

Lung Content Analysis of Cases Occupationally Exposed to Chrysotile Asbestos

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The lung contents of six workers who had been occupationally exposed to chrysotile asbestos were examined. Five were lung cancer cases from Quebec, Canada. The sixth, an American worker who had developed pleural mesothelioma, was particularly interesting, with the lung content strikingly distinct from the Canadian cases; chrysotile, the predominant fiber in his lung, was present at a concentration 300 times that of the average total fiber content in the Canadian cases. The fiber length distribution of the chrysotile recovered from the U.S. mesothelioma case was indistinguishable from that of chrysotile specimens known to produce mesotheliomas in rats. It was also found that the characteristics of the calcium-magnesium-iron silicate fibers present in all six cases were not readily comparable to tremolite asbestos specimens known to induce mesotheliomas in animals. — *Environ Health Perspect* 102(Suppl 5):245-250 (1994)

Key words: chrysotile, lung content, mesothelioma

Introduction

Human epidemiological studies and animal experiments to determine the biological effects of chrysotile present a complex pattern of results which are by no means consistent (1,2). Mortality due to mesothelioma in chrysotile-exposed individuals is significantly lower than that for those exposed to crocidolite, although the experimental animal studies do not clearly support that conclusion (3,4). Individuals with similar occupational exposure indices to chrysotile experience markedly different rates of developing lung cancer, depending on the industry in which the exposures occurred. Both Canadian chrysotile miners and millers and American friction product manufacturing workers have a much lower risk than asbestos textile workers with similar levels of exposure (5,6). Such results indicate that the information required to predict the disease outcome is not provided by the exposure indices used to compare different groups.

Even if exposure indices required to compare the groups were known precisely,

other methods would still be needed where the exposure history was not available. Examination of the lung content of individuals exposed in chrysotile-related industries has provided a different index of exposure applicable to individual cases (7-9).

In this report, the lung contents of five workers in Canada and one in the United States, who had been occupationally exposed to asbestos, were compared with those of some other industrially exposed groups reported elsewhere. In addition, several chrysotile specimens, the carcinogenicity of which had been determined separately in experimental animals (10,11), were compared by size distribution with the chrysotile obtained from the lung contents of a human case of mesothelioma.

Origin of Lung Tissue Specimens and Rationale for Selection

Lung specimens were obtained from six cases. Five comprised formalin-fixed bulk tissues taken at autopsy from male lung cancer cases, who had been exposed occupationally to chrysotile asbestos, although not exclusively, in Quebec, Canada. The sixth specimen, from the U.S. worker (Case 6), was taken during a surgical procedure. Two samples (called 6a and 6b) were fixed in paraffin wax. This U.S. case was a 59-year-old male with an occupational history of asbestos exposure, who had been diagnosed as having mesothelioma of the pleura. Tissue from a Canadian chrysotile miner presented as a grid preparation (provided by RF Dodson,

University Health Center at Tyler, TX) was used to evaluate whether the calcium-magnesium-iron silicate fibers, commonly referred to as tremolite, found in the five Canadian cases were comparable with silicate fibers reported by others. In this study, the [Ca,Mg,Fe] silicate fibers were not characterized in sufficient detail to justify identification as tremolite; this is to be done in further studies.

Origin of the Chrysotile Reference Specimens

Six mineral specimens were selected as standards for comparison with the fibers found in the human lung contents:

- i) Chrysotile No. 1026, a Paperbestos No. 5, a Canadian fiber that was air-fractionated with an Alpine fiberizer to Grade 7 (provided by J.-M. Lalandette, University of Sherbrooke, Canada). The carcinogenicity of a similar specimen was studied in an earlier animal experiment (10).
- ii) UICC chrysotile A (Zimbabwe) and B (Canada) (provided by the Pneumoconiosis Research Unit, Llandough Hospital, Penarth, Wales, UK).
- iii) Chrysotile, Calidria RG-144, chrysotile from New Idria, CA (commercially available from Union Carbide, New York, NY). The carcinogenicity of a similar specimen had been studied earlier in animal experiments (10).
- iv) Short Chrysotile IOM, a water-fractionated Grade 4-T-30 Canadian chrysotile, the pathogenicity of which was evaluated in an experimental animal study (11).

