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Chronic Inhalation of Short Asbestos: Lung Fiber Burdens and Histopathology for Monkeys Maintained for 11.5 Years after Exposure

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In an earlier report, Platek et al. (1985) presented the results of an 18-month inhalation exposure of rats and monkeys to short chrysotile asbestos. The mean chamber exposure level was 1.0 mg/m^3 with an average of 0.79 fibers/ml >5 μm in length. Gross and histopathological examination of exposed and control rats indicated no treatment-related lesions. Asbestos bodies adjacent to the terminal bronchioles, but no fibrosis, were found in lung biopsy tissue taken from the exposed monkeys at 10 months post-exposure. Fifteen monkeys (9 exposed and 6 controls) from this study were maintained for 11.5 years following exposure. Lung fiber burdens were determined by transmission electron microscopy. The mean lung burden ($\pm$ standard deviation) for 59 samples from exposed monkeys was 63 $\pm$ 30 $\times$ 10^6 fibers/g dry lung (range, 18–139 $\times$ 10^6). The geometric mean fiber length was 3.5 μm with 35% of the fibers being >5 μm in length. These data indicate some chrysotile fibers are durable *in vivo* for a significant period of time. Lungs were examined grossly and microscopically. No lesions attributable to the inhalation exposure were noted. Asbestos bodies were seen in the lungs of treated monkeys, primarily in the interstitium near bronchioles or small pulmonary blood vessels (which also may have been near to bronchioles just out of the plane of section).

INTRODUCTION

In 1978, the National Institute for Occupational Safety and Health began a study to investigate the health effects of short chrysotile asbestos inhalation in rats and monkeys. The inhalation study was conducted under NIOSH Contract 210-77-0151 by the International Research and Development Corporation, Mattewan, Michigan. Most of the results of this chronic inhalation study were presented in an earlier report (Platek et al., 1985). Briefly, male Sprague-Dawley rats and Cynomolgus monkeys were exposed for 18 months, 7 hours/day, 5 days/week to short chrysotile which had been prepared by ball milling. The bulk chrysotile was type 7TF1 obtained from the Johns Manville Sales Corporation (Denver, Colorado). Over the course of the study, the mean chamber chrysotile mass concentration ($\pm$standard deviation) as determined by gravimetric sampling was 1.0 ($\pm$0.28) mg/m^3, with a mean chamber concentration of fibers >5 μm in length of 0.79 ($\pm$0.41) fibers/ml as determined by phase contrast optical microscopy. Interim sacrifices of rats were conducted at 1, 3, 6, 12, and 18 months, with the terminal sacrifice performed at 24 months (6 months after completion of the inhalation exposures). Based upon gross and histopathologic examinations, the only treatment-related alterations seen in the rat lungs were a few and scattered macrophages in the pulmonary alveoli. No pulmonary fibrosis or pulmonary tumors were seen. Lung biopsies were performed on the monkeys at 10 months post-exposure (28-month biopsies). Again, microscopic examination 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.

In the present study, monkeys were maintained for 11.5 years following completion of the inhalation exposures (13 years from the commencement of exposures). Fifteen (9 exposed, 6 controls) of the original 20 monkeys (10 exposed, 10 controls) remained when necropsies were conducted in 1991. In addition to gross and microscopic evaluation of the monkeys' lungs, lung chrysotile burden determinations for each of the monkeys were

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performed. The results of the histopathological evaluations and the lung fiber burden determinations are presented in this report.

METHODS

Animal Care

Following the 10-month post-exposure period, the monkeys were transferred to contractors for long-term observation. The animals subsequently were transferred to the NIOSH animal facility in 1986 where they were observed until 1991. The NIOSH animal facility was fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC).

Necropsy and Pathology

The remaining nine exposed and six controls male monkeys were sacrificed by intravenous injection of sodium pentobarbital and necropsied. The respiratory tract was removed, weighed, and then left and right lungs were separated from the trachea by cutting each primary bronchus just distal to the hilus. The right lung was inflated with Karnovsky's fixative (in cacodylate buffer) to 30 cm H_2O pressure via the primary bronchus. The primary bronchus was ligated and the lung was placed in the same fixative for at least 1 week. A wedge of lung from near the surface, with attached pleura, was dissected from each of the upper, middle and lower lobes and was removed and processed for light microscopy. After sectioning at 6 μm, lung tissues were stained with hematoxylin and eosin (H&E), Masson's trichrome, and reticulin stain. Lungs were evaluated microscopically. In addition, adrenal glands, brain, sternum (with bone marrow), colon, epididymis, gall bladder, heart, kidneys, liver, lymph nodes (tracheobronchial and mesenteric), nasal cavity, pancreas, pituitary gland, prostate, seminal vesicles, spleen, stomach, testes, thyroids/parathyroid, urinary bladder and grossly-identified lesions were collected, processed to microslides and evaluated microscopically. Microscopic findings were given a severity score of minimal, mild, moderate, marked or severe to allow comparison between groups. The 28-month lung biopsies (10 month recovery period) from the same fifteen monkeys were evaluated to allow microscopic comparison with lungs after the 11.5 year recovery period. [Note — The results of the 28-month evaluation of these biopsies were reported earlier (Platek et al., 1985).]

Lung Fiber Burden Determinations

Pieces of lung tissue adjoining those taken for histopathology, and other non-adjacent samples, for which the lobe location was not known, were trimmed for fiber analysis. The tissue samples were of two types: 1) pieces with pleura (approximately 10 mm × 5 mm) on one side and containing approximately 5 mm of subpleural tissue and 2) when possible, adjacent pieces (approximately 10 mm × 5 mm × 5 mm) of subpleural tissue (near the surface, but not including pleura). The wet weights of the tissue pieces were recorded. Pieces of tissue immediately adjacent to those taken for fiber analyses were weighed and dried to constant weight to determine wet-to-dry weight ratios.

The wet tissues were digested with sodium hypochlorite (5.25%) using a modification of the procedure described by Coin et al. (1992). All reagents were triple filtered using a 0.45 μm pore size vinyl filter. A tissue specimen was placed in a small bottle and 10 ml of sodium hypochlorite solution added. The resulting suspension was gently shaken using a reciprocating shaker for 30–45 minutes and then filtered onto a 0.2 μm pore size polycarbonate filter. The filter with digestate was then treated twice with the following sequence of reagents: 10 ml of isopropanol, 10 ml of 7% oxalic acid, 10 ml of sodium hypochlorite and 10 ml of filtered, de-ionized water. Filter and reagent blanks were also prepared with each sample set. After carbon coating, pieces of the filters were placed on 200 mesh copper transmission electron microscope grids, and cleared with chloroform. Grids were prepared for 59 samples of digested lung tissue from chrysotile-exposed monkeys, 30 digested lung samples from control monkeys, and 32 reagent blanks.

