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Asbestos-Related Disease Associated With Exposure to Asbestiform Tremolite

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Tremolite is nearly ubiquitous and represents the most common amphibole fiber in the lungs of urbanites. Tremolite asbestos is not mined or used commercially but is a frequent contaminant of chrysotile asbestos, vermiculite, and talc. Therefore, individuals exposed to these materials or to end-products containing these materials may be exposed to tremolite. We have had the opportunity to do asbestos body counts and mineral fiber analysis on pulmonary tissue from five mesothelioma cases and two asbestosis cases with pulmonary tremolite burdens greater than background levels. There were no uncoated amosite or crocidolite fibers detected in any of these cases. Three patients were occupationally exposed to chrysotile asbestos; two patients had environmental exposures (one to vermiculite and one to chrysotile and talc) and one was a household contact of a shipyard worker. The tremolite burdens for the asbestosis cases were one to two orders of magnitude greater than those for the mesothelioma cases. Our study confirms the relationship between tremolite exposure and the development of asbestos-associated diseases. Furthermore, the finding of relatively modest elevations of tremolite content in some of our mesothelioma cases suggests that, at least for some susceptible individuals, moderate exposures to tremolite-contaminated dust can produce malignant pleural mesothelioma. © 1994 Wiley-Liss, Inc.

Key words: asbestosis, chrysotile, mesothelioma, tremolite, vermiculite

INTRODUCTION

Tremolite is a hydrated magnesium silicate ($Ca_2Mg_5Si_8O_{22}(OH)_2$) that belongs to the amphibole group of minerals. Collectively, actinolite, anthophyllite, and tremolite are often referred to as noncommercial amphiboles. Although the noncommercial amphiboles are, geologically, the most common amphiboles, they are of little industrial importance and not mined commercially [Pooley, 1987]. Thus, tremolite and other noncommercial amphibole exposure levels are not routinely monitored, and, until recently, were not usually considered in the epidemiology or etiology of asbestos-related disease [Weill et al., 1990]. However, recent attention has focused on the health effects of exposure to tremolite asbestos.

Although tremolite is not mined commercially, it is a common contaminant of

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other mineral deposits, such as chrysotile asbestos (which accounts for 90–95% of commercial asbestos in the United States), vermiculite, and talc [McDonald et al., 1989]. Workers may be exposed to tremolite asbestos from mining or manufacturing processes involving these minerals. Additionally, some end-products contain these minerals (such as cosmetic talc or spackling compound, which contains chrysotile) and may present a potential source of tremolite exposure. At Canadian chrysotile mines, tremolite is present in only trace amounts in the deposits [Gibbs and Lachance, 1972]. Nevertheless, several studies suggested that most cases of malignant mesothelioma observed in these miners were probably attributable to tremolite, rather than to chrysotile asbestos. This conclusion is based on the observation that the majority of uncoated fibers detected in the lungs of the miners with mesothelioma was tremolite, and pulmonary tremolite contents for the chrysotile miners exceeded control levels [Churg, 1988; McDonald et al., 1982, 1989]. One study concluded, because chrysotile represents 90–95% of all asbestos used, as many as 20% of all malignant mesotheliomas in North America may be due to tremolite [McDonald et al., 1989]. Several studies regarding vermiculite miners demonstrated a dose-related increase in asbestos-associated diseases and suggested, according to the American Thoracic Society, that tremolite exposure "represents health consequences similar to other forms of asbestos exposure, including lung cancer and mesothelioma" [Amandus and Wheeler, 1987; McDonald et al., 1986; Weill et al., 1990]. Also, in Turkey and the Metsovo region of Greece, where naturally occurring asbestiform tremolite is used for whitewashing and for stucco, studies have demonstrated an increased mesothelioma risk for residents with no history of occupational exposure and no identifiable history of exposure to other fiber types [Baris et al., 1988; Langer et al., 1987].

Collectively, these studies suggest that tremolite asbestos exposure represents a potential health risk not only to miners and manufacturers of tremolite-contaminated mineral products but also to those exposed to tremolite-contaminated end-products. This report presents seven cases of asbestos-related disease, for which scanning electron microscopic (SEM) pulmonary mineral fiber analyses support an etiologic role for tremolite.

