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Chronic Inhalation Toxicity of Fibrous Glass in Rats and Monkeys

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ABSTRACT

Numerous reports have shown that fibrous glass has the potential to cause fibrogenic and carcinogenic responses in test animals. The experiments producing significant response have, however, used unrealistic routes of exposure. The increased demand for fibrous glass for insulating purposes where respirable fibers may exist presents considerable concern for potential health problems. Therefore, a long-term inhalation study was conducted with F344 rats and cynomolgus monkeys exposed in treatments characterized by fibers of varying geometry and mass concentrations. A workweek type of exposure (7 hours/day, 5 days/week) was maintained for 18 months with monkeys and 21 months with rats, which were subsequently held to 80% mortality. The evaluation of response included life table analysis, body weights, clinical signs, hematological testing, respiratory function, ophthalmic examinations, clinical biochemical analysis, and gross and microscopic pathological examinations. Both species demonstrated pulmonary macrophage aggregates and granulomas containing fibrous glass. The rats had grossly visible pleural plaques, which were not seen in the monkeys. There was no evidence of pulmonary or mesothelial carcinogenicity or fibrogenicity in either species. There were no other significant responses with the exception of a statistically increased mononuclear cell leukemia in each fiber-exposed rat group.

INTRODUCTION

THE FIBROUS GLASS INDUSTRY IS a little less than 50 years old, and within one generation fibrous glass has become one of the most versatile products manufactured. Much of the growth of this industry has related to energy conservation through the use of fibrous glass insulating materials. In 1982, the annual production was approximately 4 billion pounds, with clear indications of continued growth. This, in part, relates to the ability of fibrous glass, a less hazardous material, to replace asbestos. NIOSH estimates that 200,000 persons are occupationally exposed to fibrous glass products. With increased usage, the expectation of increased occupation exposure has emphasized the need to further evaluate the potential health effects.

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Animal experimentation has well served the role of demonstrating potential biological responses to glass fibers. Stanton and Wrench,[1] Pott et al.,[2] Wagner et al.,[3] and Davis[4] have employed implantation techniques with fibers of varying sizes that demonstrate that biological activity relates to fiber geometry. Long, thin, respirable fibers have been shown to produce tumors. Wright and Kuschner[5] and Johnson and Wagner[6] have shown that similar size glass fibers produce pulmonary fibrosis. Sincock[7] has demonstrated cellular genetic damage in cell cultures.

The above responses all result from fiber exposures administered in highly unlikely doses and by artificial routes. Animal exposures by inhalation of well-characterized atmospheres offer appropriate dose-response information of greater application to probable human responses. However, because of the technical difficulties and costs, few inhalation toxicity studies have been conducted. In 1955, Schepers and Delanant[8] and, in 1970, Gross et al.[9] reported results of long-term inhalation studies in rodents. Other than dust-laden macrophages, there were no significant pathological results, and the investigators concluded that fibrous glass exposures should be classified as "biologically inert" or nuisance dusts. A 1979 study reported by Hardy[10] also revealed no significant pulmonary function or histopathological response to short-term, high level exposures. These inhalation studies suggest that responses after exposures are similar to those exhibited by nuisance dusts.

Until recently, epidemiological investigations have not demonstrated excess mortality or morbidity from occupational exposures to fibrous glass. Bayliss et al.[11] revealed a significant excess of nonmalignant respiratory disease in workers exposed to large-diameter glass fibers and a "borderline statistical" excess in respiratory malignancy in workers exposed to special-process, small-diameter fibers. In one of the largest industrywide epidemiological studies, Enterline et al.[12] found a significant excess in nonmalignant respiratory disease deaths in exposed workers.

In response to the inconsistencies in the research findings and recommended research need, the National Institute for Occupational Safety and Health initiated a chronic inhalation toxicology study to assess the character of pulmonary responses with relation to the current federal standard.

MATERIALS AND METHODS

Experimental animal procedures

Sixty young adult male cynomolgus monkeys (Primate Imports, Inc., Long Island, NY) and 500 Fischer 344 rats (Charles River Laboratories, Wilmington, DE) were used for this chronic exposure study. The monkeys were quarantined and examined for tuberculosis and enteric pathogens before they were released for study. During the week before the start of exposure, all monkeys' eyes were examined for possible lesions or abnormalities.

The rats were 5 weeks of age when received. They were housed five per cage in conventional polycarbonate cages and quarantined. They were observed twice daily by a veterinary technician for 7 days after arrival and then released for study.

All animals were randomized separately by computer-generated group assignment. The group composition was structured so that mean body weights were statistically similar across groups and so that there was no intergroup heterogeneity of variance. Rats were randomly assigned to individual cage compartments, alternating female and male animals in adjacent compartments within each cage.

During the quarantine and setup period before exposure, the rats were housed in polycarbonate cages containing bottle waterers. At the time of randomization, the rats were placed in stainless steel wire mesh cages for the duration of the study. The monkeys were individually housed in stainless steel cages with automatic watering.

During the study, animals in their respective cages and racks were housed in environmentally controlled animal holding rooms for approximately 17 hours per day. Of the 17 hours in the holding rooms, 12 hours were in darkness and 5 hours were in light. All test animals were housed separately from control animals. The rooms were identical in every respect. The animals were provided with a minimum of 15 fresh air changes per hour, with conditioned air programmed for a temperature of 70 ± 2°F and a relative humidity of 45 ± 5%.

On an exposure day, racks were wheeled from holding rooms to the inhalation exposure room for loading into the exposure chambers according to a standard operating protocol. Exposure followed for a period of approximately 7 hours, including start-up and shut-down time.

All holding rooms and racks were washed down daily while the animals were in the exposure chambers, and all cages and racks were changed and washed each weekend. Exposure chambers were washed daily after the exposure period ended and the animals were removed. Positive identification was provided for each animal. Rats were tagged and monkeys were tattooed.

The rats were fed feed blocks of Purina Rodent Chow 5001 (Ralston Purina, St. Louis, MO). It was placed in troughs fixed to the front of the compartments at the end of the daily exposure period. The feed troughs were left in place over the weekends.

The monkeys were fed Purina Monkey Chow 5038. The biscuits were given to the monkeys in stainless steel cups at the end of the daily exposure period and in the morning and evening on weekends.

All animals (monkeys and rats) were observed twice daily throughout the pretest and study periods by experienced technicians. The observations were made between 6 and 9 AM before the animals were loaded into the chambers and between 3 and 6 PM after the animals were removed from the chambers. On weekends, the observations were made before and after cage changing, feeding, weighing, and room cleaning.

Mortality was recorded on daily record sheets, in the project death record log, and in the computerized body weight data file.

Preexposure and postexposure eye examinations were performed by a trained veterinarian on all monkeys in this study using a Welch-Allyn direct ophthalmoscope for funduscopic examination and an American Optical slit-lamp biomicroscope for examination of the iris, lens, cornea, and conjunctivae.

The weights of all animals were recorded at the beginning of the study, weekly for the first month, and biweekly thereafter for the duration of the study. The rats were weighted individually using an automatic capture and recording system. Monkeys were individually weighed in their cages on a table balance.

Exposure environmental procedures

Method of exposure. The animals were exposed for 7 hours per day, 5 days per week, for periods of 72 weeks (monkeys) and 86 weeks (rats), excluding holidays, in Hinners' type chambers.[13] These chambers are constructed of stainless steel and are 6 feet square, designed with a pyramidal top and bottom with a nominal volume of 5.4 m^3. For this study, chambers were modified to accommodate both rats and monkeys. The interior of the chambers was cleaned daily after each animal exposure with a waterwash ring with high pressure nozzles.

Air was drawn through the chamber by a large blower mounted on the roof of the facility. The air entering each chamber was room air that had been filtered with an absolute filter mounted on the intake to each chamber and was monitored by measuring the pressure drop across calibrated orifice placed in the air inlet. The room air had previously been cleaned by an electrostatic precipitator and a bacteriostatic lithium chloride solution and conditioned to an average 68.8°F and an average 50% relative humidity.

To assure randomization of exposure, all cages were rotated from top to bottom and left to right by one position each new exposure day.

Fiber preparation and generation. The fibers used for these studies were prepared by grinding standard batches of commercially available fiber glass. After grinding, the fibers were classified in the desired size ranges by a combination of filtration, sedimentation, and air elutriation.

The long-term generation of glass fibers (with and without binder) at a specific rate was very challenging because of their electrostatic and physical properties. The fibers used for each exposure condition had their unique handling characteristics. All of the standard powder generation techniques were evaluated and failed to meet the desired criteria. A specially designed rotary platform feeder

met these requirements for three exposure conditions, and a two-fluid spray system of a glass fiber dispersion was used for the fourth.

Exposure groups. The study involved five groups of animals, including four fiber-exposed groups and a filtered air control group. Fifty male and 50 female rats, and 15 male cynomolgus monkeys, were used in each dosage group. The exposures were conducted in 5.4 m^3 chambers with air circulation of 1 m^3/minute. Exposure conditions are shown in Table 1.

TABLE 1. EXPOSURE CONDITIONS FOR BOTH SPECIES

Chamber	*Animal code*	*Fiber*	*Target concentration (mg/m^3)*
1	FO1	4–6 μ glass fiber >20 μ long with red binder	15
2	FO2	0.5–3.5 μ glass fiber >10 μ long with yellow binder	15
3	FO3	<3.5 μ glass fiber >10 μ long	5
4	FO4	<3.5 μ glass fiber <10 μ long	5
5	FO5	Control	0

Hematology and clinical chemistry. Monkey's blood for hematological and serum chemical analyses was collected twice before the initiation of exposures, at weeks 16, 32, 48, and 64 postexposure, and immediately before necropsy. The parameters that were evaluated at each interval included the following.

Hematology. Hematocrit, hemoglobin, RBC count, WBC count, reticulocyte count, platelet count, and differential count.

Serum chemistry. BUN, glucose, creatinine, inorganic phosphorus, calcium, total bilirubin, cholesterol, LDH, SGOT, sodium, and potassium. Blood samples were obtained from the femoral vein following a fast of approximately 12–16 hours. Results were evaluated by qualitative examination of group means for baseline and terminal sampling periods.

