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Chronic Inhalation of Short Asbestos Fibers[1,2,3]

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Chronic Inhalation of Short Asbestos Fibers. PLATEK, S. F., GROTH, D. H., ULRICH, C. E., STETTLER, L. E., FINNELL, M. S., AND STOLL, M. (1985). *Fundam. Appl. Toxicol.* 5, 327-340. An animal inhalation study was initiated to study the chronic biological effects of inhalation of short chrysotile asbestos fibers. Rats and monkeys were exposed for 18 months, 7 hr/day, 5 days/week to a specially prepared, chrysotile asbestos aerosol. Based upon daily chamber measurements, the mean concentration of fibers in the chamber air was 1.0 mg/m^3. By phase contrast microscopy, the number of fibers > 5 μm in length was determined to be 0.79 fiber per cubic centimeter. Rats were autopsied for pathological and histochemical examination at 1, 3, 6, 12, 18, and 24 months after initiating exposures. No significnt differences in the histochemical data were seen between the exposed and control groups. Gross and histopathologic examination of exposed and control groups of rats indicated no compound-related lesions, including fibrosis. Open lung biopsies were performed on the chrysotile-exposed and the control monkeys 28 months after initiating exposures. Histopathologic evaluation of the lung biopsy tissue showed the presence of asbestos bodies adjacent to the terminal bronchioles of the asbestos-exposed monkeys. There was no observed fibrosis in pulmonary tissue. All monkeys are being maintained for an indefinite period and observed for signs of latent pulmonary disease.

Asbestos contains numerous insulating as well as chemical- and wear-resistant properties which make it an extremely valuable mineral in the production of textiles, cement and tile products, gaskets and friction materials, coatings and automotive brake linings (Hendry, 1965). However, asbestos has been shown by numerous investigators to cause the debilitating human diseases of asbestosis, bronchogenic carcinoma, and mesothelioma (Newhouse and Thompson, 1965; Kannerstein *et al.*, 1977; McDonald and Liddell, 1979). Results of rather extensive animal research have shown that fiber length and diameter are the most important determinants in the induction of mesotheliomas. Fibers that are >8 μm in length and <1.25 μm in diameter are much more potent than those of other sizes (Stanton *et al.*, 1981). It is not known, however, and little research has been done to prove whether fiber size is an equally important determinant in the induction of asbestosis or lung cancer. A few authors have mentioned the potential hazards and problems associated with short fiber asbestos (Yeager *et al.*, 1983; Gross, 1974; Selikoff *et al.*, 1972; Holt *et al.*, 1965).

The present Occupational Safety and Health Administration (OSHA) standard for airborne asbestos fiber exposure is not to exceed two fibers longer than 5 μm per cubic

[1] A preliminary report of this material was presented at the First NCI/EPA/NIOSH Collaborative Workshop at Rockville, Md., May 7, 1980.

[2] This study was conducted under contract with The International Research and Development Corporation, Mattawan, Mich. under NIOSH Contract 210-77-0151.

[3] This study was supported by the National Cancer Institute (NCI) via an Interagency Agreement between NCI and NIOSH.

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centimeter of air over an 8-hr, time-weighted average period (OSHA, 1978). Although the OSHA standard regards airborne asbestos fibers less than 5 μm as a nuisance dust with exposure to 5 mg/m^3 permissible, it has been shown that for every fiber greater than 5 μm there may be more than 100 fibers less than 5 μm in length (Holt *et al.*, 1965). Of these smaller fibers, most are probably beyond the resolution of the optical light microscope (~0.25 μm) and may be seen only by electron microscopy. Thus, the several million Americans employed in the use of asbestos-containing products, including automotive brake and clutch relining as well as reinforcing pipe and tile manufacturing (DHEW, 1978; Bruckman, 1978; Lynch, 1968), are exposed to potentially high levels of short (<5 μm) asbestos fibers.

The purpose of this study was to determine the chronic biological effects after inhalation of chrysotile asbestos fibers less than 5 μm in length in laboratory rats and monkeys.

MATERIALS AND METHODS

Chrysotile (short fiber) preparation. Type 7TF1 chrysotile was obtained from the Johns-Manville Sales Corporation in Denver, Colorado. Four-hundred-gram batches of the chrysotile were dried in an oven for 24 hr at 191°C, milled in a ceramic ball mill for 24 hr, and then dried again for 24 hr at 191°C. Each batch was examined by scanning electron microscopy to determine fiber size. Five batches were randomly selected and quantitatively analyzed by induction coupled plasma emission spectroscopy for their elemental content.

Inhalation chambers, dust generation, and characterization. Experimental exposures were conducted in two 16-m^3 glass and stainless steel, dynamic air flow exposure chambers. Air for both control and exposure chamber ventilation was filtered with HEPA filters to remove particulates and controlled for temperature and humidity. The mean temperature within the two exposure chambers was 23.6°C (74.5°F) with a mean relative humidity of 61.5%. The chamber air flow rate for both study groups was maintained at 1500 liters per minute (53 cfm). The control inhalation chamber was supplied only with the filtered and temperature- and humidity-controlled air. The asbestos inhalation chamber used a specially designed dust-feed elutriator system (International Research and Development Corporation, Mattawan, Mich.) to generate the short fiber asbestos atmosphere (Fig. 1). The operational characteristics of the device were as follows: a known weight of prepared asbestos was placed in the reservoir of the generator and stirred by a stirring mechanism. A disk containing cups around the perimeter was rotated through the reservoir permitting the cups to be filled with the chrysotile dust. The filled cups then passed over a blowout port where a metered flow of desiccated air "puffed" the asbestos into a vertical elutriator column fitted between the generator and the chamber air inlet.

