Environmental and Occupational Exposures to Chrysotile Asbestos: A Comparative Microanalytic Study[1-3]

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ABSTRACT. Previous light microscopic analysis of lung tissue from persons living close to a large open-pit asbestos mine demonstrated asbestos body counts intermediate between those of referents and those of miners and millers. In this study, we examined via electron microscopy and x-ray energy dispersive spectrometry autopsy lung specimens from individuals ascertained to be environmentally or occupationally exposed and from a referent group. Environmental group concentrations of chrysotile fibers longer than 5 μm were significantly higher than those of referents, and 50% lower than those observed in the occupational group. Tremolite was markedly increased in the occupational group, but only marginally greater in the environmentally exposed. Electron-microscopy-derived concentrations of amphibole fibers longer than 5 μm correlated well with light microscopic asbestos body counts in the occupational group but not in the environmental or referent groups. Chrysotile concentration was not related to asbestos body concentration in any group. Crocidolite fiber, a commercial amphibole not native to the region, was nonetheless identified in lung tissue from 15 of 23 chrysotile miners and millers. Environmental exposure to asbestos fiber as a result of residence within 40 km of the mines results in increased lung chrysotile content.

CHRYSOTILE ASBESTOS is the most important commercial form of that mineral in use in North America. An important source is the Québec mining region centered around the towns of Thetford and Asbestos.[1] The Jeffrey mine in Asbestos is a large open pit situated within the city itself, with tailings extending well into the surrounding countryside.[2] In previous work by our group, air samples obtained systematically over a 1-yr period (1984) in Asbestos showed a fifty-fold excess of chrysotile fibers longer than 5 μm, compared to the general urban environment of Montréal.[2,3] Tremolite, not detectable in Montréal air samples, was also present in small quantities in Asbestos.[3] It has been suggested that prevailing wind patterns favor dispersion of asbestos fiber over the rural region around Asbestos.[4] Water samples from the mining communities have also shown increased fiber levels suggesting airborne and/or groundwater environmental contamination.[5]

These observations led us to measure and characterize asbestos content in the autopsy lung tissue of individuals resident in the Asbestos mining area who had never worked in the mines or mills: the "environmentally exposed." This evaluation of tissue burden could reflect cumulative and/or current exposure of persons exposed to low or intermediate levels of chrysotile dust. In initial work,[2,6] we observed via light microscopy increased asbestos body (AB) concentrations in lung tissue from area residents never employed in the mines or mills and living within 40 km of the mine at Asbestos. In the current study, we characterized specific lung mineral fiber content in the mining region using electron microscopic analysis of lung tissue.[7]

Methods

Identification and selection of study subjects. Identification of subjects for Asbestos environmental and occupational groups, and for a referent group, has been described previously.[2] Briefly, we reviewed 1,300 autopsies over the 5-yr interval 1979-1983 in the Centre Hospitalier Universitaire de Sherbrooke. We identified 97 persons born between 1891 and 1920, living in a 35 km × 35 km area largely east (downwind) of the Jeffrey mine *or* in a comparable "reference" region along the Québec-U.S. border. To determine lifetime primary asbestos trade work history, we checked identifying data for the 97 persons eligible against a master list of all persons in this birthdate range ever employed for 1 month or more in the Québec chrysotile mining/milling/products fabrication industries (N = 30,000).[1] Twenty-six men were positively identified as the Asbestos "occupational" group; lung samples were available for 23. The remaining 71 subjects consisted of 29 (20 males) living in the Asbestos region ("environmental" group) and 42 (22 males) in the reference region (Table 1). The 17 environmental group males for whom lung samples were available were matched for age (± 5 yr) to referent group males. In addition, 6 randomly selected environmental group women were matched by age to 6 referent group women, giving a total of 23 subjects in each group.