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Table 1. Distribution of fiber lengths of four chrysotile specimens used as reference standards and of the lung content of U.S. Case 6 of malignant mesothelioma of the pleura

Chrysotile specimen/final magnification	No. fibers sized	Percentage within each interval of fiber length			
		≤1.0 μm	1.1–5.0 μm	5.1–10.0 μm	>10 μm
ii a UICC A, Zimbabwe/×	3523	88.8%	10.6%	0.4%	0.2%
ii b UICC B, Canada/× 5000	2903	84.9%	13.5%	0.6%	0.4%
iii California/× 5000	2939	77.2%	20.5%	1.8%	0.5%
iv Short IOM/× 15,000	1523	72.8%	26.6%	0.6%	ND ^c
Case 6, United States/×7500	442 ^a	76.3 ± 13.3%	22.2 ± 11.8%	1.5 ± 1.4%	ND

ND, none detected. ^a Average for specimens 6a and 6b. ^b None detected.

Table 2. Comparison of the size distribution of two short chrysotile reference specimens and the residue of Canadian chrysotile sample 5.

Specimen/ final magnification	Average no. of fibers sized	Percentage within each interval of fiber length					
		<0.25 μm	0.25–0.49 μm	0.50–0.99 μm	1.00–1.99 μm	2.00–4.99 μm	>5.00 μm
i Chrysotile No. 1026 ^a α5000/10,000/25,000	5260 1020 ^b	61.1 ± 5.3 —	19.5 ± 5.0 —	9.8 ± 5.3 50.5	6.8 ± 1.6 35.1	2.6 ± 0.6 13.4	0.2 ± 0.1 1.0
iv Short chrysotile IOM 15 α000	1727 843 ^b	21.4 ± 3.5 —	27.4 ± 2.9 —	27.6 ± 1.4 56.6	16.8 ± 3.4 34.4	6.3 ± 1.6 12.9	0.5 ± 0.1 1.0
v Residue of Sample 5 × 7500	327	0.0	0.0	15.6	39.5	33.9	11.0
Diameters (μm) ^c	327	0.0	0.0	0.17 ± 0.06	0.17 ± 0.12	0.34 ± 0.23	0.43 ± 29

^a Average of three preparations by two analysts; six fibers showing diameters >0.25 μm (0.11%). ^b Calculated for fiber length distribution, not counting <0.5 μm fibers. ^c Mean diameter with standard deviation for each fiber length interval.

Table 3. Weight of tissue and number of fibers per gram of dry lung tissue^a obtained from six occupationally exposed cases.

Specimen	Type of lung tissue	Wet weight of tissue digested, mg	No. of fields counted	Counting method	No of fibers/ grid opening ^b	No of. fibers (in millions) g of dry lung
Case 1 Canadian	Parenchyma	2700	5	On screen	21.5 ± 5.2	23 ± 6
Case 2 Canadian	Parenchyma with pleura ^b	1640	5	On screen	4.2 ± 2.2	7 ± 4
Case 3 Canadian	Parenchyma with pleura	2060	5	On screen	27.0 ± 4.5	48 ± 8
Case 4 Canadian	Parenchyma with pleura	2030	5	On screen	30.2 ± 8.1	49 ± 13
Case 5 Canadian	Parenchyma with pleura	1990	5	On screen	8.2 ± 2.3	41 ± 11
Case 6 ^a US	Parenchyma	12.3 12.3	3 1	On screen Photomontage	121 ± 17.2 240	13 900 ± 7900 27600
^b US	Parenchyma	15.0 15.0	3 1	On screen Photomontage	60.7 ± 11.0 241	6620 ± 1100 27600

^a On screen counting and photomontages were sized at ×20,000 and ×7500 final magnification, respectively. Only fibers ≥0.5 μm in length were counted. ^b Of visceral pleura.