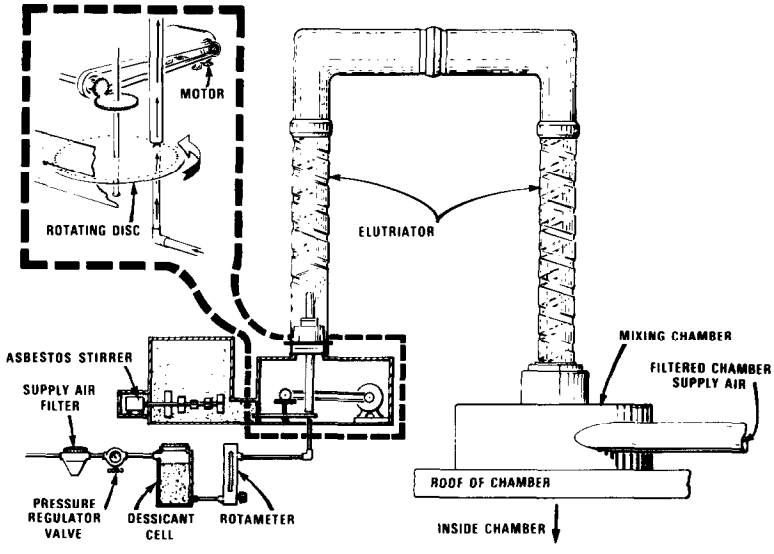


FIG. 1. Diagram of system used to generate short asbestos fibers.

The chamber's incoming air flow diluted the aerosol to the desired concentration. The elutriator column only passed the desired smaller fibers (<5 μm) while a sediment of larger fibers and particles was collected on the bottom and side convolutions of the column.

During the experiment, the inhalation chamber atmospheres were routinely characterized by three different methods. Mass concentrations were determined three times daily during exposures by drawing known volumes of chamber air through preweighed glass fiber filters. The filters were then reweighed and the mass concentration was calculated as the difference in weight divided by the total volume of air sampled.

Measurements of the chamber concentration of fibers greater than 5 μm in length were also made three times daily during exposures. In this procedure, chamber samples of known volume were collected on 0.8-μm pore-size cellulose ester membrane filters. The filters were then mounted and counted by phase contrast light microscopy at a magnification of 400× using NIOSH Physical and Chemical Analysis Method No. 239 (Taylor, 1977).

Particle size distributions were also determined daily during the exposures utilizing an Andersen cascade impactor (Andersen Samplers, Atlanta, Ga.) operating at 28.3 liters per minute. The weight of particles on each stage was determined gravimetrically.

Scanning electron microscopy was also used to monitor the size distribution of particles in the exposure chambers. Nine asbestos chamber samples which were collected on 0.1-μm pore-size Nuclepore filters were sized. Scanning electron micrograph enlargements of individual fields of view at a magnification of 5000× were used in the sizing procedure. Nonfibrous particles were sized using a Zeiss Model TCG-3 particle size counter (Carl Zeiss, Germany). The lengths and widths of fibrous particles were determined manually using a ruler.

Animal study. Three hundred male Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) with an average body weight of 109 g were randomly divided into two groups of 150 each. The rats were housed individually in suspended wire-mesh stainless-steel cages and given food (Purina Laboratory Chow) and water *ad libitum* throughout the study except during the daily exposures.

Twenty male cynomolgus monkeys, *Macaca fascicularis* (Primate Imports Corporation, Port Washington, N.Y.), weighing from 3.8 to 3.96 kg were randomly divided into exposure and control groups of 10 each. The monkeys were housed individually in suspended stainless-steel cages and given food (Purina Monkey Chow) and water *ad libitum* (except during the daily exposures) plus supplementary fruit throughout the study.

All animals were exposed in the inhalation chambers for 7 hr/day, 5 days/week for 18 months. At the end of each daily exposure, all animals were removed from the chambers. The chambers were then cleaned. The monkeys were then returned to the chambers while the rats remained in the adjacent holding room until the next exposure period. At the end of the 18-months exposure period, all surviving rats were maintained for an additional 6-month postexposure observation period prior to final sacrifice. The monkeys were maintained for a 10-month postexposure period and then transferred to a contractor for long-term observation.

Pathological analyses. Rat sacrifice intervals and on-study mortality are indicated in Table 1. At each sacrifice period, the rats were killed with an overdose of sodium pentobarbital administered by intraabdominal injection and then necropsied for gross and histopathological evaluation. The lungs were weighed and then inflated with phosphate-buffered, neutral formalin. Sections of the following organs were also fixed in phosphate-buffered, neutral formalin:

brain	mammary gland
pituitary	lung
trachea	liver
nasal turbinates	kidney
larynx	pancreas
adrenal (2)	spleen
thyroid	mesenteric lymph nodes
urinary bladder	tracheobronchial lymph node
skin	stomach
sternal bone marrow	colon (ascending and descending)
salivary gland	testis (2)
mammary gland	prostate
any other grossly abnormal tissue	

TABLE 1

EXPERIMENTAL RAT SACRIFICE AND MORTALITY SCHEDULE

	Sacrifice (study month)						Mortality[a] (month interval)					
Treatment	1	3	6	12	18	24	1–3	3–6	6–12	12–18	18–24	Total
Asbestos	5	15	15	15	20	38	2	2	3	12	23	150
Control	5	15	15	15	20	45	0	4	2	14	15	150

[a] Died in extremis or sacrificed moribund (unscheduled deaths).

