

## The Effect of Amosite and Chrysotile Asbestos on the Clearance of $\text{TiO}_2$ Particles from the Lung<sup>1</sup>

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Particle clearance from the lungs, a complex nonrespiratory function of the respiratory system, involves protective mechanisms whereby the respiratory surfaces are kept clear of substances which otherwise could interfere with gas exchange. To observe and measure lung clearance, a test substance has to be introduced into the respiratory system. The tracking of this substance and the eventual change of clearance patterns under various conditions is then the basis for retention and clearance assessments.

It is obvious that depending on the substance utilized, different clearance mechanisms may be in fact studied. It can be expected that most particles will evoke a response that involves more than one clearance pathway and mechanism. However, as evidence suggests (Ferin, 1975), there are particles, such as  $\text{TiO}_2$ , which are preferentially handled by one component and pathway at least in some part of the post exposure period. Such substances can be used as specific probes of clearance. Our lung clearance test using particles of  $\text{TiO}_2$  will yield information mainly on the alveolo-bronchial route and on the involvement of alveolar macrophages (Ferin and Leach, 1975).

In an effort to broaden the battery of testing methods in studies related to health effects of air pollutants we have performed experiments in which lung clearance has been tested in rats after various exposures of  $\text{SO}_2$  or  $\text{NO}_x$  (Ferin and Leach, 1973, 1975). In this paper we will report on amosite or chrysotile exposures and their effect on the particle clearance function of the lung as tested by the  $\text{TiO}_2$  method.

### METHOD AND MATERIALS

We used male hooded rats of Long-Evans descent, weighing about 250 g each at the beginning of experimentation. Amosite or Canadian chrysotile from the U.I.C.C. Standard Reference Asbestos Samples were used at a concentration ranging from about 1 to 14  $\text{mg}/\text{m}^3$  for a duration from 1 hour to 22 days. The dust concentration was monitored by the membrane filter technique, particle size esti-

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mates were made by use of the Hexhslet Elutriator and the Mercer Cascade Impactor. After the asbestos exposure in a 2m<sup>3</sup> chamber, the rats were challenged usually by a 7 hour inhalation exposure of TiO<sub>2</sub> at a concentration of about 15 mg/m<sup>3</sup>. This exposure resulted in a TiO<sub>2</sub> load of about 130 μg/lung. Rats in groups of about ten were sacrificed the day following the TiO<sub>2</sub> exposure, designated as Day 1, and at Days 25 and 130, postexposure. Control rats, exposed to a 7 hour TiO<sub>2</sub> exposure only, were sacrificed at the same time intervals. In order to check the possible mass effect of the asbestos load on clearance, special control groups of rats were used in some experiments. These special control rats were exposed to TiO<sub>2</sub> prior to the 7 hour test exposure in such a way that their total lung load would be comparable to the total particle load of the asbestos and the TiO<sub>2</sub> exposed animals. The lung content of TiO<sub>2</sub> was determined chemically by a photometric method using 4,4-diantipryl methane monohydrate. The TiO<sub>2</sub> retention values from the experimental animals were then compared with corresponding control values for statistically significant difference. Differences at probability levels of 0.05 or lower were considered significant. At higher probability not significant (N.S.) is indicated in the tables, in which the arithmetic mean and standard deviation resulting from about ten individual analyses are given.

For aerosol generation of both asbestos and TiO<sub>2</sub>, the Wright Dust Feed was used. The respirable fraction of the TiO<sub>2</sub> aerosol was about 78%, of the amosite aerosol about 72%, and of the chrysotile aerosol about 23%. The mass median aerodynamic diameter (MMAD) of the TiO<sub>2</sub> particles was 1.48 μm, the mean geometric standard deviation 3.26, the specific surface area of the particles 7 m<sup>2</sup>/g, and the *in vitro* solubility was ~10<sup>-2</sup> g/cm<sup>2</sup>/day. The *in vivo* dissolution tested by im injection of a TiO<sub>2</sub> suspension in rats confirmed the very low solubility; the muscle load change being negligible at 8, 15, 25, 57 and 118 days postinjection (Ferin, 1975).

## RESULTS

### *Effect of Amosite on Clearance*

The amosite experiments involved mostly short term exposures at high (~10 mg/m<sup>3</sup>), medium (~5 mg/m<sup>3</sup>) and low concentrations (~1 mg/m<sup>3</sup>). The latter is the concentration estimated to be about 10× the Threshold Limit Value of 5 fibers/ml. This computation is based on a model fiber of a 1 μm diameter, 5 μm length and a density of 3. Two experiments at this concentration involved our longest exposures, the number of exposures being 11 or 22, each lasting 5½ hours.

