Asbestos Fibers Contributing to the Induction of Human Malignant Mesothelioma

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ABSTRACT: To elucidate the features of the asbestos fibers contributing to the induction of human malignant mesothelioma, we used high-resolution analytical electron microscopy to determine the type, number, and dimensions of asbestos fibers in lung and mesothelial tissues in 168 cases of mesothelioma. Results: 1. Asbestos fibers were present in almost all of the lung and mesothelial tissues from the mesothelioma cases. 2. The most common types of asbestos fibers in lung were either an admixture of chrysotile with amphiboles, amphibole alone, and occasionally chrysotile alone. In mesothelial tissues, most asbestos fibers were chrysotile. 3. In lung, amosite fibers were greatest in number followed by chrysotile, crocidolite, tremolite/actinolite, and anthophyllite. In mesothelial tissues, chrysotile fibers were 30.3 times more common than amphiboles. 4. In some mesothelioma cases, the only asbestos fibers detected in either lung or mesothelial tissue were chrysotile fibers. 5. The average number of asbestos fibers in both lung and mesothelial tissues was two orders of magnitude greater than the number found in the general population. 6. The majority of asbestos fibers in lung and mesothelial tissues were shorter than 5 µm in length. Conclusions: 1) Fiber analysis of both lung and mesothelial tissues must be done to determine the types of asbestos fibers associated with the induction of human malignant mesothelioma; 2) short, thin asbestos fibers should be included in the list of fiber types contributing to the induction of human malignant mesothelioma; 3) Results support the induction of human malignant mesothelioma by chrysotile.

KEYWORDS: asbestos, fibers, mesothelioma, chrysotile, amphiboles

INTRODUCTION

It is well accepted that asbestos fibers are the cause of virtually all cases of human malignant mesothelioma.[1–4] It is also known that all asbestos types, including chrysotile and amphiboles, have been shown in epidemiological and toxicological studies to be fully capable of inducing the tumor.[5–9] In addition to heavy (occupational) asbestos exposure, milder asbestos exposure (bystanders and family contact) can also induce the tumor.[10–12] Presently, no data are available to support a threshold limit for exposure to asbestos below which there is no risk of malignant mesothelioma.[4]

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Human malignant mesothelioma develops after a long latency period: it takes fifteen years or more from the first asbestos exposure to death from malignant mesothelioma. Latency periods greater than 40 years have been reported.[2–5] There is no cure for human malignant mesothelioma.

Asbestos fibers are durable in nature and are not easily digested or dissolved by either phagocytic cells or the tissue fluid. Asbestos fibers are identified as asbestos bodies by light microscope or as naked asbestos fibers by an electron microscope.

Some inhaled asbestos fibers translocate from lung into regional lymph nodes,[13–15] pleural and peritoneal mesothelial tissues,[16–24] and other organs.[13,25] Fibers may pass from lung to other organs by direct migration[26,27] via lymphatic capillary system,[13–15,19] and by hematogenous spread.[28,29]

Up to now, most investigators have focused exclusively on asbestos fibers in the lung for identification of asbestos fibers that contribute to induction of human malignant mesothelioma.[30–34] We questioned the adequacy of such an approach because the primary site of malignant mesothelioma is not the lung but the mesothelial tissue and because the type and number of asbestos fibers in lung may not be identical to those in mesothelial tissue owing to possible translocation of lung fibers to other organs including mesothelial tissues.

Short, thin asbestos fibers, i.e., 0.06 µm long and 0.02–0.03 µm wide, can easily be identified by the high resolution analytical electron microscope, a transmission electron microscope with an energy dispersive X-ray spectrometer, but not by scanning electron microscope with resolution of 0.3 to 0.4 µm. It is noteworthy that the number and dimensions of asbestos fibers obtained from a high resolution analytical electron microscope will be different from those obtained by a scanning electron microscope.

It has been proposed on the basis of animal studies that long (greater than 8 µm in length) and thin (less than 0.25 µm in width) mineral fibers were strongly carcinogenic for the induction of malignant mesothelioma in rats (Stanton's hypothesis)[35] and that shorter fibers pose less risk. Stanton's hypothetical dimensions were derived from his experimental studies using direct administration of heavy doses of various mineral fibers of different dimensions into rats pleural cavities. Stanton stated that direct application of his results to the problem in man would be unwise.[36] However his hypothetical model of asbestos fibers' relative carcinogenicity has been directly applied to the counting of the asbestos fibers in man.

To evaluate airborne fibrous dusts in industrial atmospheres, the current Occupational Safety and Health Administration (OSHA) method by light microscopy (phase microscopy) counts only those asbestos fibers that are longer than 5 µm with an aspect ratio larger than 3 to 1, assuming that fibers shorter than 5 µm are not carcinogenic. Even using the electron microscopic level, using the same assumption, some investigators have neglected to count short asbestos fibers (≤ 5 µm).[37–40]

Our previous tissue burden studies[22] using high resolution analytical electron microscopy revealed that the majority of asbestos fibers from human lung and mesothelial tissues of human mesothelioma patients were less than 5 µm long (81.4%) and less than 0.25 µm in diameter. The narrow width of asbestos fibers (≤ 0.25 µm in diameter, another parameter in Stanton's hypothesis) has been emphasized as an important parameter not only for the fibers' carcinogenicity, but also for the fibers' ability to penetrate into the peripheral part of the lung by an aerodynamic mechanism.[35,36,41–46] Both OSHA's method using light microscopy and some asbestos tis-

sue burden studies using electron microscopy did not pay serious attention to fiber diameter when counting of asbestos fibers. It was noteworthy that our study[22] revealed that only 4% of all asbestos fibers detected in the lung and mesothelial tissues from mesothelioma patients fit Stanton's criteria.

