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ASBESTOS FIBER TYPE AND LENGTH IN LUNGS OF CHRYSOTILE TEXTILE AND PRODUCTION WORKERS: Fibers Longer Than 18 μm

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Excess lung cancer risk for a cohort of chrysotile textile plant workers was many times the risk observed in a cohort of chrysotile miners/millers. The latter had greater exposure to chrysotile/tremolite. A previous lung burden study confirmed this excess exposure in miners/millers and showed little difference in fiber length. Selection of too short a fiber length cut-off (5 μm or more) in the previous study could have masked differences in lung-retained fiber length. In this follow-up, we counted only those intrapulmonary fibers exceeding 18 μm in length. Long fiber concentration and dimension were assessed by transmission electron microscopy (TEM) and energy-dispersive x-ray spectrometry (EDS) for autopsy samples from 64 textile workers and 43 chrysotile miners and millers. These long fibers were significantly more concentrated in the lungs of chrysotile miners and millers, consistent with their greater exposure. However, when only these longest fibers were compared, there was a somewhat greater mean and median intrapulmonary fiber length for chrysotile textile workers (mean fiber length, all fiber types combined, 25.2 ± 10.2 μm vs. 22.9 ± 6.6 μm in miners/millers, $p < .001$; medians 21.6 vs. 20, $p < .05$). Despite their lesser apparent lung cancer risk, chrysotile, tremolite, total amphibole, and total long fiber asbestos concentrations were all highest in the lungs of miners/millers. Twenty-two of 64 textile workers had lung content of crocidolite and/or amosite (32.5% of 508). These amosite/crocidolite fibers were present in the lungs of workers who ceased employment prior to the first use of such fibers recorded in this industry. The results suggest that (1) asbestos fiber length differences cannot explain the difference in lung cancer risk excess and slope between cohorts and (2) the experience of textile workers should not be used to assess risk of lung cancer in miners, cement workers, and friction product workers, regardless of fiber type.

Animal inhalation studies (Miller et al., 1999) and previous theoretical calculations (Lippmann, 1990) have suggested a role for fiber length in the genesis of lung cancer. Indeed, recent practice for the assessment of carcinogenicity of both natural and synthetic fibers has concentrated analytical efforts on fibers longer than 20 μm and of diameter less than 1 μm. Human studies of lung-retained fibers have been less convincing (Becklake & Case, 1994), but can be criticized for their universal concentration on shorter fibers, usually defined either as all fibers longer than 5 μm (Sébastien et al., 1989) or most often all fibers of any length resolvable by the electron microscopic magnifica-

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tion in use (e.g., Green et al., 1997). This approach fails to assess adequately the longest fibers, since they comprise a relatively small proportion of fibers present in the lung, and length distributions in any analysis are heavily skewed toward the lower end.

Of particular interest in this regard is the difference in lung cancer attributable risk and exposure–diseaseslope for asbestos-exposed cohorts where fiber length might also differ. One example is the large difference in lung cancer risk between two well-studied chrysotile asbestos industries. Miners and millers of chrysotile in Quebec have had very heavy exposures but a shallow slope of lung cancer risk, with lung cancer excess mortality becoming apparent only at exceptionally high exposures, estimated recently as over 300 million particles/foot^3/yr (over 1000 fiber-years, implying exposures such as an average of 50 fibers/cm^3 for 20 yr) (Liddell et al., 1997, 1998; McDonald et al., 1980, 1993). One of the industries to which these milled fibers were shipped was a chrysotile textile plant in South Carolina studied by McDonald et al. (1983a, 1983b) and by Dement et al. (1983, 1994). The textile workers had much lower levels of exposure to asbestos according to both groups of investigators. These workers nonetheless had exceptionally high lung cancer risk: at least one order of magnitude higher than that for the Quebec miners and millers. This textile plant has been cunsidered "an almost pure chrysotile operation" (WHO, 1998), based on observations that "Chrysotile asbestos received from Quebec, British Columbia, and Zimbabwe was the only type of asbestos processed as raw fiber" (Stayner et al., 1997). Several authors have also suggested that the only use of commercial amphibole (amosite and crocidolite) in this textile manufacturing cohort was extremely small quantities of crocidolite yarn generating very little exposure in a solitary location in the plant and only after 1950 (McDonald et al., 1983a). Also, "a very small quantity of amosite (was used) for experimental purposes in the late 1950's" (WHO, 1998). In two other chrysotile textile operations in which similar excesses were recorded, there was also a record of exposure to crocidolite (McDonald et al., 1983b; Peto et al., 198.5).