To determine the effect of tissue sample preparation on fiber burden and fiber length distribution, five additional lung samples from chrysotile-exposed monkeys were prepared by the low temperature ashing procedure used by Platek et al. (1985).

The grids were examined in a JEOL 100CX transmission electron microscope at a magnification of 20,000X. All fibers found in 10 grid spaces or a minimum of 100 fibers (whichever came first) for two grids from each sample were counted. All fibers not touching a grid bar were sized. Fibers touching a grid bar were counted ($^1/_2$ fiber), but not sized. Fibers were defined as particles with parallel sides having aspect ratios > 3:1. Asbestos bodies present in the samples also were counted and sized. Fibers were classified as chrysotile by inspection, based on the presence of an internal capillary. Rare fibers not having an internal capillary were analyzed using energy dispersive x-ray analysis and classified based on this qualitative analysis. Fiber length and diameter measurements were determined directly from the screen using the scanning transmission image. Diameters for most fibers were measured at a magnification of 150,000X. The magnifications used to measure lengths varied depending on actual fiber length. Final lung fiber concentrations were calculated from the relationship between the wet weight of the lung tissue, wet-to-dry weight ratio, area of the filter analyzed, the total filter area and the total number of fibers counted.

Statistical Analyses for Fiber Burden Determinations

Prior to the analyses, the values from the two grids for each sample were averaged. Occasionally there were multiple samples from the same monkey and lobe location which were also averaged for the statistical analysis. Linear mixed models were used to test for effects of lung location and tissue preparation on fiber burden (total fibers per gram of dry lung). These models were estimated using restricted maximum likelihood. All calculations were performed with SAS® (Release 6.12, SAS Institute, Inc., Cary, North Carolina).

The first model was $Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ijk}$, where μ is the grand mean, α_i is a random effect of monkey, β_j is a

fixed effect of lobe (upper, middle, lower), γ_k is a fixed effect of surface (pleural, subpleural), and ε_{ijk} is a normally distributed, independent error.

Another model was also used to test for an effect of surface and included samples where the lobe was not known, $Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$. Here μ is the grand mean, α_i is a random effect of monkey, β_j is a fixed effect of surface (pleural, subpleural), and ε_{ij} is a normally distributed, independent error.

Finally, a model was used to test for preparation effects using samples from the pleural surfaces of five monkeys. In this case the model was $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where μ is the grand mean, α_i is the fixed effect of preparation technique (ashing, digestion), and the errors, ε_{ij}, were modeled with and unstructured covariance matrix.

RESULTS

Pathology

On necropsy, lungs had normal gross appearance, with the exception of the previous biopsy sites (middle and lower lobes of left lung), where fibrosis of the parietal pleura and adhesions between the visceral and parietal pleura were evident. There were no treatment-related effects on lung weight or lung-to-body-weight ratios. The mean lung weight and mean lung-to-body-weight ratio for controls were 27.8 grams and .0045, respectively; for treated animals they were 25.7 grams and .0045.

Microscopically, the only treatment-related effect in lungs after the 11.5 year recovery period was the presence of minimal numbers of asbestos bodies (observed in 0/6 controls, 9/9 treated animals). Asbestos bodies appeared as linear fibers with a golden-brown coating (some beaded), or as small fragments or globules of golden-brown pigment, which were presumed to be fragments of asbestos bodies. Asbestos bodies were primarily observed in the inter-alveolar or interlobular septa near bronchioles or small pulmonary blood vessels (Figure 1). Less frequently, asbestos bodies were observed in the pleura, in alveolar macrophages, or in the walls of bronchioles (Figure 2). Among all treated animals, a few asbestos bodies were observed in alveolar duct bifurcations.

In 28-month lung biopsies (taken 10 months post-exposure), minimal (6/9 treated animals) to mild (3/9 treated animals) numbers of asbestos bodies were observed in locations similar to those where necropsy fibers were observed. Asbestos bodies were more frequently observed in alveolar macrophages or multinucleated giant cells in biopsied lungs (Figure 3) than in lungs from necropsies.

In the lungs of all monkeys (biopsied and necropsied monkeys; both control and treated) a black, finely granular pigment and, less frequently, a colorless-to-brown anisotropic (birefringent with polarized light) crystalline pigment were observed in the interstitium near pulmonary blood vessels and bronchioles. These pigments were typical of mite pigment and indicative of past infection with *Pneumonyssus simicola*, a common lung mite in monkeys. Some of these deposits may also have been indicative of anthracosilicosis. Deposits were observed in similar, and often the same, locations in the lung where asbestos bodies were observed in treated animals. The asbestos bodies and fragments were readily distinguishable from the incidental pigment observed in the lungs of all animals. Lung mite pigment was slightly more severe in the lung biopsies. In a few of the biopsies, lung mites and associated granulomatous inflammation were observed.

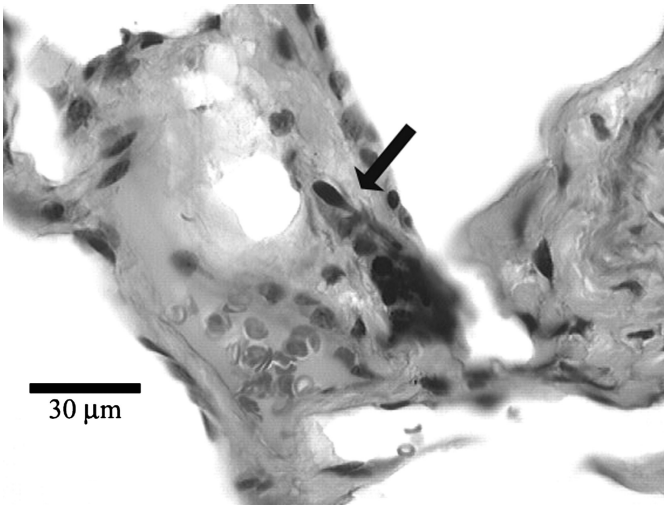

FIG. 1. Light micrograph of an asbestos body (arrow) in the interstitium near a pulmonary blood vessel from a monkey 11.5 years post-exposure. Hematoxylin and eosin stain.