MATERIALS AND METHODS

Case Selection

The seven cases selected for this study were obtained from one of the author's (V.L.R.) consultation files. The criteria for selection included 1) a diagnosis of asbestos-related disease, 2) no uncoated amosite or crocidolite fibers detected by SEM, and 3) pulmonary uncoated tremolite fiber content greater than control levels. The upper limit of the control level was 2,540 uncoated fibers (5 μm or greater in length) per gram of wet lung. This value was based on SEM mineral fiber analyses for 19 control cases, as defined in earlier reports (i.e., macroscopically normal lungs at autopsy, no evidence of asbestos-related disease, and no documented history of asbestos exposure) [Roggli et al., 1986, 1992a,b].

Five of the cases represented in this study were malignant pleural mesotheliomas. The remaining two cases had diagnoses of asbestosis; one of these also had adenocarcinoma of the lung. The diagnosis of malignant mesothelioma was confirmed by one of the authors (V.L.R.) using our previously published criteria. These criteria include gross distribution of the tumor, histologic appearance, and, when

TABLE I. Demographic, Pathologic, and Occupational Information for Seven Patients With Asbestos-Related Diseases*

Case[a]	Age/sex	Diagnosis	Occupation	Exposure duration	Smoking history
1	74/M	Asbestosis	Manufactured asbestos blankets/gaskets	7 yr	Smoker (PY unknown)
2	44/M	Asbestosis/Lung adenocarcinoma	Lived near vermiculite plant during childhood	20 yr	Ex-smoker, (1-2 PY), (quit 20 years)
3	56/M	BPL	Painter/spackler	38 yr	Smoker (70 PY)
4	62/M	EPL	Engineer at power plant; insulator	N/A	Smoker (6 PY)
5	44/F	EPL	Assembler at dry cleaner	N/A	N/A
6	57/F	EPL	Housewife of shipyard worker	1–2 yr	Smoker (40 PY)
7	58/F	DPL	Teacher's aide in building with tremolite in tiles	18 yr	Nonsmoker

*BPL = biphasic pleural mesothelioma; DPL = desmoplastic pleural mesothelioma; EPL = epithelial pleural mesothelioma; PY = pack-years; N/A = not available.

[a]Data for the 19 control cases are summarized in the text of this report and are detailed in earlier as yet unpublished observations.

indicated, the findings of histochemical, immunohistochemical, and/or ultrastructural studies [Roggli et al., 1992b]. In all cases, the diagnosis of mesothelioma was made independently of asbestos exposure history or tissue mineral fiber content. The diagnosis of asbestosis was confirmed by one of the authors (V.L.R.) using the histologic criteria set forth by the Pneumoconiosis Committee of the College of American Pathologists and the National Institute for Occupational Safety and Health, which defines the minimum criteria permitting the diagnosis of asbestosis as "demonstration of discrete foci of fibrosis in the wall of respiratory bronchioles associated with accumulations of asbestos bodies" [Craighead et al., 1982].

Tissue Digestion Technique and AB (by LM) Analysis

Formalin-fixed lung parenchyma was prepared for analysis by digestion in a 5.25% sodium hypochlorite solution (commercial bleach), as detailed in previous reports [Roggli, 1992b; Roggli et al., 1986]. Asbestos body (AB) counts for two mesothelioma cases (cases 6 and 7; see Table I) were quantified using the technique of Smith and Naylor for approximately 5 g samples of lung tissue [Roggli, 1992b; Smith and Naylor, 1972]. For three cases (cases 1,2, and 5; see Table I), limited lung tissue (<1.0 g) was available. For these cases, our laboratory developed a hypochlorite digestion procedure [modified from Williams et al., 1982] which is suitable for smaller sample sizes (0.1–0.4 g wet weight). Our laboratory has shown that, for small sample sizes, AB counts obtained by this technique are, on average, within 10% of values determined by the Smith and Naylor procedure [Roggli et al., 1986].