Rat's blood for hematology and serum chemistry analyses was collected just before termination. Blood was collected by cardiocentesis after anesthetization of the rats with pentobarbital sodium injected intraperitoneally. The parameters evaluated were the same as those described above for the monkeys. Ten rats per sex were selected randomly for these evaluations.

Results were evaluated by qualitative examination of group means and standard deviations of exposed rats as compared to the control group.

Pulmonary function evaluation. Lung mechanics evaluations were determined using the techniques of Neergaard and Wirz[14] after direct recording of transpulmonary pressure, lung airflow rate, and respired gas volume onto an Electronics for Medicine VR-6 recorder. Dynamic lung volumes were obtained from recordings of volume excursions during required breathing patterns. Re-

spiratory maneuvers necessary for these evaluations were induced by a variable-pressure plethysmograph. The design and operation of this device is described in detail by Moorman et al.[15] Nitrogen washout and closing volume were measured by the method of Buist et al.[16] The volume of helium isoflow was assessed using the techniques described by Hutcheon et al.[17]

Pulmonary function evaluations were performed at three time periods during the course of this study: (1) before the initiation of exposure, (2) after 9 months of exposure, and (3) after 18 months of exposure. All testing was done with the monkey anesthetized using a mixture of ketamine and xylazine dosed to an effect.[13] Table 2 lists the pulmonary function parameters studied.

TABLE 2. PULMONARY ASSESSMENT PARAMETERS

Parameter	*Abbreviation*	*Units*
Dynamic airways resistance	R_L	cm H_2O/l/sec
Dynamic lung compliance	C_L	ml/cm H_2O
Forced expiratory volume in 0.5 second normalized to FVC	FEV.5/FVC	%
Forced expiratory volume in 1 second normalized to FVC	FEV1/FVC	%
Peak expiratory flow rate	PEFR	ml/sec
Forced expiratory flow at n% of lung volume	FEF @n%	ml/sec
Total lung capacity	TLC	
Forced vital capacity	FVC	ml
Inspiratory capacity	IC	ml
Expiratory reserve volume	ERV	
Residual volume	RV	
Closing volume ratio to vital capacity	CV	%
Sum of CV and RV normalized to TLC	CV+RV/TLC	%
Anatomical dead space	VADS	ml
Slope of phase III N_2 washout	$\%N_2$/100 ml	%
Volume of helium isoflow	Viso	ml
Viso normalized to FVC	Viso/FVC	%

Gross and microscopic pathology. Monkeys. Detailed gross examinations were conducted on all monkeys, and pertinent observations were recorded. Monkeys were killed by anesthetization with pentobarbital sodium, followed by exsanguination. The following tissues were removed and fixed in 10% neutral formalin:

Larynx	Urinary bladder
Trachea	Testes
Lung (section from each lobe)	Prostate gland
Heart	Ovaries
Liver	Uterus
Esophagus	Spleen
Stomach	Pancreas
Ileum	Kidneys
Colon	Adrenal glands
Pleural membrane	Pituitary gland
Nasal passages	Brain
Paranasal sinus	Thyroid gland
Tracheobronchial lymph node	Lesions or masses
Mesenteric lymph node	Eyes (fixed in 3% buffered glutaraldehyde)

The tissues listed on the previous page were processed in the routine manner, embedded in paraffin, cut at 5 μ, stained with hematoxylin and eosin, and examined microscopically. Histochemical stains were used on specific tissues at the discretion of the pathologist to aid in interpretation of changes.

Rats. Detailed gross examinations were conducted on all rats, and pertinent observations were recorded. Rats were killed by intraperitoneal injections of pentobarbital sodium followed by exsanguination (if blood was collected for hematology and serum chemistry studies) or by CO_2 inhalation followed by exsanguination (if blood was not used for clinical laboratory procedures). Tissues removed for fixation and subsequent histological examination were the same as those listed on the previous page for monkeys.

Statistical analysis

Body weights. The statistical analyses consist of body weight and weight gain analyses across dose groups at 0, 9, and 18 months for monkeys, and at 0, 9, and 21 months each for male and female rats. Percentage of weight changes over the entire measurement period, over the first 9 months, and over the remaining months were also analyzed across dose groups for monkeys and for each sex in rats. A survival analysis for rats only was performed, as only 2 deaths occurred among the 60 monkeys. These 2 early deaths were excluded from monkey body weight analyses as outliers because their weights were much lower than the weights of the other monkeys.

Survival analysis. Mortality data for rats were analyzed by the actuarial life table method developed by Berkson and Gage.[18] For each group, survival information was printed, and a nonparametric test devised by Desu and Lee[19] was used to compare groups.

Chamber concentrations. For each day, chamber concentration levels were measured a minimum of three times. Based on the measured reading obtained each time, the concentration level in the chamber was then manually adjusted toward a target level for the chamber. The data collected for each day and chamber included the measured concentration levels and the time period covered by each reading.

Statistical measures were computed to globally describe the concentration levels present in the chamber for each day. These measures included a time-weighted average of the concentration levels, a weighted measure of the variability in concentration levels, and the minimum and maximum levels.

An average concentration level for each chamber over each quarter was computed by averaging the values for each day in the quarter. In addition, the lengths of test time for all the days were summed to get a total value for each quarter.

Pulmonary function. The one-way analysis of variance (ANOVA) and the Kruskal-Wallis one-way rank analysis of variance were used to evaluate the group statistics for the pulmonary data. Bartlett's test was used to validate the ANOVA. Those parameters that demonstrated nonhomogeneous variance (Bartlett's $P > 0.005$) were tested by the nonparametric Kruskal-Wallis evaluation. For all procedures, the 95% level of significance was used.

RESULTS

Exposure environment results

Temperature and relative humidity. Throughout this study, only slight variations of relative humidity or temperature were detected for the inhalation chambers. Actual readings varied somewhat, but in accordance with the room environment. The average temperature throughout the study was 68.8 ± 1.9°F and the relative humidity was 50 ± 6.4%.

Size characteristics of glass fibers. In addition to the measurement of the fiber glass aerosols with a cascade impactor, samples were collected on absolute filters, and subsequent particle size measurements were made of photomicrographs taken of these collections. Figures 1, 2, 3, and 4 are typical scanning electron micrographs of filters used to obtain size distribution of the powder cloud in the four chambers. Figures 5, 6, 7, and 8 are three-dimensional plots of typical particle size data obtained.

Exposure concentrations. Because of the potentially large variations in daily chamber concentrations, four measurements typically were made. Table 3 summarizes the concentrations for the initial time period in which only rats were exposed and the following six quarters in which both rats and monkeys were exposed. In addition, Table 3 shows the overall exposure concentrations for both rats and monkeys. Spot checks for ammonia levels showed 0.2 ppm or less.

Hematology and clinical chemistry. There were no changes in mean values for hematology or clinical chemistry parameters that were outside the expected range, nor were there any biologically significant variations from control values. There were occasional fluctuations in individual values for a given parameter, which is expected, but there was no evidence that such changes were related to fibrous glass exposures.

In some rats the decreases of erythrocyte parameters were noted, resulting from moderately depressed values in two rats in which mononuclear cell leukemia (MCL) occurred with attendant bone marrow suppression. Such variations in erythrocyte parameters were most prominent in rats with MCL, a common finding in aging Fischer 344 rats.

Pulmonary function results. The results of the pulmonary function evaluation for monkeys are summarized in Tables 4, 5, 6, 7, and 8, which list the means and standard deviations of all pulmonary function parameters that were calculated for this program at each of the three evaluation periods. Each table represents one exposure group.

FIG. 1. SEM photograph of glass fibers collected from chamber 1. ×210.

FIG. 2. SEM photograph of glass fibers from chamber 2. ×700.

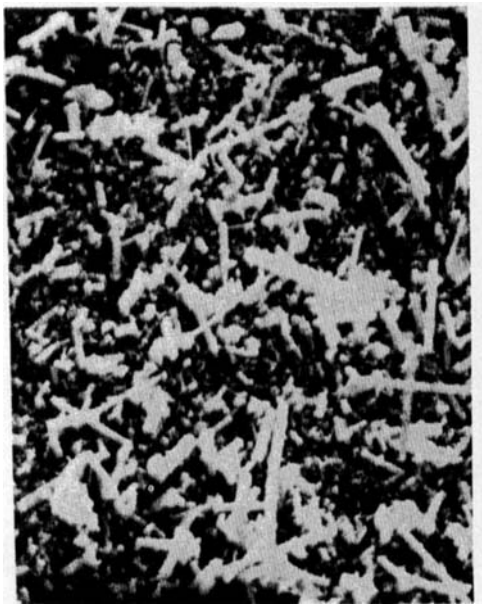

FIG. 3. SEM photograph of glass fibers from chamber 3. ×700.

FIG. 4. SEM photograph of glass fibers from chamber 4. ×2100.

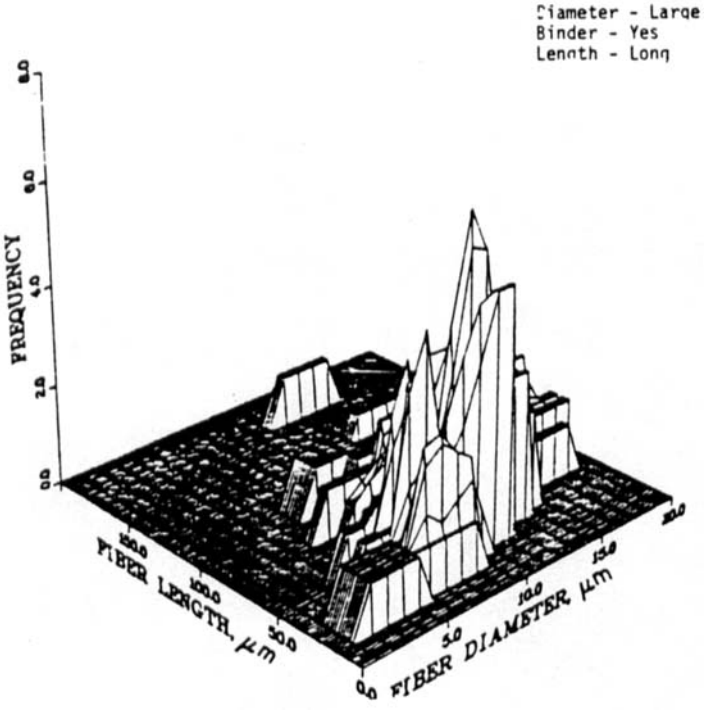

FIG. 5. Size distribution of glass fibers collected on filter from chamber 1.