Asbestos body pigment (golden-brown globules) suggestive of asbestos body fragments were also observed within macrophages in tracheobronchial lymph nodes (0/6 controls, 6/9 treated animals).

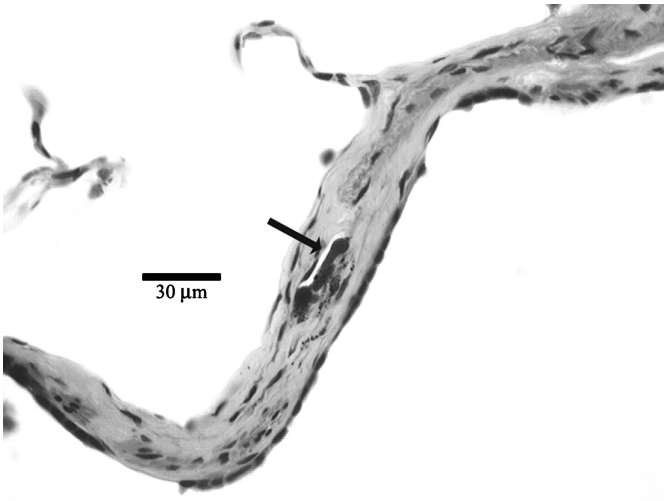

FIG. 2. Light micrograph of an asbestos body (arrow) in the wall of a respiratory bronchiole from a monkey 11.5 years post-exposure. Hematoxylin and eosin stain.

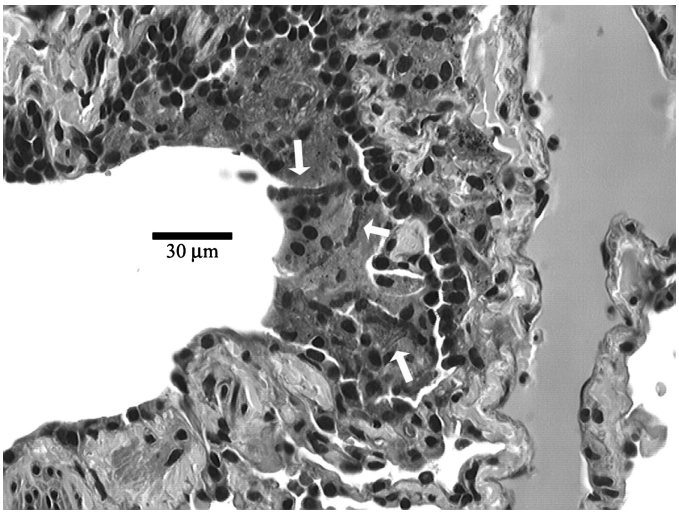

FIG. 3. Light micrograph of asbestos bodies (white arrows), in macrophages and multinucleated giant cells, in the lumen of a bronchiole from a biopsy taken 10 months post-exposure. Hematoxylin and eosin stain.

Lung Fiber Analyses

The results of lung chrysotile burden determinations for the exposed monkeys after 11.5 years are summarized in Table 1. The lung chrysotile concentration data are based on total fibers counted, i.e., the sum of the chrysotile fibers and asbestos bodies. The mean (±standard deviation) chrysotile concentration for the 59 samples of lung tissue processed through the digestion procedure was $63 \pm 30 \times 10^6$ fibers/g dry lung (range, 18–139×10^6). The mean chrysotile concentrations for the nine exposed monkeys ranged from 27 to 95×10^6 fibers/g of dry lung. Asbestos bodies accounted for 4.0% of the total chrysotile lung burden.

Chrysotile size data for the lung digests are summarized in Table 2. The geometric mean lengths of the chrysotile fibers for exposed monkeys were very similar, ranging from 3.2 to 3.7 μm. The geometric mean length of the asbestos bodies was 10.9 μm. The bivariate size distribution for all of the fibers (asbestos bodies excluded) is shown in Figure 4. Fiber lengths, but not diameters, were log-normally distributed. The maximum fiber length noted was 110 μm. The count median fiber diameter was 0.03 μm. The maximum fiber diameter observed was 1.1 μm. The bivariate size distribution for the asbestos bodies is shown in Figure 5. The body diameters were measured, whenever possible, on the largest non-coated segment. The maximum asbestos body length observed was 74.5 μm.

A high magnification transmission electron micrograph of two single fibrils from a terminal sacrifice monkey is shown in Figure 6. These fibrils have an irregular coating on their outside surface. This irregular surface coating was seen on most of the fibers from the terminal sacrifice monkeys.

Non-chrysotile fibers accounted for 1.7% of the exposed monkey total lung fiber burden. The vast majority of these non-chrysotile fibers were <5 μm in length. Aluminum silicates, iron silicates, calcium magnesium silicates and rutile were the most common non-chrysotile fibers seen. A total of 17 calcium magnesium silicate fibers, whose qualitative chemistry was consistent with tremolite, were found (0.2% of fibers examined). An average of 1.8 and 1.7 fibers per 20 grid spaces were found in the control monkey lungs and reagent blanks, respectively. The median lengths of these fibers were small, 1.1 μm and 1.0 μm, for the control lung and reagent blank preparations, respectively.

Data for the effect of lung tissue preparation method on fiber size and concentration is summarized in Table 3. Clearly, the lung tissue preparation method affects both fiber burden results and fiber length distributions. The mean chrysotile fiber

TABLE 1
Lung chrysotile burdens

Exposed monkey number	Number of lung digests analyzed	Number of chrysotile fibers sized	Number of asbestos bodies sized	Chrysotile[a] concentration [mean ± SD] (10^6/g dry lung)
91-18	5	700	60	54 ± 16
91-20	5	846	13	71 ± 26
91-21	6	972	37	67 ± 20
91-23	6	992	93	95 ± 30
91-24	9	1263	37	52 ± 14
91-26	8	1231	37	51 ± 21
91-27	4	535	19	46 ± 17
91-29	7	848	15	27 ± 8
91-30	9	1437	67	94 ± 31
Sum of All Digests	59	8824	378	63 ± 30

[a]Concentration is based on the sum of all chrysotile fibers and asbestos bodies counted.