Digested lung tissue was collected on 0.4 μm pore size polycarbonate filters. One filter was mounted on a glass slide, and the entire filter was examined by light microscopy (LM, at a magnification of ×200) for AB. Ferroprotein-coated fibers with broad yellow or black cores were considered to be nonasbestos ferruginous bodies (pseudoasbestos bodies) and were not counted in these analyses [Roggli, 1992a]. The detection limit of this analysis was one AB per filter. The equivalent detection limit in AB/g wet lung depends on the weight of the sample (e.g., 0.2 AB/g for a 5.0 g sample and 3.0 AB/g for a 0.3 g sample of lung tissue).

In two cases (cases 3 and 4; see Table I), formalin-fixed lung tissue was not available. For these cases, the tissue was obtained from paraffin blocks by dissolving the paraffin in xylene and rehydrating to 95% ethanol, as described in earlier reports [Roggli et al., 1982, 1986]. Tissue digestion and microscopic analyses were performed as described above. The results were multiplied by 0.7 to correct for the wet weight difference between formalin-fixed lung and paraffin-embedded lung [Roggli, 1992b; Roggli et al., 1986].

Mineral Fiber Analysis (by SEM)

All samples for SEM analysis were prepared by digesting lung tissue (0.25–0.35 g, wet weight) in hypochlorite using the method developed by our laboratory for small samples, as discussed above [Roggli, 1992b; Roggli et al., 1986]. The residue was then collected on 0.4 μm pore size polycarbonate filters. The filter was mounted on a carbon disc with colloidal graphite and then sputter coated with gold. A JEOL JSM 35C scanning electron microscope operated at 20 kV accelerating voltage, a screen magnification of × 1,000 and a scan rate of 5 sec per frame was used to count total uncoated fibers (UF) and AB (ferroprotein-coated fibers) [Roggli, 1991]. Fibers were defined as particles with an aspect ratio (length:diameter) of at least 3:1 and having approximately parallel sides. Only particles that met both of these criteria and had a length of 5 μm or greater were counted. At a screen magnification of × 1,000, most of the fibers counted were 0.2 μm in diameter or greater. A total of 100 fields, approximately 2.53 mm^2 in total area, was counted for each sample. The detection limit for the SEM analysis was 140 AB or UF per filter, which corresponds to approximately 440 AB or UF/g wet lung for an average 0.3 g sample. Blank filters were also examined and all reagents were prefiltered to avoid contamination with fibers [Roggli, 1991].

The chemical composition of mineral fibers (asbestos and nonasbestos) was determined by energy dispersive X-ray analysis (EDXA). The asbestos fibers were classified as amosite, crocidolite, tremolite, anthophyllite, actinolite, or chrysotile based on their morphology and X-ray spectra [Roggli et al., 1986, 1992a]. Nonasbestos mineral fibers (NAMF) were classified based on the morphology and elemental content by X-ray spectra and included, among others, talc, rutile, miscellaneous silicates, and fibrous glass [Roggli, 1989; Roggli et al., 1992a]. For each case, 5–30 fibers (average of 16 fibers) were classified by EDXA. The detection limit for each fiber type will vary somewhat from case to case, depending on the concentration of fibers in the lung tissue and the actual number of fibers analyzed by EDXA for that case.

Case Studies

The demographic, pathologic, and occupational data for each case are presented in Table I.

The three patients with documented occupational exposure (cases 1,3, and 4) were employed in a diverse group of occupations associated with exposure to either asbestos directly or to asbestos-containing end-products. Three cases had nonoccupational exposures to asbestos. One (case 6) was the wife of a shipyard worker and one (case 7) was a teacher's aide who worked for 18 years in a building containing chrysotile and tremolite in the acoustical tile [Roggli and Longo, 1991]. The other (case 2) was a man who lived near a vermiculite processing plant during the first 20 years of his life and, as a child, sometimes played in the piles of vermiculite tailings. The longest tremolite fibers detected in this study were in this patient, with many greater than 100 μm in length. Representative scanning electron micrographs from this case are shown in Figure 1, and a typical EDXA spectrum for tremolite in Figure 2.

Very long (>30 μm) tremolite fibers were also detected in case 1, a man who worked manufacturing chrysotile asbestos blankets and gaskets from 1939 to 1945. Although he had no subsequent documented exposure to asbestos, pulmonary tissue analysis 42 years later revealed numerous very long tremolite and chrysotile fibers.

