Fiber Size and Number in Amphibole Asbestos-Induced Mesothelioma

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Numbers and sizes of fibers from the lungs of 10 patients who had an amphibole asbestos-induced malignant pleural mesothelioma were analyzed. Amosite was found in 10 lungs and crocidolite in 9; the average ratio of amosite to crocidolite was approximately 14:1. In the 8 patients who were not long-time asbestos insulators, the mean number of amosite fibers was 2.3 × 10^6 fibers/g dry lung, and of crocidolite fibers, 0.2 × 10^6/g; these values represent an approximately 250-fold increase over those found in the general population. Crocidolite fibers were significantly narrower than amosite fibers (mean width, 0.13 versus 0.23 μ), were significantly shorter (mean length, 4.0 versus 5.8 μ), and had a significantly higher mean aspect (length to width) ratio (48 versus 34). Aspect ratios in general increased with increasing fiber length and decreasing fiber width, but the highest values were found for thin amosite fibers at about 13 μ in length, and thin crocidolite fibers at 8 or 15–17 μ in length. Comparison with data from other asbestos-exposed populations indicates that mesothelioma can be induced by relatively small numbers of amphibole fibers and also indicates that amosite is an effective mesothelial carcinogen in humans. Comparison of these data with epidemiologic and experimental predictions of carcinogenic size ranges for mesothelioma induction implies that either the carcinogenic size range is much broader than has been claimed (in particular, fibers considerably shorter than 8 μ and broader than 0.05 μ can produce mesothelioma), or, alternately, that extraordinarily small absolute numbers of fibers in certain size ranges can induce tumors in humans. (Am J Pathol 1984, 115: 437–442)

ALTHOUGH the association of mesothelioma and exposure to asbestos is well documented, there is considerable controversy regarding the relative carcinogenic effects of different types of asbestos fibers. Amphibole fibers are probably more carcinogenic than chrysotile fibers, and there is some suggestion that crocidolite is worse than amosite in this regard.[1–7] Animal studies imply that not only numbers of fibers but sizes and shapes of fibers are critical in the genesis of mesothelioma,[8] and such studies, combined with measurements of mine dust size and epidemiologic associations, have led to predictions of carcinogenic size ranges in human.[9,10] In this paper we examine numbers and sizes of fiber in lung in a series of amphibole asbestos-associated human mesothelioma cases.

Materials and Methods

All cases of pleural mesothelioma in our files were reviewed, and 10 cases were selected for which 1) histologic review confirmed the diagnosis of pleural mesothelioma; 2) initial analysis had shown only increased (compared with the general population[11]) numbers of amosite and/or crocidolite fibers in lung (cases with increased numbers of chrysotile fibers—and increased numbers of tremolite, actinolite, or anthophyllite fibers which accompany chrysotile as part of the ore—were excluded); 3) A piece of formalin-fixed wet lung tissue weighing at least 5 g was available for mineralogic analysis. All patients had worked in the Pacific Northwest (Oregon, Washington, British Columbia).

For each case in the study, a 3–5-g (wet weight)

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Table 1—Effects of Preparatory Procedures on Fiber Lengths*

	Size range (μ)				
	<1	1-3	3-5	5-10	>10
Treated crocidolite	1	23	31	30	15
Untreated crocidolite	1	47	20	20	12
Treated amosite	0	14	16	42	28
Untreated amosite	0	15	18	33	34

* Values are percentages of fibers in each size range.

piece of lung was taken from a 0.5-cm deep subpleural strip of lung, and a separate piece was taken at a distance of 3 cm or more from the pleura. For 2 cases, sufficient tissue was available for only 1 sample. We dried a portion of each sample to a constant weight at 60 C to allow expression of the final results as fibers per gram dry lung; the remaining tissue was dissolved in bleach, and the sediment was treated with hydrogen peroxide to further destroy organic matter, as previously described.[11] The residual inorganic material was collected on a Millipore filter with a 0.45-μ pore size, and the particulates were transferred from the filter to coated electron microscope grids. Details of the procedure can be found in Churg and Warnock.[11]