- v) The residue from Canadian Chrysotile Sample No. 5, from which the chrysotile had been removed by chemical digestion (12).

- vi) Tremolite asbestos from Jamestown, CA.

Fiber Length Distribution of the Chrysotile Reference Specimens

Only fiber length was determined, since the diameter has been found to be consis-

tently uniform at about 0.06 μm (8). The electron microscope grids used to determine the length distributions of the five reference chrysotile specimens were prepared by two methods. In the first, the wipe-out method, the fibers were dispersed between two glass slides into a thin film of nitrocellulose and amylacetate. In the second procedure specimens were prepared by dispersion in water with ultrasound, and 10 μl of the suspension were placed on a

carbon-coated formvar grid. In all cases, 200-mesh nickel locator grids were used. Fiber length distributions of specimens prepared by either method yielded similar results.

Electron microscopy grids of reference standards ii and iii were prepared by the wipe-out method. Fiber length distributions were determined by direct measurement on photomontages of 110 μm × 110 μm grid openings, magnified ×5000. Reference stan-

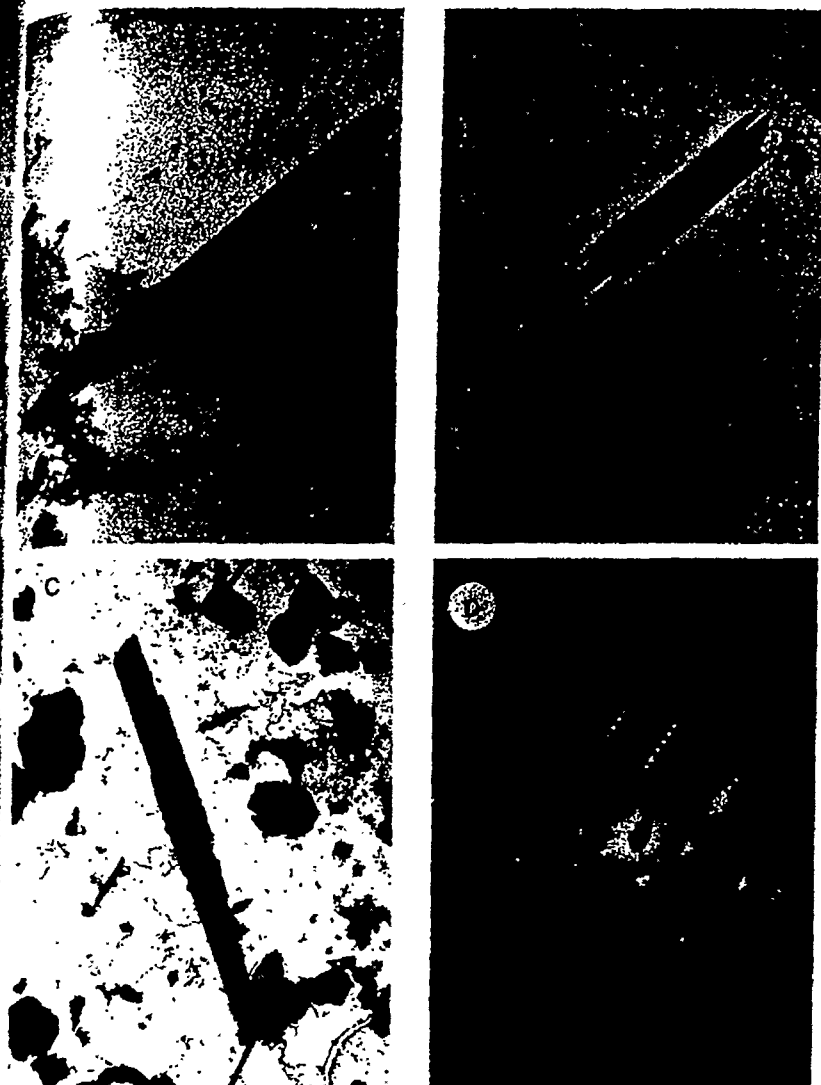


Figure 1. Transmission electron photomicrographs of representative fibers found in the lung tissues of the Canadian cases. (A) A magnesium silicate fiber found in Case 2, approximately $7.5 \mu\text{m} \times 0.83 \mu\text{m}$, morphologically composed of individual "fibrils". Fibrils may represent mineral intergrowths. (B) A typical $[\text{Ca},\text{Mg},\text{Fe}]$ silicate fiber found in Case 4, approximately $2.4 \mu\text{m} \times$ approximately $0.2 \mu\text{m}$. The fiber is composed of individual "fibrils" $3.03\text{--}0.04 \mu\text{m}$ in diameter. (C) A typical $[\text{Ca},\text{Mg},\text{Fe}]$ silicate fiber found in a Canadian worker (grid provided by RF Dodson). The fiber is approximately $25 \mu\text{m} \times 2.5 \mu\text{m}$ and composed of "fibrils". (D) The selected area electron diffraction pattern of the fiber shown in (C). It contains Kikuchi lines indicating a thick fragment, characteristic of a cleavage fragment, not an amphibole fiber asbestos (13,14).

standard iv was prepared for electron microscopy by the water dispersion method. The length distribution was determined at magnification $\times 15,000$. The four reference specimens were found to have very similar fiber length distributions (Table 1).

Of the total 10,888 fibers, whose lengths were determined, $98.7 \pm 0.8\%$ were $< 5 \mu\text{m}$. One of these, reference specimen iii, often referred to as a naturally occurring short fiber, had a fiber length