Specimen collection and preparation. For each subject, a single subpleural formalin-fixed lung sample without gross evidence of disease was obtained from autopsy stock jars. A portion of tissue was weighed and chemically digested with filtered fresh commercial bleach. The density (dry weight/wet weight ratio) of an adjacent portion was used to convert into dry weight the wet weight of the digested portion.[8,9] Bleach volume was adjusted to a concentration of 1 mg dry weight lung/L bleach. Aliquots of 25 ml (for environmental and referent samples) or 10 ml (for occupational group samples) were filtered through an 0.45 μm pore size 25 mm diameter Millipore® membrane filter. Filters were (low-temperature) ashed overnight together with blank filters, and the ash resuspended in 50 ml Millipore Milli-Q® ultra-clean water. One hour later, suspensions were refiltered through 0.2 μm pore size 25 mm diameter Nuclepore® filters and mounted on 200 mesh copper grids using a carbon replica technique.[10] All preparations were performed in a Clean Room. Blanks, processed in tandem with each group of four samples, contained only rare, short (< 5 μm) chrysotile fibers. Technicians were blind as to the origin of the samples.

Analysis. Grids were examined in a graphite sample holder mounted in a JEOL 100 CX® transmission electron microscope (TEM) equipped with a PGT System IV® energy dispersive x-ray spectrometer (EDS) for elemental analysis. Counts were performed at 10,000 X screen magnification under 80 KV accelerating voltage. Each visible particle longer than 5 μm and having an aspect ratio greater than 3:1 was identified individually on the basis of morphology, EDS spectrum, and electron diffraction if necessary.[7,11,12] For chrysotile and tremolite fibers, fiber length (to nearest .20 μm) and diameter (to nearest .03 μm) were measured directly from the screen

Table 1.—Subject selection and characteristics

	Referent Group	Environmental Group	Asbestos miners/millers
Number eligible for study*	42	29	26
Age	73 ± 8	72 ± 7	63 ± 14†
Sex	(22 M, 20 F)	(20 M, 9 F)	(all male)
Number with lung samples available	39	25	23
Sex	(21 M, 18 F)	(17 M, 8 F)	
Number selected for study‡	23	23	23
Sex	(17 M, 6 F)	(17 M, 6 F)	
Age	75 ± 8	73 ± 8	67 ± 7
Number analyzed	19§	22§	23

*All subjects (a) born between 1891 and 1920, and who (b) died in either the study area or reference area between 1979 and 1983, and who (c) had autopsy at Sherbrooke University Hospital.
†$p < .05$ vs. environmental and referent groups (mean years ± *SD*).
‡All male and six female "environmental group" subjects having autopsy lung samples available were selected and matched for age and sex to the closest available referent group subject. All 23 occupational group subjects with tissue available were selected.
§Samples for three males and one female in the reference group and one environmental group female could not be analyzed due to technical difficulties.

using a calibrated eyepiece graticule and a system of concentric circles. Number-size distributions were condensed into three classes: "optical" fibers (length > 5 μm, diameter > 0.25 μm); "Stanton" fibers[13] (length > 8 μm, diameter < 0.25 μm); and "short" fibers (all others). For each sample, up to 50 fibers or all fibers in 30 randomly selected fields were counted, to a detection limit of 0.06 fibers/μg dry lung. Total fibers observed for the three groups using these criteria were: referent group, 206; environmental group, 341; and occupational group, 747.

Representation of results and statistical methods. Fiber concentrations showed extreme non-normal variation from case to case. Fiber concentration data are therefore presented as geometric means and as median concentrations. Where no fibers of a given type were seen, the concentration was set at one-half the detection limit to allow geometric mean calculations. Other results are presented as arithmetic means ± standard deviation or standard error. Significance tests were not appropriate for comparing fiber type distributions, which are presented as mean percent values of total fibers present per individual. Significance tests used for other comparisons were the two-sample *t* test with separate estimates of variance[14] and Wilcoxon rank sum.[15]

Results

Characteristics of study subjects. Age, sex, and number of subjects in each group before and after selection for study are depicted in Table 1. All occupational group members were male, on average younger at the time of death than environmental and referent group members. Mean duration of chrysotile mining or milling employment was 32 yr (± 13) and mean interval between last exposure and death 11 yr (± 18).

Tissue fiber concentrations. Lung samples from the environmental group showed statistically significant excesses in geometric mean and median concentrations of chrysotile fiber longer than 5 μm, when compared to referents (Table 2). A small excess of tremolite was not significant. As expected, total asbestos fiber concentrations longer than 5 μm were about one order of magnitude less in the environmental group than in the occupational group. This excess was composed principally of tremolite: indeed, chrysotile levels in occupational group members were not significantly different from those in the environmental group.