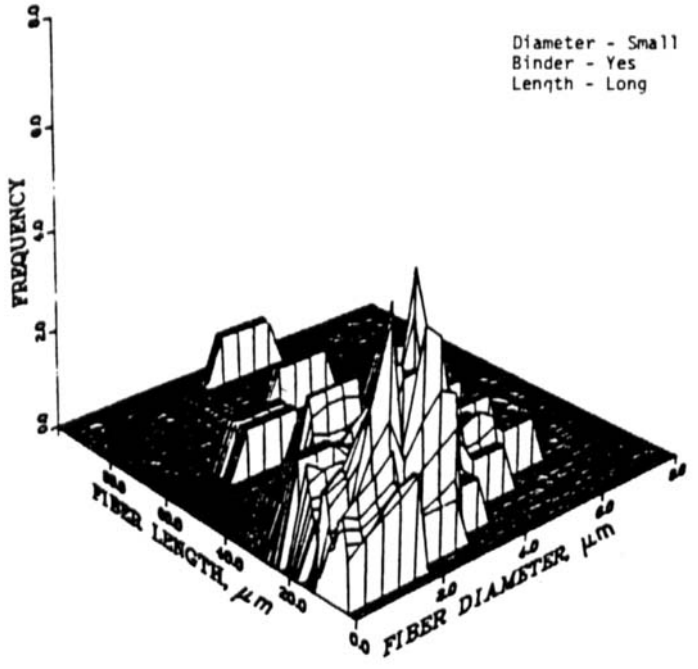

FIG. 6. Size distribution of glass fibers collected on filter from chamber 2.

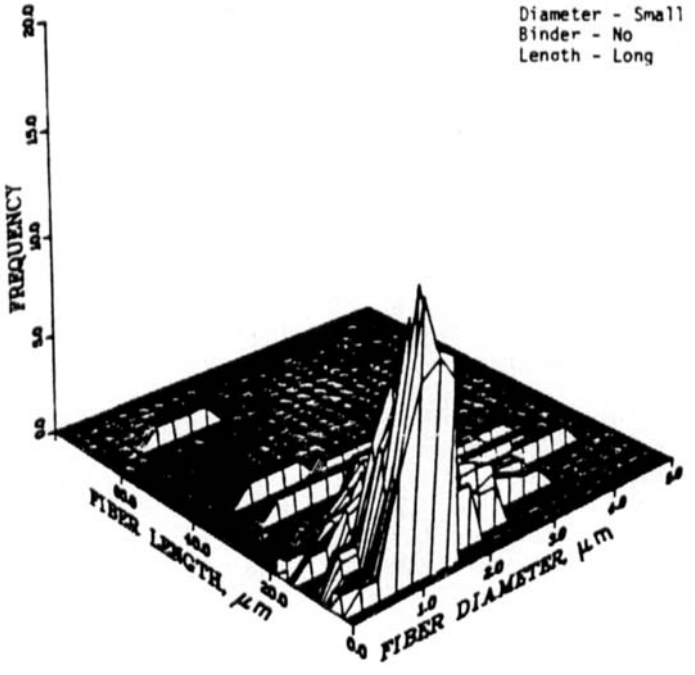

FIG. 7. Size distribution of glass fibers collected on filter from chamber 3.

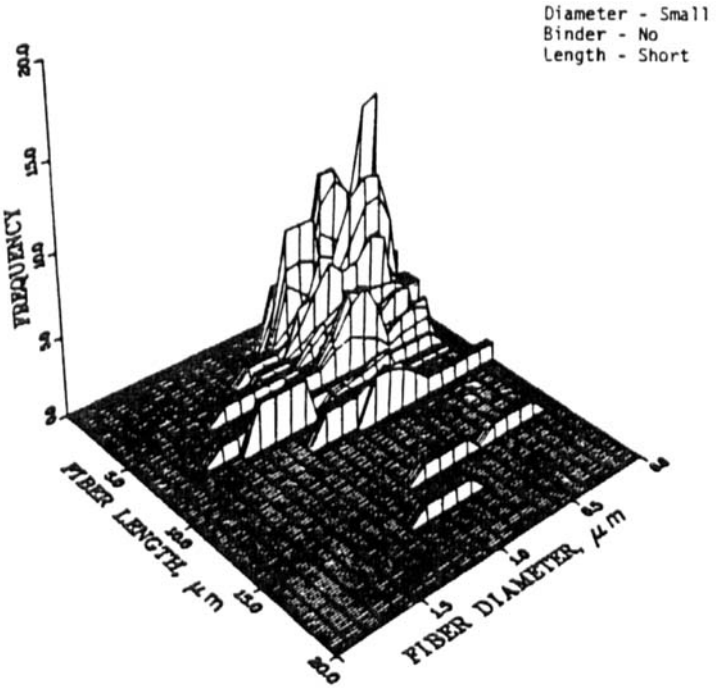

FIG. 8. Size distribution of glass fibers collected on filter from chamber 4.

At the 9-month evaluation, several parameters appeared to be different when compared statistically ($P < 0.05$) with controls. Dynamic compliance was higher in the 15 mg/m³ > 10 μ, 15 mg/m³ > 20 μ, and the 5 mg/m³ > 10 μ groups.

Of the parameters tested for this study, only expiratory reserve volume (ERV) and forced expiratory volume at 0.5 second normalized to forced vital capacity (FEV.5/FVC) showed significant differences ($P < 0.05$) after the 18-month evaluations. ERV was reduced significantly in both the 15 mg/m³ exposure groups but not in either of the 5 mg/m³ groups. Additionally, the FEV.5/FVC parameter was higher in the 15 mg/m³ > 20 μ exposure group when compared to controls. Non-normally distributed parameters did not show significant ($P > 0.05$) difference by Kruskal-Wallis after 18 months of exposure.

The limited number of significant differences demonstrated by pulmonary function analysis does not provide a clear basis for the diagnosis of any pattern of respiratory compromise. As all calculated parameters in both of the 5 mg/m³ (FO3 and FO4) exposure groups were not significantly ($P > 0.05$) different from the control group, it must be concluded that, within the capabilities of our evaluation, no substantiated changes occurred in the 5 mg/m³ groups as a result of their exposure to fibrous glass. The reduction of ERV alone, as in the case of the 15 mg/m³ treatments or in conjunction with an increased FEV.5/FVC, as in the FO1 group exposed to 15 mg/m³ (>20 μ) fibers, is not a typical pattern of ventilatory impairment. An indication of altered inflation levels is suggested by a trend to increased inspiratory capacity and a significant decrease in ERV. The reduced ERV constitutes a mild restrictive type of functional impairment. Volume restrictions are consistent with interstitial lung disease. In conclusion, no profound changes could be attributed to exposure to the fibrous glass as determined by our pulmonary function evaluation in monkeys.

Gross and microscopic pathology. Monkeys. The only unequivocal microscopic changes that resulted from exposure to fibrous glass occurred in the lungs and tracheobronchial lymph nodes of

monkeys in all exposure groups. Lesions in the tracheobronchial lymph nodes consisted of minimal to moderate amounts of fibrous glass in macrophages, usually in the medulla of the lymph node (Figs. 9 and 10). Changes in the lungs were generally mild and consisted of single macrophages or small aggregates of macrophages that contained fibrous glass fibers (Figs. 11, 12, and 13). A few free fibrous glass fibers were visible in alveoli, interstitium, or other areas in the lungs of monkeys exposed to fibrous glass fibers. Macrophages containing fibrous glass were usually located in peribronchiolar, peribronchial, or perivascular areas as well as within alveoli and in pleural and subpleural locations. There were no obvious differences in the lobar distribution of these fibrous glass-containing macrophages or free fibrous glass fibers. In many instances, the macrophages that contained fibrous glass also contained pigments and debris typical of that resulting from infestation with lung mites (*Pneumonyssus simicola*). Although fibrous glass-containing macrophages and free fibers occurred in pleural and subpleural locations and fibrous glass-containing macrophages were prominent in tracheobronchial lymph nodes, there was no further evidence of translocation of fibrous glass fibers in monkeys. There was no evidence of fibrous glass in organs other than lungs and tracheobronchial lymph nodes.

The only other change that was apparent in fibrous glass-exposed monkeys occurred solely in group FO1. This change consisted of mildly increased numbers of lymphoid nodules or aggregates in peribronchiolar and perivascular areas. There were no other associated changes, nor did this change occur in other fibrous glass-exposed groups or in controls.

There were differences in the extent of involvement among the various exposure groups. This was observed as a substantially less extensive involvement of animals from the FO1 group (15 mg/m^3 > 20 μ with binder) as compared to the other exposed groups. There were minimal variations in qualitative or quantitative fibrous glass-related changes in lungs or tracheobronchial lymph nodes among animals from the other three exposure groups. Numbers and qualitative severities of the fibrous glass-induced pulmonary lesion and tracheobronchial lymph node lesion, that is, macrophage aggregates with fibrous glass deposition (lung) and fibrous glass deposition (tracheobronchial lymph node), are shown in Table 9. The only difference was a slightly greater general severity for groups FO3 and FO4 as compared to group FO2 for the tracheobronchial lymph node lesion. The fibrous glass-containing macrophages, in the lungs of virtually all monkeys, occupied less than 5% of the total area of the lung sections.