The remaining patient (case 5) was a woman employed as an assembler in a dry cleaning plant, an occupation with no known exposure to asbestos.

The data for the 19 control cases are detailed in an earlier study (unpublished data, summarized in Table II). The age range of the controls was 28–85 years with a median age of 62 years, and all were men. These patients died from a variety of nonpulmonary causes. They were employed in a diverse group of occupations, none of which was associated with potential exposure to asbestos, either directly or indirectly.

RESULTS

The results of AB counts by LM and pulmonary mineral fiber analyses by SEM are shown in Table II. The AB counts by LM for three cases were within our normal range of 0–20 AB/g wet lung, which is based on 84 cases with no history of asbestos exposure nor evidence of asbestos-related disease [Roggli et al., 1982, 1986]. The asbestosis cases (cases 1 and 2) were associated with AB counts two orders of magnitude greater than those for the mesothelioma cases.

The tremolite asbestos fiber counts by SEM also are reported in Table II. Paralleling the trend noted with the AB counts by LM, the tremolite fiber burdens for the asbestosis cases also were approximately one to two orders of magnitude greater than those for the mesothelioma cases. The highest counts occurred in the patient who was employed for 7 years manufacturing asbestos gaskets and blankets (case 2). The detection of more asbestos fibers in the asbestosis cases than in the mesothelioma cases is consistent with previous studies indicating higher pulmonary tissue asbestos burdens are required to produce asbestosis than to initiate malignant mesothelioma [Roggli, 1990]. However, this result is contrary to studies of Canadian chrysotile miners, in which the median tremolite fiber count in mesothelioma cases was similar to or exceeded that of asbestosis cases [Churg and Wright, 1989; Churg et al., 1993].

For each of the seven cases, the predominant fiber type detected by SEM was tremolite. In two cases (cases 5 and 7; see Table II), the only uncoated asbestos fibers (≥5 μm) detected by SEM were tremolite. The vast majority of tremolite fibers detected had an aspect ratio greater than 5:1. Uncoated commercial amphiboles (i.e., amosite or crocidolite fibers ≥5 μm) were not detected in any of these seven cases. Uncoated chrysotile fibers ≥5 μm were present in two cases, and the noncommercial amphiboles, anthophyllite and actinolite, were detected in two cases each.