For each sample site, approximately 50 amphibole fibers were measured and identified with the use of electron diffraction and energy-dispersive X-ray spectroscopy. UICC Standard Reference Asbestos Samples of amosite and crocidolite were used for reference identification purposes. Using an algorithm relating numbers of grid squares examined in the electron microscope and weight of sample, values were converted to fibers per gram dry lung. Because the total number of crocidolite fibers found was relatively small (see Results), no attempt was made to report central and peripheral samples separately, and the values given in this paper represent the average of the two sites.

To investigate the possible effects of the preparatory treatment on fiber size, a sample of the UICC Standard Reference Amosite and another sample of the Standard Reference Crocidolite were prepared in distilled water. One aliquot of each was allowed to dry on a coated electron microscope grid, and another aliquot was run through the bleach/hydrogen peroxide procedure used for tissue. One hundred sequentially encountered fibers were measured for each sample.

For evaluation of any possible contamination of samples from fibers in air, water, or other laboratory specimens, a blank sample, which was prepared by running the entire procedure without tissue, was created and examined for each case. No amosite or crocidolite fibers were found in any blank.

Results

Fiber Size Changes Induced by the Preparatory Procedure

Table 1 shows a comparison of the fiber sizes for each reference sample with and without the bleach/peroxide treatment. Statistically, there is no difference in the length distribution with or without treatment, indicating that our preparative procedure does not artifactually alter fiber length (χ^2 for amosite groups = 2.99, P = NS; χ^2 for crocidolite groups = 7.46 P = NS).

Fiber Number

Table 2 shows patient ages, sex, and exposures as well as numbers of fibers of amosite and crocidolite per gram dry lung. Amosite was found in all 10 cases, with values ranging from 0.1×10^6 to 75.7×10^6 fibers/g dry lung; crocidolite was found in 9 of 10 cases, with values ranging from 0.1 to 17.2×10^6 fibers/g dry lung. Except for 1 case, amosite fibers

Table 2—Patient Data and Numbers of Fibers*

Case	Age/sex	Exposure	Amosite	Crocidolite	A/C ratio†
1	74/M	Shipyard 1941–1945	0.1	0	
2	55/F	Shipyard 1943–1945	3.7	0.1	37.0
3	70/M	Asbestos insulator 1938-1975	75.7	9.5	8.0
4	57/M	Shipyard 1946–1948	6.1	0.2	30.5
5	48/M	Shipyard 1951–1953	1.6	0.7	2.3
6	64/M	Office worker, shipyard, 1950–1980	0.4	0.1	4.0
7	66/M	Asbestos siding installation, 1937–1945	4.9	0.4	12.2
8	66/M	Asbestos insulator 1945-1980	0.7	17.2	0.04
9	80/M	Sheet metal worker, shipyards, 1942-1965	3.0	0.2	15.0
10	53/M	Sheet metal worker, shipyards, 17 years	2.3	0.1	23.0
Mean			9.9	2.9	14.3

* All fibers $\times 10^6$/g dry lung.
† Ratio of amosite to crocidolite.

Table 3 – Mean Sizes for All Fibers

	Amosite	Crocidolite
Total number of fibers examined	766	133
Mean length ± SD (μ)	5.8 ± 6.0	4.0 ± 4.3
Mean width ± SD (μ)	0.23 ± 0.18	0.13 ± 0.13
Mean aspect ratio ± SD	34 ± 39	48 ± 55
Geometric mean length (μ)	3.9	2.8
Geometric mean width (μ)	0.19	0.10

were always present in considerably greater number than crocidolite fibers.