Fifty-nine of the sixty monkeys used in this study had lesions consistent with lung mite infestation. These lesions ranged from the presence of lung mite pigment and debris in macrophages to extensive lesions, including bronchiolitis that was usually eosinophilic or granulomatous. Bronchiectasis was present in a few animals, and parasites or remnants of parasites were observed in the lungs of some monkeys. Fibrosis or smooth muscle hyperplasia was common in the lungs of animals from all groups. These changes were nearly always focal or multifocal and were qualitatively and quantitatively similar among all dosage groups. The smooth muscle hyperplasia, fibrosis, pleural adhesions, and most inflammatory changes in the lungs of these monkeys were apparently related to the lung mite infestations. The distribution of the smooth muscle hyperplasia suggested that it occurred primarily as a result of local changes in ventilatory mechanics. Lung mite pigments and debris usually coexisted with fibrous glass in the pulmonary macrophages of monkeys from groups exposed to fibrous glass. The numbers of single macrophages and macrophage aggregates were, however, mildly to moderately increased in some animals from each of the exposure groups.

Inflammatory lesions of the nasal passages, trachea, and paranasal sinuses were slightly more prominent in monkeys from group FO4 as compared to controls. However, the diversity of these changes and their mild intensity does not make a definitive interpretation possible.

There was no evidence that any of the changes observed at necropsy were associated with exposure to fibrous glass. Many of the lesions observed grossly were consistent with parasite infestations. The anthracosilicosis that was described grossly in monkeys from all groups resulted from the black or green-black depositions of lung mite pigments that are sometimes seen in cynomolgus monkeys.

Early death rats. During the course of this study, 187 rats died spontaneously or were killed in a moribund condition. Many of the spontaneous deaths were due to MCL or pituitary adenomas,

TABLE 3. AVERAGE CHAMBER CONCENTRATIONS

Quarter		*Chamber* 1	2	3	4
1 Rats only	Date:	3/12/79 to 7/9/79	3/12/79 to 7/16/79	3/12/79 to 7/2/79	3/12/79 to 6/25/79
	Concentration (mg/m^3):	9.43 ± 6.44[a]	10.84 ± 7.47	3.71 ± 2.08	4.80 ± 2.00
	Exposure days:	64.5	86.4	77.1	74.2
2 Rats & monkeys	Date:	7/10/79 to 10/9/79	7/17/79 to 10/16/79	7/3/79 to 10/2/79	6/26/79 to 9/25/79
	Concentration (mg/m^3):	12.39 ± 4.17	14.04 ± 4.76	4.52 ± 1.96	4.85 ± 1.47
	Exposure days:	64.6	64.7	63.9	63.7
3 Rats & monkeys	Date:	10/10/79 to 1/9/80	10/17/79 to 1/16/80	10/3/79 to 1/2/80	9/26/79 to 12/25/79
	Concentration (mg/m^3):	15.24 ± 4.55	15.73 ± 7.70	4.77 ± 2.84	4.06 ± 2.44
	Exposure days:	59.7	59.6	59.7	60.4
4 Rats & monkeys	Date:	1/10/80 to 4/9/80	1/17/80 to 4/16/80	1/3/80 to 4/2/80	12/26/79 to 3/25/80
	Concentration (mg/m^3):	13.57 ± 4.36	16.23 ± 4.62	4.71 ± 1.66	4.77 ± 1.82
	Exposure days:	64.3	64.2	64.2	62.2

5	Date:	4/10/80 to 7/9/80	4/17/80 to 7/16/80	4/3/80 to 7/2/80	3/26/80 to 6/25/80
Rats & monkeys	Concentration (mg/m³):	14.33 ± 4.19	15.51 ± 2.75	5.13 ± 1.46	4.55 ± 1.31
	Exposure days:	63.1	63.1	64.0	67.0
6	Date:	7/10/80 to 10/9/80	7/17/80 to 10/16/80	7/3/80 to 10/2/80	6/26/80 to 9/25/80
Rats & monkeys	Concentration (mg/m³):	16.23 ± 3.23	15.46 ± 2.45	5.60 ± 0.88	5.34 ± 0.82
	Exposure days:	64.5	64.1	64.2	64.8
7	Date:	10/10/80 to 1/14/81	10/17/80 to 1/17/81	10/3/80 to 1/1/81	9/26/80 to 12/24/80
Rats & monkeys	Concentration (mg/m³):	16.56 ± 2.20	16.77 ± 2.06	5.50 ± 0.72	5.10 ± 0.82
	Exposure days:	60.9	60.0	59.0	61.0
TOTAL	Concentration				
RAT	(mg/m³):	13.96 ± 4.16	14.94 ± 4.55	4.85 ± 1.66	4.78 ± 1.52
EXPOSURE	Exposure days:	441.5	462.0	452.0	453.1
TOTAL	Concentration				
MONKEY	(mg/m³):	14.72 ± 3.78	15.62 ± 4.06	5.04 ± 1.58	4.78 ± 1.44
EXPOSURE	Exposure days:	377.0	375.6	374.9	378.9

[a]Mean ± standard deviation.

TABLE 4. PULMONARY FUNCTION ANALYSIS OF CONTROL GROUP MONKEYS (FO5)

Physiology parameter	*Baseline*	*9 Months*	*18 Months*
Mechanics			
R_L (cm H_2O/l/sec)	5.2 ± 2.2[a]	9.1 ± 7.2	7.9 ± 2.6
C_L (ml/cm H_2O)	28.2 ± 10.6	24.1 ± 10.7	20.2 ± 5.3
Dynamic lung volumes			
FVC (ml)	304 ± 46	315 ± 60	403 ± 68
FEV.5/FVC (%)	74.2 ± 15.6	80.8 ± 8.6	71.5 ± 7.8
FEV1/FVC (%)	94.4 ± 4.2	96.1 ± 3.3	94.5 ± 3.3
PEFR (ml/sec)	990 ± 232	1020 ± 160	1101 ± 189
FEF 50% (ml/sec)	877 ± 356	841 ± 244	995 ± 206
FEF 25% (ml/sec)	590 ± 374	431 ± 226	617 ± 327
FEF 10% (ml/sec)	195 ± 209	134 ± 72	145 ± 74
FEF 50%/FVC (FVC/sec)	2.82 ± 1.01	2.71 ± 0.82	2.49 ± 0.50
FEF 25%/FVC (FVC/sec)	1.97 ± 1.16	1.4 ± 0.76	1.48 ± 0.63
FEF 10%/FVC (FVC/sec)	0.65 ± 0.62	0.43 ± 0.26	0.36 ± 0.18
Lung volumes			
IC (ml)	130 ± 2	132 ± 34	153 ± 44
ERV (ml)	163 ± 33	180 ± 69	252 ± 59
N_2 washout			
CV (ml)	24.7 ± 22.2	28.8 ± 16.4	34.1 ± 13.8
CV/VC (%)	8.22 ± 6.31	9.13 ± 4.62	8.31 ± 3.82
% N_2/100 ml	0.7 ± 0.5	0	0.85 ± 0.7
Viso (ml)	40.6 ± 36.7	40.0 ± 19.0	44.1 ± 16.5
Viso/FVC (%)	12.7 ± 11.8	12.8 ± 5.0	11.1 ± 4.0

[a]Mean ± standard deviation.

both of which are commonly seen in aged Fischer 344 rats. Other animals were sacrificed because of large skin tumors, such as fibroadenomas of the mammary gland. With the exception of MCL, there appears to be no increased incidence of these lesions in the fibrous glass test groups when compared to the control group of animals.

Plaquelike lesions of the pleura were the only lesions observed at necropsy that were attributed to fibrous glass exposure. These plaquelike lesions consisted of gray to tan, elevated, firm areas of various sizes on the surface of the lung lobes. These lesions occurred in 1 of 19 females from the FO1 group (15 mg/m^3 > 20 μ fiber with binder), in 5 of 23 females from the FO2 group (15 mg/m^3 > 10 μ fiber with binder), in 2 of 12 females and in 4 of 18 males from the FO3 group (5 mg/m^3 > 10 μ without binder), and in 5 of 26 females from the FO4 group (5 mg/m^3 < 10 μ without binder). The superficial lung lesions in the animals from the FO4 group were "gritty" or "granular" in character.

Histomorphological lesions induced by fibrous glass were limited to the lungs, pleura, and lymph nodes (thymic and tracheobronchial) in rats from all exposure groups.

The lung lesions consisted of small to large aggregations of macrophages containing a few to many nonpolarizable, needle-shaped fibers (fibrous glass), readily seen under reduced light and located in peribronchiolar, peribronchial, or perivascular areas as well as within alveoli and in pleural and subpleural locations.

TABLE 5. RESULTS OF PULMONARY FUNCTION ANALYSIS FOR MONKEYS FOR EXPOSURE LEVEL 15 MG/M³ AND FIBER LENGTH GREATER THAN 10 μ (FO2)

Physiology parameter	*Baseline*	*9 Months*	*18 Months*
Mechanics			
R_L (cm H_2O/l/sec)	4.99 ± 2.38[a]	6.3 ± 4.4	8.3 ± 2.3
C_L (ml/cm H_2O)	32.85 ± 13.15	33.3 ± 12.1[b]	24.8 ± 6.3
Dynamic lung volumes			
FVC (ml)	308.9 ± 60.5	357 ± 63.8	379.8 ± 83.3
FEV.5/FVC (%)	78.7 ± 10.3	78.7 ± 7.7	75.0 ± 6.42
FEV1/FVC (%)	95.5 ± 4.4	94.2 ± 3.98	97.0 ± 2.58
PEFR (ml/sec)	964 ± 163	980 ± 130	1050 ± 188
FEF 50% (ml/sec)	897 ± 189	830 ± 248	906 ± 233
FEF 25% (ml/sec)	567 ± 258	530 ± 302	577 ± 257
FEF 10% (ml/sec)	209 ± 182	203 ± 177	170 ± 121
FEF 50%/FVC (FVC/sec)	2.97 ± 0.70	2.33 ± 0.58	2.46 ± 0.42
FEF 25%/FVC (FVC/sec)	1.86 ± 0.81	1.45 ± 0.73	1.51 ± 0.48
FEF 10%/FVC (FVC/sec)	0.67 ± 0.51	0.42 ± 0.20	0.43 ± 0.27
Lung volumes			
IC (ml)	122 ± 23.5	152 ± 42.2	183 ± 38.1
ERV (ml)	175 ± 40.9	204 ± 33.3	196 ± 53.1[b]
N_2 washout			
CV (ml)	20.8 ± 12.41	25.5 ± 11.3	40.2 ± 15.5
CV/VC (%)	6.8 ± 3.6	7.0 ± 2.8	10.9 ± 4.9
% N_2/100 ml	0.69 ± 0.34	0	0.52 ± 0.39
Viso (ml)	39 ± 31	31.3 ± 21.7	46.5 ± 13.6
Viso/FVC (%)	11.6 ± 9.1	9.6 ± 6.6	13.1 ± 5.9

[a]Mean ± standard deviation.
[b]Significant difference from control values at the same time period ($P < 0.05$).