Tissue fiber type distributions. Asbestos fibers constituted, on average, 59.6% of all fibers counted in the environmental group vs. 43.3% in the referent group (Table 3). The excess was due entirely to chrysotile fibers. Other fiber types were relatively common, particularly mica and titanium. Most samples from both groups contained at least some chrysotile, while 15 of 22 environmental and 10 of 19 referent samples contained some tremolite. Comparison of environmental group fiber type distributions to those in occupational groups shows a striking difference in the types of asbestos fiber present (Table 3). Nearly 80% of fibers longer than 5 μm in the occupational group, on average, were asbestos. The contribution of tremolite was particularly high (38.5% [mean] and 35% [median] vs. 13.4% and 10% in the environmental group). A surprising finding was the presence of crocidolite fibers in a large number of workers—15 of 23 examined—constituting, on average, over 10% of all fibers counted in this group. Crocidolite was identified in lung tissue from only one environmental case and in none of the referents.

Fiber size distributions for chrysotile and tremolite. Most chrysotile fibers sized were shorter than 8 μm and under 0.25 mm in diameter (64% of all chrysotile fibers in environmental group, 61% in referent group, and 55% in occupational group [Table 4]). Mean length for chrysotile was 8.3 ± 5.6 μm (environmental) vs. 7.6 ± 3.1 μm for referents (not significant [NS]). Interestingly, chrysotile fiber length distribution in the occupational group (8.4 ± 5.4 μm) was closest to environmental group values, although there was wide variation. Chrysotile fibers in the "Stanton"[13] size range (length > 8 μm, diameter < 0.25 μm) were most frequent in occupational group samples (34%), followed by the environmental group (28%), and referents (23%). Chrysotile fibers had finer

Table 2.—Tissue fiber concentrations/μg dry lung* in referent, environmental, and occupational groups

	Referent group		Environmental group		Occupational group	
Fiber type	Geometric mean	Median	Geometric mean	Median	Geometric mean	Median
Chrysotile	0.08	0.06	0.28†	0.28‡	0.65§	0.50†
Tremolite	0.06	0.05	0.08	0.06	1.18§	1.30§
Amosite	0.03	0.03	0.03	0.03	0.08†	0.03
Crocidolite	0.03	0.03	0.03	0.03	0.19§	0.10§
All asbestos	0.26	0.22	0.57†	0.40†	3.30§	3.70§
All nonasbestos	0.36	0.37	0.35	0.34	0.62†	0.59†
All fibers	0.71	0.62	1.05	1.08	4.48§	4.70§

*All fibers longer than 5 μm. Zero values converted to one-half the detection limit (0.03 f/μg).
†$p < .05$.
‡$p < .01$.
§$p < .001$ vs. referent group corresponding value.

Table 3.—Mean and median autopsy lung tissue fiber type proportions* in referent, environmental, and occupational group members

Fiber type	Referent group Mean % (SE %)	Median %	N† (of 19)	Environmental group Mean % (SE %)	Median %	N† (of 22)	Occupational group Mean % (SE %)	Median %	N† (of 23)
Chrysotile	28.6% (7.6)	12%	16	44.6% (7.3)	42%	19	27.1% (5.8)	17%	19
Tremolite	14.3% (4.3)	4%	10	13.4% (3.6)	10%	15	38.5% (5.7)	35%	21
Amosite	0.4% (6.1)	0%	1	1.5% (1.3)	0%	2	2.8% (1.1)	0%	9
Crocidolite	0.0% —	0%	0	1.5% (1.3)	0%	1	11.1% (3.0)	4%	15
All asbestos	43.3% (7.9)	37%	16	59.6% (6.7)	65%	20	79.5% (4.7)	89%	23
Mica	16.1% (4.6)	10%	13	16.1% (4.6)	10%	13	2.9% (1.4)	0%	6
Titanium	8.5% (2.9)	4%	7	9.9% (2.9)	4%	11	6.4% (3.1)	0%	8
Talc or anthophyllite	8.7% (3.7)	1%	7	5.4% (2.3)	0%	7	3.5% (1.2)	0%	10
Other fibers‡	13.8% (6.5)	1%	6	6.6% (2.4)	1%	9	4.3% (1.2)	0%	9
Unidentified fibers	2.3% (1.3)	0%	3	2.4% (1.1)	0%	3	3.9% (1.2)	0%	10

*Expressed as percentage of all fibers counted; calculated from percentage fiber type distributions for each subject. Results are arithmetic mean percentages (± SE) and median of individual observations.
†Number of individuals having *any* fiber(s) of given type in their autopsy lung sample.
‡More than 20 different fiber types were observed.