Fiber Size and Aspect Ratio

The actual number of fibers of amosite examined was 766; of crocidolite, 133. The mean sizes and aspect (length to width) ratios are shown in Table 3. The mean amosite fiber length, 5.8 μ, is longer than the mean crocidolite length, 4.0 μ ($T = 3.31$, $P < 0.001$), while the mean fiber width is slightly narrower for crocidolite – 0.13 μ – than for amosite – 0.23 μ ($T = 6.13$, $P < 0.001$). The mean aspect ratio for crocidolite – 48 – is higher than that for amosite – 34 ($T = 3.57$, $P < 0.001$). Because fiber size distribution is frequently log-normal, we also transformed the values into logarithms and repeated the statistics; length and width differences were even more significant when this was done. For the same reason, we also calculated the geometric mean sizes: the geometric mean lengths were 3.9 μ for amosite and 2.8 μ for crocidolite, while the geometric mean widths were 0.19 μ for amosite and 0.10 μ for crocidolite.

Table 4 shows the length and width distributions in defined size ranges. Statistical examination of the original data used in compiling Table 4 shows that the major length differences are for fibers with lengths <1 μ and in the 2–3-μ range, which contain proportionately more crocidolite, and the >5-μ range, which contains proportionately more amosite. Similarly, there are statistically greater numbers of crocidolite fibers in the sizes ranges below 0.1 μ and greater numbers of amosite fibers above 0.2 μ.

The effects of these size differences are seen more clearly in Table 5, which shows cumulative length and width distributions within these ranges. Fifty-five percent of the crocidolite fibers were shorter than 3 μ, compared with 38% of amosite fibers; 77% were shorter than 5 μ, compared with 60% of amosite fibers; and 94% were shorter than 10 μ, compared with 88% of amosite fibers. Similarly, 55% of the crocidolite fibers were less than 0.1 μ in width, compared with 21% of the amosite fibers.

The effects of length and width on the aspect ratio are illustrated in Figures 1 and 2. These figures show that the aspect ratio tends to decrease as a function of width and to increase as a function of length, up to a point. The maximum value for the aspect ratio occurs at about the 13-μ length for amosite, whereas at least two major peaks are seen for crocidolite at about 8 μ and at about 15–17 μ. Table 6 shows the cumulative percentage of fibers of greater than a given aspect ratio; there are proportionately more crocidolite than amosite fibers for any chosen value.

Table 7 shows the percentages of amosite and crocidolite fibers falling into specified length and width ranges; in general, there are proportionately more amosite fibers in the broader width and greater length categories. These data are considered further below.

Discussion

The data presented above can be considered in terms of fiber size and fiber number. Hypotheses concerning carcinogenic sizes ranges for mesothelioma in humans have been based largely on the animal work of Stanton and colleagues[8] and on estimates of size from samples of fibers which do or do not cause mesothelioma in humans.[9,10] Stanton and co-workers[8] demonstrated that many types of mineral fibers can produce mesotheliomas in animals if they are 1) insoluble, and 2) sufficiently small and long. In their experiments, fibers of a length greater than 8 μ and width less than 0.25 μ (and hence with an aspect ratio

Table 4 – Fiber Length and Width Distributions*

	Amosite	Crocidolite
Length (μ)		
<1	5.6	12.8
1–2	22.0	25.6
2–3	10.8	16.5
3–4	13.0	12.0
4–5	9.0	10.5
5–7.5	8.7	6.8
7.5–10	18.9	9.8
10–15	7.2	3.8
15–20	2.3	1.5
20–25	1.0	0
25–50	0.9	0.8
>50	0.3	0
Width (μ)		
<0.02	0	0
0.02–0.03	0.6	6.0
0.03–0.04	0.9	1.5
0.04–0.05	2.2	6.7
0.05–0.1	17.6	40.6
0.1–0.2	34.9	33.1
0.2–0.3	15.9	6.0
0.3–0.4	12.1	3.8
0.4–0.5	9.0	0.8
0.5–0.6	3.3	0
>0.6	3.9	1.5

* Values are percentages of all fibers of a given type.