In many animals, there was granulomatous inflammation of the lung and pleura that was minimal to severe and apparently associated with fibrous glass deposition. This inflammatory response consisted of fibrous glass-laden macrophage aggregates that were surrounded by varying numbers of lymphocytes, plasma cells, and, at times, neutrophils. There were no obvious differences in the lobar distribution of these pulmonary lesions in the affected rats. The fibrous glass-containing macrophages, in lungs of virtually all rats, occupied less than 5% of the total area of the lung sections.

The thymic and tracheobronchial lymph nodes contained various amounts of fibrous glass fibers, usually in the medulla of the lymph node.

Although fibrous glass-laden macrophages occurred in pleural and subpleural locations and were present in thymic and tracheobronchial lymph nodes, there was no evidence of translocation of fibrous glass fibers to other organs in these rats.

There were substantial variations in qualitative and quantitative fibrous glass-related changes among the various exposure groups in the lungs and thymic and tracheobronchial lymph nodes. These changes were least pronounced in the FO1 group, became progressively more pronounced in the FO2 and FO3 groups, and were most pronounced in the FO4 group. Table 10 depicts the numbers of animals affected per exposure group and the qualitative severities of the fibrous glass-induced lesions in the lung and lymph nodes (thymic and tracheobronchial).

TABLE 6. RESULTS OF PULMONARY FUNCTION ANALYSIS FOR MONKEYS FOR EXPOSURE LEVEL 5 MG/M³ AND FIBER LENGTH GREATER THAN 10 μ (FO3)

Physiology parameter	*Baseline*	*9 Months*	*18 Months*
Mechanics			
R_L (cm H_2O/l/sec)	4.9 ± 2.4[a]	6.3 ± 2.9	7.4 ± 1.6
C_L (ml/cm H_2O)	30.5 ± 11.5	33.4 ± 5.6[b]	23.0 ± 7.6
Dynamic lung volumes			
FVC (ml)	313 ± 53	348 ± 62	390 ± 76
FEV.5/FVC (%)	80.4 ± 9.4	81.2 ± 4.4	77.7 ± 11.9
FEV1/FVC (%)	96.9 ± 2.0	95.5 ± 2.6	96.7 ± 2.0
PEFR (ml/sec)	1069 ± 123	919 ± 184	1021 ± 130
FEF 50% (ml/sec)	1036 ± 125	902 ± 157	924 ± 159
FEF 25% (ml/sec)	738 ± 209	468 ± 109	675 ± 227
FEF 10% (ml/sec)	189 ± 153	148 ± 53	181 ± 83
FEF 50%/FVC (FVC/sec)	3.37 ± 0.63	2.67 ± 0.64	2.46 ± 0.67
FEF 25%/FVC (FVC/sec)	2.42 ± 0.88	1.35 ± 0.22	1.79 ± 0.73
FEF 10%/FVC (FVC/sec)	0.65 ± 0.64	0.42 ± 0.13	0.46 ± 0.18
Lung volumes			
IC (ml)	136 ± 33	150 ± 42	178 ± 47
ERV (ml)	161 ± 30	196 ± 36	212 ± 49
N_2 washout			
CV (ml)	16 ± 6.8	25 ± 11.1	39 ± 17.7
CV/VC (%)	5.2 ± 1.8	7.6 ± 2.6	10.2 ± 2.8
% N_2/100 ml	0.85 ± 1.0	0	0.51 ± 0.3
Viso (ml)	27.6 ± 11.7	40.1 ± 21.9	49.7 ± 13.5
Viso/FVC (%)	12 ± 13.1	11.0 ± 4.9	12.9 ± 4.2

[a]Mean ± standard deviation.
[b]Significant difference from control values at the same time period ($P < 0.05$).

Four of twenty male rats from the FO4 group had mesotheliomas that primarily involved the testis (no other early death animals had this lesion). However, we believe that this neoplastic process was spontaneous because this tumor commonly occurs in aged Fischer 344 rats and there was no evidence of fibrous glass about or within (translocation) the serosal surfaces of the testis.

Many early death rats in this study had MCL (Fischer rat leukemia) (Table 11). The male rats in the FO5 (control) group appeared to have a lower incidence of MCL than the male and female rats in the other test groups.

Scheduled sacrifice rats. A total of 313 rats were killed by group, beginning with the FO1 group (15 mg/m³ > 20 μ fiber with binder) and ending with the control group (FO5). Plaquelike lesions of the pleura were the only lesions observed at necropsy that were attributed to fibrous glass exposure. Essentially no gross lesions were observed in the respiratory tracts of rats from the control group (FO5) or in rats from the FO1 group (Fig. 14).

Plaquelike lesions of the pleura occurred in 25 of 27 females and in all 31 males from the FO2 group (15 mg/m³ > 10 μ fiber with binder). These lesions were multifocal, gray to tan, elevated, firm plaques that measured 1–6 mm or more in diameter. They were on the surface of the lung lobes

TABLE 7. RESULTS OF PULMONARY FUNCTION ANALYSIS FOR MONKEYS FOR EXPOSURE LEVEL 15 MG/M³ AND FIBER LENGTH GREATER THAN 20 μ (FO1)

Physiology parameter	*Baseline*	*9 Months*	*18 Months*
Mechanics			
R_L (cm H_2O/l/sec)	5.1 ± 2.2[a]	5.5 ± 2.0	6.0 ± 3.7
C_L (ml/cm H_2O)	29.0 ± 12.1	34.0 ± 11.5[b]	23.6 ± 5.6
Dynamic lung volumes			
FVC (ml)	312 ± 74	338 ± 55	377 ± 68
FEV.5/FVC (%)	79.6 ± 8.0	78.6 ± 6.4	78.1 ± 6.0[b]
FEV1/FVC (%)	94.7 ± 5.2	95.2 ± 2.0	96.4 ± 1.8
PEFR (ml/sec)	1034 ± 163	990 ± 116	1053 ± 165
FEF 50% (ml/sec)	968 ± 211	919 ± 143	983 ± 154
FEF 25% (ml/sec)	702 ± 210	419 ± 150	701 ± 212
FEF 10% (ml/sec)	186 ± 121	119 ± 62	158 ± 69
FEF 50%/FVC (FVC/sec)	3.26 ± 1.0	2.76 ± 0.49	2.63 ± 0.31
FEF 25%/FVC (FVC/sec)	2.41 ± 1.0	1.22 ± 0.33	1.88 ± 0.54
FEF 10%/FVC (FVC/sec)	0.59 ± 0.35	0.34 ± 0.15	0.41 ± 0.16
Lung volumes			
IC (ml)	133 ± 33	144 ± 18	178 ± 45
ERV (ml)	167 ± 38	193 ± 45	199 ± 40[b]
N_2 washout			
CV (ml)	21 ± 11.7	34 ± 18.4	43 ± 12.6
CV/VC (%)	7.2 ± 3.6	10.4 ± 6.1	11.7 ± 3.7
% N_2/100 ml	0.67 ± 0.36	0	0.55 ± 0.25
Viso (ml)	29.6 ± 16.3	37.4 ± 24.9	37.9 ± 13.6
Viso/FVC (%)	8.8 ± 4.7	10.5 ± 7.2	9.7 ± 3.0

[a]Mean ± standard deviation.
[b]Significant difference from control values at the same time period ($P < 0.05$).

and primarily involved the dorsal aspect of the diaphragmatic lobes. In one male rat, there were extensive fibrous adhesions between the left diaphragmatic lung lobe and both the diaphragm and adjacent thoracic wall.

Plaquelike lesions of the pleura occurred in 34 of 38 females and in 27 of 32 males from the FO3 group (5 mg/m³ > 10 μ plain fiber). These pulmonary lesions were similar to those seen in the FO2 group; however, they were smaller and there were fewer plaquelike lesions per lung lobe (Fig. 15).

Plaquelike lesions of the pleura occurred in all 24 females and in 26 of 30 males from the FO4 group (5 mg/m³ < 10 μ plain fiber). Thirty-six animals (18 females and 18 males) from this group had superficial lung lesions as previously described. However, the plaquelike lesions were less extensive than those from animals in the FO3 group. Most lesions were about 1 mm in diameter, and there were often only a few plaques per lung lobe. Fourteen animals (6 females and 8 males) from this group had plaquelike lesions that were less than 1 mm in diameter or a pleural surface that was granular in character. The granular lesions of the pleura were about equally distributed in all lung lobes in these animals (Fig. 16).

Fibrous glass-induced histomorphological lesions were primarily limited to the lungs, pleura, and thymic and tracheobronchial lymph nodes in rats from all exposure groups.