In Table 1 the results of two high concentration (11 to 14 mg/m<sup>3</sup>) experiments are shown. In both experiments a control and a special control (mass control effect check) group was used. There were no significant differences in terms of TiO<sub>2</sub> retention between these control groups, therefore no special control group will be referred to in subsequent tables. The TiO<sub>2</sub> retention was in both amosite groups, exposed for 1 or 3 hours, significantly elevated, when compared to their control or special control group, indicating a suppression of clearance by about 54% in Experiment 1 and about 37% in Experiment 2.

Results of two experiments at a low concentration (0.9 to 1.0 mg/m<sup>3</sup>) are shown in Table 2. Although the number of exposures had been increased to three or five, respectively, no effect on TiO<sub>2</sub> retention was observed.

TABLE 1  
TiO<sub>2</sub> RETENTION AFTER AMOSITE EXPOSURE AT HIGH CONCENTRATIONS

Rat group	Amosite exposure		TiO <sub>2</sub> Exposure		TiO <sub>2</sub> Load			
	(Mg/m <sup>3</sup> )	(Hour)	(Mg/m <sup>3</sup> )	(hour)	Day 1 (μg/lung)	Day 25 (μg/lung)	(%)	(P<)
Experiment 1								
Control	—	—	11	1 × 7	121 ± 15	61 ± 10	50	0.01 0.05
Experimental	11	1 × 1	11	1 × 6	92 ± 12	72 ± 26	78	
Special control	—	—	11	1 × 1 & 1 × 6	123 ± 16	70 ± 18	57	
Experiment 2								
Control	—	—	16	1 × 7	129 ± 25	70 ± 15	54	0.01 0.05
Experimental	14	1 × 3	16	1 × 6	108 ± 9	79 ± 15	73	
Special control	—	—	16	1 × 3 & 1 × 6	149 ± 27	90 ± 13	60	

The next two experiments (Table 3), one at medium (5 mg/m<sup>3</sup>) and one at low (0.9 mg/m<sup>3</sup>) concentration give results of retention analysis beyond Day 25 post-exposure. No effect on clearance was observed at the low concentration (Experiment 5) either at Day 25 or Day 130 postexposure. However, at medium concentration (Experiment 6), a significant difference in TiO<sub>2</sub> retention at Days 25 and 130 postexposure indicate a suppression of clearance by about 50%.

Retention analysis of the last two experiments (Table 4) at low concentration (1 mg/m<sup>3</sup>) and extended exposure revealed no effect on clearance as seen at Day 25 postexposure. At Day 130 postexposure a statistically significant difference between the amosite and control groups in terms of TiO<sub>2</sub> retention indicate a suppressive effect on clearance. However, as clearance at Day 25 shows normal values, and because the analytical results in terms of absolute values were low, the results of these experiments point, in our judgement, more toward no effect on clearance than clearance suppression.

#### *Effect of Chrysotile on Clearance*

Results of three chrysotile experiments are on hand at the present. They indicate a similar pattern in terms of clearance effects as the amosite experiments do.

TABLE 2  
TiO<sub>2</sub> RETENTION AFTER AMOSITE EXPOSURE AT LOW CONCENTRATIONS

Rat group	Amosite exposure		TiO <sub>2</sub> exposure		TiO <sub>2</sub> Load			
	(mg/m <sup>3</sup> )	(hr)	(mg/m <sup>3</sup> )	(hr)	Day 1 (μg/lung)	Day 25 (μg/lung)	(%)	(P<)
Experiment 3								
Control	—	—	15	7	147 ± 30	86 ± 22	59	N.S.
Experimental	1.0	3 × 5½	15	7	152 ± 19	78 ± 21	51	
Experiment 4								
Control	—	—	15	7	147 ± 30	86 ± 22	59	N.S.
Experimental	0.9	8 × 5½	15	7	135 ± 12	92 ± 23	68	

TABLE 3  
TiO<sub>2</sub> RETENTION AT DAYS 25 AND 130 POSTEXPOSURE AFTER AMOSITE EXPOSURES  
AT MEDIUM OR LOW CONCENTRATION