TABLE 2
Chrysotile and asbestos body size data

Exposed monkey number	Geometric mean length (μm)	Geometric standard deviation (σ_g)	Percentage of fibers having length >		
			5 μm	10 μm	20 μm
91-18	3.4	2.5	33	13	1.6
91-20	3.2	2.6	32	12	2.1
91-21	3.7	2.5	36	15	2.1
91-23	3.7	2.3	37	12	1.8
91-24	3.6	2.5	36	13	3.2
91-26	3.5	2.4	34	11	2.3
91-27	3.5	2.7	37	15	3.7
91-29	3.2	2.4	32	9	1.5
91-30	3.6	2.6	37	14	2.4
Sum of chrysotile fibers from all digests	3.5	2.5	35	13	2.3
Sum of asbestos bodies from all digests	10.9	2.1	84	55	21

concentration for the five ashed samples (202×10^6 fibers/gram) is significantly greater ($p = 0.0087$) than for the five adjacent lung digests (43×10^6 fibers/gram). This increased fiber burden is a direct result of fiber breakage during the ashing procedure as noted by the drastic decrease in the percentage of fibers >5 μm in length, 1.4% for the ashed samples versus 35% for the digested samples. Fiber diameters do not appear to be greatly affected by the tissue preparation method as the count median diameters for both the ashed and adjacent digested samples were 0.03 μm. Bivariate size distributions for the ashed samples, adjacent

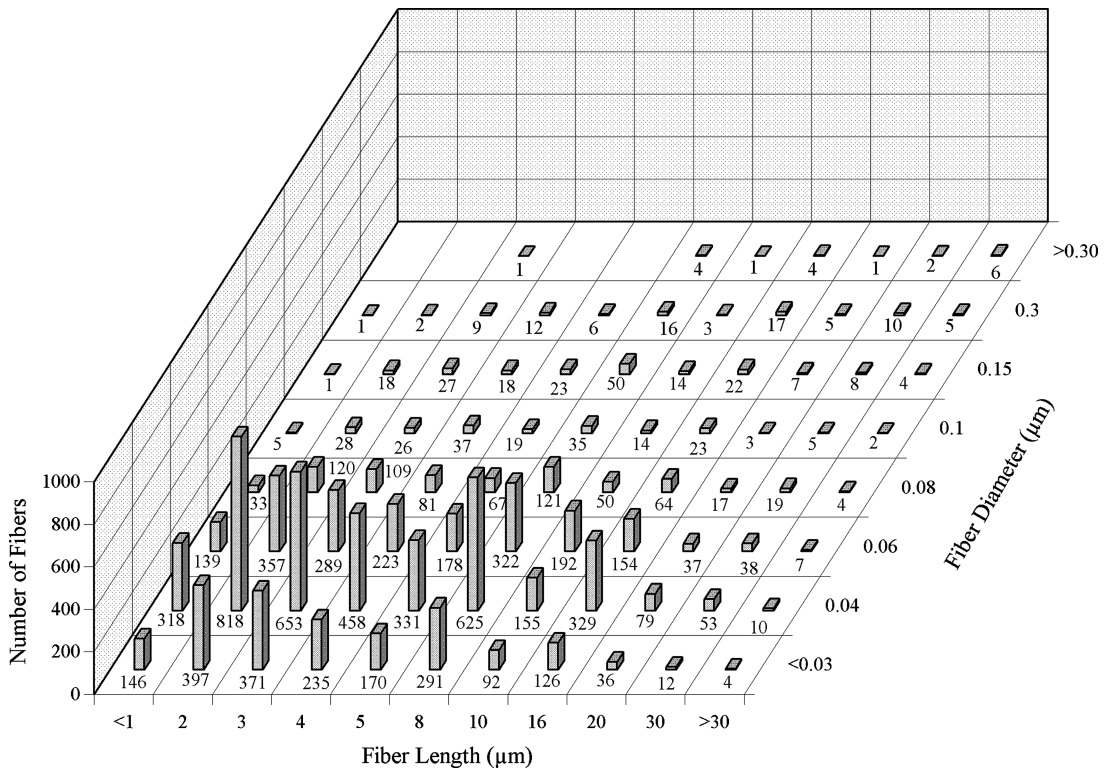

FIG. 4. Bivariate fiber size distribution for 59 digested monkey lung samples from the terminal sacrifice.

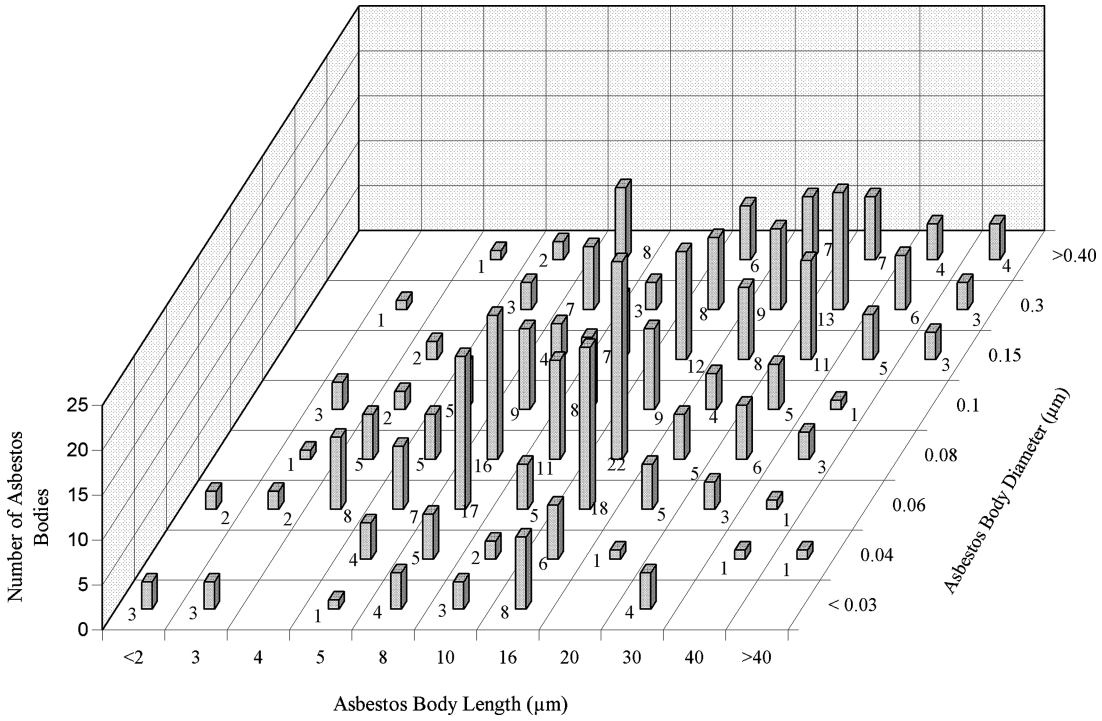

FIG. 5. Bivariate size distribution of the asbestos bodies from the 59 digested monkey lung samples.

digests, and for the ashed lung biopsy samples (Platek et al., 1985) are shown in Figures 7–9.

