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Relationships Between Phase Contrast Microscopy and Transmission Electron Microscopy Results of Samples from Occupational Exposure to Airborne Chrysotile Asbestos

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Transmission electron microscope (TEM) and phase contrast microscope (PCM) fiber counts from the same filter were compared. Industrial hygiene samples from operations within a chrysotile mine, crusher, mill and tailings site, a brake manufacturing industry, and a taping products manufacturer were used. A total of 10 318 individual fibers were analyzed from 65 filter samples. The aim of the study was to derive the relationships between TEM counts and PCM counts that could be used in the extrapolation of risk estimate from occupational exposure to low-level nonoccupational exposure to asbestos. The results show that ratios of EM-PCM equivalent to PCM counts vary between 1.4 to 3.2. The proportion of long thin fibers ($L > 8$ μm, $D < 1.5$ μm) increased as the asbestos operation moved from primary sector (mining) to end use sector (manufacturing).

Asbestos has long been known to be a health hazard to people occupationally exposed. Miners, millers, insulators, and workers using asbestos in industries (e.g., asbestos cement [transite pipe] industry, brake manufacturing, etc.) have suffered numerous exposure-related diseases (e.g., asbestosis, lung cancers, and mesothelioma).[1–4]

The health effects are well documented, and exposure levels for occupational settings have been determined since the late 1960s by the membrane filter method.[4] While this method is a good index of exposure in occupational settings, it does not provide the necessary information to be applicable to nonoccupational or even paraoccupational settings. To assess the true asbestos fiber exposure in such situations, electron microscopy (EM) (preferably transmission electron microscopy [TEM]) must be used. The phase contrast microscope/membrane filter methods can neither distinguish between asbestos and nonasbestos fibers, nor between the different types of asbestos. Also, due to different regulations respecting asbestos, not all fibers are counted. Established methods define a fiber as greater than 5 μm in length with an aspect ratio of 3 to 1 or greater.[4–5] Some methods also exclude any fibers with a diameter greater than 3 μm.[6–7] These fiber definitions include many nonasbestos fibers as well as excluding many asbestos fibers present in the air.

As an index of the concentration of asbestos fibers in the air, the phase contrast microscope (PCM) method is adequate when the predominant fiber type is asbestos; however, in nonoccupational and paraoccupational settings where many types of fibers are present, electron microscopy techniques that positively identify the types of fibers present must be used.[8]

The fiber counts obtained by PCM differ greatly from EM. It has been suggested that counts obtained by EM could be 50 to 70 times higher than those obtained using PCM on the same air sample.[9–10] This difference essentially relates to the issue of fiber dimensions; that is, fibers of sizes visible by EM and those visible by PCM. It is known that PCM cannot detect fibers smaller than 0.25 μm (about 0.3 μm in practice) while EM, with its superior resolution, can detect unit fibrils of diameters as small as 0.01 μm. This is graphically depicted in Figure 1. The fibers that are thin, generally with diameters less than 1.5 μm (variously from 0.25 μm to 1.5 μm), and lengths longer than 8 μm, are thought to be most carcinogenic.[11–14] A vast majority of such long thin fibers cannot be detected by PCM and can only be seen by EM (see Figure 1). The relative number of fibers in the different size ranges likely depends on the type of asbestos and operations involved. Furthermore, PCM methods may include nonasbestos fibrous material when it conforms to the definition of a fiber. Accurate identification and quantification of asbestos in nonoccupational and paraoccupational situations can only be reliably obtained by using EM methods.

It is generally accepted that the health risks associated with fiber levels typical of nonoccupational and paraoccupational exposure are very low.[2,3,15] At present, data does not exist that relates low asbestos fiber levels obtained by EM to health effects;[2,3,15] however, a grading of relative hazard potential can be developed if the following data is accumulated: (1) an inventory of fiber levels in outside air by EM, and (2) in selected occupational exposure situations, air samples analyzed by both EM and PCM.