The types of NAMF (≥5 μm) detected by SEM in each of the seven cases is

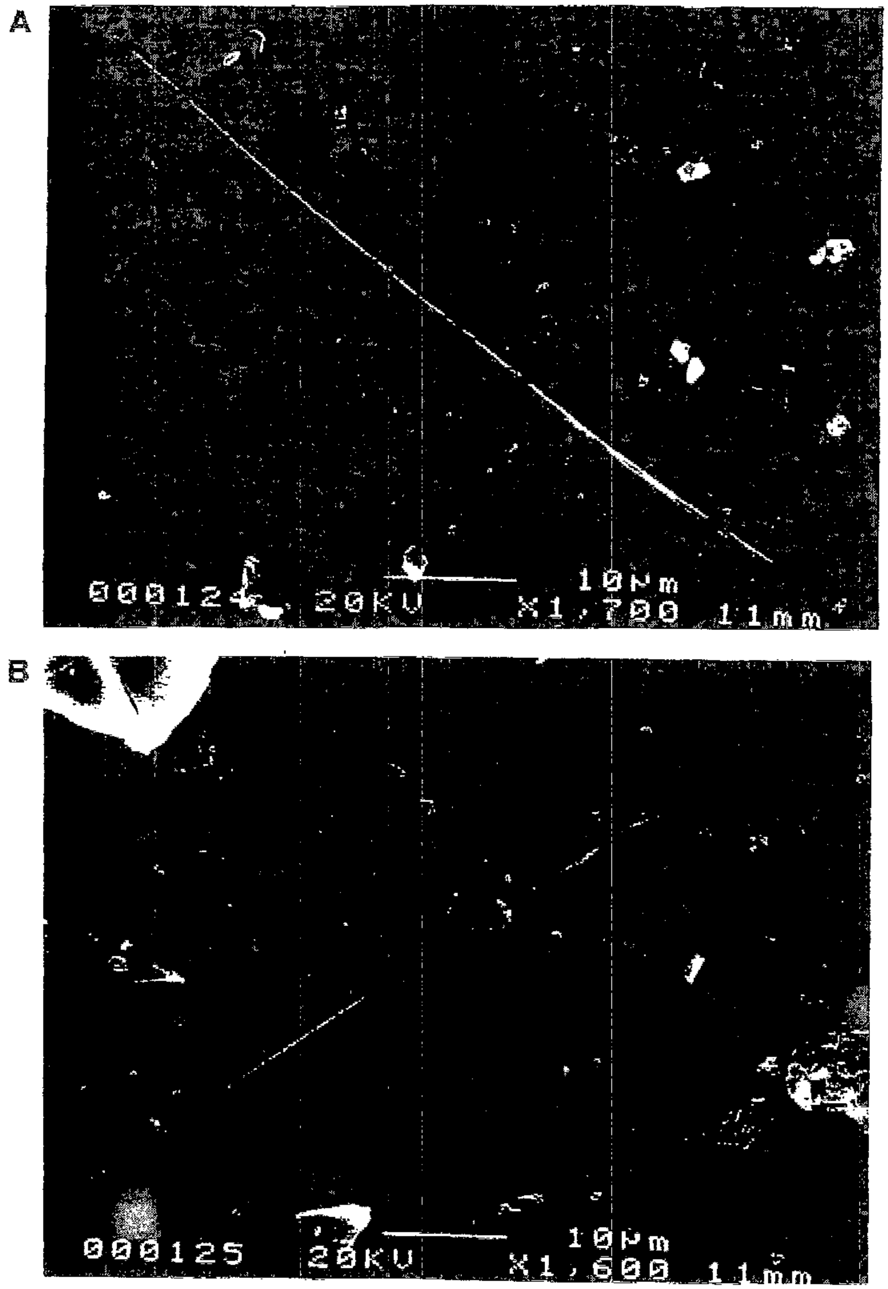

Fig. 1 **A,B:** Long tremolite fibers, each exceeding 70 μm in length, demonstrating high aspect ratio. Fibers were isolated from the lungs of case 2 using sodium hypochlorite digestion procedure. (SEM: A, × 1,700; B, × 1,600.)

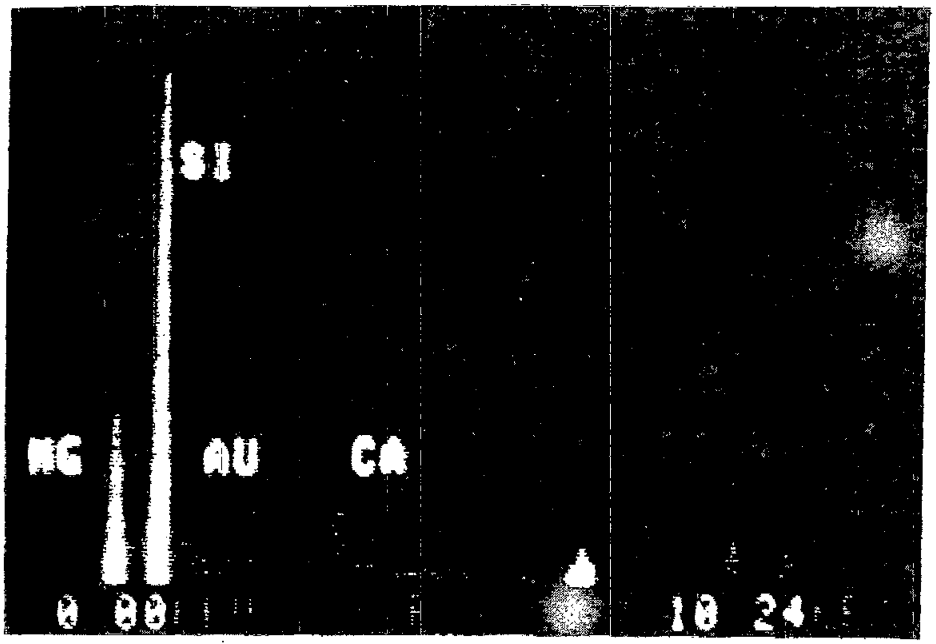

Fig. 2. Typical EDXA spectrum for tremolite showing prominent silicon, calcium and magnesium peaks, consistent with its composition as a calcium magnesium silicate.