An asbestos tissue burden study is an effective approach to clarify whether chrysotile fibers are capable of inducing human malignant mesothelioma. If the asbestos fiber type seen in both the lung and mesothelial tissues is solely chrysotile, such mesothelioma cases can be considered to have been caused by chrysotile exposure. Indeed, such cases have been reported elsewhere.[21,22,47]

Our objective in this study was to characterize the features of the asbestos fibers contributing to the induction of human malignant mesothelioma. To achieve this goal, the type, number, and dimensions of the asbestos fibers in both lung and mesothelial tissues taken from human malignant mesothelioma cases were investigated.

MATERIALS AND METHODS

Both lung and mesothelial tissues (the mesotheliomatous and/or fibroplastic serosal tissues) from 168 cases of human malignant mesothelioma (164 males and 4 females; 156 pleural and 12 peritoneal; definite or probable diagnostic certainty) were used. The mesotheliomatous tissue was selected from the primary serosal tumor where the tumor was intimately associated with fibrosis and/or hyaline plaque. Asbestos fibers were studied in both the lung and mesothelial tissues in 74 of the 168 cases, exclusively in lung in 45 of the 168 cases, and exclusively in mesothelial tissue in the remaining 49 cases.

Patients' occupational history was diverse and included asbestos insulators, pipe fitters, electricians, shipyard workers, U.S. Navy servicemen, sheet metal workers, power plant workers, boiler men, brake lining mechanics, fire fighters, family members of asbestos workers, etc.

To prepare electron microscopic specimens, bulk tissues were digested using bleach or KOH solution, or a low temperature ashing technique of 25 μm thick section, or both were used. Details of these techniques have been reported elsewhere.[18,47–49]

A high-resolution analytical electron microscope (JEOL 100CX equipped with an EDX spectrometer) was used for the identification and characterization of asbestos fibers in these tissues; ultrastructure, energy dispersive X-ray spectrophotometry and, in a limited number of cases, selected area electron diffraction were utilized. Asbestos fibers were measured in printed electron micrographs, and those with an aspect ratio of 3:1 and greater were counted even if they were shorter than 1 μm in length.

RESULTS

1. Types of asbestos fibers in lung and mesothelial tissues in the 168 malignant mesothelioma cases studied are outlined below.

TABLE 1. Type of asbestos fibers in lung and mesothelial tissues in 168 malignant mesothelioma cases

A. Asbestos tissue burden study was performed in both the lung and mesothelial tissues: 74 of 168 cases

Lung tissue	Mesothelial tissue	No. of cases
C + A	C	19
C	C	18
A	C	16
C + A	C + A	9
A	C + A	4
A	–	3
–	C	2
C	C + A	2
A	A	1
		Total 74

B. Asbestos tissue burden study was performed in the lung tissues alone: 45 of 168 cases

Lung tissue	No. of cases
A	19
C + A	15
C	11
–	0
	Total 45

C. Asbestos tissue burden study was performed in mesothelial tissues alone: 49 of 168 cases

Mesothelial tissue	No. of cases
C	35
C + A	7
–	6
A	1
	Total 49

C = chrysotile; A = amphibole(s); C + A = chrysotile and amphibole(s); – = not detected.

A. In 74 of the 168 cases, asbestos fiber analysis was performed in both the lung and mesothelial tissues, using digested bulk samples, ashed sections or both. Results are summarized in TABLE 1A.

1) Types of asbestos fibers detected in the lung were quite often different from those in the mesothelial tissue. The combination of asbestos type between the lung and mesothelial tissues was as follow:

(i) chrysotile plus amphibole(s) in lung, and chrysotile alone in mesothelial tissues: 19/74 (25.7%);

(ii) chrysotile in lung, and chrysotile in mesothelial tissues: 18/74 (24.3%);

(iii) amphibole(s) in lung, and chrysotile in mesothelial tissues: 16/74 (21.6%);

(iv) chrysotile plus amphibole(s) in lung, and chrysotile plus amphibole(s) in mesothelial tissues: 9/74 (12.2%);
(v) amphibole(s) in lung, and chrysotile plus amphibole(s) in mesothelial tissues: 4/74 (5.4%);
(vi) amphibole in lung, and no asbestos fibers in mesothelial tissues: 3/74 (4.0%);
(vii) no asbestos fibers in lung, and chrysotile in mesothelial tissues: 2/74 (2.7%);
(viii) chrysotile in lung, and chrysotile plus amphibole(s) in mesothelial tissues: 2/74 (2.7%); and
(ix) amphibole in lung, and amphibole in mesothelial tissues: 1/74 (1.4%).

In summary, a disproportion of the type of asbestos fibers between the two tissues was seen in the majority (46 of 74; 62.2%) of cases.

2) Asbestos types identified in lung were chrysotile (49/74; 66.2%) and amosite (49/74; 66.2%), followed by tremolite (15/74; 20.3%), crocidolite (13/74; 17.6%) and anthophyllite (12/74; 16.4%).