The lung lesions consisted of small to large aggregations of macrophages containing various

TABLE 8. RESULTS OF PULMONARY FUNCTION ANALYSIS FOR MONKEYS FOR EXPOSURE LEVEL 5 MG/M³ AND FIBER LENGTH GREATER THAN 10 μ (FO4)

Physiology parameter	*Baseline*	*9 Months*	*18 Months*
Mechanics			
R_L (cm H_2O/l/sec)	4.3 ± 1.7[a]	9.1 ± 7.0	9.0 ± 2.5
C_L (ml/cm H_2O)	25.7 ± 9.5	27.4 ± 11.1	22.2 ± 7.5
Dynamic lung volumes			
FVC (ml)	312 ± 49	356 ± 65	379 ± 80
FEV.5/FVC (%)	78.2 ± 15.4	75.8 ± 8.3	70.2 ± 12.6
FEV1/FVC (%)	92.3 ± 11.4	93.8 ± 5.2	95.1 ± 2.1
PEFR (ml/sec)	1025 ± 178	1029 ± 141	1034 ± 149
FEF 50% (ml/sec)	948 ± 231	906 ± 207	896 ± 222
FEF 25% (ml/sec)	695 ± 335	455 ± 215	555 ± 270
FEF 10% (ml/sec)	326 ± 333	133 ± 71	210 ± 179
FEF 50%/FVC (FVC/sec)	3.09 ± 0.90	2.60 ± 0.70	2.41 ± 0.63
FEF 25%/FVC (FVC/sec)	2.28 ± 1.2	1.29 ± 0.65	1.43 ± 0.57
FEF 10%/FVC (FVC/sec)	1.06 ± 1.2	0.37 ± 0.17	0.51 ± 0.34
Lung volumes			
IC (ml)	129 ± 31	161 ± 43	167 ± 47
ERV (ml)	169 ± 22	195 ± 26	212 ± 46
N_2 washout			
CV (ml)	21 ± 12.3	33 ± 16.8	44 ± 19.2
CV/VC (%)	7.3 ± 4.2	9.5 ± 4.8	12.2 ± 5.8
% N_2/100 ml	0.91 ± 0.39	0	0.36 ± 0.19
Viso (ml)	34.7 ± 21.0	46.4 ± 18.5	42.7 ± 20.0
Viso/FVC (%)	12.3 ± 6.2	13.4 ± 4.9	11.2 ± 6.0

[a]Mean ± standard deviation.

amounts of nonpolarizable, needle-shaped fibers (fibrous glass), readily seen under reduced light and located in peribronchiolar, peribronchial, or perivascular areas as well as within alveoli and in pleural and subpleural locations. In many animals, there was granulomatous inflammation in the lung and pleura that was of minimal to severe intensity (Figs. 17 and 18). This inflammatory response consisted of fibrous glass-laden macrophage aggregates that were surrounded by varying numbers of lymphocytes, plasma cells, and, at times, neutrophils (Fig. 19). These pulmonary lesions appeared to be more severe and prevalent in the diaphragmatic areas of the lung lobes in the affected rats. The fibrous glass-containing macrophages, in lungs of virtually all rats, occupied less than 5% of the total area of the lung sections.

The thymic and tracheobronchial lymph nodes contained small to large aggregations of macrophages containing various amounts of fibrous glass fibers that were usually in the medulla of the lymph node (Figs. 20 and 21). Granulomatous inflammation was present in some sections of lymph node in a few rats exposed to fibrous glass.

Although fibrous glass-laden macrophages occurred in pleural and subpleural locations and were present in thymic and tracheobronchial lymph nodes, there was little evidence of translocation of fibrous glass fibers in other organs in the rats in this study. However, two male rats in the FO1 group had a few small macrophage aggregates laden with fibrous glass in their mesenteric lymph nodes.

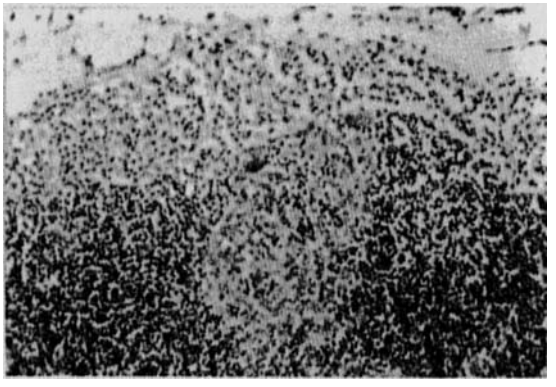

FIG. 9. Tracheobronchial lymph node, male monkey, FO4 group. A large macrophage aggregate is present in the medulla and contains moderate amounts of fibrous glass and lung mite pigment. ×35.

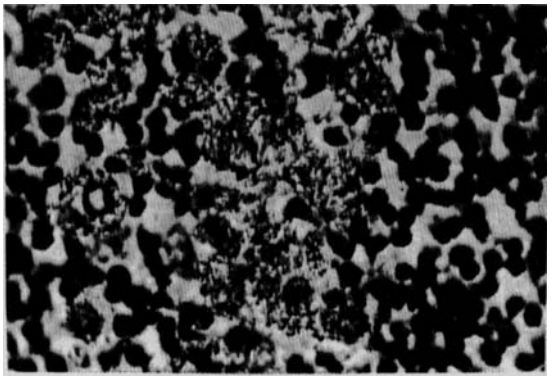

FIG. 10. Tracheobronchial lymph node, male monkey, FO4 group. Fibrous glass particles are clearly depicted in the macrophage aggregate. ×175. Reduced light.

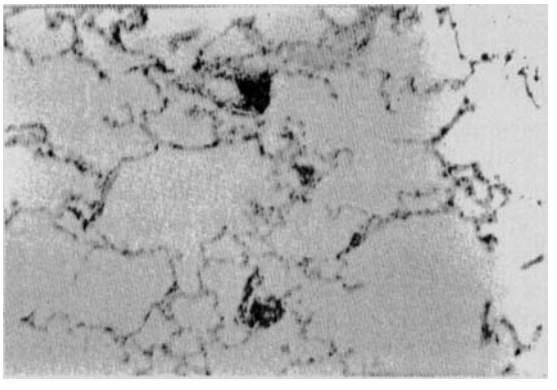

FIG. 11. Lung, male monkey, FO4 group. Macrophage aggregates are present in the alveoli and about blood vessels, which contain fibrous glass and lung mite pigment. ×17.5.

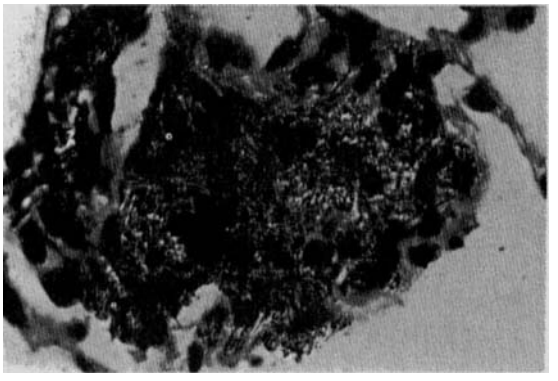

FIG. 12. Lung, male monkey, FO4 group. Fibrous glass particles and lung mite pigment are clearly depicted in this alveolar macrophage aggregate. ×175. Reduced light.

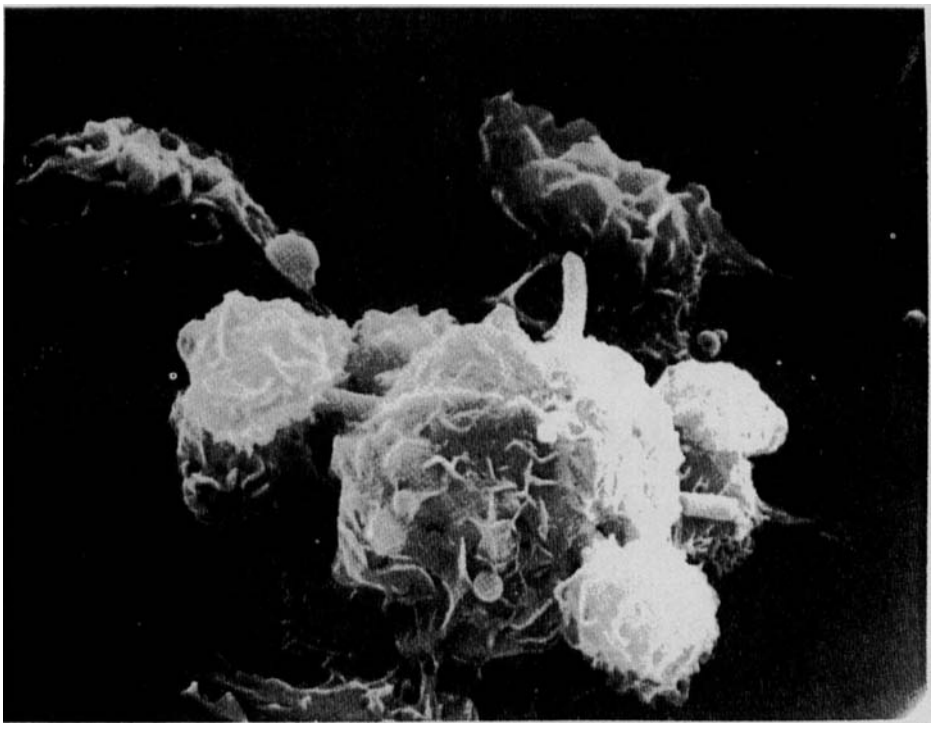

FIG. 13. This SEM photograph depicts several fibrous glass-laden alveolar macrophages that were obtained by pulmonary lavage from a male monkey (group FO2) that died spontaneously. ×1875.

There were rather significant histomorphological variations in qualitative and quantitative fibrous glass-related changes among the various exposure groups in the lungs and thymic and tracheobronchial lymph nodes. These changes were least pronounced in the FO1 group, became progressively more pronounced in the FO2 and FO3 groups, and were most pronounced in the FO4 group. In contrast to this, the gross changes seen in the lung lobes at the time of necropsy were most severe in the FO2 group, were somewhat less severe in the FO3 group, and were least severe in the FO4 group, that is, in relation to size of the plaquelike lesions of the pleura. The pleural lesions seen mi-

TABLE 9. DISTRIBUTION OF LESIONS

Lesion	*Severity*	*Group FO1*	*Group FO2*	*Group FO3*	*Group FO4*	*Control FO5*
Pulmonary	Minimal	5	1	0	0	0
	Mild	—	10	11	12	0
	Moderate	1	1	1	0	0
	No lesion	6	0	0	0	0
Tracheo-bronchial lymph node	Minimal	2	5	1	1	0
	Mild	2	3	6	4	0
	Moderate	0	2	5	5	0
	No lesion	7	2	0	2	0

croscopically in many of the FO4 group animals may have been relatively more significant (more lesions per unit area of pleura) than those seen in the FO2 and FO3 group animals because there were more lesions per unit area of pleura, that is, the pleura was granular in character in many of the animals at necropsy. Hence, a larger number of pleural lesions would be present per microscopic field, resulting in a more severe lesion designation.