TABLE II. Results of LM and SEM Analyses in Seven Patients With Asbestos-related Diseases*

LM data		SEM data (uncoated fibers/g)[b]					
Case[a]	AB/g	Total	Tremolite	Anthophyllite	Actinolite	Chrysotile	NAMF
1	6,200	554,000	405,000	63,800	<21,300	63,800	21,300
2	2,900	140,000	124,000	<8,200	16,500	<8,200	<8,200
3	<15.0	101,000	8,100	8,100	<4060	<4060	85,200
4	42	10,700	5,940	<1200	<1200	1,200	3,570
5	64	6,800	5,440	<680	<680	<680	1,360
6	2	24,300	4,860	<4860	4,860	<4,860	14,600
7	2.8	13,000	4,330	<870	<870	<870	8,670
Controls	0.2–22.0	420–12,700	<160–2,540	<2,540	<80–1,310	<80–1,000	210–10,160

*AB/g = asbestos bodies per gram of wet lung; total = total uncoated fibers per gram of wet lung NAMF = nonasbestos mineral fibers

[a]Case numbers for cases in this study (also see Table I); controls refers to 19 control cases detailed in earlier, as yet unpublished, observations

[b]Uncoated fibers (≥5 μm in length) only; no uncoated amosite or crocidolite fibers (≥5 μm) were detected.

detailed in Table II. Note that four of the cases contained fibrous talc, which is frequently contaminated with tremolite asbestos. None of the other nonasbestos mineral fibers listed in Table III are known to be contaminated with tremolite.

TABLE III. Uncoated NAMF From Seven Patients With Asbestos-related Diseases*

Case[a]	Talc	Rutile	Aluminum silicates	Other silicates
1	21,300	<21,300	<21,300	<21,300
2	<8,200	<8,200	<8,200	<8,200
3	60,800	16,200	<4,060	8,100
4	<1,190	2,380	<1,190	1,190
5	680	680	<680	<680
6	<4,860	<4,860	<4,860	14,600
7	6,930	<870	870	870
Controls	<80–10,200	<80–5,300	<80–1,110	<80–1,800

*Number of uncoated NAMF (≥5 μm) per gram of wet lung detected by SEM.
[a]See footnote to Table II.

DISCUSSION

Tremolite is nearly ubiquitous and represents the most common amphibole fiber in the lungs of urbanites [Churg and Wiggs, 1986]. However most of these fibers are short, low-aspect-ratio fibers, which studies have suggested are not associated with increased mesothelioma risk except at perhaps high concentrations, whereas longer, high-aspect-ratio tremolite fibers are considered a potent mesothelioma initiator even at low concentrations [Langer et al., 1987; Yazicioglu et al., 1980]. Studies have shown that if all tremolite fibers detected by electron microscopy are counted, the geometric mean length is less than 2 μm, with a geometric mean aspect ratio of 8:1 to 10:1 [Churg, 1987]. However, if only fibers greater than 5 μm are counted, the geometric mean aspect ratio increases to greater than 20:1 [Weill et al., 1990]. In the study presented here, only tremolite fibers greater than or equal to 5 μm were counted during SEM analysis. Differentiating between asbestos fibers and cleavage fragments can be difficult because of the overlapping morphology between asbestiform and nonasbestiform fibers [Weill et al., 1990]. However, there is general agreement that asbestos fibers greater than or equal to 5 μm are the most pathogenic [Davis et al., 1978; Lippmann, 1988; Schneider and Skotte, 1990], which encompasses all fibers detected in this study.