Fiber burdens were determined for pieces of tissue taken from various lung locations: upper, middle and lower lobes, including samples with pleura and samples consisting solely of subpleural tissue. Data showing fiber burden with respect to sampling location is summarized in Table 4. Excluded from Table 4 is data for a number of samples for which the lobe location was not available. There were no statistically significant differences in mean fiber burdens for samples with pleura from the upper, middle and lower lung lobes. There were not enough subpleural samples to make meaningful comparisons for fiber burdens between lobe locations. When data for all pleura and subpleural samples were compared, fiber burdens in the samples with pleura were significantly greater ($p = .0183$) than in the subpleural samples.

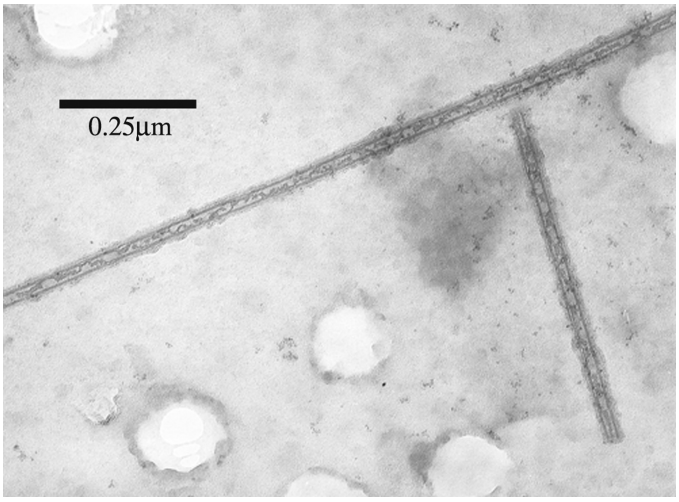

FIG. 6. Transmission electron micrograph showing two chrysotile fibrils from a terminal sacrifice monkey. Both fibrils have an irregular coating on their exterior.

DISCUSSION

The histopathology results from this study are the same as those in the earlier report on rat exposures and monkey biopsies (Platek et al., 1985). No treatment-related effects such as pulmonary fibrosis were observed in monkeys held for 11.5 years following an 18-month exposure to predominantly short chrysotile asbestos. The only definitive evidence of the chrysotile exposure was the presence of asbestos bodies in the lungs of exposed monkeys. Asbestos bodies in both 10-month post-exposure biopsies and in 11.5 year post-exposure lungs were most commonly observed in the interstitium near pulmonary blood vessels and bronchioles, in the areas where lymphatic drainage would be expected. Brody et al. (1981) in a chrysotile inhalation study using the rat noted accumulation of asbestos fibers at bifurcations of alveolar ducts. Asbestos bodies were only rarely observed in this location in the present study, and thickening of alveolar duct bifurcations was not observed. In another study (Bernstein et al., 2004) of chrysotile exposure in rats, fibers were observed to translocate to the

TABLE 3
The effect of lung preparation on fiber burden and size

Sample type	Number of samples	Number of fibers sized	Chrysotile[a] concentration [mean ± SD] (10^6/g dry lung)	Geometric mean length (μm)	Geometric standard deviation (σ_g)	Percentage of fibers having length >		
						5 μm	10 μm	20 μm
Ashed lungs	5	1080	202 ± 135	1.4	1.7	1.4	0.1	0
Adjacent lung digests	5	629	43 ± 19	3.6	2.5	35	12	3.0
28-Month biopsies (ashed)	10	3209	327 ± 53	2.0	1.8	6.3	1.2	—
Sum of all lung digests	59	8824	63 ± 30	3.5	2.5	35	13	2.3

[a]Concentration is based on the sum of chrysotile fibers and asbestos bodies.

broncho-associated lymphoid tissue (BALT) subjacent to bronchioles. While BALT was not especially prominent in the monkeys in the current study, some of the asbestos bodies were located in or near BALT. Only a few asbestos bodies were observed in the pleurae.

The lack of pathologic findings with low-dose exposure is consistent with other studies of chrysotile exposure in animals. In several recent studies, rats exposed to chrysotile fibers at higher doses than in the present study produced no pathology (Bernstein et al., 2003, 2004, 2005a, 2005b). Bernstein et al., 2006, in exposing rats to Brazilian chrysotile for 90 days with a 92-day recovery period, noted that 3413 fibers/ml (76 fibers > 20 μm/ml) did not produce lung pathology, while 8941 fibers/ml (207 fibers > 20 μm/ml) produced only slight fibrosis. The duration of exposure for these recent studies was 90 days or less, versus 18 months in the present study.