At all sacrifice periods, the left lungs from five randomly selected rats from each exposure group were analyzed for the presence of an inflammatory response by measuring the following enzymes: lactic dehydrogenase (Zugibe, 1970), acid phosphatase (Barka and Anderson, 1963), and β-glucoronidase (Thompson, 1966). At the 3-, 6-, 12-, and 18-month and terminal sacrifices, the left lungs from 10 randomly selected rats from each exposure group were taken for subsequent hydroxyproline and elemental silicon analyses. These lungs were sectioned longitudinally along the main stem bronchi such that each contained portions of the apex and base. One section was then analyzed for hydroxyproline and the other for elemental silicon by plasma emission spectroscopy. In addition, blood was taken from these same rats for elemental silicon determination. Blood was also drawn from all monkeys at these sacrifice intervals for elemental silicon quantitation.

Hematoxylin and eosin stained sections of the remaining lung tissue and of each of the above tissues were prepared for and examined by light microscopy.

Twenty-eight months after the initiation of exposures (10 months after completion of exposures), open lung biopsies were performed on all monkeys for histopathological evaluation and for lung tissue fiber content determinations. Approximately 2 g of tissue was taken from the anterior margin of the right lower lobe of each monkey.

The procedure for determining lung fiber content is as follows. Pieces of lung tissue were freeze-dried to constant weight using a Labconco Freeze Dry 5 freeze dryer and then ashed in a low-temperature asher (International Plasma Corporation, Model 1PC 1005-148AN) using an oxygen plasma. The ash from each lung was then added to 50 ml of a 0.05% solution of Aerosol OT (Fisher Scientific) in filtered, deionized water and then sonicated for 10 min in an ultrasonic bath. The resulting suspensions were then diluted to 100 ml and stirred magnetically for 10 min. Aliquots of the suspensions were then filtered through 0.1-μm pore-size Nucleopore filters. The filters were then carbon coated in a vacuum evaporator (Edwards High Vacuum, Model 306). Pieces of these filters were then placed on 200-mesh transmission electron microscope grids and cleared with chloroform using a modified Jaffe–Wick technique (Jaffe, 1948; Chatfield and Dillon, 1978).

The resulting grids were examined in a transmission electron microscope (JEOL, Model 100CX) equipped with an energy dispersive X-ray spectrometer system (EG&G Ortec, Model EEDS II). All of the fibers found in 10 grid spaces for two grids of each sample were counted and sized. Every 10th fiber was analyzed by energy dispersive X-ray analysis and selected area electron diffraction to confirm that it was chrysotile. Lung fiber concentrations were calculated from the fiber densities on the filter preparations and the original dry lung weights.

All of the monkey lung biopsies, both exposed and controls, were analyzed for fiber content. In addition, lungs from five of the 18- and 24-month (terminal sacrifice) rats from each exposure group were also analyzed for fiber content.

RESULTS

Five batches of the ball-milled chrysotile preparations used in the exposure chamber were chemically analyzed. The results of these elemental analyses are summarized in Table 2. Of interest are the relatively high concentrations of aluminum (0.88–1.1%).

Each batch of the ball-milled asbestos was also examined using the scanning electron microscope (JEOL, JXA 50A) at magnifications of 400× and 5000× to ensure that the vast majority of the prepared fibers were less than 5 μm. An unavoidable by-product of the ball-milling procedure was the preparation of agglomerated asbestos "balls" or bundles of asbestos fibers. Figure 2a is a scanning electron micrograph showing a typical ball-milled preparation. A high magnification enlargement of one of the asbestos "balls" is shown in Fig. 2b.

The results of the exposure chamber monitoring were as follows: the mean mass concentration as determined by gravimetric sampling was 1.0 mg/m^3 with a standard deviation of ±0.28. The range of mass values over the entire experiment was 0.4 to 1.8 mg/m^3. The mean number of asbestos fibers greater than 5 μm in length as determined by light microscopy (Taylor, 1977) was 0.79 fiber/cm^3 with a standard deviation of ±0.41. The values ranged from 0.08 to 1.5 fibers/cm^3. The mean equivalent aerodynamic diameter for the particles in the exposure chamber as determined by using the Andersen cascade impactor was 5.0 μm with a geometric standard deviation of 2.9.

A total of 14,558 particles (fibrous and nonfibrous) in 138 fields of view from nine samples of the asbestos chamber atmosphere collected on Nucleopore filters were counted and sized by SEM. Of these particles, 6940

TABLE 2

CONCENTRATION OF INORGANIC ELEMENTS IN 7TF1 CHRYSOTILE[a]

	Batch No.					
	21	29	39	61	84	Mean
Al	0.880	0.960	0.904	1.10	0.909	0.95
Ca	0.425	0.396	0.416	0.429	0.420	0.42
Cr	0.086	0.079	0.095	0.088	0.093	0.088
Fe	3.65	3.33	3.59	3.59	3.64	3.56
Li	.001	0.001	.001	0.001	.001	.001
Mg	18.3	16.4	15.5	14.4	16.9	16.3
Mn	0.068	0.063	0.067	0.067	0.068	0.067
Mo	0.005	.001	0.005	0.005	0.005	0.004
Na	0.466	0.279	0.544	0.614	0.515	0.483
Ni	0.132	0.102	0.132	0.131	0.134	0.126
P	0.018	0.017	0.018	0.019	0.021	0.019
Ti	0.039	0.034	0.034	0.036	0.034	0.035
Tl	0.003	0.002	0.003	0.003	0.003	0.003
V	0.003	0.003	0.003	0.003	0.003	0.003
Zn	0.004	0.004	0.006	0.005	0.005	0.005
Zr	0.003	0.005	0.005	0.006	0.004	0.005