3) Chrysotile was the most common asbestos type detected in mesothelial tissues. It was present in 70 of the 74 cases (94.6%); chrysotile was exclusively detected in 55 of the 74 cases (74.3%).

4) When chrysotile was exclusively seen in the lung, the asbestos type detected in mesothelial tissues was also exclusively chrysotile in 18 of 20 cases (90%).

5) When amphibole(s) was exclusively found in lung, the asbestos type(s) in mesothelial tissues was exclusively amphibole(s), although this occurred rarely (1/24; 4.2%). Other asbestos types found in mesothelial tissues were chrysotile alone (16/24; 66.7%), chrysotile plus amphibole(s) (4/24; 16.7%), and none (3/24;12.5%).

B. In 45 of the 168 cases, an asbestos tissue burden study was carried out exclusively in the lung using digested bulk samples, ashed tissue section, or both. Results are summarized in TABLE 1B.

1) Asbestos types detected in lung of the 45 cases varied. They were amphibole(s) alone (19/45; 42.2%) followed by chrysotile plus amphibole(s) (15/45; 33.3%), and chrysotile only (11/45; 24.5%).

2) The subtype of amphiboles in the lung of 34 of the 45 cases was amosite alone (18/34; 52.9%), followed by amosite plus tremolite/actinolite (5/34; 14.7%), crocidolite alone (4/34; 11.8%), tremolite alone (2/34; 5.9%), amosite plus crocidolite (2/34; 5.9%), amosite plus crocidolite plus anthophyllite (2/34; 5.9%), and amosite plus anthophyllite (1/34; 2.9%).

C. In 49 of the 168 cases, an asbestos tissue burden study was done on mesothelial tissues only, using digested bulk samples, ashed sections, or both. Results are summarized in TABLE 1C.

1) Again, chrysotile fibers were the major asbestos type detected in mesothelial tissues.

2) Asbestos types seen in mesothelial tissues were chrysotile alone (35/49; 71.4%) followed by chrysotile with amphibole (7/49; 14.3%), no asbestos fibers detected (6/49; 12.3%), and amphibole alone (1/49; 2.0%).

Findings presented in 1, A, B and C are summarized as follows.

(1) Asbestos fibers were present in almost all of the lung tissue (117/119; 98.3%) as well as in the mesothelial tissue (114/123; 92.7%).

TABLE 2. Type and number of asbestos fibers in lung parenchyma, pleural plaque, and mesothelomatous tissues among 22 cases of mesothelioma

Case #	Occupation	Site	Disease	Chry	Amos	Croc	Anth	Tr/Ac	DL	Total #
1	insulation	L	pl meso	28.3	125	<DL	2.83	<DL	2.83	156.1
		P		12.1	1.29	<DL	<DL	<DL	0.16	13.4
2	insulation	L	pl meso	28.6	194	<DL	3	3	1.5	228.6
		P		39.2	0.6	<DL	<DL	<DL	0.6	39.8
		T		62.1	<DL	<DL	<DL	<DL	1.27	62.1
3	insulation	L	pe meso	24	139	7.37	<DL	11.4	1.26	181.8
		P		36.3	6.34	<DL	<DL	<DL	0.58	42.6
		T		14.8	<DL	<DL	<DL	<DL	0.76	14.8
4	insulation	L	pe meso	111	282	25.6	4.3	<DL	2.13	422.9
		P		31.8	6.81	<DL	<DL	<DL	0.76	38.6
		T		16.5	0.52	<DL	<DL	<DL	0.17	17
5	insulation	L	pe meso	25.5	120	<DL	<DL	<DL	0.77	145.5
		P		29.4	1.8	<DL	<DL	<DL	0.6	31.2
		T		12.6	1.76	<DL	<DL	<DL	0.44	14.4
6	insulation	L	pe meso	91.9	213	86.4	<DL	3.68	1.84	395
		T		50.1	1.79	<DL	<DL	<DL	0.6	51.9
		T		43.7	<DL	<DL	<DL	<DL	0.48	43.7
7	insulation	L	pe meso	18.8	415	11.3	<DL	11.3	3.75	456.44
		T		90	14	<DL	<DL	<DL	1.42	104
8	insulation	L	pe meso	1.5	7.1	<DL	<DL	<DL	0.29	8.5
		T/P		17	<DL	<DL	<DL	<DL	0.26	17
9	engineer	L	pl meso	<DL	2.5	0.53	<DL	<DL	0.18	3
		T		22.5	<DL	0.22	0.22	<DL	0.22	22.94
10	aircraft inspector	L	pl meso	61	<DL	<DL	<DL	0.7	0.35	61.7
		T		120	<DL	<DL	<DL	<DL	0.35	120
11	power plant	L	pl meso	<DL	47	<DL	<DL	<DL	2.9	47
		T		240	<DL	<DL	<DL	<DL	2.9	240
12	shipyard/ power plant	L	pl meso	<DL	2.6	<DL	<DL	<DL	0.22	2.6
		T		51.3	<DL	<DL	<DL	<DL	0.27	51.3
13	power plant	L	pl meso	<DL	1.3	<DL	0.15	0.15	0.15	1.6
		T		2.6	0.3	<DL	<DL	<DL	0.15	2.9
14	welder	L	pl meso	0.62	<DL	<DL	0.26	<DL	0.26	0.88

TABLE 2. Type and number of asbestos fibers in lung parenchyma, pleural plaque, and mesotheliomatous tissues among 22 cases of mesothelioma (*Continued*)