Table 5 – Cumulative Length and Width Distributions*

	Amosite	Crocidolite
Length (μ)		
1	5.6	12.8
2	27.6	38.4
3	38.4	54.9
4	51.4	66.9
5	60.4	77.4
7.5	69.1	84.2
10	88.0	94.0
15	95.2	97.8
20	97.5	99.3
25	98.5	99.3
50	99.4	100.1
Width (μ)		
0.02	0	0
0.03	0.6	6.0
0.04	1.5	7.5
0.05	3.3	14.3
0.1	20.9	55.1
0.2	55.8	88.2
0.3	71.7	94.2
0.4	83.8	98.0
0.5	92.8	98.8
0.6	96.1	98.8

* Values are cumulative percentages for all fibers of a given type.

of 32 or greater) appeared to be the most carcinogenic, regardless of mineral type; lesser degrees of carcinogenicity were seen with shorter or wider fibers.

As noted by Harington,[9] these experiments are highly artificial because large numbers of fibers are actually implanted in the pleural or peritoneal cavities, as opposed to the situation in man, where (presumably) smaller numbers and perhaps different sizes of inhaled or ingested fibers manage to reach the same locations. Harington made an estimate of the carcinogenic diameters of asbestos fibers using measured dimensions of fibers from an anthophyllite mine and two different crocidolite mines. The two types of fibers (anthophyllite and Transvaal crocidolite) which do not appear to produce mesotheliomas in humans were relatively wide, with only about 1% of the total narrower than 0.1 μ. By comparison, it was estimated that 60% of the Northwest Cape crocidolite (an effective inducer of mesotheliomas) fibers were narrower than 0.1 μ. Harington suggested that the carcinogenic range for humans is narrower than this, probably less than 0.05 μ in diameter.

Similar considerations of fiber lengths led him to conclude that the carcinogenic length range must be shorter than 8 μ, probably in the range of 3–5 μ. Wagner[10] has taken this hypothesis further and proposed that fibers narrower than 0.5 μ and 5–30 μ in length tend to induce mesotheliomas, while fibers narrower than 2 μ and 10–50 μ in length induce carcinomas.

The data presented in this report confirm and contradict some of these hypotheses. There are distinct size differences between amosite and crocidolite fibers recovered from human lung. Crocidolite fibers are on average shorter, with more than half the crocidolite fibers shorter than 3 μ and three-quarters shorter than 5 μ, as opposed to corresponding values of 38% and 60% for amosite. Crocidolite fibers are also thinner: almost 90% of crocidolite fibers are narrower than 0.2 μ, and 55% are narrower than 0.1 μ (a value close to Harington's estimate of 60%, cited above), compared with values of 56% less than 0.2 μ and 21% less than 0.1 μ for amosite.

It has generally been surmised, in large measure because of the work of Stanton et al,[8] that fibers with a higher aspect ratio are more dangerous than fibers with a lower aspect ratio, and that the highest aspect ratios are seen with the longest fibers. Figures 1 and 2 make it clear that the question of the aspect ratio is complex. Overall, the crocidolite fibers have a higher mean aspect ratio than the amosite fibers; and, as a general trend, the aspect ratio increases for fibers of both types as width decreases and length increases. However, as illustrated in Figures 1 and 2, very long fibers actually have lower aspect ratios than shorter fibers; amosite fibers reach peak aspect ratios in the 10–17-μ range of length and <0.2-μ width; whereas peak crocidolite ratios occur at the same widths but at lengths of around 8 μ and then again at lengths between approximately 15 and 17 μ. There is also considerable variation in the rate at which aspect ratios decrease as width increases for any given length. If,