Pulmonary tissue AB counts for three of the seven cases were within our normal range of 0–20 AB/g wet lung [Roggli et al., 1982, 1986]. Nonetheless, based on this study and a previous study, these three cases are probably asbestos related because the tremolite-uncoated fiber counts exceeded the upper limit of the control range of 2,540 uncoated fibers/g of wet lung. Additionally, although the other two mesothelioma cases had AB counts outside the normal range, the values (42 and 65 AB/g wet lung) were still considerably lower than the geometric mean value of 1,000 AB/g wet lung observed for an earlier study of mesothelioma cases due to occupational exposure to asbestos [Roggli et al., 1992a]. An earlier report by McDonald et al. [1989] indicated that the proportion of longer, and thus, more carcinogenic tremolite fibers increases at lower fiber concentrations. Therefore, electron microscopic analysis of pulmonary mineral fibers may be required to differentiate asbestos-related mesotheliomas from nonasbestos-related cases when AB counts by LM are within the range of background values.

It is of interest to compare the levels of tremolite fibers in our five patients with mesothelioma with those of the commercial amphiboles amosite and crocidolite in our overall series of mesothelioma cases. Among 84 cases of mesothelioma for which SEM results of uncoated fiber counts and fiber types were available, uncoated amosite or crocidolite fibers 5 μm or greater in length were detected in 72 (86%) with a range of values from 122 to 2,890,000 fibers/g of wet lung. In 22 of these cases (31%), the concentration ranged between 1,000 to 10,000 fibers/g, which is similar to the range of tremolite values found in the five mesothelioma cases in the present study (Table II). In three additional cases, amosite fibers were detected at levels below 1,000 fibers/g. Uncoated amosite or crocidolite fibers 5 μm or greater in length are generally below the limits of detectability for our technique in our control cases.

One might argue that, because our technique primarily detects fibers that are 0.2 μm or greater in diameter, we could have overlooked substantial numbers of fibers that are 5 μm or greater in length and 0.1 μm or less in diameter. We think that this possibility is unlikely to alter our results or conclusions, because substantial numbers of amosite or crocidolite fibers less than 0.1 μm in diameter should be accompanied by substantial numbers of fibers 0.2 μm or greater in diameter. This opinion is supported by the observations of Churg and Wiggs [1984] and Warnock [1989], since these investigators used transmission electron microscopy (TEM) and found ratios of amosite to crocidolite among U.S. mesothelioma cases indistinguishable from those found in our previously reported study [Roggli et al., 1993]. It is more likely that our analysis would underestimate the numbers of chrysotile fibers 5 μm or greater in length, because chrysotile tends to undergo longitudinal splitting with many fibers consequently having diameters less than 0.1 μm [Roggli, 1991; Roggli et al., 1993].

Women represent 43% (3/7) of the cases in this study vs. only 8% of the 153 mesotheliomas cases, with tissue asbestos analyses, in one of the authors' (V.L.R.) consultation files. A potential source of exposure for these three cases was cosmetic talc, which may be contaminated with tremolite asbestos. The 19 control cases used to determine the upper limit of control tremolite levels were all men. Therefore, it is not known whether women in the general population have pulmonary tremolite levels greater than men. However, the literature includes the results of one TEM analysis which showed no significant difference in pulmonary amphibole levels between men and women [Churg and Warnock, 1980].

One of the unresolved questions is the role of chrysotile vs. tremolite in producing mesotheliomas and other asbestos-related disease in patients with a history of tremolite-contaminated chrysotile asbestos exposure. Some authors believe that tremolite is the main culprit [Churg, 1988; McDonald et al., 1982, 1989]; however, at least one group of authors suggests that chrysotile may be the more responsible agent in some cases [Bégin et al., 1992]. In the study presented here, uncoated chrysotile fibers (≥5 μm) were detected in only two of the seven cases, and the tremolite concentrations for those two cases were four to five times the chrysotile concentrations.

Our study confirms the relationship between tremolite exposure and the development of asbestos-associated diseases and suggests that certain types of exposure are likely to be associated with elevated tissue levels of tremolite asbestos. Furthermore, the finding of relatively modest elevations of tremolite content in some of our mesothelioma cases suggests to us, that at least for some susceptible individuals, moderate exposures to tremolite-contaminated dust can produce malignant pleural mesothelioma.

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