Case #	Occupation	Site	Disease	Chry	Amos	Croc	Anth	Tr/Ac	DL	Total #
		T		0.7	0.4	<DL	0.3	<DL	0.09	1.4
15	US Navy	L	pl meso	27	<DL	<DL	<DL	<DL	4.4	27
		T		22	<DL	<DL	<DL	<DL	0.88	22
16	electrician	L	pl meso	<DL	19.2	2.9	<DL	<DL	1.45	22.1
		T/P		228.2	1.8	<DL	<DL	<DL	2.9	230
17	firefighter	L	pl meso	32.5	1.4	<DL	<DL	<DL	1.77	33.9
		T/P		16.6	<DL	<DL	<DL	<DL	0.22	16.6
18	US Navy/ railroad	L	pl meso	<DL	0.08	<DL	<DL	<DL	0.02	0.08
		T		0.06	<DL	<DL	<DL	<DL	0.03	0.06
19	US Navy	L	pl meso	<DL	0.52	<DL	<DL	<DL	0.03	0.53
		T		2.6	<DL	<DL	<DL	<DL	0.11	2.6
20	sheetmetal	L	pl meso	0.49	<DL	<DL	0.04	<DL	0.04	0.53
		T		0.19	<DL	<DL	0.04	<DL	0.04	0.23
21	roofer	L	pl meso	1.5	0.03	<DL	<DL	<DL	0.03	1.53
		T		0.3	<DL	<DL	<DL	<DL	0.03	0.3
22	boiler mechanic	L	pl meso	<DL	0.54	0.07	<DL	<DL	0.02	0.6
		T		<DL	0.15	<DL	<DL	<DL	0.03	0.15

NOTE: Figures represent asbestos fibers $\times 10^6$/gram (dry tissue).
ABBREVIATIONS: L, lung; P, plaque; T/P, tumor/plaque; DL, detection limit; <DL, under detection limit (no detection); Chry, chrysotile; Amos, amosite; Croc, crocidolite; Anth, anthophyllite; Tr/Ac, tremolite/actinolite; pl, pleura; pe, peritoneum; meso, mesothelioma.

(2) A disproportion in the types of asbestos fibers between lung and mesothelial tissues was common and was present in 49 of 74 cases (66.2%).
(3) The most common asbestos types in lung were an admixture of chrysotile with amphiboles (43/119 36.1%) or amphiboles alone (43/119; 36.1%). Chrysotile alone was seen occasionally (31/119; 26.1%). Rarely, no asbestos fibers were seen (2/119; 1.7%).
(4) In mesothelial tissues, the major asbestos type was chrysotile alone (90/123; 73.2%) followed by chrysotile plus amphibole (22/123; 17.9%), no asbestos fibers detected (9/123; 7.3%), and amphibole alone (2/123; 1.6%). The amphiboles included anthophyllite mixed with chrysotile in 15 cases and amphiboles alone in 1 case, followed by tremolite mixed with chrysotile in 4 cases, amosite mixed with chrysotile in 3, and amosite alone in 1 case.

2. Quantitative analysis of asbestos fibers in the tissues (number of fibers/dry gram) was performed in both digested lung and digested mesothelial tissues taken from 22

TABLE 3. Type and number of asbestos fibers in lung parenchyma in 27 ac .nal cases of mesothelioma

Case #	Occupation	Site	Chry	Amos	Croc	Anth	Tr/Ac	DL	Total #
1	electrician	L (L)	0.04	0.08	0.02	0.02	<DL	0.02	0.16
		L (R)	12.1	1.29	<DL	<DL	<DL	0.03	13.4
2	US Navy	L	<DL	3.3	<DL	<DL	<DL	0.17	3.3
3	insulation	L	<DL	0.6	<DL	<DL	0.9	0.26	1.5
4	family contact	L-1	<DL	0.11	<DL	0.11	0.33	0.17	0.55
		L-2	<DL	<DL	<DL	0.31	0.31	0.29	0.62
5	jet plane mechanic	L	260	<DL	<DL	<DL	<DL	0.22	260
6	mechanic	L	76	0.98	<DL	0.16	<DL	0.12	77.1
7	construction	L	<DL	9.9	<DL	<DL	<DL	0.13	9.9
8	US Navy	L	<DL	2.78	<DL	0.22	<DL	0.11	3.0
9	insulation	L	<DL	7.06	<DL	<DL	<DL	0.11	7.0
10	insulation	L	<DL	26	<DL	<DL	<DL	0.22	26.0
11	construction	L	36	7.5	<DL	<DL	0.75	0.75	44.3
12	electrician	L	1.5	1	0.5	<DL	<DL	0.25	3.0
13	pipe fitter	L	1.26	0.63	2.8	0.63	<DL	0.33	5.32
14	US Navy	L	16	<DL	0.22	<DL	<DL	0.22	16.2
15	insulation	L	88	<DL	<DL	<DL	<DL	0.44	88.0
16	US Navy	L	<DL	1.64	0.12	0.5	<DL	0.12	2.26
17	shipyard	L	0.66	1.32	<DL	<DL	<DL	n/a	1.98
18	US Navy	L	0.94	0.38	<DL	<DL	<DL	n/a	1.32
19	boiler repair	L	<DL	0.35	<DL	0.07	0.97	0.02	1.39
20	pipe fitter	L (R)	3	<DL	<DL	<DL	<DL	0.05	3.0
		L (L)	0.03	<DL	<DL	<DL	<DL	0.03	0.03
21	boiler repair	L	2.9	<DL	<DL	<DL	0.07	0.04	3.6
22	shipyard	L	<DL	0.08	<DL	<DL	<DL	0.03	0.08
23	shipyard	L	<DL	0.11	0.1	<DL	0.08	0.02	0.29
24	machinist	L	<DL	0.06	<DL	<DL	<DL	0.02	0.06
25	boiler worker	L	<DL	0.1	<DL	0.04	0.04	0.02	0.18
26	shipfitter	L	<DL	1.99	<DL	<DL	0.07	0.07	2.06
27	pipe coverer	L	<DL	<DL	0.58	<DL	<DL	0.05	0.58

