawn from this study with respect to pulmonary carcinogenicity of talc in exposed humans.

With respect to the experimentally observed increased lung tumor incidence in female rats, some other studies have also reported a greater pulmonary carcinogenic response in female rats than in male rats when exposed chronically to particles, e.g., Sb_2O_3 (Groth *et al.*, 1986), volcanic ash (Wehner *et al.*, 1986), or a prevalence of malignant tumors in females was found compared to males, e.g., TiO_2 (Lee *et al.*, 1985), although TiO_2-exposed males also showed a high incidence in adenomas which probably will develop into malignant tumors. Other studies did not find a sex-specific tumorigenic response and lung tumors were induced in both male and female rats, e.g., diesel soot and carbon black (Mauderly

TABLE 7
Average Pulmonary Retention Halftimes and Average Clearance Rates for Talc in Mice Estimated from Observed Pulmonary Talc Burdens in the Chronic NTP Study

	(mg/m^3)	$T_{1/2}$, days	Clearance rate/day
Males	6	380	1.82×10^{-3}
	18	850	8.15×10^{-4}
Females	6	400	1.73×10^{-3}
	18	1000	6.93×10^{-4}

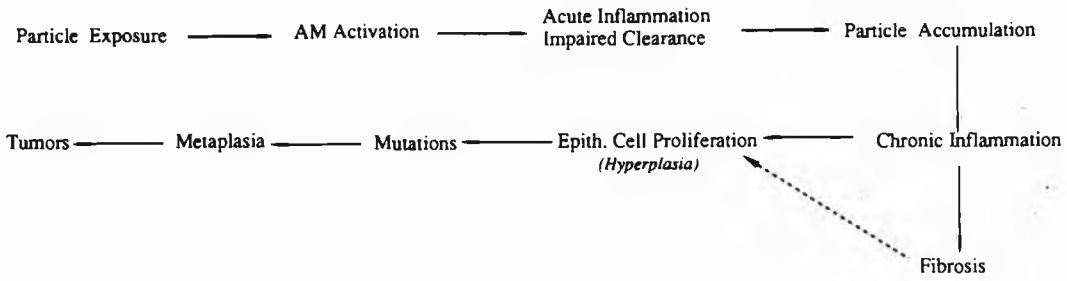

FIG. 4. Hypothetical sequence of pulmonary events in rats induced by chronic exposure to high concentrations of highly insoluble low-toxicity particles (AM, alveolar macrophages).

et al., 1987; Heinrich *et al.*, 1992). Thus, the tumor response to high particulate lung burdens cannot be viewed as a sex-specific phenomenon. Perhaps the prevalence of tumors in female rats in some studies can be explained by an earlier response in females compared to males. There may be only a gradual rather than principal difference between males and females regarding tumor induction, yet further studies are needed for clarification.

Hyperplastic and interstitial fibrotic responses occurred in both sexes of talc-exposed rats to a significant degree, and these responses may be mechanistically linked to tumorigenesis (Dungworth, 1994). A chain of events can be postulated starting with inflammatory processes which develop into chronic inflammation maintained by high burdens of particles accumulating in the lung due to impaired clearance; inflammation-associated target cell proliferation and potentially inflammatory cell-induced mutational events may result in metaplasia and the eventual development of tumors (Fig. 4). It appears that these mechanistic linkages are especially prevalent in rats but do not occur to the same degree in other laboratory rodents which respond with a much lower degree of inflammation and hyperplasia to inhaled particles (Dungworth, 1994).

Is the tumorigenic response in the talc study a consequence of impaired lung clearance, i.e., lung overload, due to mechanisms suggested above? We should recall that impaired particle clearance per se may not always be a symptom of lung particle overload. For example, as was shown in Fig. 3, exposure to SiO_2 particles clearly leads to a prolongation of particle clearance at much lower lung burdens (either gravimetric or volumetric) than those of other more benign particles. Likewise, SiO_2 has been shown to induce lung tumors in chronic rat inhalation studies at much lower concentrations (Muhle *et al.*, 1989) than those inducing tumors with more benign particles, i.e., TiO_2 or carbon black; thus, with respect to particle-induced lung tumors we should evaluate not only an effect on particle clearance but also the cytotoxicity of the inhaled particles.

With respect to a particle effect on lung clearance talc resembles more the nuisance dust TiO_2 and not the cytotoxic SiO_2 (Fig. 3). Obviously, the impairment of particle clearance is highly important since it contributes significantly to the increase of dose in the lung in general and in specific target cells, and the observed tumor-response of talc is likely to be a secondary effect mechanistically attributable to the chronic inflammatory and cell-proliferative events (Fig. 4). Although the basic underlying mechanisms and pathogenic sequence of particle-induced lung tumors may be quite similar for cytotoxic (such as SiO_2) and more benign particles (such as TiO_2)—i.e., chronic inflammation, cell proliferation, fibrosis, tumors—one crucial difference lies in the dose levels which induce the effect. Clearly, crystalline SiO_2 is most effective in this regard, i.e., effects occur at low lung burdens, whereas talc may be comparable to TiO_2.

Although Fig. 3 implies that talc particles fit very well into the category of low-toxicity particles, the clearance rates for talc derived for this figure are only crude estimates based on the pulmonary talc accumulation data of the NTP report; it cannot be excluded that the actual clearance rates might have been different. Furthermore, the results of the 4-week talc study indicate that at lung talc burdens as low as 130–180 μg significant effects occurred on lung clearance function. This would not be consistent with a low-toxicity particle but would be indicative of a high-toxicity particle—like crystalline SiO_2.