The first explanation for the divergence between results in these two groups of workers (low lung cancer rates in the more highly exposed miners and millers) was that exposure must have been misclassified: too high an estimate for miners/millers, too low an estimate for textile workers, or both. This was refuted by a previous lung-retained fiber study (Sébastien et al., 1989). Lung samples taken at autopsy from 89 chrysotile miners/millers and 74 chrysotile textile manufacturing workers at the plant in question were compared for lung fiber content for all fiber types, longer than 5 μm. Chrysotile and tremolite concentrations were highest in miners/millers, and fiber dimensions were closely similar in the two groups. Exposure data were also available for each subject, and analyses matched for duration of employment and time from last employment to death demonstrated that ratio of chrysotile fiber concentrations in lungs from miners/millers to those of textile workers were "even

higher than the corresponding ratios of estimated exposure intensity. Non-trivial concentrations of amosite and crocidolite were present in 32% of textile workers' lung samples versus only 9% for miners/millers. The latter finding was reconfirmed by Green et al. (1997), who found amosite and/or crocidolite in the lungs of 28% of a smaller group ($n = 35$) of textile workers from the same plant. Neither group of investigators assigned much significance to this finding, however, as concentrations were low relative to those for chrysotile and tremolite (geometric mean 0.14 fibers of amosite or crocidolite/μg dry lung as compared with 0.63 fibers/μg for chrysotile and 0.38 fibers/μg for tremolite; Sebastien et al., 1989).

The authors speculated that the possible presence of cocarcinogens in the textile plant—specifically mineral oil, sprayed onto asbestos stock to facilitate spinning and reduce breakage-could have played a role. By 1998, none of the suggested explanations (misclassification of exposure, greater fiber length in textile manufacture, mineral oil use) had enough support to explain the huge risk difference, and it was suggested that this important paradox should be investigated further by looking at that fraction of lung-retained fibers longer than 20 μm (McDonald, 1998). This follow-up study addresses that issue.

METHODS

Samples in the current study were derived from those obtained in the previous work (Sébastien et al., 1989). Subject selection, sample collection, and preparation are described in that study. Chrysotile mining subjects were drawn from the Thetford Mines portion of the Quebec cohort as constituted in 1976 (McDonald et al., 1980; Sébastien et al., 1989). Chrysotile textile workers were drawn from the cohort described by McDonald et al. (1983a).

Usable electron-microscopic grids were obtained for 64 of the original 74 textile workers (50 members of the original cohort of McDonald et al. (1983a) as well as 14 women fulfilling the same cohort definition and having full exposure histories available] and for a random selection of 43 of the 89 Thetford Mines male chrysotile miners/millers reported by Sébastien et al. (1989). Lung samples consisted either of paraffin blocks (which were deparaffinized) or formalin-fixed tissue. Samples were chemically digested in fresh, filtered commercial bleach, and the volume of bleach was adjusted to achieve a digest concentration of 1 mg dry weight/ml. Then 15 ml was filtered through a standard Millipore 25-mm, 0.45-μm pore size membrane filter, which was plasma-ashed overnight. Suspended residue was filtered through 25-mm Nucleopore membrane filters (0.2 μm pore size), with final mounting on transmission electron microscopy (TEM) number 200 copper mesh grids using a carbon replica technique.

In the current study, these grids were examined in a JEOL 100CX TEM at 10,000×. For each case, four grids were examined. For each grid, grid openings having an area of 6400 μm^2 were randomly selected for counting of

fibrous particles. From the four grids, up to 90 such openings (total), or 30 fibers (whichever occurred first), were counted. The length and diameter of all fibers were measured to the nearest 0.045 μm (0.5 screen mm) directly on the fluorescent screen, using 2 concentric circles (10 mm and 50 mm screen diameter). To be counted, fibers had to have a minimum length of 18 μm, minimum diameter of ,045 μm, and aspect ratio at least 3:1. Because such long fibers may overlap TEM grid bars, only those overlapping fibers in the upper and right quadrants were counted. Fiber type was identified from both morphological features and energy-dispersive spectrometry (EDS), with selected area electron diffraction where necessary. Over 15 types of fiber were identified, but the current analysis considers only 4 individual asbestos fiber types; other fibers, which were rare, are grouped together as "nonasbestos." For geometric mean fiber concentration calculations, zero values were transformed to one-half the detection limit (0.018 fibers/μg dry lung) prior to log transformation.