Table 12 depicts the numbers of animals affected per exposure group and qualitative severities of the fibrous glass-induced lesions in the lung and lymph nodes (thymic and tracheobronchial).

Many scheduled sacrifice rats in this study had MCL (Fischer rat leukemia) (Table 13). Both the male and the female rats in the FO5 (control) group had a lower incidence of MCL than the male and female rats in the other test groups. When the males and females, early death and scheduled sacrifice, were considered together and a chi-square analysis was applied, the difference between the control group (FO5) and each of the test groups was significant ($P < 0.05$) (Table 14).

DISCUSSION

The response to fibrous glass inhalation was qualitatively similar in both rats and monkeys, in that the changes induced consisted of macrophage responses to the inhaled fibers. In both species, there was translocation of fibers from the lung to draining lymph nodes, and, with the exception of two rats in which fiber-laden macrophages were observed in mesenteric lymph nodes, there was no further evidence of fiber translocation in rats or monkeys from this study. There was no evidence of fibrous glass-induced pulmonary carcinogenicity or carcinogenicity of serosal surfaces. The only question regarding carcinogenicity arose in the increase seen in the incidence of MCL in exposed versus control rats. There was no evidence that the inhaled fibrous glass induced fibroplasia or any additional change other than the macrophage response in the lung or lymph nodes of either rats or monkeys. There was no evidence that the pleural adhesions observed at necropsy in one rat from the FO1 group were associated with fibrous glass inhalation.

Explanation of the increased incidence of MCL in the Fischer 344 rat could simply be that aged Fischer 344 rats have an increased spontaneous incidence of this disease. However, only the exposed rats in this study demonstrated a significantly increased incidence of MCL. Therefore, an alternate hypothesis could be that the presence of fibrous glass in the pulmonary or lymphoid tissues may have resulted in altered cellular immune function due to mononuclear phagocytic inhibiting and promoting lymphocytic factors, with an enhanced incidence of spontaneously occurring MCL.

TABLE 10. NUMBER OF RATS WITH FIBROUS GLASS-INDUCED LESIONS BY EXPOSURE GROUP (EARLY DEATHS)

		Number of animals							
		Group FO1		*Group FO2*		*Group FO3*		*Group FO4*	
Lesion	*Severity*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
Lymph node-thymic, macrophage aggregates, multifocal	Minimal	1	0	1	0	0	0	0	0
	Mild	1	0	4	2	2	3	8	3
	Moderate	0	0	4	3	9	7	15	12
	Severe	0	0	0	0	0	0	0	0
	No lesion	31	31	22	22	21	28	7	9
Lymph node-tracheobronchial, macrophage aggregates, multifocal/focal	Minimal	3	4	0	0	10	6	1	5
	Mild	2	0	3	2	8	14	11	8
	Moderate	0	0	0	1	2	2	6	7
	Severe	0	0	0	0	0	0	0	0
	No lesion	28	27	28	24	12	16	12	4
Lung, macrophage aggregates, multifocal/focal	Minimal	23	17	14	18	30	35	23	21
	Mild	1	3	16	9	2	3	6	2
	Moderate	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	No lesion	9	11	1	0	0	0	1	1
Lung, pneumonia, granulomatous, multifocal/focal	Minimal	10	7	19	19	17	28	10	11
	Mild	3	2	6	1	15	7	20	13
	Moderate	0	0	1	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
	No lesion	20	22	5	7	0	3	0	0
Pleura, pleuritis, granulomatous, multifocal/focal	Minimal	0	0	5	3	19	27	15	12
	Mild	0	0	3	0	6	7	13	9
	Moderate	0	0	0	0	0	0	1	2
	Severe	0	0	0	0	0	0	0	0
	No lesion	33	31	23	24	7	4	1	1

TABLE 11. MONONUCLEAR CELL LEUKEMIA (MCL) IN SPLEEN OF EARLY DEATH RATS

Group	*MCL (males)/ Total examined*	*% MCL males*	*MCL (females)/ Total examined*	*% MCL females*	*MCL (males + females)/ Total examined*	*% MCL males + females*
FO1	9/17	53.9	14/19	73.7	23/36	63.9
FO2	10/19	52.6	11/23	47.8	21/42	52.6
FO3	9/18	50.0	5/12	41.7	14/30	46.7
FO4	13/20	65.0	12/25	48.0	25/45	55.0
FO5	3/14	21.4	10/18	55.6	13/32	40.6

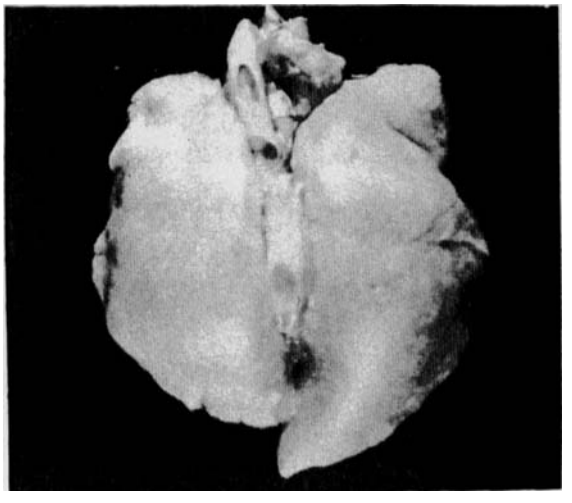

FIG. 14. Lung, male rat, control group. Normal left and right lung lobes.

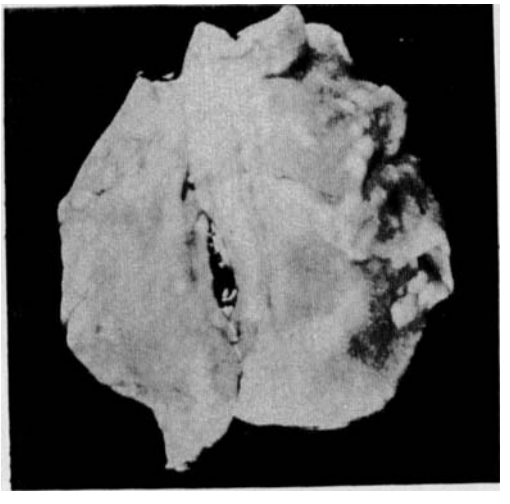

FIG. 15. Lung, female rat, FO3 group. All lung lobes have multifocal gray to tan, elevated, firm plaques, measuring 1–3 mm in diameter.

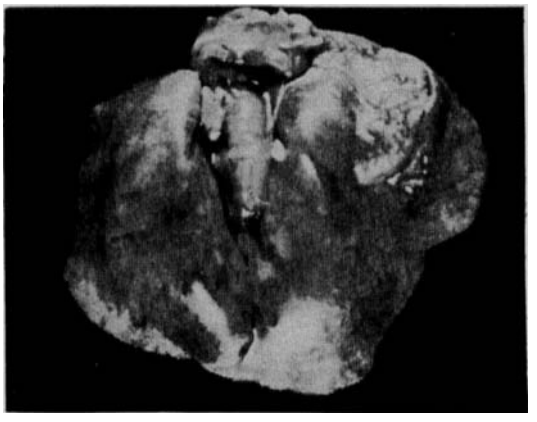

FIG. 16. Lung, female rat, FO4 group. The pleura has a granular surface involving all the lung lobes.

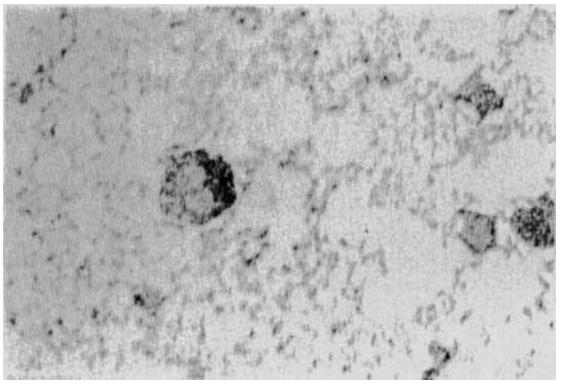

FIG. 17. Lung, male rat, FO3 group. Many granulomas are present in the alveoli that are mild to moderate in intensity. The macrophages are laden with numerous fibrous glass particles. ×17.5.

FIG. 18. Lung, pleura, male rat, FO3 group. A granuloma is present in the pleura that is moderate in intensity. The macrophages are laden with many fibrous glass particles. ×17.5.

FIG. 19. Lung, male rat, FO4 group. The granulomas are characterized by fibrous glass-laden macrophage aggregates, which are surrounded by varying numbers of lymphocytes, plasma cells, and, at times, neutrophils. ×70.

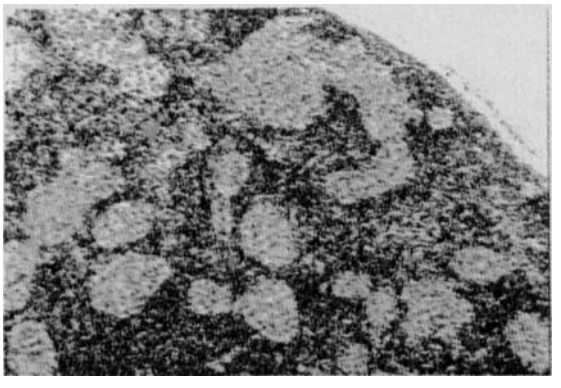

FIG. 20. Thymic lymph node, male rat, FO4 group. Many small to large macrophage aggregates are present in the medullary area. The macrophages are laden with many fibrous glass particles. ×17.5.

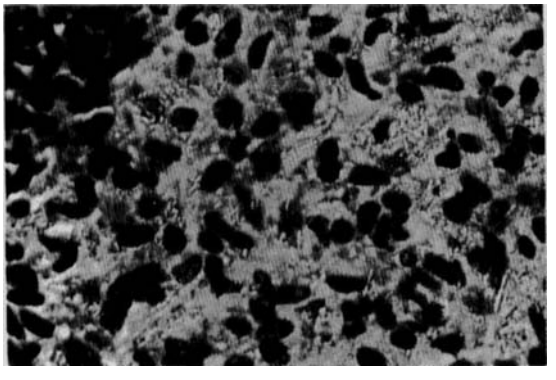

FIG. 21. Thymic lymph node, male rat, FO4 group. Fibrous glass particles are clearly depicted in this macrophage aggregate. ×175. Reduced light.