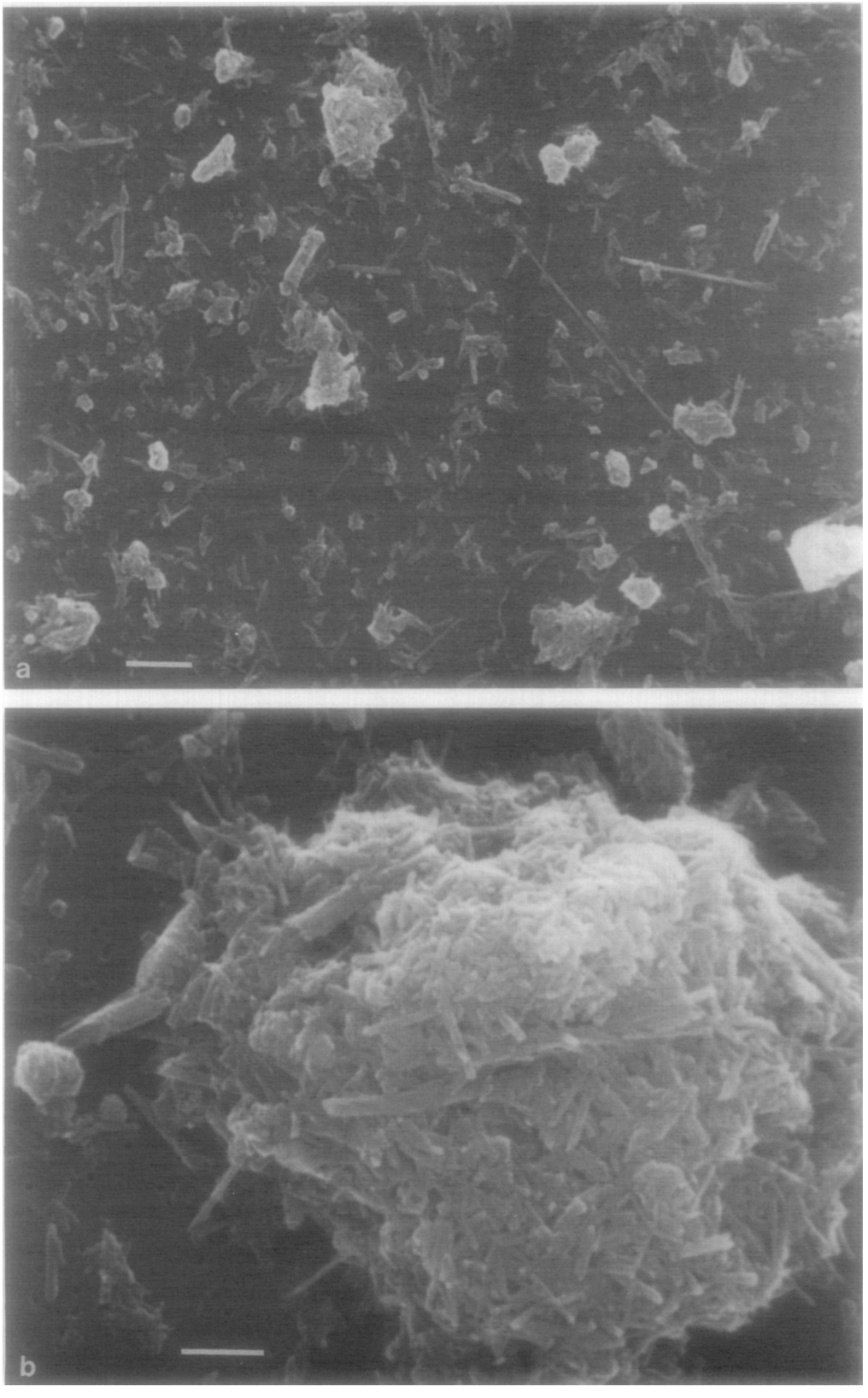
Note. The following elements were less than 0.001% in all of the samples: Ag, As, Be, Cd, Co, Cu, Pb, Pt, Se, Sn, Te, W, and Y.

[a] Values are percentages.

(48%) were fibers and 7618 (52%) were nonfibrous. The majority of the nonfibrous particles were the "asbestos balls" as shown in Fig. 2(b). The fiber size distribution is shown in Fig. 3 for eight of the nine samples. The count median length of the fibers was 0.67 μm (geometric standard deviation = 1.87), the count median diameter was 0.09 μm, and the median aspect ratio (length:width) was 12. Only 46 (0.66%) of the 6940 fibers were greater than 5 μm in length. Only 0.35% of the fibers were both greater than 5 μm in length and greater than 0.3 μm in diameter. Since the volume of chamber air pumped through these nine filters was measured at the time of collection (2.1 liters/min for 60 min) the number of fibers per cubic centimeter of chamber air was also calculated. The mean number of fibers greater than 5 μm in length found by this method was 3.0/cm^3 of air (range, 0.4–7.5). This is 3.8 times the mean number of fibers found by phase contrast light microscopy. Since the number of fibers greater than 5 μm in length and greater than 0.3 μm in diameter represented about 52% of all fibers greater than 5 μm in length, their concentration in the chamber air was estimated to be 1.6 fibers/cm^3 of air. This is about two times the mean number of fibers found by phase contrast light microscopy. The mean number of fibers less than 5 μm in length in these nine samples was 493/cm^3 of air (range, 144–1226).

Table 1 shows the on-study mortality of the experimental rats between scheduled sacrifice intervals. Table 3 shows the rat mean body and lung weights at the designated sacrifice intervals. Both tables illustrate that no significant difference was observed in group mortality or body and lung weights over the course of the experiment. No significant body weight differences were observed in the monkey groups at regular weighing intervals except at the 3-month period.