Table 4.—Fiber size distribution in environmental, referent, and occupational groups

	Group Referent	Environmental	Occupational
Chrysotile	(*N* = 49)	(*N* = 195)	(*N* = 121)
Optic* fibers	8 (16%)	16 (8%)	13 (11%)
Stanton* fibers	11 (23%)	54 (28%)	41 (34%)
Short* fibers	30 (61%)	125 (64%)	67 (55%)
Mean fiber length (in μm ± *SD*)	7.6 ± 3.1	8.3 ± 5.6	8.4 ± 5.4
Mean fiber diameter (in μm ± *SD*)	.15 ± .18	.13 ± .25	.13 ± .16
Tremolite	(*N* = 28)	(*N* = 30)	(*N* = 158)
Optic* fibers	21 (75%)	25 (83%)	101 (64%)
Stanton* fibers	1 (4%)	1 (3%)	10 (6%)
Short* fibers	6 (21%)	4 (14%)	4 (14%)
Mean fiber length (in μm ± *SD*)	7.6 ± 2.8	7.7 ± 3.4	6.9 ± 3.6
Mean fiber diameter (in μm ± *SD*)	.66 ± .48	.62 ± .74	.30 ± .25

*Definitions: short fiber = length < 8 μm (but > 5 μm), diameter < 0.25 μm; Stanton fiber = length > 8 μm, diameter < 0.25 μm; and optic fiber = all other fibers, having diameter > 0.25 μm.

mean diameter in environmental and occupational groups, although there was marked intragroup variation. Tremolite fibers were on average shorter and thinner in the occupational group.

Correlation of fiber concentrations with asbestos body counts. In previous work,[2,6] we described light microscopic asbestos body (AB) concentrations for the groups outlined in this paper, showing statistically significant differences between the three groups, with environmental values intermediate between occupational and referent data. As expected, EM tissue fiber concentrations correlated well with optical AB counts in the occupational group for tremolite ($r = .69$; $p < .01$), crocidolite ($r = .82$; $p < .01$), and for all amphiboles ($r = .83$; $p < .01$). Electron-microscopy-derived chrysotile fiber concentrations were not related to optical asbestos body counts in miners and millers ($r = .11$, NS) or in other groups. There was no correlation of light microscopic AB concentrations with EM-derived amphibole concentrations for environmental and referent groups. This is not surprising given the very small numbers of amphiboles present (Table 2).

Autopsy findings. Autopsy findings commonly associated with asbestos exposure were recorded for all persons eligible for the study (Table 5). Mesothelioma was confirmed by the Canadian Tumor Reference Center. Cases of lung cancer and asbestosis in chrysotile miners and millers were reviewed by an expert Compensation Panel. Asbestosis, pleural plaques, mesothelioma, and asbestos bodies in routine histologic sections were not observed by pathologists in any persons from the reference area or in the environmentally exposed. Lung and gastrointestinal cancer were uncommon in both groups. All of the above findings except gastrointestinal cancer were increased in the occupational group, as expected from previous work.[1]

Table 5.—Autopsy findings* in the environmentally exposed

	Referent group (N = 42)	Environmental group (N = 29)	Occupational group (N = 36)
Age	73 ± 8	72 ± 7	63 ± 14
Sex	(22 male)	(20 male)	(all male)
Findings:			
Lung cancer	2 (5%)	1 (3%)	6 (23%)
Mesothelioma	0	0	1 (4%)
GI cancer	3 (7%)	2 (7%)	3 (12%)
(colon)	1	0	3
(stomach)	2	1	0
(other)†	0	1	0
Asbestosis	0	0	10 (39%)
Pleural plaques	0	0	15 (58%)
Asbestos bodies in routine sections	0	0	13 (50%)
Pulmonary fibrosis: any type other than asbestosis‡	1 (2%)	2 (7%)	3 (12%)

*Diagnoses (*not* causes of death) recorded from autopsy reports for all subjects *eligible* for the study in each group (See Methods, Part 1). Subjects are unselected and unmatched. With the exception of "Pulmonary fibrosis . . . other than asbestosis," only diagnoses potentially and directly caused by asbestos exposure are included. Subjects may have more than one diagnosis.
†Duodenal endocrine tumor. There were no pancreatic cancers in any group.
‡See text for details. One case from the occupational group may have been asbestosis which was misclassified.