distribution indistinguishable from the other reference specimens.

Approximately 1% of the fibers in the four specimens were $> 5 \mu\text{m}$ in length. Specimens i and iv were selected to determine the fiber length distribution of two short reference samples of known pathogenicity, and length intervals were selected to describe this fiber population. An average fiber length distribution of reference specimen i was determined from direct sizing on photographic plates of grids prepared by the

wipe-out technique. Each size determination was performed by two analysts and the data sets were averaged to reduce interobserver error and variance. Enlargement of the photographic plates for the analysis of the fibers gave final magnifications of $\times 5,000$, $\times 10,000$, and $\times 25,000$ (Table 2).

Low magnification photographs were best for counting long fibers; higher magnification was required for the resolution of fibrils with lengths $< 0.5 \mu\text{m}$. The shortest chrysotile fibers detected were approximately $0.1 \mu\text{m}$ in length, with a unit fibril diameter of approximately $0.02 \mu\text{m}$. Reference specimen iv, was similarly analyzed and counted at a final magnification of $\times 15,000$. Specimen i contained a larger proportion of fibers $< 0.25 \mu\text{m}$ than specimen iv, but if the fibers with lengths $< 0.5 \mu\text{m}$ were excluded, fiber length of the two specimens were indistinguishable (Table 2).

The length and diameter of the fibers in reference specimen v, predominantly $[\text{Ca},\text{Mg},\text{Fe}]$ silicates, were determined. None of the 327 fibers was $< 0.5 \mu\text{m}$ in length. The number of fibers $\geq 5 \mu\text{m}$ was approximately 13 times higher in this specimen than in the other five. Fiber diameters were determined for each length interval, with standard deviations. Diameters increased with increasing fiber length and were considerably larger than the diameters of $0.06 \mu\text{m}$ of chrysotile (Table 2).

Preparation of the Lung Tissue Specimens

Xylene was used to remove paraffin from the fixed tissues, which were then dried and weighed. After weighing, each tissue was added to 30 ml of 5% potassium hydroxide and digested for several hours at approximately 80°C . The insoluble particles were then pelleted by centrifugation at $10,000g$ and the pellet was redispersed in a known volume of distilled water with a 10-sec burst of ultrasound. A 10- μl drop of the dispersed suspension was placed on a carbon-coated formvar 200-mesh nickel locator grid, two or three of which were prepared for each specimen. The type and wet weight of lung tissue are reported in Table 3.