RESULTS

Results of the study are summarized in Tables 1–3. Overall, 1186 fibers were counted in this study: 679 in the lungs of 43 chrysotile miners/millers (chrysotile 356, tremolite 286, amosite 22, crocidolite 8, all others 7) and 507 fibers in lung samples from 64 chrysotile textile manufacturing workers (chrysotile 256, tremolite 47, amosite 122, crocidolite 64, all others 39).

In textile workers, geometric mean lung chrysotile fiber concentrations were highest and most closely related to cumulative exposure measured in million particles per cubic foot-years (MPCFY), with a correlation coefficient of .50 (Pearson $r, p < .001$). For miners and millers, exposure was most closely related to tremolite fiber concentration (Pearson $r = .39$; $p < .01$).

Fiber length distributions, which were nonnormal and skewed toward the lowest (18 μm) value, showed increasing percentages of amosite and crocidolite with increasing fiber length in textile workers' lungs but not in those of miners and millers. Overall, in textile workers, of 95 fibers over 30 μm,

TABLE 1. Comparison of the Two Lung Tissue Groups

Study subjects (N)	Miners/millers (43 of 89 reported by Sébastien et al., 1989; all from Thetford Mines)	Textile manufacture (64 of 74 reported by Sébastien et al., 1989)
Median birth year	1913	1915
Median year of hire	1936	1941
Median year termination	1971	1950
Median death year	1978	1972
Mean years worked (SD)	30 ± 16 ($p < .001$)	13 ± 14
Time since end exposure	Median 8 yr ($p = .08$)	Median 20 yr
Geometric mean exposure	186 MPCFY ($p < .001$)	3.63 MPCFY

TABLE 2. Geometric Mean Fiber Concentration/µg Dry Lung and Geometric Mean Fiber Length (Fibers >18 µm; Aspect Ratio >3:1)

	Miners/millers (n = 43)		Textile manufacture (n = 64)	
	Concentration	Fiber length	Concentration	Fiber length
Chrysotile	0.231[a]	22.6 µm	.054	23.3 µm
Tremolite	0.325[b]	21.7 µm	.027	21.9 µm
Amosite/crocidolite	0.024	Amosite 24.0 µm Crocidolite 22.6 µm	.037[b]	Amosite 25.7 µm Crocidolite 24.3 µm
Total amphiboles	0.294[a]	21.8 µm	.053	24.4 µm[a]
Total asbestos	0.728[a]	22.2 µm	.113	23.8 µm
Total nonasbestos	0.036	22.8 µm	.027	23.9 µm
Total all fibers	0.834[a]	22.2 µm	.140	23.8 µm[a]

[a]Significant at $p < .001$ (two-sample test).
[b]Significant at $p < .01$ (two-sample test).

TABLE 3. Geometric Mean Fiber Diameter and Median Aspect Ratio (Fibers >18 µm)

	Miners/millers		Textile manufacture	
Chrysotile	Diameter .08[a]	270:1[a]	Diameter .11	220:1
Tremolite	Diameter .41	53:1	Diameter .46	47:1
Total asbestos	Diameter .16	205:1	Diameter .16	205:1
Total nonasbestos	Diameter .95[a]	21:1	Diameter .63	40:1[b]

[a]Significant at $p < .001$ (two-sample test).
[b]Significant at $p < .01$ (two-sample test).

42 were amosite or crocidolite and 31 chrysotile; of 37 fibers over 40 µm in length, 20 were commercial amphibole and 12 chrysotile; and of the 19 fibers longer than 50 µm, 11 were amosite or crocidolite and 5 chrysotile. In the same length categories in chrysotile miners and millers, 100% of these longest fibers were chrysotile (32 fibers from 30 to 40 µm; 8 from 41 to 50; 2 over 50) or tremolite (12 from 30 to 40 µm, 7 from 41 to 50 µm, and 1 over 50 µm).