We also recorded notation on the autopsy report of any form of pulmonary fibrosis *other* than asbestosis. Three such cases were present among the 26 chrysotile workers. One of these had "small foci of pulmonary fibrosis, associated with emphysema, with rare asbestos bodies"; one "anthraco-silicosis"; and one "interstitial pulmonary fibrosis" (without asbestos bodies). Lung samples were available for the latter two cases. In the case of "anthracosilicosis," we found 1.7 asbestos fibers (length > 5 μm) per μg dry lung (0.7 crocidolite, 0.6 tremolite, 0.4 chrysotile). In the case of diffuse interstitial fibrosis not considered asbestosis by the pathologists on the basis of absence of asbestos bodies in routine lung sections, chrysotile was not found but tremolite fibers were present in increased concentration (1.2 fibers/μg dry lung). This underlines the potential diagnostic importance of tissue digestion/concentration studies.

In the environmental group, there were two cases of pulmonary fibrosis other than asbestosis. One was a case of pulmonary fibrosis secondary to sero-positive rheumatoid disease (no lung sample was available for analysis in this case). The second case was described as having bilateral fibrous pleural adhesions. No asbestos bodies were described in routine autopsy lung sections. Our own electron microscopic examination identified no chrysotile fibers longer than 5 μm. A single tremolite fiber was present (0.06 fibers/μg dry lung). One referent had silicosis with "a few" asbestos bodies noted by pathologists in autopsy lung sections. Chrysotile and tremolite were absent from our lung tissue digest in this case.

Discussion

Two methodological refinements in this study were the limitation of analyses to fibers longer than 5 μm and the use of a uniform strategy to avoid tissue site selection bias. Fiber analytic studies measure a very small fraction of what is actually present in tissue and create, by extrapolation, an "index" of total lung content.[16-22] In most studies,[8,16,20-22] 80-90% of asbestos fibers counted are between a length detection limit determined by final magnification and 5 μm. Investigators who count "all fibers" implicitly devote most of their analytic effort to this short fiber range. Fibers longer than 5 μm are poorly evaluated. Our design choice makes the opposite concession: fibers longer than 5 μm are more accurately assessed, but the more numerous short fibers are not examined. Both methods introduce a bias, but our method has several practical advantages: fibers are more reliably observed, counted and identified, and the "index" of lung content obtained is in line with air monitoring standards employed by industrial hygienists. Comparison of our results to those of investigators implicitly concentrating their efforts on short fibers should be performed with caution, in the absence of interlaboratory comparisons using the same study population.

Selection bias in a study of this nature comes from two potential sources: selection of cases for autopsy and selection of tissue site for analysis. Autopsy selection bias is evident in our "occupational group." Due to current compensation standards, chrysotile miners and millers in Québec are more likely to have an autopsy if they have a potentially asbestos-related disease and if they are of working age at the time of death. This translates into a younger age for this group (Table 1) and a greater frequency of lung cancer and asbestosis (Table 5) than that observed in the same cohort in epidemiologic studies.[1] Site-selection bias was avoided by our use of uniform selection of lung samples without gross evidence of disease. Pathologists select lung tissue from grossly diseased areas (tumor, pneumonia, grossly visible scars), or randomly in lung lobes where gross disease is absent.

Selection of "diseased" lung for our study would lead to marked bias, since fibers are absent from tumor tissue and lung tissue weight (the "denominator" for concentrations) is increased by pneumonia and other consolidative lesions. Use of lung sections preselected for disease could thus provide near-zero concentrations for cases with tumor, and lower concentrations in cases with severe bronchopneumonia. The result would be a very large and very artificial decrease in "fiber concentration" in the (more "diseased") occupational group.