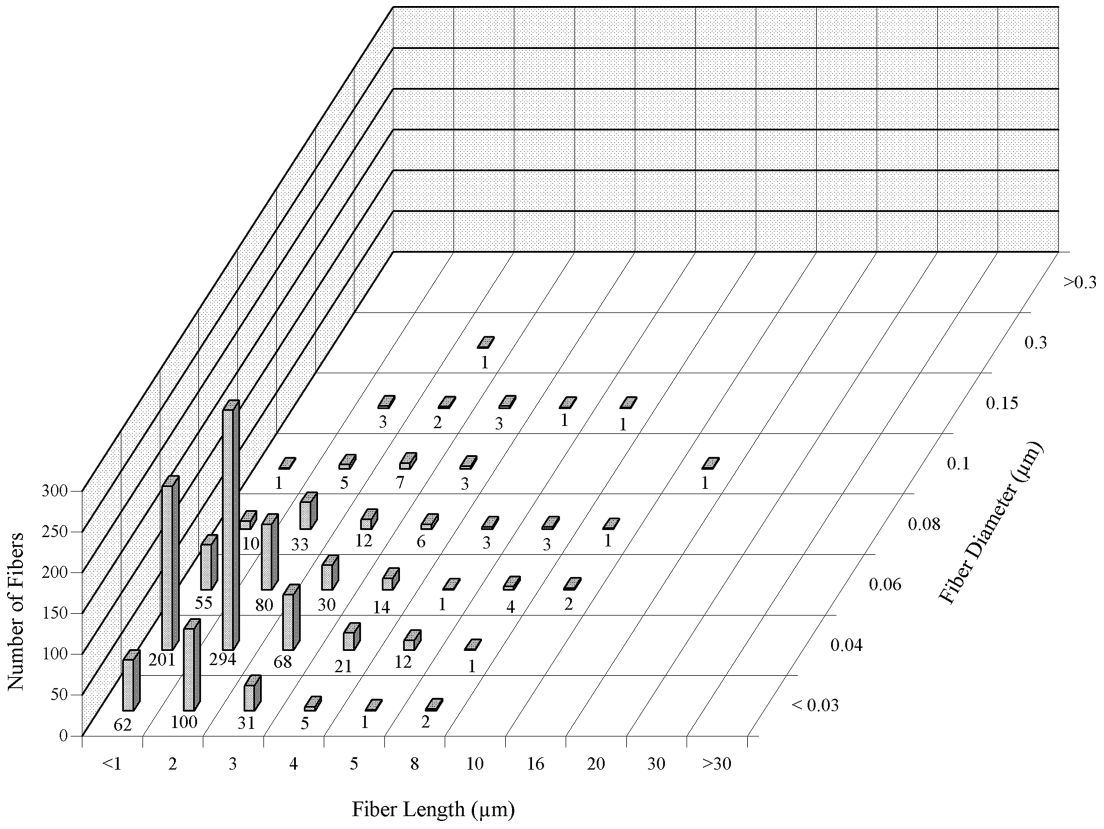

FIG. 7. Bivariate fiber size distribution for 5 ashed lung samples from the terminal sacrifice.

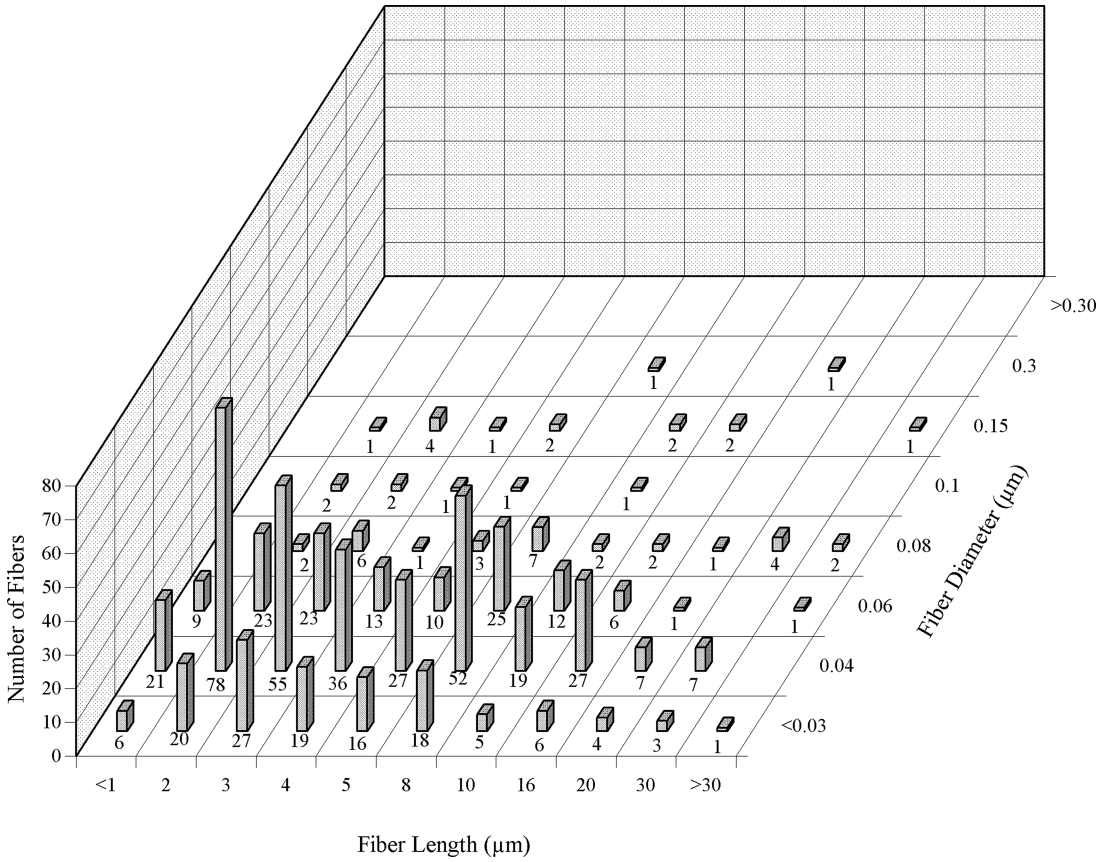

FIG. 8. Bivariate fiber size distribution for 5 adjacent (to the ashed samples in Figure 7) digested lung samples.

Currently, factors thought to be related to the pulmonary toxicity of respirable fibers include fiber dose, dimension, surface chemistry and biodurability (Warheit et al., 1995). Stanton et al. (1981) in a series of experiments with rats dosed by intrapleural implantation noted fiber length, particularly fibers >8 μm in length and ≤ 0.25 μm in diameter, as an important parameter with respect to carcinogenicity. Inhalation studies with rats also have shown fiber length to be related to carcinogenicity and fibrosis (Davis et al., 1986; Davis and Jones, 1988). Various *in vitro* studies also have shown the importance of longer fibers with respect to toxicity (e.g., Mossman, 1990; Hesterberg and Barrett, 1984; Blake et al., 1998). Given the considerable body of evidence that fiber length plays an important role in toxicity, the negative results obtained in this study are not surprising since the average concentration of fibers >5 μm in length in the inhalation atmosphere was only 0.79 fibers/ml. This average fiber level was less than the OSHA standard in effect at the time of the study (not to exceed two fibers longer than 5 μm per cm^3 over an 8-hr time weighted average period (OSHA, 1978)), but approximately 8 times the current OSHA PEL of 0.1 fiber/cm^3 (29 CFR 1910.1001).