DISCUSSION

Lung-retained fiber study is limited in inference, as the results represent only the fraction of internal dose that is retained until death. Further, comparison of groups of individuals using this technique is valid only insofar as those studied are representative of the larger groups (epidemiological cohorts, in this case) from which they are derived. We cannot be certain to what degree our groups of chrysotile miners/millers and textile workers are representative of the cohorts from which they are derived; there is some evidence that miners and millers with lung tissue available may have a higher disease incidence

than the overall mining/milling cohort (Sébastien et al., 1989; Case, 1994).The situation is less certain for the textile workers, although lung cancer deaths in our study subjects (7 of 64 cases) were similar to proportional mortality for the whole cohort (Dement et al., 1983, 1994). In addition, the two groups differ in interval between cessation of work and death, and substantially in cumulative exposure, mean years worked, and year of termination. Each of these differences could work in the direction of underestimating exposure in the textile workers' group. One hypothesis for the excess lung-cancer slope in textile workers, the idea that mineral oils could have played some role, cannot he addressed by the present study and remains to he tested either epidemiologically or experimentally.

Lung-retained fiber analysis is most useful in exposure assessment and in cross-disciplinary validation of that assessment as it has been used in epidemiological protocols. In the current study, the results may help us answer three questions. First, are the findings supportive of those in previous studies, both epidemiological and of lung tissue, in showing greater exposure for the cohort with less lung cancer (miners and millers)?Second, are the findings consistent with a role lor greater fiber length as an explanatory factor for the higher risk slope in textile workers (McDonald, 1998)? Finally. to what degree do the findings support or refute the claim that the textile cohort was exposed to "pure chrysotile" and is therefore an appropriate reference for chrysotile risk assessment (Stayner et al., 1997; WHO, 1998)?

Our results closely parallel those reported by Sébastien et al. in 1989. Any other result would he surprising, since subjects were drawn from the latter study, although the population of fibers examined is not only longer hut of a completely different size range with almost no overlap. Indeeci, direct correlation of filher concentrations in the two studies was very high: For the textile workers the two studies have a Pearson r of .94, .97, and .67 for amosite/crocidolite, chrysotile, and tremolite, respectively (all $p < .000$). We cannot directly compare our results on an individual basis to those of Green et al. (1997), who examined an even shorter iiher length range than that assessed by Sébastien et al. It is nonetheless clear from the two previous studies as well as this one that exposure to chrysotile and tremolite is indeed greater in the miners and millers, although the difference is perhaps less than that which would he suggested by the much larger difference in both cumulative exposure and total years of employment (Table 1).

Our results do not support a role for fiber length alone in the genesis of greater lung cancer risk in textile workers. Direct comparison of fiber concentrations in this analysis of 1186 fibers longer than 18 μm for all ashestos, chrysotile, and tremolite fibers show all of these to he significantly higher in the miners and millers. Furthermore, chrysotile fihers were significantly thinner and aspect ratios were significantly greater in the miners and millers' lungs, again consistent with the previous findings by Sébastien et al. When all fibers are consiclered together (including nonasbestos iiher types), geometric mean, median, and mean iiher length are greater ($p < .001$) in the textile workers'

lungs, even though concentrations of these longest fibers are lower in that group. Total amphiboles also had significantly greater geometric mean fiber length in textile workers, due largely to the excess of the shorter tremolite fibers in the mining population. This nonetheless confirms the suggestion by many investigators that environmental exposures in the textile plant were, at leastoverall and for total amphiboles, to somewhat longer fibers. Unless there is a very precise and specific "critical length" for lung-cancer genesis (rather than the total length of all long fibers combined), fiber length alone must he questioned as the explanation for the high lung-cancer risk excess in this group of textile workers.

Finally, as in two previous studies, we found that the "chrysotile only" textile workers had a high proportion of individuals with lung tissue containing amosite and/or crocidolite. Sebastien et al. had noted that these fibers were present in "nontrivial" concentrations in 32% of workers examined. Green et al. found commercial amphibole in 28% of 35 textile workers' lungs examined. Our findings are similar for the proportion of workers exposed: 19 of 50 males and 3 of 14 females, and 32% of all fibers counted in the lungs of textile workers were amosite or crocidolite. Finally, 12 of the 22 textile workers having commercial amphibole in their lungs stopped working between 1938 and 1947, long before any such documented exposure in the textile plant. For 15 of the 22, amosite and crocidolite formed the majority of all fibers present. This subset of the Charleston textile workers does not support the hypothesis that this is a "pure chrysotile" cohort (WHO, 1998). More generally, the exposure experience of textile workers is clearly unique and should not be used to assess risk of lung cancer in miners, cement workers, or friction product workers, regardless of fiber type.

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