Characterization of Fiber Content of the Lung Tissues

Each tissue preparation was examined by transmission electron microscope to determine the number of fibers per gram of dry lung tissue. Two criteria were used to define a fiber: the aspect ratio had to be

Table 4. Fiber types present in lung tissue obtained from six cases of exposed workers examined by analytic transmission electron microscopy.

	Case 1 Canadian	Case 2 Canadian	Case 3 Canadian	Case 4 Canadian	Case 5 Canadian	Case 6a U.S.	Case 6b U.S.	Jamestown, California (Ref. spec. vi)	Residue chrysotile sample no. 5 (Ref. spec. vi)
No [Mg,Ca,Fe] silicate fibers/total nonchrysotile fibers	57/63	5/20	56/76	14/49	31/33	~2/240	~2/241	ND ^a	ND
Mg/Si	0.34 ± 0.03	0.42 ± 0.04	0.33 ± 0.01	0.32 ± 0.01	0.32 ± 0.05	0.16 ± 0.08	0.34 ± 0.03	0.32 ± 0.06	
Ca/Si	0.40 ± 0.06	0.25 ± 0.13	0.41 ± 0.16	0.32 ± 0.04	0.32 ± 0.05	0.33 ± 0.04	0.38 ± 0.03	0.32 ± 0.05	
Fe/Si	0.10 ± 0.04	0.05 ± 0.03	0.19 ± 0.15	0.13 ± 0.03	0.11 ± 0.13	0.43 ± 0.13	0.03 ± 0.02	0.14 ± 0.06	
Commercial asbestos	Chrysotile	Chrysotile	Chrysotile	Chrysotile	Chrysotile	Chrysotile	Chrysotile	ND	Trace of chrysotile
Types present talc	Anthophyllite- Crocidolite	Amosite Anthophyllite	Amosite talc	Crocidolite					

^a Not detected.**Table 5.** Length distribution of chrysotile fibers isolated from two lung parenchymal specimens from the U.S. pleural mesothelioma case (6a,b).

Tissue specimen	No. fibers sized	Percentage within each interval of fiber length					
		<0.50 µm	0.50–0.99 µm	1.00–1.99 µm	2.00–4.99 µm	5.00–7.99 µm	≥ 8.00 µm
Case 6a ^a	311	22.8	44.1	22.5	8.0	1.9	0.6
	240 ^b	—	57.1	29.2	10.4	2.5	0.8
Case 6b ^a	572	57.9	27.8	10.1	3.7	0.5	0.0
	241 ^b	—	66.0	24.1	8.7	1.2	0.0

^a Fiber lengths were determined at a magnification × 7500; all fibers and fibers ≥ 0.50 µm counted. ^b Length distribution not counting <0.5 µm fibers.

greater than 3:1, and the fiber length had to be ≥ 0.5 µm. The weight of wet tissue used prior to digestion ranged from 12.3 to 2700 mg.

The fiber content of the tissue specimens was determined by counting the number of fibers in a 110 µm × 110 µm grid opening on the fluorescent screen at a

magnification × 20,000. Three to five fields were randomly selected for counting. The average number of fibers per grid opening varied among the six cases from 4.2 ± 2.2 to 121 ± 17 with a precision of 26 ± 12% (Table 3).

Assuming the fibers are randomly located on the grid, and the number of

fibers in a 110 µm × 110 µm grid opening are known, as well as the total surface area of the grid, then the total number of fibers present on the grid can be calculated. Since this total is equivalent to the number of fibers in the 10 µl of suspension, and since the mass of the digested tissue and the volume of water in which the insoluble particles were resuspended are known, then the number of fibers per gram of wet weight of tissue can be calculated. The dry tissue weight is simply assumed to be 10 times less than the wet weight. By the above procedures, it was found that the fiber concentration of the six tissue specimens varied from 7 ± 4 × 10⁶ to 13,900 ± 2900 × 10⁶ fibers of dry lung (Table 3).