A correlation between EM and PCM fiber counts could be established for specific occupational settings and fiber types. The

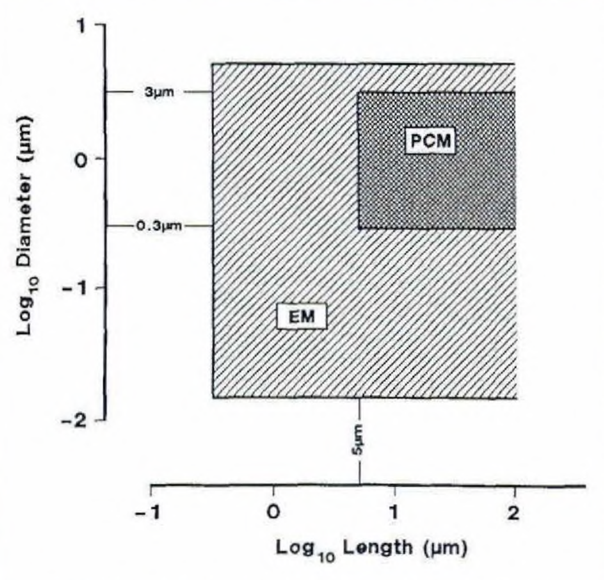

FIGURE 1. Ability of electron microscopy (EM) and phase-contrast microscopy (PCM) to detect asbestos based on fiber length and diameter

health effects based primarily on PCM counts could then be related to EM fiber counts. It is postulated that the relationship between asbestos concentration measured by PCM and by EM is:

$$C_{PCM} = f(C_{EM}, \text{type of asbestos, operation, process, ?})$$

In this equation the question mark represents unknown factors such as the source of the asbestos—for example, Canadian or Rhodesian chrysotile. Using a linear dose response relationship assumption, the magnitude of health risk of low-level exposure could be estimated by extrapolation as conceptually shown in Figure 2, where the Y axis represents health risk in terms of morbidity or mortality.

Chesson et al.[16] realized the difficulties and uncertainty involved in determining the quantitative relationship between levels of exposure and the risk of disease at the levels associated with nonoccupational asbestos exposure. They presented a mathematical model for investigating asbestos risk estimates based on measurement by one method (PCM) to an equivalent measurement by another method (EM). The model suggests approaches for obtaining conversion factors that will allow TEM measurements to be used in a PCM-based risk equation. Of the three ingredients in the model the most crucial is the distribution of asbestos fibers/structures and the ability of the measurement methods to detect the size of each structure. The relationships between fiber counts obtained by PCM to those obtained by EM in occupational environments is crucial to the determination of risk estimates at low levels. This study attempts to provide needed data relating PCM fiber counts to those obtained by EM for use in risk estimates. More specifically, the objectives of this study were (1) to obtain data relating PCM counts to the TEM counts from mining, milling, brake manufacturing, and a dumping and loading operation, and (2) to compare this study's findings to those previously reported.

MATERIALS AND METHODS

A total of 65 samples, consisting of 52 personal and 13 area samples, taken for routine industrial hygiene monitoring of airborne asbestos concentrations, were analyzed by phase contrast microscopy and by transmission electron microscopy. Chrysotile was the only type of asbestos in all of the operations sampled.

The air samples were collected on 37-mm diameter, 0.8 µm-pore size, cellulose ester filters at a sampling flow rate of 2 L/min. Air monitoring of occupational exposure to asbestos in Canada routinely used 37-mm diameter filters as per NIOSH P&CAM 239 (predecessor of NIOSH 7400 method) at the onset of this project. This filter size was used throughout the project to avoid introducing another variable, and also because there was a larger area available for eight good-size wedges for replicate analyses. Most of the samples were long-term samples. The sampling time was varied to accommodate the different operations and dust levels. The details of samples are given in Table I.