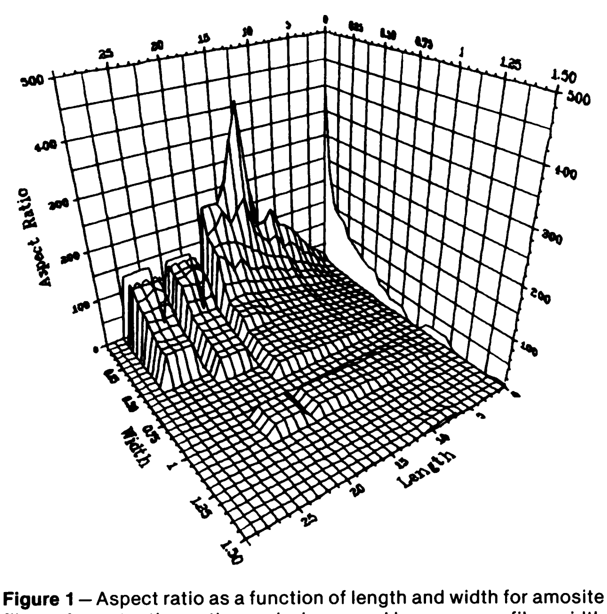

Figure 1 – Aspect ratio as a function of length and width for amosite fibers. Aspect ratio, on the z axis, in general increases as fiber width decreases and as fiber length increases, but very long fibers actually have lower aspect ratios than shorter ones.

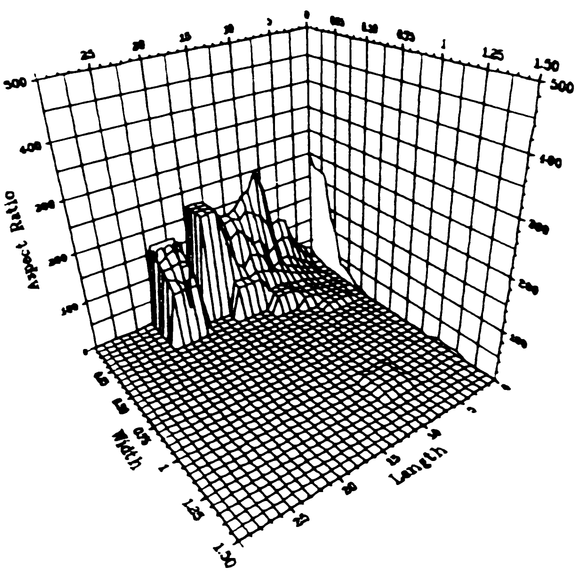

Figure 2 – Aspect ratio as a function of length and width for crocidolite fibers. The general trend of increase of the aspect ratio is similar to that for amosite.

therefore, any given specific aspect ratio (for example, Stanton's value of 32) is an absolute or relative requirement for carcinogenicity in this setting, a variety of length and width combinations might be found that produce fibers of the chosen aspect ratio. Hence, long fibers are not necessarily more carcinogenic than short ones; thin fibers are not necessarily more carcinogenic than wide ones; and, on the basis of aspect ratio, the carcinogenic size range could be very broad indeed.

It is unlikely from our data that width less than 0.05 μ is a good estimate of carcinogenic size for mesothelioma. Much of the argument advanced by Harington[9] depends on the presence of such a large total dose that the absolute number of fibers within the range of less than 0.05 μ is quite high. But only 3.3% of our amosite fibers and 14% of the crocidolite fibers fell into this size range. Combining these values with the rather modest total numbers (Table 1) of fibers seen in most of these cases, a size cutoff of 0.05 μ implies induction of mesotheliomas by an extraordinarily small number of fibers. A similar conclusion holds for fibers longer than 8 μ and narrower than 0.25 μ (ie, the size range suggested by Stanton et al[8]). Only 11% of amosite and 7.5% of crocidolite fibers fall into this size range (Table 7); and if this is the critical size cutoff, then again, very small number of fibers produce disease.

Although it is not strictly provable from out data, it appears more likely that both shorter and broader fibers are important in the genesis of these tumors; this conclusion is consistent with Stanton's observation that fibers of a width up to 1.5 μ and length greater than 4 μ also produce mesotheliomas, although not with the efficiency of longer and thinner fibers.[8] Determination of whether the size differences between amosite and crocidolite are important in humans will require epidemiologic studies showing clear-cut differences in carcinogenic potential between these two fibers. However, our data do indirectly confirm the importance of fiber size in mesothelial carcinogenesis. Mesothelioma is rare in chrysotile miners, despite exposure to large amounts of asbestos dust. In a group of chrysotile miners who had no parenchymal lung disease and no mesotheliomas, the mean number of tremolite, anthophyllite, and actinolite fibers found was 58 × 10^6/g dry lung.[12] These amphibole fibers, which are natural components of the chrysotile ore, had a mean length of 2.3 μ, a mean width of 0.23 μ, and a mean aspect ratio of only 14.