In the five Canadian cases amosite, anthophyllite-talc, chrysotile, crocidolite, and [Ca,Mg,Fe] silicate fibers were identified by energy dispersive X-ray spectroscopy (EDS). Chrysotile was present in all five but was predominant in none. The nonchrysotile fibers were analyzed sequentially by EDS. In Cases 1 and 5, where no commercial amphibole asbestos was present, over 90% of the fibers were [Ca,Mg,Fe] silicates (Figure 1).

The anthophyllite-talc fibers in Cases 1 and 3 were assumed to have originated from a source other than exposure to anthophyllite asbestos. In the three cases

Table 6. Mesotheliomas induced in rats by the intraperitoneal injection of reference specimens of chrysotile asbestos^a.

	Dose, mg	Mesothelioma- bearing animals		Average latency, weeks
		No.	%	
iiib, Canadian, UICC B	25	32	80.0	92.2
iiia, Zimbabwe, UICC A	25	33	82.5	89.7
iii, New Idria, California	25	29	72.5	85.3
i, Short chrysotile, Grade 7 ^b	25	29	72.5	76.4
iiib, Canada UICC B	30	30	75.0	76.1
i, Short chrysotile, Grade 7 ^c	10	11	27.5	57.0
	5	4	10.0	59.7
	1	0	—	—
iiib, Canadian, UICC B	10	14	35.0	61.6
	5	11	27.5	60.3
	1	0	—	—
iv, Canadian chrysotile, Grade 4-T-30 ^d	25	24	95.8	51.6
	2.5	24	91.7	73.0
	0.25	24	66.7	105.1
iv, Short chrysotile IOM	25	24	91.7	72.0
	2.5	24	33.3	96.4
	0.25	24	0	—

^a Data from Maltzoni and Minardi (10) and Davis and Jones (11). ^b Water fractionated from Paperbestos 5 similar to specimen No. 1026. ^c Results available after 76 weeks' study. ^d Start material for preparation of Short Chrysotile IOM.