To resolve this uncertainty a further evaluation of the study results is warranted. A quantitative comparison of the cell-proliferative and fibrogenic responses observed in the talc study with those found in other chronic particle studies (TiO_2, toner, carbon black) would be very useful for this purpose. However, such comparison would require a side-by-side evaluation of histological lung sections of the different studies. Another sensitive indicator of a pulmonary response to particles in the alveolar space is the increase of PMNs in bronchoalveolar lavage as a marker of inflammation to differentiate particles of varying cytotoxicity. Results from Muhle *et al.* (1991) and Bellmann *et al.* (1991) from a chronic study in rats with toner, TiO_2, and crystalline SiO_2 particles are compiled in Fig. 5. Added to this figure are the results of the chronic NTP talc study on lung lavage PMNs for lung burdens measured at 24 months of exposure. The dose

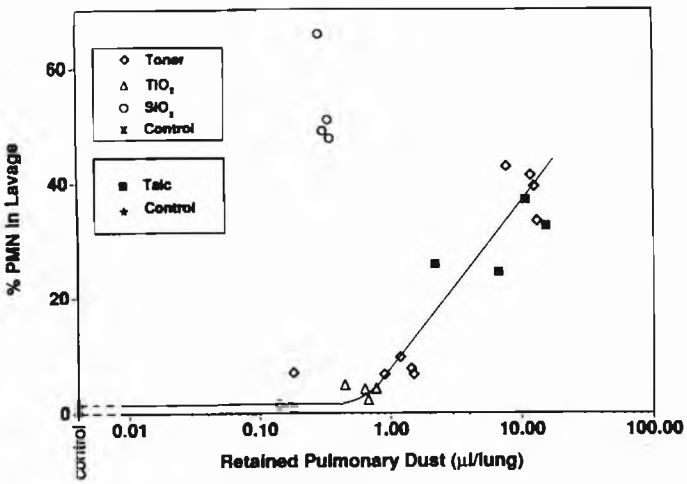

FIG. 5. Increase of PMN in pulmonary lavage of rats in chronic particle inhalation studies (toner, TiO_2, and SiO_2: Muhle *et al.*, 1991; Bellmann *et al.*, 1991; talc: NTP, 1993) as a function of the volumetric lung burdens of different particulate compounds. Data are expressed as percentages of the total lavaged cells rather than as absolute cell numbers since the lavage techniques used in the different studies might have been different. (Line was drawn through the points).

parameter is the same as that used in Fig. 3, i.e., retained particle volume in the lungs. Two conclusions are obvious from this graph: One is that talc fits extremely well into the overall dose–response curve for TiO_2 and toner particles. The other is that crystalline SiO_2 is very different from the rest of the particles, providing further evidence for the suggestion that indeed talc can be categorized similar to TiO_2 and toner as a low-toxicity particle.

Further comparisons of the effects of talc with diverse particulate material are scanty. Results of an *in vitro* study on the cytotoxicity of talc for macrophages showed that several different talc preparations were slightly more toxic to these cells than magnetite (Fe_3O_4, used as surrogate for a nonfibrogenic dust), whereas crystalline silica was significantly more cytotoxic in this study (Davies *et al.*, 1983).

Thus, with respect to the classification of talc particles we can conclude that they have a significantly lower biological activity than crystalline silica and may be slightly more active than other low-toxicity particles, such as Fe_3O_4 or TiO_2. A lower TLV for talc compared to TiO_2 or iron oxide appears to be justified (ACGIH, 1994). However, based on reported results of several chronic animal inhalation studies and on our own study results, the present TLV of 5 mg/m^3 for the former category of nuisance particles (now termed PNOC) in general appears to be too high and a lowering would be justified to avoid potential particle overload effects on lung clearance. Extrapolation from results of rat studies to humans using rat- and human-specific data for alveolar macrophage morphometry and numbers and for particle kinetics shows that on the basis of a volumetric overload effect a TLV of about 1 mg/m^3 would prevent overload-induced impaired particle clearance (Oberdörster, 1994). This value is for PNOC of unit density, for PNOC of higher density the TLV could be higher. For particles of materials with greater biological activity the TLV should be lower than one would derive from their density.

Justification for a lower TLV for PNOC is based on the following reasoning: Excess lung tumors in chronic rat inhalation studies with particles occurred only when lung clearance was impaired and even then only in the higher exposure groups; excess tumors were never seen when lung clearance remained unaffected. Since the rat appears to be more sensitive with respect to tumor responses after nonfibrous particle exposure than other species—including humans—the setting of occupational exposure limits aimed at preventing lung overload-impaired lung clearance would also protect from any other effects, including any potential tumorigenic effect.

Both concentrations of talc used in the chronic study resulted in lung overload and exceeded the MTD or MFTD. Since any highly persistent particulate compound of low cytotoxicity has a carcinogenic potential in rats when chronically inhaled at high enough concentrations the classification of such particles with respect to human pulmonary carcinogenicity must be carefully considered. A classification as "Animal Carcinogen" according to the ACGIH (1994) categories for carcinogenicity would be appropriate. Compounds in this category would not be likely to cause cancer in humans except under uncommon or unlikely levels of exposure (ACGIH, 1994). Thus, the conclusion of the NTP report (1993) that there was clear evidence of carcinogenicity in female rats needs to be qualified by a statement that lung tumors were most likely produced secondary to particle overload and related chronic toxicity.

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