As expected, mean and median lung chrysotile fiber concentrations are significantly increased in environmentally exposed individuals vs. referents. Fiber type distributions also show a relative increase in chrysotile in the mining communities. Tremolite showed only a small excess in the environmental group, which was not statistically significant. This is consistent with our previous observation that tremolite in air samples in the Asbestos region is present just above the limit of detection, while chrysotile is markedly increased.[3] This result should not be generalized to other mining communities such as Thetford, where tremolite levels in air samples are much higher.[3]

Results observed for chrysotile concentrations in the environmentally exposed group are less than one order of magnitude below values recorded for our group of miners and millers from Asbestos. In addition, lung tissue from environmental group members appears to contain chrysotile fibers which are similar in length and in diameter to those present in the lungs of asbestos miners and millers, and longer and thinner than those in the lung tissue of referents.

Commercial amphiboles, which are not native to the region, were virtually absent in environmental and referent groups but relatively common in low concentrations in Asbestos miners and millers (amosite 9/23; crocidolite 15/23). We attribute much of this excess to the historical fact of asbestos products fabrication and asbestos quality control testing using imported amphiboles in Asbestos.[1]

The similarities and differences between the occupationally exposed and the environmentally exposed may be explained partially by the current state of knowledge of asbestos fiber deposition and clearance.[16-20] Lung chrysotile content appears to best indicate current and recent (< 6 yr) exposure at the time of death.[20] The long interval between cessation of employment and death (11 ± 18 yr) in the miners and millers appears to have brought their lung chrysotile concentrations into the same range as those in the environmental group. Amphiboles accumulate continuously in lung tissue, as evidenced again by the behavior of tremolite and crocidolite in the lungs of our occupational group. Information on years lived in the region, distance of homes from the mine, and domestic exposures were not available for this retrospective study, but the exact relationship between these factors and fiber accumulation will be explored in a prospective study in the same area.

The relative paucity of amphiboles, particularly tremolite, in our environmental group must be treated with some caution. Electron microscopic analysis, while highly sensitive in the detection of chrysotile fibers, is inferior by several orders of magnitude to lung digest optical asbestos body (AB) counts in the detection of amphibole fibers, if one assumes that most ABs are formed on amphibole cores. Indeed, our previous study[2,6] showed a clear excess of optical AB counts in the environmental group, which are not correlated with the EM fiber concentrations obtained in the current study. While tremolite content is low in the air around Asbestos, its gradual accumulation could result in moderately increased levels in lung tissue not detectable using electron microscopic analysis.

Our finding of increased AB concentrations[2,6] and chrysotile fiber concentrations in environmentally exposed individuals leads to important questions concerning the validity and clinical applicability of the results. We tested the validity of our system for identifying chrysotile mining or milling occupation from the "master list"[1] using a systematic review of 371 autopsy records in another Québec mining community, where complete work histories are known and recorded with the pathologic reports. The review identified 124 miners and millers born between 1891 and 1920, 100% of whom were on our master list. We cannot exclude household exposure as a possible confounding factor in our analysis in an area where a large proportion of the male population has been engaged in mining and milling asbestos. Similarly, occupational exposure outside the asbestos industry cannot be ruled out, although this is equally likely in referent and environmental groups.

It now appears that asbestos fiber concentrations are increased in mining area air samples,[3,4] water,[5] and the lung tissue of those never employed in the mines,[2,6] as reported in this paper. The latter finding has potential application to other situations in which low level exposure to chrysotile asbestos is encountered, such as residence or work in asbestos-insulated buildings.

The health implications of increased lung chrysotile content in the environmentally exposed remain to be assessed. Examination of autopsy reports for our environmental group showed no definite asbestos-related disease or lesions, but the numbers exposed are too small for proper epidemiologic analysis (Table 5). Evaluation of the problem by conventional epidemiologic surveys is difficult, due to the high prevalence of work in the mines and mills.[16,23-26] Repeated findings of respiratory cancer excess in the area are usually attributed to occupational exposure. A preliminary epidemiologic study of mining community residents never occupationally exposed showed no definite respiratory cancer excess,[27] but more definitive work is needed before this risk can be excluded.

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