For analysis each filter was divided into eight equal wedges. A one-eighth wedge was cut from the filter and prepared for PCM analysis by the standard acetone/triacetin method[17] and analyzed according to the Ontario Ministry of Labour regulation respecting asbestos made under the Occupational Health and Safety Act, revised July 1987.[7] This method is essentially the NIOSH 7400 method[5] (counting rules A) with an upper diameter cutoff of 3 µm. The wedge cut for PCM was assigned number 8. Of the remaining seven wedges, four were randomly selected and prepared for TEM analysis. The selection of filter

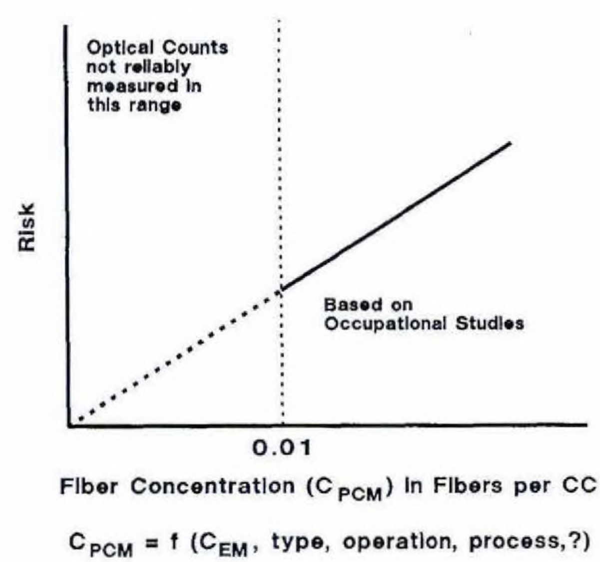

FIGURE 2. Conceptual relationship between risk and asbestos concentrations

TABLE I. Origin of Industrial Hygiene Samples and Number of Samples and Fibers Analyzed

Industry	*Operation*	*Type*[A] *and Number of Samples (filters)*	*Range of Air Volumes (L)*	*Total Number of EM Fibers*
Mining	Driller	3-P	80–440	852
	Shovel operator	5-P	280–510	573
	Pit electrician	4-P	170–428	200
	Pit laborer	3-P	250–260	58
Crusher	Primary	5-A	118–125	843
	Secondary	3-P	170–310	780
	Crusher and dryer	2-A	216–218	632
Milling	Baghouse	5-P	120	438
	Floors 2 to 6	5-A	120	1103
	First floor (packaging)	5-P 1-A	120–238	1351
Tailings	Helper and operator	3-P	290–456	638
Taping products manufacturer	Dumping and loading	8-P	37–52	1236
Brake manufacturer	Various tasks	13-P	10–446	1614
Total		65 (13-A + 52-P)		10 318

[A] A = area samples, P = personal samples

wedges and the direct transfer technique used have been described previously.[18] Since all samples received were from occupational settings where chrysotile was the only type of asbestos used and the only type of fiber expected, identification of asbestos fibers during TEM analysis was by morphology and tubular structure alone. All fibers were sized with respect to length and diameter, and the results entered into a program written for a VAX 8530 minicomputer (Digital Equipment of Canada, Ontario, Canada) and size distributions generated. A total of 10 318 EM fibers were sized.

RESULTS

Table I gives the industries from which the samples were taken, the operation being performed during sampling, the number of samples, the air volume sampled, and the total number of EM fibers analyzed.

In Table II the distribution of fiber concentrations of various size ranges for individual samples of the mining operations are given. The data in Table II will permit calculation of different ratios of EM to PCM for each sample. For example, all fibers (all lengths, all diameters) or all asbestos fibers (all lengths, all diameters) or fiber concentrations of defined size ranges can be ratioed to the PCM concentrations. The EM all-fiber counts include all fibers as defined by an aspect ratio of 3 to 1 irrespective of length and diameter, and includes nonasbestos fibers. The EM confirmed asbestos fibers are presented as all lengths, all diameters and lengths greater than 5 μm, and lengths smaller than 5 μm. The asbestos fibers greater than 5 μm in length have been subdivided into all diameters, diameters less than 3 μm, and diameters between 0.3 μm and 3 μm. The EM fibers greater than 5 μm in length, with diameters between 0.3 μm and 3 μm (limited by PCM resolution and the counting rules), should theoretically be visible by PCM and equivalent to PCM counts. For convenience these EM fibers are termed EM-PCM equivalent (EM_{PCME}). Likewise, the fibers greater than 5 μm in length with diameter less than 3 μm that were detected by EM are termed $EM_{PCME\,plus}$, since this group contained those with diameters less than 0.3 μm in addition to EM_{PCME} fibers.