Results of histochemical tests, which included β-glucuronidase, acid phosphatase,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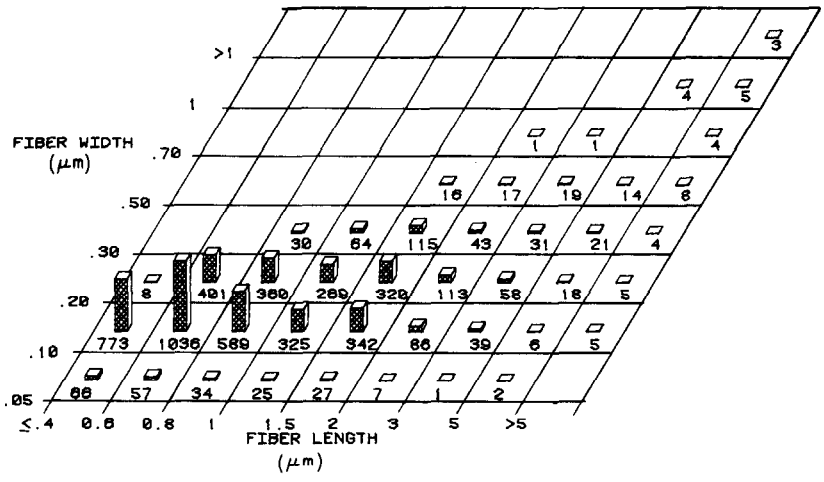

FIG. 3. Aerosol chamber fiber size distribution. The data shown are the sum of nine filter analyses.

and lactic dehydrogenase, indicated no difference between the control and exposed group. The silicon content of the experimental rat lung and blood serum for each sacrifice interval is shown in Table 4. Because of the broad range of silicon values in both the rat and monkey groups as well as varying limits of detectability, no conclusions may be drawn with regard to silicon content.

The hydroxyproline determination of the experimental rat lung as seen in Table 5 revealed no significant difference between the exposed and control groups at sacrifice intervals as well as over the course of the study (analysis of variance, $p > 0.5$).

Pathology

Based upon gross and microscopic observations the only pulmonary alterations seen in rats that could be attributed to exposure to chrysotile were few and scattered macrophages in the pulmonary alveoli. Scanning–transmission electron microscopy of these macrophages revealed the presence of chrysotile. No pulmonary fibrosis or pulmonary tumors were seen. An unexpected finding was gastritis in 12/38 exposed and 2/45 control rats at the terminal sacrifice. Microscopically the majority of these lesions consisted of focal mucosal ulcerations and submucosal inflammation in the nonglandular portion of the stomachs.

Microscopic examination of the lung biopsies from the monkeys revealed a few scattered macrophages in alveoli and a few asbestos bodies adjacent to the terminal bronchi. No fibrosis or tumors were seen in these biopsies.

Lung Fiber Analyses

The results of the fiber size analyses for the fibers recovered from the lungs of five rats from the 18-month exposure group and five rats from the 6-month postexposure group (terminal sacrifice) appear in Figs. 4 and 6 and Table 6.

In the 18-month group, a total of 2141 fibers were sized. The mean number of fibers per gram of dried lung for these rats was 294 $\pm$ 31 $\times$ 10^6 (range, 257–327 $\times$ 10^6). The mean

FIG. 2. (a) Scanning electron micrograph of a typical ball-milled asbestos preparation. Bar = 2.0 μm. (b) A scanning electron micrograph showing a typical asbestos "ball." Note that individual short fibers are aggregated together to form the ball. Bar = 1.0 μm.

TABLE 3

MEAN VALUES FOR RAT LUNG AND BODY WEIGHTS

Exposure interval (months)	Group	Body weight (g)	Lung weight (g)
1	Control	330 ± 33.5[a]	1.58 ± 0.25
	Asbestos	332 ± 21.9	1.66 ± 0.13
3	Control	454 ± 45.4	1.65 ± 0.11
	Asbestos	463 ± 47.0	1.81 ± 0.22[b]
6	Control	558 ± 50.5	1.88 ± 0.15
	Asbestos	565 ± 59.0	1.92 ± 0.16
12	Control	634 ± 61.4	2.01 ± 0.20
	Asbestos	656 ± 85.3	2.03 ± 0.16
18	Control	710 ± 95.4	2.09 ± 0.19
	Asbestos	693 ± 101.1	2.07 ± 0.19
24[c]	Control	765 ± 185.2	2.34 ± 0.22
	Asbestos	756 ± 152.4	2.31 ± 0.20

[a] Mean ± SD.
[b] Statistically different from the control group ($p < 0.05$).
[c] Six months postexposure.

concentration of fibers less than 5 μm in length was 272 ± 31 × 10^6 (range, 241–308 × 10^6), and the mean concentration of fibers greater than 5 μm in length was 23 ± 4.9 × 10^6 (range, 16–28 × 10^6). The mean percentage of fibers greater than 5 μm in length in these lungs was 7.7% (range, 5.8–9.6%). A scanning electron micrograph of a typical ashed lung preparation is shown in Fig. 5. Note the individual fibers as well as one of the asbestos "balls."

In the 6-month postexposure group (Fig. 6), a total of 919 fibers were sized. The mean number of fibers per gram of dried lung for these rats was 192 ± 27 × 10^6 (range, 143–209 × 10^6). This value is significantly lower than the same value obtained at 18 months ($p < 0.001$). The mean concentration of fibers less than 5 μm in length was 164 ± 28 × 10^6 (range, 117–189 × 10^6). This value is significantly lower than the same value obtained at 18 months ($p = 0.001$). The mean concentration of fibers greater than 5 μm in length was 27 ± 9.3 × 10^6 (range, 18–41 × 10^6). This value is not significantly different from that seen at 18 months ($p > 0.05$). The mean percentage of fibers greater than 5 μm in length in the 6-month postexposure lungs was 12.6%.