The actual short fiber exposure dose in the present study was quite small. The mean number of fibers <5 μm long as determined by scanning electron microscopy (SEM) for nine chamber filter samples was only 439 per ml (Platek et al., 1985). Although the mean chamber chrysotile mass concentration was 1.0 mg/m^3, 52% of all particles examined by SEM were nonfibrous, primarily clumps of small chrysotile fibers produced by the ball milling of the bulk chrysotile. These clumps of chrysotile remained intact as shown in SEM micrographs of ashed preparations of rat lungs (see Platek et al., 1985). In addition, it should be remembered that the short chrysotile was prepared by ball milling. Other investigators have noted that mechanical milling changes the crystalline structure and surface chemistry of chrysotile (Langer et al., 1978; Spurny et al., 1980). Since surface chemistry is thought to play an important role in fiber-related lung fibrosis and carcinogenicity (Jaurand 1991; Bonneau et al., 1986), changes induced by ball milling may have affected the biological activity of the chrysotile. It should be noted that the design of the present study allowed for only a small number of animals and low exposure levels and duration (relative to human exposures). Hence the ability to draw inferences from these data is limited. Nonetheless, our data indicate that despite significant lung fiber burden, short chrysotile fibers at the dose used in this inhalation study were non-toxic 11.5 years after an 18-month exposure in the 9 monkeys that were evaluated.

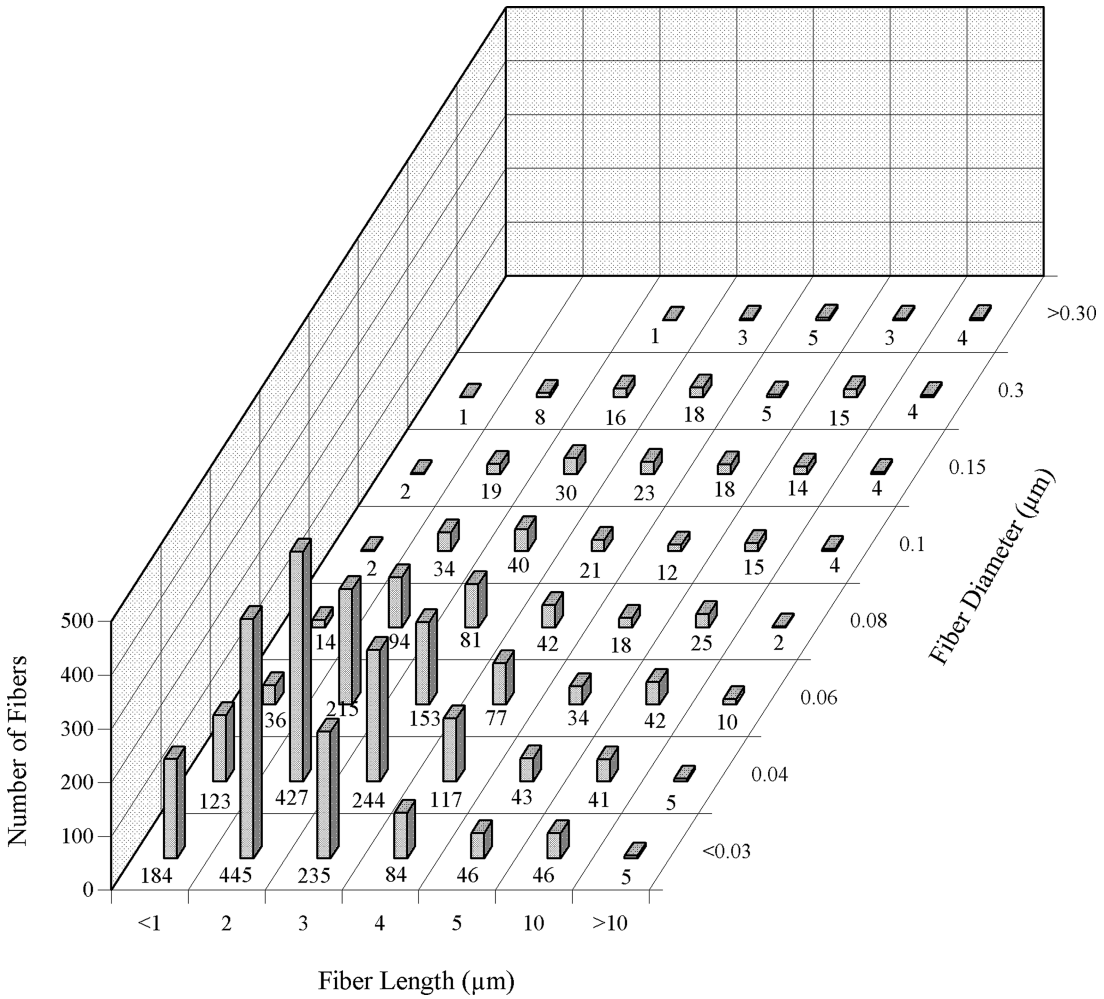

FIG. 9. Bivariate fiber size distribution for the 28-month biopsy samples.

Brody et al. (1981) in a chrysotile inhalation study using the rat noted that fibers passing through the conducting airways deposit at the bifurcations of alveolar ducts. Chrysotile fibers are taken up by alveolar macrophages and alveolar epithelial cells, with some fibers transported to the interstitium through the alveolar epithelial cells. Pinkerton et al. (1984) noted that significant clearance of fibers from the lung interstitium does not occur after cessation of exposure. Finkelstein and Dufresne (1999), studying clearance among chrysotile miners and millers, estimated the clearance rate of chrysotile fibers greater than 10 μm to be 8 years. Our fiber burden data supports a slow rate of clearance of fibers from the interstitium as we found a mean lung burden of 63×10^6 fibers per gram of dry lung 11.5 years after completion of the exposure regimen.