The data on total numbers of fibers in our study produce two important conclusions. One is that mesothelioma is found in patients with a wide range of pulmonary asbestos loads, many of which are relatively modest. Table 8 compares the data from the

Table 6 – Selected Aspect Ratios for All Fibers*

Aspect ratio greater than	Amosite	Crocidolite
10	72.9	78.9
25	45.0	51.8
50	20.6	27.8
75	11.3	20.3
100	6.1	12.8

* Values are percentages of all fibers of a given type.

Table 7 – Selected Size Distributions for All Fibers*

Length greater than (μ)	Amosite	Crocidolite
Width <0.25 μ		
2	50.0	59.3
4	31.6	30.8
6	18.8	14.2
8	11.3	7.5
10	5.7	5.2
Width <0.1 μ		
2	8.7	24.0
4	5.0	14.2
6	2.3	6.8
8	1.4	3.8
10	0.6	2.2
Width <0.05 μ		
2	2.6	10.5
4	1.1	6.0
6	0.2	2.2
8	0.1	0.8
10	0	0

* Values are percentages of all fibers of a given type.

Table 8 – Numbers of Amosite and Crocidolite Fibers in Different Reports and Populations*

Group	No cases	Amosite	Crocidolite	Ratio†
General population[13]	25	0.01‡		
Pleural plaques[13]	29	0.26	0.24	50
Present report	10	9.9	2.9	1280
Same group minus 2 insulators	8	2.3	0.2	250

* Mean values, $\times 10^6$/g dry lung.
† Ratio of total amosite plus crocidolite to values found in the general population.
‡ Combined amosite and crocidolite.

present report with that from previous studies from our laboratory of patients with only "background" nonoccupational exposure and patients with pleural plaques.[13] If one compares the 8 workers in the present report who were not long-term insulators, the mean value for amosite and crocidolite (total 2.5×10^6 fibers/g) is increased only about 250-fold over the values seen in a nonexposed background population.[11,13] Similar conclusions can be drawn from studies of mesothelioma cases performed in other laboratories, although comparison is sometimes difficult because of differences in sampling, technique, and method of expressing results. The study of Wagner et al[14] on mesothelioma cases in the United Kingdom demonstrated that these cases had on average about 100 times more amphibole (amosite and crocidolite) than controls from a general population. A study of mesothelioma cases from a textile factory[15] showed that crocidolite content ranged from 0.6 to 335×10^6 fibers/g dry lung, values which are reasonably similar to ours. McDonald et al also found widely varying fiber content in North American cases.[16] By contrast, the report of Ashcroft and Heppleston on workers with asbestosis[17] suggested that this process was seen with fiber burdens greater than 100×10^6 fibers/g. Presumably the relatively small fiber load seen in some cases reflects a relatively small exposure, a notion which would be in accord with epidemiologic studies of mesothelioma.

The second conclusion is that our data confirm the carcinogenic potential of amosite, as suggested by recent epidemiologic and mineralogic studies of mesothelioma in North America.[4,7,16] In our cases, the average amosite/crocidolite ratio is 14:1 (Table 2); unless one again postulates that very small numbers of crocidolite fibers are disproportionately dangerous, amosite must be a relatively effective pleural carcinogen. Conversely, comparison of the numbers of fibers of amphibole asbestos seen in our patients with mesothelioma with the much larger numbers of chrysotile and tremolite fibers found in chrysotile miners without asbestos-induced disease[12] tends to confirm the rather weak carcinogenic potential (for inducing mesothelioma) of chrysotile and tremolite in humans.

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