Only one fiber was found in the lung of one out of four control rat lungs at the 18-month sacrifice. The concentration of fibers in the lung of that rat was calculated to be 0.16 × 10^6 per gram of dried lung. Only one fiber was found in the lung of one out of five control rats at the 24-month sacrifice. The concentration of fibers in the lung of that rat

TABLE 4

SILICON CONCENTRATIONS IN RAT LUNGS AND BLOOD[a]

Group	Sac. interval (months)	Dry lungs			Serum		
		Range	Mean	SD	Range	Mean	SD
Cont.	3	60–300	115.0	±98.6	2–4	2.9	±0.60
Exp.	3	60–200	79.0	±43.6	2–30	6.4	±8.44
Cont.	6	40– 90	59.0	±13.7	2–270	32.8	±83.70
Exp.	6	40– 70	54.0	±10.8	2–5	2.8	±1.03
Cont.	12	70–450	86.1	±142.02	0.8–120	14.6	±37.18
Exp.	12	20–110	35.9	±28.90	0.8–240	24.9	±75.60
Cont.	18	70– 80	36.0	±19.55	1–13	2.8	±3.61
Exp.	18	20–110	54.6	±28.25	1–3	1.9	±0.60
Cont.	24	20–120	67.7	±27.7	1–24	5.4	±7.42
Exp.	24	30–130	59.5	±31.91	1–485	5.9	±6.34

[a] mg/g.

TABLE 5

HYDROXYPROLINE DETERMINATION IN EXPERIMENTAL RAT PULMONARY TISSUE

Exposure interval (months)	Group	Hydroxyproline (mg/g dry lung tissue)
3	Control	28.38 ± 2.29[a]
	Asbestos	28.84 ± 3.24
6	Control	31.08 ± 2.48
	Asbestos	32.20 ± 2.84
12	Control	35.39 ± 2.81
	Asbestos	33.69 ± 6.92
18	Control	Laboratory error—sample lost
	Asbestos	Laboratory error—sample lost
24[b]	Control	24.53 ± 7.38
	Asbestos	26.73 ± 10.63

[a] Mean ± SD.
[b] Six months postexposure.

was calculated to be 0.19×10^6/gram of dried lung.

The results of the fiber size analyses for the fibers recovered from the lungs of the 10 monkeys at the 28-month biopsy are summarized in Fig. 7. A total of 4124 fibers were sized. Of these fibers, 239 or 5.8% were greater than 5 μm in length. The number of fibers per gram of dry lung for these monkeys was determined to be $327 \pm 53 \times 10^6$. The values for the 10 biopsy samples ranged from 228 to 356×10^6 fibers/g.

DISCUSSION

The results of our study show that inhalation exposures of male rats to ball-milled chrysotile at a concentration of 1 mg/m^3 and 0.79 fiber > 5 μm in length/cm,^{3} 7 hr/day, 5 days/week for 18 months is insufficient to produce pulmonary fibrosis or tumors within 24 months. It also shows that a concentration of 23×10^6 chrysotile fibers > 5 μm in length/g of dry lung, 272×10^6 chrysotile fibers < 5 μm in length/g of dry lung, or a combination of the two are insufficient to product pulmonary fibrosis or tumors in a 6-month period (18–24 months after initiating exposures) in male rats. There are no other comparable studies reported in the literature.

However, there are several inhalation studies reported which have shown that a variety of types of asbestos can produce pulmonary fibrosis and tumors in rats. In 1977, Gross *et al.* reported the induction of lung tumors in male rats (10/41) exposed to ball- and hammer-milled Canadian chrysotile at an average weekly concentration of 86 mg/m^3,

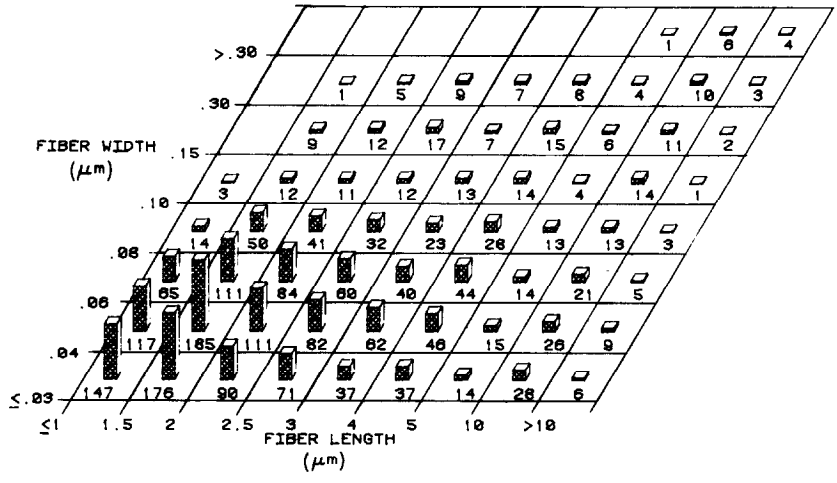

FIG. 4. Fiber size distribution for fibers extracted from five rats of the 18-month sacrifice.

TABLE 6

NUMBERS AND LENGTHS OF CHRYSOTILE FIBERS IN RAT LUNGS BY TEM[a]

Rat No.	Fiber length					
	All lengths	<5 μm	>5 μm	>8 μm	>10 μm	>15 μm
			18 Months of exposure			
80-217	302	277	25	14	5.6	3.2
80-218	291	263	28	14	6.0	3.0
80-219	257	241	16	7.3	2.4	0.61
80-220	294	269	25	8.8	5.1	2.2
80-221	327	308	19	11	5.0	2.9
Mean (SD)	294 (31)[b]	272 (31)[b]	23 (4.9)	11	4.8	2.4
			6 Months postexposure			
80-554	201	183	18	11	5.8	5.0
80-555	209	189	20	12	4.8	2.4
80-556	143	117	26	9.5	1.9	1.9
80-557	201	170	31	9.8	4.4	2.2
80-558	204	163	41	24	8.4	4.8
Mean (SD)	192 (27)	164 (28)	27 (9.3)	13	5.1	3.3

[a] Values are No. of fibers/g dry lung/10^6.