NOTE: Figures represent asbestos fibers $\times 10^6$/gram (dry tissue).
ABBREVIATIONS: L, lung; DL, detection limit; <DL, under detection limit (no detection); Chry, chrysotile; Amos, amosite; Croc, crocidolite; Anth, anthophyllite; Tr/Ac, tremolite/actinolite; (L), left; (R), right; n/a, not available.

mesothelioma cases (TABLE 2) and from the digested lung alone in an additional 27 mesothelioma cases (TABLE 3).

A. As shown in TABLE 2, the total number of asbestos fibers detected in lung tissue was 456.4×10^6 fibers/dry gram maximum, and 0.08×10^6 fibers/dry gram minimum, and 99.9×10^6 fibers/dry gram on average. The average number of intrapulmonary asbestos fibers seen in the 22 cases was greatest for amosite (71.4×10^6 fibers/dry gram) followed by chrysotile (20.6×10^6 fibers/dry gram), and crocidolite (6.1×10^6 fibers/dry gram), tremolite/actinolite (1.37×10^6 fibers/dry gram and 0.48×10^6 fibers/dry gram).

B. In the mesothelial tissues, the total number of asbestos fibers was 240×10^6 fibers/dry gram maximum, 0.06×10^6 fibers/dry gram minimum, and 46.5×10^6 fibers/dry gram on average. The average number of intramesothelial asbestos fibers was greatest for chrysotile (45.2×10^6 fibers/dry gram) followed by amosite (1.3×10^6 fibers/dry gram), anthophyllite (0.03×10^6 fibers/dry gram), crocidolite (0.01×10^6 fibers/dry gram), and tremolite (0.0×10^6 fibers/dry gram). Total number of chrysotile fibers was compared with that of amphiboles in the mesothelial tissues from 22 cases. The total number of chrysotile fibers was 30.3 times greater than the total number of amphiboles fibers in mesothelial tissues

C. Asbestos fiber analysis was done exclusively in the lung in an additional 27 mesothelioma cases (TABLE 3). The total number of asbestos fibers detected in lung was 260×10^6 fibers/dry gram maximum, 0.08×10^6 fibers/dry gram minimum, and 21.0×10^6 fibers/dry gram on average. The average number of intrapulmonary asbestos fibers among asbestos types seen in these 27 cases was greatest for chrysotile (18.2×10^6 fibers/dry gram) followed by tremolite/actinolite (3.18×10^6 fibers/dry gram), amosite (2.46×10^6 fibers/dry gram), crocidolite (0.16×10^6 fibers/dry gram), and anthophyllite (0.06×10^6 fibers/dry gram).

Combined data for both the type and number of intrapulmonary asbestos fibers in the 49 mesothelioma cases (22 from TABLE 2 and 27 from TABLE 3) was as follows:

(i) Total number of asbestos fibers detected in lung of the 49 cases was 456.4×10^6 fibers/dry gram maximum, 0.08×10^6 fibers/dry gram minimum and 56.4×10^6 fibers/dry gram on average.

(ii) The most common asbestos type(s) seen in lung in the 49 cases was amphibole alone (23/49; 47.0%), followed by amphibole(s) plus chrysotile (22/49; 44.9%) and chrysotile alone (4/49; 8.1%).

(iii) Among the various asbestos type seen in lung of the 49 cases, amosite fibers were greatest in number (36.9×10^6 fibers/dry gram on average) followed by chrysotile (19.4×10^6 fibers/dry gram on average), crocidolite (3.13×10^6 fibers/dry gram on average), tremolite/actinolite (0.69×10^6 fibers/dry gram on average) and anthophyllite (0.27×10^6 fibers/dry gram on average).

Findings obtained from 2A, B and C (based on TABLES 2 and 3) are summarized as follows:

(1) Except for 3 cases, the number of asbestos fibers in lung tissue of 49 mesothelioma cases (22 from TABLE 2 group and 27 from TABLE 3 group) was greater than the average number in lung in the general population (0.44×10^6 fibers/dry gram).[22]

(2) The number of asbestos fibers in mesothelial tissues taken from 22 mesothelioma cases (TABLE 2 group) was also greater in the majority of cases (18/22) than the average number of the general population (0.41×10^6 fibers/dry gram).[22]

(3) The average number of each type of asbestos fibers in lung (49 cases) was greatest for amosite (36.9×10^6 fibers/dry gram), followed by chrysotile (19.4×10^6 fibers/dry gram), crocidolite (3.13×10^6 fibers/dry gram, tremolite/actinolite (1.69×10^6 fibers/dry gram) and anthophyllite (0.27×10^6 fibers/dry gram). In contrast, in mesothelial tissues (22 cases), the average number of asbestos fibers was greatest for chrysotile (45.2×10^6 fibers/dry gram) followed by amosite (1.3×10^6 fibers/dry gram), anthophyllite (1.03×10^6 fibers/dry gram), crocidolite (0.01×10^6 fibers/dry gram) and tremolite/actinolite (0×10^6 fibers/dry gram). It was obvious that a disproportion of the average number of asbestos types was present between lung and mesothelial tissues in the 22 cases of malignant mesothelioma.