The individual raw data has been ratioed (EM/PCM) and grouped by location, operation, and industry in Table III.

In Figure 3 the long thin fibers as percent of the total EM fibers are shown for various operations under the broad industrial classification of primary and end-use sectors. In Figure 4 the data showing ratio of EM to PCM counts has been presented.

DISCUSSION

There are numerous indices from which to choose a criterion to determine the health hazards due to asbestos exposure. Long thin fibers, sometimes inappropriately referred to as Stanton fibers, have been considered most carcinogenic. Lippman[19] has proposed asbestos exposure indices relating to asbestosis, mesothelioma, and lung cancer. Unfortunately, the indices proposed relate mainly to amphibole asbestos and are expressed as total surface area of fibers within a specific size range for asbestosis. These fibers are greater than 2 μm in length and greater than 0.15 μm in diameter. For mesothelioma and lung cancer, Lippman's exposure indices are expressed in number of fibers with minimum lengths of 5 μm and 10 μm and diameters less than 0.1 μm and greater than 0.15 μm, respectively. These indices are based on animal as well as human studies.

When analyzing airborne asbestos by TEM, many different results can be obtained, such as total fibers present, PCM equivalent fibers, long thin fibers, and ratios of TEM to PCM results. Depending on which result is used to assess the asbestos health hazard, one can arrive at different conclusions. When comparing

TABLE II. Distribution of Fiber Concentrations of Individual Samples from Mining Operations

Operation	Fibers Per Millimeter Squared: All Fibers, All Lengths, All Diameters	EM Fibers, Asbestos: All Lengths, All Diameters	Length > 5 μm: All Diameters	Length > 5 μm: D < 3 μm	Length > 5 μm: 0.3 μm < D < 3 μm	L < 5 μm: All Diameters	PCM
Mining							
Tractor operator	1596	1496	278	272	167	1218	35
Shovel operator	557	531	149	149	91	382	10
Shovel operator	401	386	21	21	14	365	13
Shovel operator	400	393	28	28	21	366	10
Shovel operator	600	592	105	98	42	488	9
Mine electrician	798	732	200	188	172	532	45
Pit electrician	103	91	19	13	6	71	6
Pit electrician	187	162	32	26	19	129	17
Pit electrician	71	65	0	0	0	65	9
Secondary driller	8900	8860	2228	2201	1237	6631	52
Secondary driller	1856	1802	271	257	176	1531	47
Primary driller	406	379	142	122	95	237	25
Pit laborer	186	172	14	7	7	158	5
Pit laborer	169	141	49	35	21	92	9
Pit laborer	49	49	7	0	0	42	2
Tailings helper	457	450	14	14	7	436	11
Tailings helper	3714	3714	1386	1344	748	2328	141
Tailings operator	4060	4060	1402	1336	494	2658	331