[b] Significantly higher than the same-sized fiber concentration obtained 6 months postexposure ($p \leq 0.001$).

6 hr/day, 5 days/week for 62 weeks and observed for up to 34 months following initiation of exposures. He also observed pulmonary fibrosis and one mesothelioma in the exposed rats. No information on the number of fibers per cubic centimeter in the chambers or fibers per gram of lung tissue was given. In 1974, Wagner *et al.* reported the induction of pulmonary fibrosis, lung tumors, and mesotheliomas in rats exposed for a variety of intervals ranging from 3 to 24 months to amosite, anthophyllite, crocidolite, Canadian chrysotile, or Rhodesian chrysotile. The exposures were for 7 hr/day, 5 days/week. The mean respirable dust concentrations varied from 10.1 to 13.5 mg/m^3. No information on the number of fibers per cubic centimeter in the chambers or the concentrations of fibers in the lung tissue was given.

Davis *et al.* (1978) reported the induction of pulmonary fibrosis, lung tumors, and two mesotheliomas in rats exposed to concentrations of UICC chrysotile, crocidolite, and amosite ranging from 2 to 10 mg/m^3, 7 hr/day, 5 days/week for 1 year and sacrificed 16.5 months postexposure. The highest incidence (15/40) of lung tumors and the most severe fibrosis occurred in the group exposed to chrysotile (10 mg/m^3). The concentration of fibers greater than 5 μm in length in the chamber air by phase contrast light microscopy was found to be 1950/ml. The rats exposed to chrysotile at 2 mg/m^3 (390 fibers > 5 μm in length/ml) also developed lung tumors (8/42) and pulmonary fibrosis. The incidence of lung tumors in the rats exposed to crocidolite and amosite ranged from 2.5 to 4.7% and the extent of fibrosis was less than that seen in the groups exposed to chrysotile even though the mass and number of fibers > 5 μm in length per milliliter in the aerosol were comparable to those seen with the chrysotile-exposed animals. Examination of the fiber preparations by scanning electron microscopy, however, revealed many

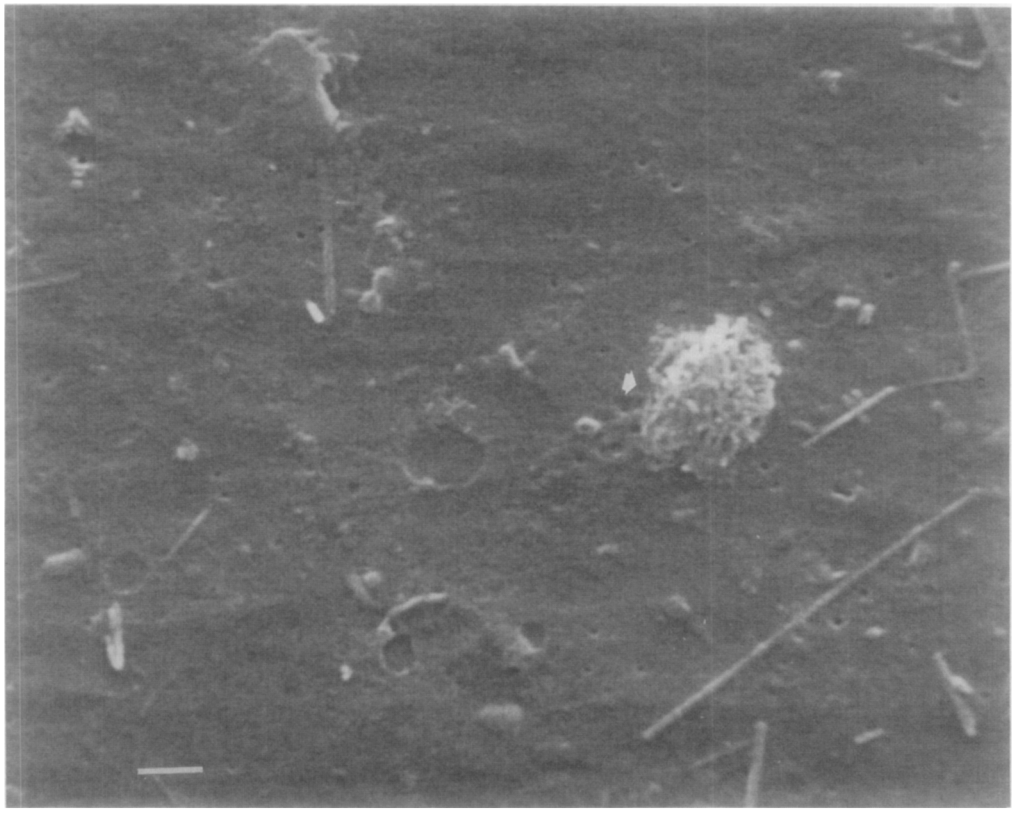

FIG. 5. A scanning electron micrograph of an ashed lung preparation for an 18-month rat. Note that an asbestos "ball" (arrow) is still intact. Bar = 1.0 μm.

more long fibers in the chrysotile preparation. It was estimated that the chrysotile aerosol (10 mg/m^3) contained 360 fibers > 20 μm in length per milliliter, whereas the crocidolite and amosite aerosols at the same mass concentrations had only 34 and 6 fibers > 20