TABLE 4. Dimensions of 10,575 asbestos fibers detected in lung and mesothelial tissues: totals for the 168 cases

	Length					Width				
Tissue	N.	G.M.	G.S.D.	Min.	Max.	N.	G.M.	G.S.D.	Min.	Max.
Amosite										
Lung	1577	5.08	3.12	0.20	82.4	1577	0.19	2.47	0.02	6.50
Plaque	48	2.38	3.62	0.15	28.0	48	0.14	2.61	0.02	1.10
Tumor	66	4.55	3.50	0.30	62.0	66	0.21	2.19	0.03	1.10
Chrysotile										
Lung	2921	0.42	2.26	0.08	18.5	2921	0.04	1.46	0.01	3.00
Plaque	1208	0.39	2.37	0.08	38.0	1208	0.04	1.40	0.01	0.20
Tumor	4412	0.35	1.97	0.07	15.0	4412	0.04	1.36	0.01	0.70
Crocidolite										
Lung	230	4.63	2.34	0.40	31.5	230	0.14	1.96	0.03	1.50
Plaque*	–	–	–	–	–	–	–	–	–	–
Tumor	2	3.53	1.09	3.33	3.75	2	0.40	1.37	0.32	0.50
Tremolite										
Lung	54	5.80	2.75	0.60	34.5	54	0.33	2.60	0.05	1.80
Plaque*	–	–	–	–	–	–	–	–	–	–
Tumor	11	1.61	2.18	0.40	3.72	11	0.19	2.37	0.03	0.80
Anthophyllite										
Lung	38	5.57	3.11	0.25	49.6	38	0.54	2.37	0.10	2.90
Plaque*	3	2.30	2.26	1.00	5.10	3	0.23	2.08	0.10	0.40
Tumor	5	4.40	3.01	1.60	26.3	5	0.39	1.78	0.24	1.00

N = number; G.M. = geometric mean; G.S.D. = geometric standard deviation.

3. From the 168 cases of human malignant mesothelioma, dimensions (length and diameter) of a total of 10,575 asbestos fibers detected in lung and mesothelial tissues (mesotheliomatous tissue and fibrotic serosa including hyaline plaque) were measured. Findings are summarized in TABLE 4 and TABLE 5.

A. 1) As shown in TABLE 4, the 10,575 asbestos fibers consisted of 8,536 chrysotile fibers (2,921 in lung, 1,203 in plaque, 4,412 in tumor), 1,691 amosite fibers (1,577 in lung, 48 in plaque, 66 in tumor), 232 crocidolite fibers (230 in lung, 0 in plaque, 2 in tumor), 65 tremolite/actinolite fibers (54 in lung, 0 in plaque, 11 in tumor), and 46 anthophyllite fibers (38 in lung, 3 in plaque, 5 in tumor).

2) The chrysotile fibers obtained were generally short in length (geometric mean [G.M.]: 0.42 μm in lung, 0.39 μm in hyaline plaque, 0.35 μm in tumor) and thin in diameter (G.M.: 0.04 μm in lung, 0.04 μm in both plaque and tumor). Amosite fibers were greater in length (G.M.: 5.08 μm in lung, 2.38 μm in plaque, 4.55 μm in tumor) and thicker in diameter (G.M.: 0.19 μm in lung, 0.14 μm in plaque, 0.21 μm in tumor). Although other amphibole fibers, such as crocidolite, tremolite/actinolite and anthophyllite fibers were much less common: crocidolite fiber length was 4.63 μm (G.M.) in lung, not available in plaque, and 3.53 μm (G.M.) in tumor; their diameter was 0.14 μm (G.M.) in lung, not available in plaque, and 0.40 μm (G.M.) in tumor. Tremolite/actinolite fiber length was 5.80 μm (G.M.) in lung, not available in plaque, and 1.61 μm (G.M.) in tumor; their diameter was 0.33 μm (G.M.) in lung, not available in plaque, and 0.19 μm (G.M.) in tumor. Anthophyllite fiber length was 5.57 μm (G.M.) in lung, 2.30 μm in plaque, and 4.40 μm (G.M.) in tumor; diameter was 0.54 μm (G.M.) in lung, 0.23 μm (G.M.) in plaque, and 0.39 μm (G.M.) in tumor.

3) The numerical distribution of each type of asbestos fiber was compared between the lung and mesothelial tissues (hyaline plaque plus mesotheliomatous tissue). Distributions were quite different for chrysotile fibers and amphibole fibers. 34.2% (2,921/8,536) of chrysotile fibers were detected in lung, and 65.8% (5,616/8,536) were present in mesothelial tissues. The majority of amphibole fibers were seen in lung (amosite: 93.3%; 1,577/1,691; crocidolite: 99.1%; 230/232; tremolite/actinolite: 83.0%; 54/65; and anthophyllite: 82.6%; 38/46). Only a small proportion of these amphibole fibers were present in mesothelial tissues. This finding supported that compared with amphibole(s) fibers, chrysotile fibers had a much stronger capacity to translocate from the lung to the mesothelial tissue.