A number of investigators have noted that short fibers are cleared from the lung more easily than long fibers resulting in increasing fiber lengths over time along with decreasing fiber diameters (e.g., Abraham et al., 1988; Coin et al., 1992; Kauffer et al., 1987; Searl, 1997; Sébastien et al., 1986; Warheit et al., 1996). In contrast, more recent animal studies (Bernstein et al., 2003, 2004, 2005a, 2005b, 2006) have shown a more rapid clearance of long fibers due to breakage and dissolution. Hence, comparison of the monkey lung fiber burdens and size distributions at 10 months (biopsy samples) and at 11.5 years post exposure would be of interest. The count median fiber diameters at 10 months and 11.5 years were similar (between 0.03 and 0.04 μm). The geometric mean length (3.5 μm) of the fibers at 11.5 years post-exposure is much greater than that at 10 months post-exposure (2.0 μm), while the mean fiber burden (63×10^6 fibers/gram of dry lung) is significantly lower than that for the biopsy samples (327×10^6 fibers/gram of dry lung). At first glance, the difference in fiber burden between the biopsy and terminal sacrifice samples would seem to indicate extensive clearance over the years. Unfortunately, such comparisons are problematic since different sample preparation techniques were used at the two time periods.

Our data for adjacent terminal sacrifice samples prepared by low temperature ashing and digestion (Table 3) clearly indicate substantial transverse fiber breakage during the ashing procedure, leading to significantly greater fiber concentrations

TABLE 4
Comparison of fiber burden with lung sampling site

Sampling site	Number of samples	Chrysotile concentration [mean ± SD] (10^6/g dry lung)	Range of concentrations (10^6/g dry lung)
Upper Lobe, with pleura	9	72 ± 34	21–130
Middle Lobe, with pleura	9	63 ± 41	20–140
Lower Lobe, with pleura	10	68 ± 18	41–96
Upper Lobe, subpleural[b]	1	68	—
Middle Lobe, subpleural	2	33 ± 21	18–49
Lower Lobe, subpleural	2	35 ± 0.2	34–35
All Pleural Samples[a]	44	67 ± 31	20–140
All Subpleural Samples[a]	15	50 ± 23	18–99

[a]There were some samples for which the lung lobe location was not available.

[b]Subpleural samples were taken from lung parenchyma near the surface of the lung; however, they did not include pleura.

(approximately a 5-fold increase) and smaller geometric mean lengths (1.4 μm vs 3.6 μm). Previously, Ashcroft and Heppleston (1973) noted that drying the tissue leads to fracture of the longer coated and uncoated fibers and results in an exaggerated fiber count. Gylseth et al. (1981) also noted that shrinkage of tissue during dry ashing affects fiber length distribution and concentration due to breakage of long fibers. Pooley and Mitha (1986) also have noted increased fiber concentrations and smaller mean lengths with plasma ashing compared to digestion with KOH. Upon re-examination of some of the monkey biopsy lung preparations, partially fractured fibers (see Figure 10) were readily found. This finding implies some fibers may have been completely fractured. Given the reports noted above, our ashing/digestion data for the terminal sacrifice samples, and the existence of partially fractured fibers in the biopsy specimens, it is concluded that the low temperature ashing procedure used for the biopsy specimens resulted in an overestimation of fiber burden and an underestimation of fiber size. The ashing/digestion data at 11.5 years post-exposure might be used to provide an upper bound to the inflation of the biopsy fiber concentration data (approximately 5-fold). Because chrysotile fibers in the biopsy samples could be less fragile than those in the 11.5 years post-exposure samples, the actual fiber inflation at 10 months could be less that that seen at 11.5 years. Unfortunately, a definitive answer to this problem can not be provided since

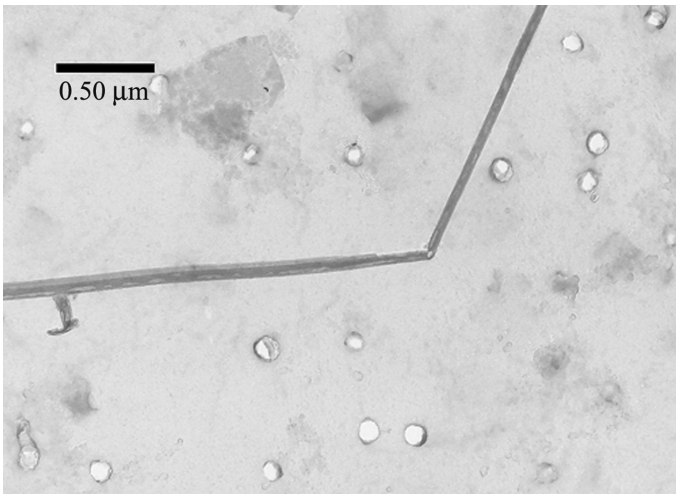

FIG. 10. Transmission electron micrograph showing a broken fiber found in a 28-month monkey biopsy sample which had been prepared by low temperature ashing.

no biopsy tissue remained for re-analysis using the digestion procedure.

The question remains as to whether the digestion method itself affects fiber count and size distribution. To definitively answer this question, one would need a fiber sample which had been aged *in vivo* for at least 11.5 years. Unfortunately, we do not have such a sample. At most, we can say that partially fractured fibers, such as those found in the biopsy samples, were not seen in the digested lung samples. However, a few of the asbestos bodies in the digested samples were very short; indicating some of the bodies may have been fractured, either while in the lung or during the digestion procedure. Ultimately, all that can be said about the digestion procedure is that very similar fiber size distributions were obtained for all of the 11.5 year post-exposure monkeys when the digestion procedure was used.

CONCLUSIONS

1. No treatment-related lesions were observed in 9 monkeys maintained for 11.5 years following an 18-month inhalation exposure to short (<5 μm long) chrysotile asbestos at a concentration level of 1.0 mg/m^3. Asbestos bodies were seen in the lungs of treated monkeys, primarily in the interstitium near bronchioles or small pulmonary blood vessels (which also may have been near to bronchioles just out of the plane of section).
2. Some chrysotile asbestos fibers were durable *in vivo* for 11.5 years or longer. The mean chrysotile lung burden (±standard deviation) for 59 samples was $63 \pm 30 \times 10^6$ fibers/g dry lung, with a geometric mean fiber length of 3.5 μm. The fibers remaining in the lung underwent some modification over the years as most had an irregular surface coating.
3. The method used to prepare lung tissue for fiber analysis can have a significant effect on lung fiber burden results and

fiber length distributions. We found that drying of the tissue followed by low temperature ashing leads to substantial transverse breakage of the fibers, resulting in elevated fiber burden levels and decreased fiber lengths.

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