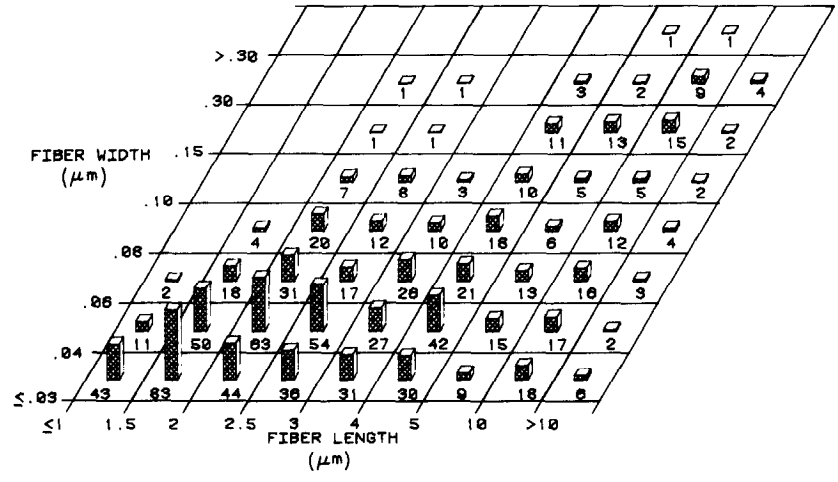

FIG. 6. Fiber size distribution for fibers extracted from five rats of the 24-month (terminal) sacrifice.

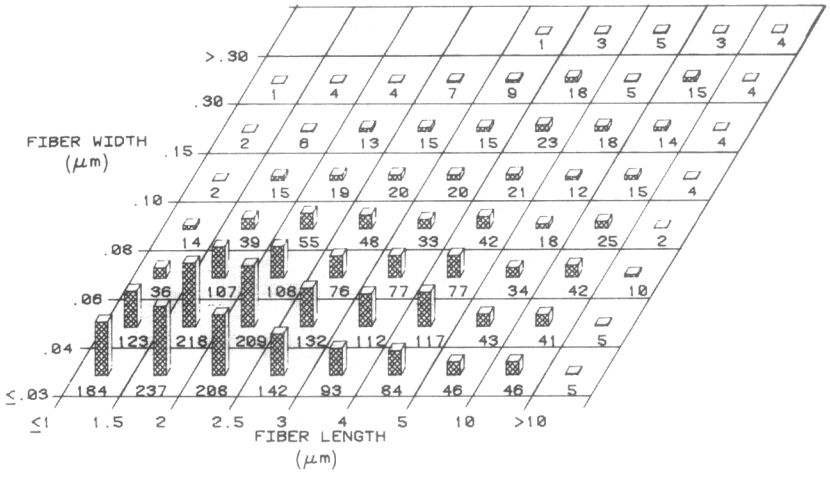

FIG. 7. Fiber size distribution for fibers extracted from 10 monkeys at the 28-month biopsy.

μm in length per milliliter. Concentrations of fibers in the lungs of the animals were not measured.

The most probable explanation for why pulmonary fibrosis and lung tumors were produced in rats by the above investigators, but not in our experiment, is the large difference in the concentration of long fibers in the aerosols. One chrysotile aerosol (10 mg/m^3) in the Davis *et al.* (1978) study contained 2538 times more fibers greater than 5 μm in length than the aerosol in our experiment and their other chrysotile aerosol (2 mg/m^3) contained 500 times more fibers greater than 5 μm in length. It is interesting to note also that Davis *et al.* (1978) found that the pulmonary fibrosis and tumor incidence correlated better with the number of fibers >20 μm in length when the preparations were examined by scanning electron microscopy.

Other methods of administering asbestos to animals have also supported the concept that fiber lengths are most important in the induction of biological effects. Wright and Kuschner (1977) injected guinea pigs intratracheally with long and short fibers of crocidolite, as well as synthetic fluoramphiboles and glass fibers. Animals were sacrificed at intervals up to 24 months. One crocidolite preparation in which 80% of the fibers were greater than 10 μm in length produced extensive interstitial pulmonary fibrosis, whereas the crocidolite preparation, in which 99% of the fibers were less than 5 μm in length, produced no fibrosis. The fiber diameters in both preparations were between 0.1 and 0.3 μm. The fact that only 4 mg of the long fibers compared to 25 mg of the short fibers had been injected per animal enhanced the significance of the results.

The most detailed studies relating fiber sizes to biologic effects were those reported by Stanton *et al.* (1981). They tested fibers of differing lengths and of several different chemical compositions. These included crocidolite, various types of fibrous glass, aluminum oxide, dawsonite ($NaAl(OH)_2CO_3$), wollastonite, tremolite, amosite, attapulgite, hallyosite, silicon carbide, and potassium octatitinate. The fibers were injected intrapleurally in rats. They found that fibers >8 μm in length and <1.25 μm in diameter were much more potent in inducing mesotheliomas than other fibers (i.e., those <8 μm in length and >1.25 μm in diameter).

In all of the above studies, asbestos was tested alone; that is, no other carcinogen was administered with the asbestos. Therefore, no comment can be made on the relative importance of short fibers versus long fibers

in acting as cocarcinogens for substances like cigarette smoke, which is most important in the occupational setting (Hammond *et al.*, 1979).

There are two other factors that might have influenced the results of our study. These are the rather high aluminum content (0.88–1.1%) and the fact that the chrysotile was ball milled. The aluminum content was about twice as much as that reported to be present in the UICC Rhodesian chrysotile (Timbrell, 1969) tested by Wagner *et al.* (1974). Aluminum compounds have been reported to modify the fibrogenic potency of silica (LeBouffant *et al.*, 1977), and it is possible that they could also modify the fibrogenic potency of silicates, e.g., asbestos. Other investigators have reported that ball milling may also cause a degradation of the crystal structure of the asbestos fibers (Spurny *et al.*, 1980) which may affect the biological activity of the asbestos. The modification of chrysotile, e.g., by leaching in 1 N HCl (without apparently modifying fiber length), has been shown to modify the biological effects both *in vitro* and *in vivo* (Morgan *et al.*, 1977; Evans *et al.*, 1983).

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