TABLE 12. NUMBER OF RATS WITH FIBROUS GLASS-INDUCED LESIONS BY EXPOSURE GROUP (SCHEDULED SACRIFICE)

		Number of animals							
		Group FO1		*Group FO2*		*Group FO3*		*Group FO4*	
Lesion	*Severity*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
Lymph node-thymic, macrophage aggregates, multifocal	Minimal	1	2	1	1	1	0	1	0
	Mild	1	2	2	5	4	5	5	5
	Moderate	0	2	3	1	4	2	7	9
	Severe	0	0	0	0	0	1	1	1
	No lesion	15	15	13	16	9	3	6	11
Lymph node-tracheobronchial, macrophage aggregates, multifocal/diffuse/focal	Minimal	1	0	0	0	1	2	5	4
	Mild	0	0	1	2	5	1	10	6
	Moderate	0	1	0	0	1	0	0	1
	Severe	0	0	0	0	0	0	0	0
	No lesion	16	18	18	21	11	9	4	14
Lung, macrophage aggregates, multifocal	Minimal	15	15	8	11	1	5	3	1
	Mild	0	2	10	8	15	7	16	23
	Moderate	0	0	0	0	1	0	1	1
	Severe	0	0	0	0	0	0	0	0
	No lesion	2	2	1	4	1	0	0	1
Lung, pneumonia, granulomatous, multifocal	Minimal	0	0	0	1	6	5	5	8
	Mild	0	0	1	0	6	2	13	13
	Moderate	0	0	0	0	0	0	1	1
	Severe	0	0	0	0	0	0	0	0
	No lesion	17	19	19	23	6	5	1	4
Pleura, pleuritis, granulomatous, multifocal/focal	Minimal	1	1	0	2	4	2	4	7
	Mild	1	1	2	0	3	5	13	10
	Moderate	0	0	0	0	1	0	0	3
	Severe	0	0	0	0	0	0	0	0
	No lesion	15	17	17	21	10	5	3	6

TABLE 13. MONONUCLEAR CELL LEUKEMIA (MCL) IN SPLEEN OF SCHEDULED SACRIFICE RATS

Group	MCL (males)/ Total examined	% MCL males	MCL (females)/ Total examined	% MCL females	MCL (males + females)/ Total examined	% MCL males + females
FO1	8/33	24.2	6/31	19.4	14/64	21.9
FO2	8/31	25.8	8/27	29.6	16/58	27.6
FO3	11/32	34.4	10/37	27.0	21/69	30.4
FO4	12/30	40.0	5/24	20.8	17/54	31.5
FO5	7/36	19.4	1/31	3.2	8/67	11.9

TABLE 14. MONONUCLEAR CELL LEUKEMIA (MCL) IN SPLEEN OF BOTH EARLY DEATH AND SCHEDULED SACRIFICE RATS

Group	MCL (males)/ Total examined	% MCL males	MCL (females)/ Total examined	% MCL females	MCL (males + females)/ Total examined	% MCL males + females
FO1	17/50	34.0	20/50	40.0	37/100	37.0[a]
FO2	18/50	36.0	19/50	38.0	37/100	37.0[a]
FO3	20/50	40.0	15/49	30.6	35/99	35.4[a]
FO4	25/50	50.0	17/49	34.7	42/99	42.4[b]
FO5	10/50	20.0	11/49	22.4	21/99	21.2

[a] $P < 0.05$ by chi-square test.
[b] $P < 0.01$.

In addition to the above hypotheses, it is known that fibrous glass can be both cytotoxic and genotoxic,[7] and, therefore, it is plausible that fibrous glass may have a direct genotoxic effect on stem leukocytes in the pulmonary or lymphoid tissues, resulting in an increased incidence or probability of MCL. Fiber-exposed workers have demonstrated increased lymphomas and leukemias.[20,21] In view of these facts, one may speculate that fibrous glass may predispose man to an increased incidence of lymphoma and leukemia.

Fibrous glass-induced pulmonary lesions in monkeys were generally mild. With the exception of the FO1 group ($>20\ \mu \times 4$ to $6\ \mu$ plus binder) in which the macrophage response was minimal and in which lymphoid aggregates increased mildly, there were no apparent differences in response among the fibrous glass-exposed groups. The significance of the increase in the lymphoid aggregates is unknown, but the most probable explanation would be a mild stimulation from an antigen, such as the binder. The response in rats increased in severity from group FO1 to group FO4 and consisted of foci of granulomatous inflammation that were not present in monkeys. It was more difficult to define precisely the response induced by the fibrous glass in monkeys because this response was generally superimposed on the lesions induced by lung mite infestations, and the aggregates of macrophages often contained both lung mite debris and fibrous glass. Lung mite-induced pulmonary lesions were prominent in many monkeys from this study and were present to some degree in nearly all monkeys. Some of these lesions were similar to those that might be expected from inhaled fibers, including such changes as pleural, subpleural, and interstitial fibrosis as well as smooth muscle hyperplasia. The presence of parasite-induced lesions in the lungs of monkeys could have masked subtle changes induced by the fibrous glass. However, the nature and distribution of the fibrous glass-induced lesions and their consistency among all groups, including monkeys with minimal lung mite-associated lesions, suggest that this was not the case. There were no fibrous glass-associated granulomas in monkeys as there were in rats.

The prescribed fiber diameter in group FO1 ($4–6\ \mu$) was of such dimension as to preclude most fibers from entering the alveoli. The presence of fibrous glass-induced lesions in this group in some animals from both species, although minimal, probably occurred as a result of the presence of smaller-diameter fibers that are inevitably present to some extent in these preparations.

A previous fiberglass tracheal instillation and inhalation study in rats used fibers measuring either $1.5\ \mu \times 5\ \mu$ or $1.5\ \mu \times 60\ \mu$.[22] The shorter fibers did not induce granulomas in the lung; instead fibers were phagocytized and cleared to the draining lymph nodes. However, the longer fibers induced granulomas and were not cleared to the lymph nodes.[22] The results in rats from this study did not follow that pattern entirely, since the most intense pulmonary response occurred in group FO4 where the fiber length was shortest. There was translocation of fibers to the pulmonary lymph nodes even in the group with the longest fibers (FO1, $>20\ \mu$), indicating that either particles of this length can be phagocytized and cleared or that the phagocytized and transported fibers were fractured fibers of shorter length. As previously described, the response in monkeys was similar to that described for fibers of $1.5\ \mu \times 5\ \mu$ in rats. However, the possibility for variation in response between species and the generally mild and consistent response in monkeys does not allow for viable comparisons with the work of Bernstein et al. in rats.[23]

Generally, the pulmonary response in rats was greater in groups with smaller-diameter fibers as would be expected. That was true in monkeys only to the extent that the larger diameter fibers in the FO1 group induced a less severe response than did fibers from the other three exposure groups.

The grossly visible plaquelike foci that occurred in rats resulted from accumulations of granulomatous foci in pleural and subpleural locations. The term "plaquelike" is used to denote a gross morphological change only and should not be confused with the characteristic asbestos plaque, which contains fibrous tissue.) The decreasing severity of these grossly observed lesions among three of the test groups (FO2 > FO3 > FO4) was in direct contrast to the severity observed microscopically (FO4 > FO3 > FO2). Although the explanation for this discrepancy was not entirely obvious, it apparently resulted from a variation in character and in pleural localization of granulomatous foci that was not directly related to the general severity of the pulmonary lesions. These lesions were limited to granulomatous foci, there was no fibrosis, and there were no growth alterations in adja-

cent tissues; therefore, there is no evidence in these animals that any further sequelae would result beyond that observed.

There was no consistent variation in occurrence and severity of fibrous glass-induced lesions among the various lobes of the lungs in monkeys. There was, however, generally a greater severity of such lesions in the posterior lobes of the lungs in rats from groups FO2 and FO3, whereas there was a more even distribution among all lobes in rats from group FO4. The residual fiber in the lung of animals from group FO1 was considerably less than the other fiber treatments, presumably as a result of the large aerodynamic diameter and poor respirability.

To summarize our findings:

1. The only unequivocal responses induced by fibrous glass inhalation in monkeys were macrophage aggregates with phagocytized fibrous glass in the lungs and tracheobronchial lymph nodes.

2. The pulmonary responses in the rat induced by fibrous glass inhalation were characterized by macrophage aggregates and granulomas that contained fibrous glass fibers. The grossly visible plaquelike foci resulted from accumulations of granulomatous foci in pleural and subpleural locations. These lesions were limited to granulomatous foci, there was no fibrosis, and there were no growth alterations in adjacent tissues. Therefore, there is no evidence in these animals that any further sequelae would result beyond that observed.

3. There was no evidence of a fibrous glass-induced fibrogenic response in either monkeys or rats.

4. The most severe lesions in rats were in the FO4 group ($< 10\ \mu \times 1\ \mu$, no binder), whereas the response in the FO1 group ($> 20\ \mu \times 4$–$5\ \mu$, with binder) was minimal.

5. The severity of response in monkeys was similar for all exposed groups except the FO1 group ($> 20\ \mu \times 4$–$6\ \mu$, with binder), in which the response was minimal. Group FO1 also included monkeys that had mildly increased numbers of lymphoid nodules or aggregates in peribronchiolar and perivascular areas. The significance of the increase in the lymphoid aggregates is unknown, but the most probable explanation would be a mild stimulation from an antigen, such as the binder.

6. The fibrous glass-induced lesions were similarly distributed among all lobes of the lung in monkeys; in rats, the lesions were most prominent in posterior lobes in all but the FO4 group, where there was more equal distribution throughout the lung.

7. The only evidence of translocation of fibers occurred in macrophage transport to draining pulmonary lymph nodes in many animals (rats and monkeys) and to mesenteric lymph nodes in two rats.

8. The MCL was statistically significant when each test group was individually compared to the control group. The importance of this exposure-related incidence should not be ruled out.

9. This study showed no evidence of pulmonary or mesothelial carcinogenicity associated with inhaled fibrous glass.

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