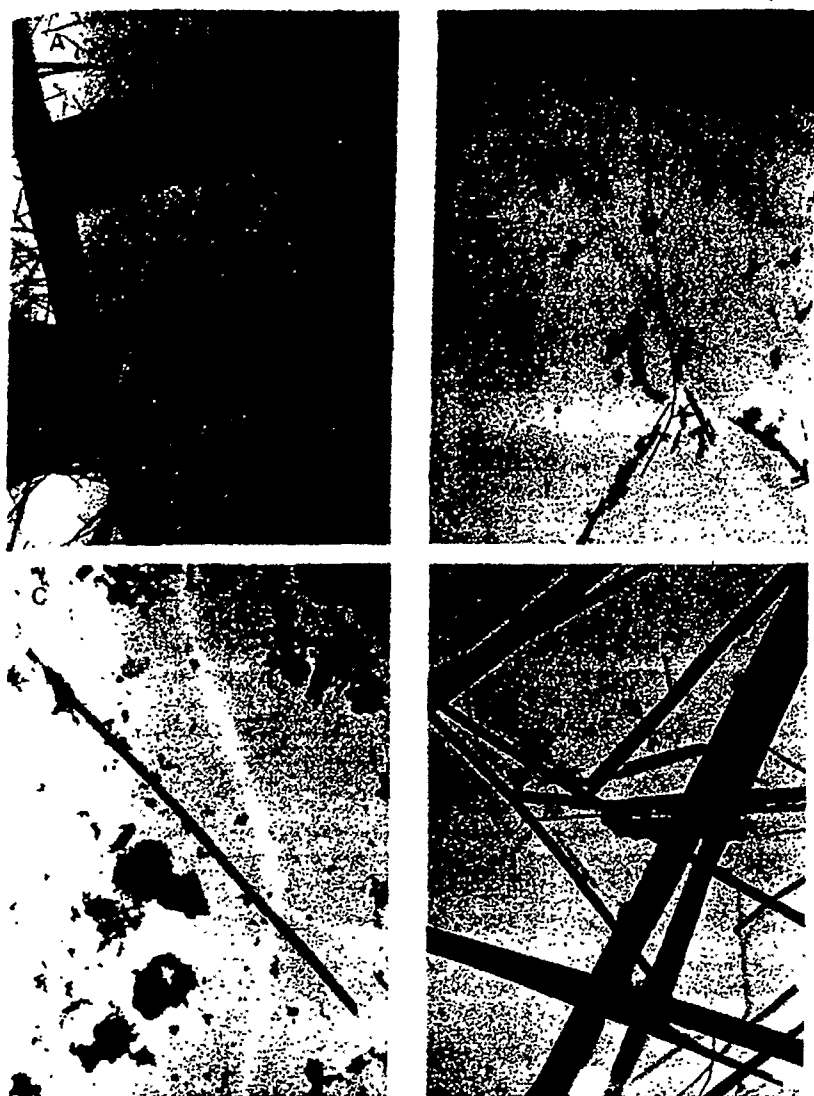


Figure 2. (A) A typical tremolite asbestos from Jamestown, CA. The linear grid opening is approximately 110 μm . Compare this with (B), a residue from specimen 5, predominantly [Ca,Mg,Fe] silicates. The long fiber is approximately 90 μm in length. The fibers in specimen no. 5 are of shorter length and greater diameter than those which characterize the tremolite asbestos specimen. (C) Fiber is a high aspect ratio [Ca,Mg,Fe] silicate fiber found in Case 4 is compared with tremolite asbestos from Jamestown, CA (D). Arrow (D) points to fiber of 2 μm diameter.

where amosite or crocidolite were found, only approximately 42% of the nonchrysotile fibers had [Ca,Mg,Fe] silicate compositions. The ratio of the EDS counts for calcium, magnesium and iron to silicon are given in Table 4.

For comparison, the analyses of the fibers present in a tremolite asbestos, reference specimen vi and in reference specimen are included. The analysis of the two parenchymal lung tissue specimens in the U.S. pleural mesothelioma, Case 6, showed that the predominant fiber type was

chrysotile, and the fiber concentration averaged approximately $10,000 \times 10^6$ fibers/g of dry lung. This is approximately 300 times greater than the average total fiber concentration in the Canadian cases.

To determine the fiber length distribution and to quantify the fiber number per field more accurately in each of the two specimens, the U.S. 6a and 6b, a series of overlapping photographs of a complete grid opening was taken and assembled into a photomontage. The length of each fiber present and the total number of fibers in

each of the two grid openings were determined from the photomontage (Table 5).

The total number of fibers sized varied by almost 2-fold between the two tissue specimens. This difference was due principally to the large number of fibers $<0.5 \mu\text{m}$ in length in Case 6b. If only fibers of lengths $\geq 0.5 \mu\text{m}$ are included, the numbers of fibers per grid opening are 240 for Case 6a and 241 for Case 6b, the two fiber length distributions are also quite similar (Table 5).

No amosite or crocidolite fibers were found among 350 fibers scanned in Case 6a at a magnification $\times 20,000$; nor among 180 fibers scanned in 6b; neither were they found in low magnification scans at $\times 5000$ for 25 fields in each preparation. The analytical sensitivity indicates that the concentration of amosite or crocidolite, if present, would be below approximately 4,600,000 fibers/g of dry lung. [Ca,Mg,Fe] silicate fibers were identified at an average concentration of 2 ± 0.71 in five fields examined. This corresponds to $223 \pm 90 \times 10^6$ fibers/g of dry lung. The average length, diameter, and aspect ratio of 17 [Ca,Mg,Fe] silicate fibers sized from photographic plates was $6.7 \pm 4.3 \mu\text{m}$, $0.36 \pm 0.2 \mu\text{m}$, and 23.8 ± 20 respectively. Fiber dimensions ranged from $15.0 \mu\text{m} \times 0.2 \mu\text{m}$ to $2 \mu\text{m} \times 0.17 \mu\text{m}$. No fibers $<2 \mu\text{m}$ were identified. Of the 11 nonfibrous particles analyzed, 10 had a morphology and elemental composition consistent with that of quartz.

