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 animals may not similarly 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. Lanercoste, University of Sherbrooke, Canada). The carcinogenicity of a similar specimen was studied in an earlier animal experiment (10).

ii) UIICC 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).
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 | ≤1.0 µm | 1.1–5.0 µm | 5.1–10.0 µm | >10 µm |
|----------------------------------------|------------------|---------|------------|------------|------|
| iia UICC A, Zimbabwe/x                 | 3523             | 88.8%   | 10.6%      | 0.4%       | 0.2% |
| iib UICC B, Canada/x 5000              | 2903             | 84.9%   | 13.5%      | 0.6%       | 0.4% |
| iii California/x 5000                  | 2393             | 77.2%   | 20.5%      | 1.8%       | 0.5% |
| iv Short IOM/x 15,000                  | 1523             | 72.8%   | 28.6%      | 0.6%       | ND*  |
| Case 6, United States/x 7500           | 442*             | 76.3 ± 13.3% | 22.2 ± 11.8% | 1.5 ± 1.4% | ND*  |

ND*, none detected. *Average for specimens 6a and 6b. *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 | <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*      | 5260                        | 61.1 ± 5.3 | 19.5 ± 5.0    | 9.8 ± 5.3    | 6.8 ± 1.6    | 2.6 ± 0.6    | 0.2 ± 0.1 |
| αx5000/10,000/25,000        | 1020*                      | —        | —            | 50.5         | 35.1         | 13.4         | 1.0      |
| iv Short chrysotile IOM     | 1727                        | 21.4 ± 3.5 | 27.4 ± 2.9    | 27.6 ± 1.4   | 16.8 ± 3.4   | 6.3 ± 1.6    | 0.5 ± 0.1  |
| 15 x5000                    | 943*                       | —        | —            | 56.6         | 34.4         | 12.9         | 1.0      |
| v Residue of Sample 5       | 327                         | 0.0      | 0.0          | 15.6         | 39.5         | 33.9         | 11.0     |
| Diameters (µm)*             | 327                         | 0.0      | 0.0          | 0.1 ± 0.06   | 0.17 ± 0.12  | 0.34 ± 0.23  | 0.43 ± 0.29 |

*Average of three preparations by two analysts; six fibers showing diameters >0.25 µm (0.11%). *Calculated for fiber length distribution, not counting <0.5 µm fibers. *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 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* | 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*          | 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   | US                  | Parenchyma                       | 12.3                 | 3               | On screen                 | 121 ± 17.2                             | 13 900 ± 2900                          |
|          |                     |                                  | 12.3                 | 1               | Photomontage              | 240                                    | 27800                                 |
|          | US                  | Parenchyma                       | 15.0                 | 3               | On screen                 | 60.7 ± 11.0                            | 6620 ± 1100                            |
|          |                     |                                  | 15.0                 | 1               | Photomontage              | 241                                    | 27800                                 |

*On screen counting and photomontages were sized at ×20,000 and ×7500 final magnification, respectively. Only fibers ≥0.5 µm in length were counted. *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 consistently 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-
The length and diameter of the fibers in reference specimen v, predominantly [Ca,Mg,Fe] silicates, were determined. None of the 327 fibers was <0.5 μm in length. The number of fibers ≥5 μm 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 μm 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

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Note: The image contains micrographs of fibers, which are not transcribed into text due to the nature of the task.
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 × 10^4 to 13,900 ± 2900 × 10^4 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 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.

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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的朋友and in reference specimen} v 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 \( \mu m \) in length in Case 6b. If only fibers of lengths \( \geq 0.5 \mu m \) 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 \( \pm 0.71 \) 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 \( \pm 4.3 \mu m \), 0.36 \( \pm 0.2 \mu m \), and 23.8 \( \pm 20 \) respectively. Fiber dimensions ranged from 15.0 \( \mu m \) \( \pm 0.2 \mu m \) to 2 \( \mu m \) \( \times 0.17 \mu m \). No fibers <2 \( \mu m \) 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 \( \mu m \) 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 \( \mu m \)—on average, only 1.5% of the 883 fibers sized were \( \geq 5 \mu m \) in length (Table 5). This fiber length distribution is comparable with that obtained from workers in a Swedish cement plant where 1.42%
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 µm (Table 2). If the <0.5 µm 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 ≥5 µm 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 ≥5 µm, while the mean of the three reference chrysotile specimens was 1.3%.

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