TABLE 5A. Total number of asbestos fibers in lung, plaque, and mesotheliomatous tissues greater than or equal to 5 μm in length from the 168 cases

Amosite	873/1691	(51.6%)
Crocidolite	112/232	(48.3%)
Tremolite	30/65	(45.5%)
Anthophyllite	23/46	(50.0%)
Chrysotile	83/8541	(1.0%)
Fibers ≥ 5 μm	1121/10,575	(10.6%)
Fibers ≤ 5 μm	9454/10,575	(89.4%)

TABLE 5B. Number of fibers found with length greater than or equal to 8 μm and diameter less than or equal to 0.25 μm from the 168 cases

	Lung	Plaque	Tumor
Amosite	171/1577 (10.8%)	2/48 (4.2%)	7/66 (10.6%)
Crocidolite	48/230 (20.9%)	0/0 (0.0%)	0/2 (0.0%)
Tremolite	4/54 (7.4%)	0/0 (0.0%)	0/11 (0.0%)
Anthophyllite	0/38 (0.0%)	0/3 (0.0%)	0/5 (0.0%)
Chrysotile	4/2921 (0.1%)	7/1208 (0.6%)	4/4412 (0.1%)
	Totals: 247/10,575 (2.3%)		

B. (1) Asbestos fibers greater than 5 μm in length were counted in the 10,575 fibers. As shown in TABLE 5A, only 10.6% (1,121/10,575) of fibers were longer than 5 μm, and 89.4% were shorter than 5 μm. The proportion of long fibers, i.e., those > 5 μm, was greatest for amosite (873/1,691; 51.6%) followed by anthophyllite (23/46; 50.0%), crocidolite (112/232; 48.3%), tremolite/actinolite (30/65; 45.5%), and chrysotile (83/8,541; 1.0%).

(2) TABLE 5B shows that of the 10,575 fibers, only 247 fibers (2.3%) fit Stanton's hypothetical dimensions (> 8 μm in length and < 0.25 μm in diameter). The proportion of asbestos fibers that fit the hypothetical dimensions among asbestos types in these tissues was greatest for crocidolite (48/232; 20.7%), followed by amosite (180/1,691; 10.6%), tremolite /actinolite (4/65; 6.2%), chrysotile (15/8,541; 0.2%) and anthophyllite (0/46; 0%). None of crocidolite (2), tremolite/actinolite (11) and anthophyllite (5) fibers seen in mesothelial tissues (hyaline plaque and mesotheliomatous tissue) fit Stanton's hypothetical dimensions.

On the basis of these findings, it was concluded that asbestos fibers detected in lung and mesothelial tissues from mesothelioma patients were predominantly short and thin; 89.4% of asbestos fibers in these tissues were shorter than 5 μm, and the percentage of asbestos fibers that confirms Stanton's hypothetical dimensions (longer than 8 μm in length and smaller than 0.25 μm in diameter) was only 2.3%.

DISCUSSION

Translocation of asbestos fibers from the lung to other organs, including the pleura and peritoneum, has been well documented.[16–24] On the light microscopic level, asbestos bodies translocated from lung of deceased asbestos factory workers have been found in various organs such as kidney, heart, liver, spleen, adrenal, pancreas, brain, prostate, and thyroid.[25] Asbestos bodies have also been documented in hilar, mediastinal and abdominal lymph nodes, peritoneal mesotheliomatous tissue, and intestinal wall taken from mesothelioma cases.[13]

On the level of electron microscopy, in 1973, LeBouffant *et al.*[16] revealed the presence of numerous uncoated short, thin chrysotile fibers in pleural hyaline plaques taken from asbestos workers using a transmission electron microscope. This was an important finding at that time because pathologists could not obviously iden-

tify coated or uncoated asbestos fibers in the hyaline plaque in routine histopathological slides under light microscopy although they knew that this unique pleural change was intimately related to exposure to asbestos. Several years later, Sébastian *et al.*[17] found an obvious disproportion in the type and number of asbestos fibers between lung and parietal pleura of 29 asbestos workers: most of asbestos fibers seen in the parietal pleura were short chrysotile fibers. Dodson *et al.*[19] also found asbestos fibers (predominantly chrysotile) in pleural hyaline plaque taken from tissues of eight shipyard workers. Boutin *et al.*[20] also found highly concentrated asbestos fibers in black spots (glomerate lymphatic capillaries stained dark from anthracitic pigmentation) in the parietal pleura and stated that amphiboles outnumbered chrysotile in the black spots. Dodson *et al.*[24] detected asbestos fibers (predominantly amosite fibers) in omentum and mesentery taken from 20 (17 pleural and 3 peritoneal) mesothelioma cases; they concluded that asbestos fibers could translocate from the lung to the peritoneal cavity.