Rat group	Amosite Exposure		TiO <sub>2</sub> Exposure		TiO <sub>2</sub> load							
	(mg/m <sup>3</sup> )	(hr)	(mg/m <sup>3</sup> )	(hr)	Day 1		Day 25		Day 130			
					(μg/lung)	(μg/lung)	(%)	P<	(μg/lung)	(%)	P<	
Experiment 5												
Control	—	—	16	7	110 ± 16	69 ± 17	62	N.S.	20 ± 10	18	N.S.	
Experimental	0.9	3 × 5½	16	7	112 ± 23	69 ± 12	62		21 ± 7	19		
Experiment 6												
Control	—	—	15	6	87 ± 20	53 ± 11	61	0.05	14 ± 6	16	0.01	
Experimental	5	1 × 2¾	15	6	70 ± 27	56 ± 19	80		21 ± 9	30		

At high concentrations (~15 mg/m<sup>3</sup>) lasting only a few hours an elevated TiO<sub>2</sub> retention is found at Day 25 postexposure. One experiment at a concentration of about 1 mg/m<sup>3</sup>, lasting 5 × 5½ hours showed no indication of TiO<sub>2</sub> clearance suppression. The effective air concentration of chrysotile may be different from that of amosite because of the much lower respirable fraction of chrysotile. A detailed report on chrysotile effect will be given after completion of more chrysotile exposures.

#### DISCUSSION

Wagner and Skidmore (1965) have shown that different forms of asbestos are cleared from the lung of rats, exposed to high concentrations of dust (~40–70 mg/m<sup>3</sup>) for 30 days, with a biological half-life of about 85 days except for Rhodesian chrysotile which has a three times greater elimination rate. A faster clearance of chrysotile from the lung has been confirmed by other investigators including Timbrell (1969) and Morris *et al.* (1967).

We studied the effect of two types of asbestos (amosite and chrysotile) on the clearance of inert TiO<sub>2</sub> particles which were used as a test substance. The experimental results showed that short exposures in terms of hours affect the alveolar clearance if the concentration is above 5 mg/m<sup>3</sup>. Concentrations around ~1 mg/m<sup>3</sup>, which were estimated to be 10× above the present Threshold Limit Value did

TABLE 4  
TiO<sub>2</sub> RETENTION AFTER 11 OR 22 AMOSITE EXPOSURES AT LOW CONCENTRATION

Rat group	Amosite exposure		Exposure		TiO <sub>2</sub> Load						
	(mg/m <sup>3</sup> )	(hr)	(mg/m <sup>3</sup> )	(hr)	Day 1		Day 25		Day 130		
					(μg/lung)	(μg/lung)	(%)	P<	(μg/lung)	(%)	P<
Experiment 7											
Control	—	—	14	1 × 7	122 ± 22	65 ± 11	53	N.S.	14 ± 18	11	0.01
Experimental	1	11 × 5½	14	1 × 7	120 ± 16	68 ± 15	57		20 ± 9	17	
Experiment 8											
Control	—	—	14	1 × 7	122 ± 22	65 ± 11	53	N.S.	14 ± 8	11	0.05
Experimental	1	22 × 5½	14	1 × 7	128 ± 20	69 ± 12	54		20 ± 8	16	

not affect the studied clearance pathway. However, extending the observation period from 25 to 130 days postexposure and increasing the number of amosite exposures from 3 to 11 or 22 showed an effect in our testing procedure. How important this late effect is can not be determined from our experience. However, the effect of high concentrations of asbestos even at exposures lasting for a short time should be considered with concern.

### SUMMARY

It is well known that the pulmonary system encompasses pathways by which foreign materials can be removed from the body. The defense mechanisms involved in this removal, are presently being examined closely in order to more fully understand the complex interactions between the lung parenchyma and airborne agents. In the experiments described here, techniques have been developed to assess the effects of air pollutants on the alveolar clearance pattern of inert particles deposited in the lungs of laboratory animals. Amosite or chrysotile asbestos exposures, relatively short in duration (1–3 hours) and high in concentration ( $\sim 10 \text{ mg/m}^3$ ) depressed the alveolar clearance of titanic oxide ( $\text{TiO}_2$ ) particles in rats. This effect was not observed when asbestos air concentrations were reduced to about  $10\times$  the Threshold Limit Value (TLV) estimated to be  $\sim 0.1 \text{ mg/m}^3$ . Extending the postexposure observation period and the number of exposures to 11 or 22 a suppressive effect on  $\text{TiO}_2$  clearance was seen. How important this late effect is can not be determined at this time. However, the effect of high concentrations of amosite or chrysotile at exposures lasting only a short time should be considered with concern.

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