Discussions and Conclusions

The lung content of Case 6, who developed a pleural mesothelioma, is of particular interest because of the extremely high content of chrysotile found. Approximately $27,600 \times 10^6$ fibers $>0.5 \mu\text{m}$ in length/g of dry lung tissue were found in each of the two parenchymal tissue specimens and more than 99% of the fibers identified were chrysotile. Using a protocol with an analytical sensitivity of approximately 4,600,000 fibers/g of dry lung, no commercial amphibole asbestos minerals were detected in either specimen of Case 6. About 0.8% of the fibers present were calcium-magnesium-iron silicates.

The fiber length distribution in each of the two specimens in Case 6 was determined. Although long chrysotile fibers were identified—the longest fiber found being 33 μm —on average, only 1.5% of the 883 fibers sized were $\geq 5 \mu\text{m}$ in length (Table 5). This fiber length distribution is comparable with that obtained from workers in a Swedish cement plant where 1.42%

of the 7849 fibers sized were $\geq 6 \mu\text{m}$ (8) but considerably shorter than the fiber length distribution obtained in an American textile plant where 7.7% of 1459 fibers sized were $\geq 6 \mu\text{m}$ (8). The average concentration in Case 6, counting fibers of all lengths, was approximately $50,600 \times 10^6$ fibers/g of dry lung. This concentration is approximately 670 times higher than the mean concentration determined for asbestos cement workers and 880 times higher than that for asbestos textile workers.

The length distribution of the fibers found in Case 6 was compared to the five reference chrysotile mineral specimens, the carcinogenicity of which had been determined earlier in experimental animal studies (10,11). On average, $1.3 \pm 0.9\%$ of the fibers were $\geq 5 \mu\text{m}$ in length in the two UICC reference specimens (iia and iib) and the commercial specimen (iii) (Table 1). For the intervals chosen, the fiber length distribution of these three reference specimens and that of Case 6 were similar. A 25 mg dose of each of the three reference specimens, when administered to rats by

intraperitoneal injection, produced a similar incidence of mesothelioma (Table 6).

The length distribution of the fibers found in Case 6 was also compared to two reference specimens. Smaller intervals were selected to better define those fiber lengths predominantly $< 5 \mu\text{m}$ (Table 2). If the $< 0.5 \mu\text{m}$ fibers are ignored, the fiber length distributions in reference specimens i and iv and Case 6 are indistinguishable. By intraperitoneal injection at high dose (25 mg), both i and iv produced high incidences of mesotheliomas in rats (72.5% and 91.7% respectively) (10,11). As doses were reduced, the two short chrysotile specimens (i and iv) produced fewer mesotheliomas than the longer reference specimens. Although this decrease in the production of mesotheliomas in the rats often is attributed to reduction in fiber length, it is important to recognize that long chrysotile fibers are generally bundles of individual fibers. Once these bundles are dispersed, the fiber length distribution of the six chrysotile specimens was very similar.

The fiber content of Case 6 differed markedly from the five Canadian cases.

The mean total fiber concentration of the Canadian cases was 300 times less than in Case 6, and the [Ca,Mg,Fe] silicate fiber concentration was 6 times less. In none of the Canadian cases was chrysotile the predominant fiber type, whereas in Case 6, approximately 99% of the fibers detected were chrysotile and approximately 0.8% were [Ca,Mg,Fe] silicates. Conversely, in the Canadian cases, on a fiber number and mass basis, [Ca,Mg,Fe] silicate fibers exceeded the chrysotile present. These [Ca,Mg,Fe] silicate fibers, commonly referred to as tremolite, frequently have been found in the lungs of Quebec miners and millers, particularly in Thetford (7,9). When reviewing the results of studies where only fibers $\geq 5 \mu\text{m}$ in length are counted, however, a bias towards tremolite may be introduced since the fiber length distributions of chrysotile and tremolite are different and thus contribute to the outcome: 11% of the [Ca,Mg,Fe] silicates in reference specimen v were found $\geq 5 \mu\text{m}$, while the mean of the three reference chrysotile specimens was 1.3%.

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