Our previous studies[18,21,22] revealed that the types of asbestos fibers were quite often different between the lung and mesothelial tissues in mesothelioma cases and that the major asbestos type seen in the pleural and peritoneal mesothelial tissues was short, thin chrysotile fibers. The capacity of such short, thin chrysotile fibers to translocate from alveoli to the pulmonary interstitium and finally to the pleura has been documented in experimental animal studies.[26,27]

Our present study based on a larger number of mesothelioma cases confirmed the same disproportion in fiber types (as shown in TABLE 1A) and number (as shown in TABLE 2) of asbestos fibers between lung and mesothelial tissues. We have already suggested that such a disproportion was caused by the strong ability of chrysotile fibers to translocate from lung to the pleura and peritoneum.[18,21,22]

We proposed that, to clarify the features of the asbestos fibers contributing to the induction of malignant mesothelioma, asbestos fiber analysis should be done of both lung and mesothelial tissues obtained from deceased mesothelioma patients. This approach is essential to grasp the total picture of asbestos exposure in malignant mesothelioma cases.

The number of asbestos fibers (per dry gram) counted in both the digested lung (49 cases) and mesothelial tissues (22 cases) varied among the mesothelioma cases. However, it was greater than the general population average number in lung (0.44×10^6 fibers/dry gram) in 45/49 (91.8%). It was also greater than the general population average number in the mesothelial tissues (0.41×10^6 fibers/dry gram) in 18/22 (81.8%). The numerical ratio between chrysotile fibers and amphibole(s) fibers in the mesothelial tissues was examined in 13 of the 22 mesothelioma cases and was found to be 30.3 (chrysotile):1 (amphibole[s]) in the mesothelial tissues.

Our present study revealed that the majority of asbestos fibers detected in the lung and mesothelial tissues were shorter than 5 μm; only 10.5% (1,115/10,575) of the fibers exceeded 5 μm in length.

Thinness of asbestos fibers has been emphasized as an important factor in their penetration from the proximal area to the peripheral part in the lung and for their translocation from the lung to the pleura.[44–46] It was also suggested that the thinness was related to the carcinogenicity of asbestos fibers.[35,36,42,43] The present study supports this concept in that the vast majority of asbestos fibers that translocated into the mesothelial tissues, the original site from which malignant mesothelioma develops, were very thin (0.04 μm in G.M.).

Our present study also revealed that asbestos fibers fitting Stanton's hypothetical dimensions (≥ 8 μm in length and ≤ 0.25 μm in width) comprised only 2.3% (247/10,575) of the fibers detected in both the lung and mesothelial tissues. From these findings, it is obvious that if we exclusively count only asbestos fibers longer than 5 μm or if we select only asbestos fibers fitting Stanton's hypothetical dimensions, a large proportion of asbestos fibers in these tissues will be omitted.

We conclude that short, thin asbestos fibers should be considered carcinogenic because they were the principal type of asbestos fiber encountered in the lung and mesothelial tissues taken from human mesothelioma cases.

It has been generally accepted, that like other asbestos types, chrysotile fibers are capable of inducing human malignant mesothelioma. This conclusion has been obtained from various sources including molecular biological studies,[50–54] animal experimental,[23,35,36,41,42,55–57] epidemiological studies,[58–70] case reports,[71–73] and asbestos tissue burden studies.[21,22,47]

The present study of asbestos tissue burden further supports the notion that chrysotile fibers are capable of inducing human malignant mesothelioma, because a) chrysotile was seen exclusively in both the lung and the mesothelial tissues in 18/74 (24.3%) cases, in the lung alone in 11/45 (24.4%), and in the mesothelial tissues alone in 35/49 (71.4%) cases; and b) chrysotile was the most common asbestos types in mesothelial tissue (112/123; 91.1%), which is the original site of the induction of malignant mesothelioma.

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Carcinogenicity and Mechanistic Insights on the Behavior of Epoxides and Epoxide-Forming Chemicals

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ABSTRACT: **Many epoxides and their precursors are high production volume chemicals that have major uses in the polymer industry and as intermediates in the manufacture of other chemicals. Several of these chemicals were demonstrated to be carcinogenic in laboratory animal studies conducted by the Ramazzini Foundation (e.g., vinyl chloride, acrylonitrile, styrene, styrene oxide, and benzene) and by the National Toxicology Program (e.g., ethylene oxide, 1,3-butadiene, isoprene, chloroprene, acrylonitrile, glycidol, and benzene). The most common sites of tumor induction were lung, liver, harderian gland, and circulatory system in mice; Zymbal's gland and brain in rats; and mammary gland and forestomach in both species. Differences in cancer outcome among studies of epoxide chemicals may be related to differences in study design (e.g., dose, duration, and route of exposure; observation period; animal strains), as well as biological factors affecting target organ dosimetry of the DNA-reactive epoxide (toxicokinetics) and tissue response (toxicodynamics). *N*7-Alkylguanine, *N*1-alkyladenine, and cyclic etheno adducts, as well as K-*ras* and *p53* mutations, have been detected in animals and/or workers exposed to several of these chemicals. The classifications of these chemical carcinogens by IARC and NTP are based on animal and human data and results of mechanistic studies. Reducing occupational and environmental exposures to these chemicals will certainly reduce human cancer risks.**

KEYWORDS: **vinyl chloride; vinyl halides; butadiene; styrene; ethylene oxide; glycidol; epoxides; carcinogenicity; toxicokinetic modeling; DNA adducts; etheno adducts; K-*ras* mutations; cancer risk; human carcinogens**

INTRODUCTION

Because of their reactivity, several epoxides are important intermediates in the chemical industry. In addition, epoxides may also be formed *in vivo* during the metabolic elimination of their respective alkene or aromatic precursors. Human exposure to epoxides is a concern because these chemicals are alkylating agents that can react *in vivo* with nucleophilic sites on proteins and DNA.

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