TABLE III. Ratio of EM to PCM for Various Operations and Locations

Sector	Operation and Location	All EM Fibers: Mean	All EM Fibers: Standard Deviation	All EM Asbestos Fibers: Mean	All EM Asbestos Fibers: Standard Deviation	EM Asbestos Fibers L > 5 μm D < 3 μm: Mean	L > 5 μm D < 3 μm: Standard Deviation	L > 5 μm 0.3 μm < D < 3 μm: Mean	L > 5 μm 0.3 μm < D < 3 μm: Standard Deviation
Primary	*mining*								
	driller	75.6	68.2	74.6	68.4	17.6	17.5	10.4	9.4
	pit electrician	13.5	4.2	12.0	3.8	2.0	1.5	1.5	1.4
	shovel operator	47.8	12.4	46.1	12.4	7.6	5.0	4.3	2.8
	pit laborer	26.7	7.7	24.8	7.7	1.8	1.6	1.2	1.0
	crusher								
	primary	31.7	4.0	29.7	6.1	2.5	0.6	1.8	0.7
	secondary crusher	32.9	7.5	32.9	7.5	7.2	1.5	4.0	0.9
	crusher and dryer	36.7	1.5	36.7	1.4	6.8	1.3	4.4	0.6
	milling								
	baghouse	145.6	130.6	144.4	128.7	3.1	2.5	1.2	1.8
	floors 2 to 6	39.6	14.5	39.5	14.5	3.7	1.5	2.7	0.9
	first floor (packaging)	47.7	12.1	46.7	12.5	5.2	3.6	2.8	1.7
	tailings								
	helper and operator	26.7	12.0	26.5	11.7	4.9	3.4	2.5	2.0
End Use	*taping products manufacturer*								
	dumping and loading	18.5	6.7	18.4	6.6	2.9	0.9	1.4	0.7
	brake manufacturer								
	various tasks	19.5	12.6	19.4	12.7	5.4	4.3	3.2	2.5

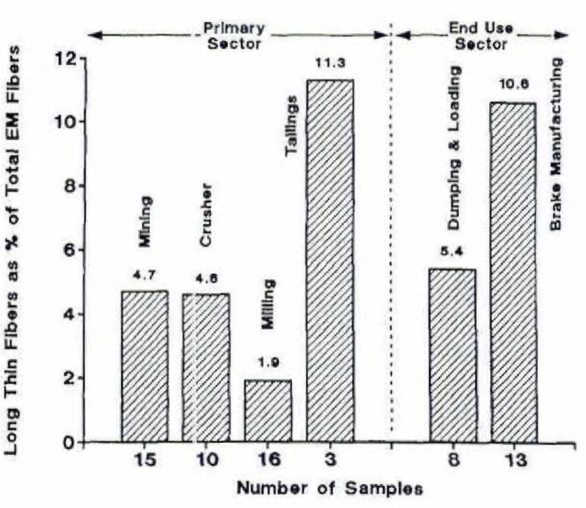

FIGURE 3. Long thin fibers (L > 8 μm and D < 1.5 μm) as percent of total EM fibers

total TEM fibers, the milling operation has the highest results when ratioed to PCM counts (see Figure 4); however, when long thin fibers are used as the criterion to assess the health hazard, the tailings operation has the greatest number, followed closely by brake manufacturing (see Figure 3).

As can be seen in Figure 4, the ratio of EM to PCM counts is highly variable, ranging between 19 and 76. The high ratio of total EM to PCM of 76 in milling is due to the high result found in the baghouse, where the dust collector bags more effectively capture the larger than the smaller fibers. This predominance of small fibers greatly increases the ratio of total EM to PCM. The ratio between EM_{PCME} and PCM is fairly consistent, ranging between 1.4 and 3.2 based on data grouped by operation (see Table IV). The small range indicates that the techniques used for counting fibers by PCM and counting and sizing fibers on EM were consistent.

The results in Tables II and III show that the airborne fibers in the operations studied were mainly asbestos. Of the total EM fibers, 93 to 100% were confirmed as asbestos. This is not unexpected considering the nature of the operations. The EM_{PCME} fibers as a percent of the total range from 4 to 18%. When EM_{PCME} is compared to PCM counts, the ratio for samples grouped by occupation/location, as shown in Table III, ranges between 1.2 to 10.4; mostly below 4.4.

Even when comparing similar operations, there seem to be differences in the results. The taping products manufacturer should be similar to the packaging operation at the mill, since one is placing the asbestos into packages and the other is dumping the contents into a hopper. The origin of the asbestos from the taping products operation (sampled) is unknown and probably came from a different source than the mill in this study, or it is possible that there were some nonasbestos fibers identified as asbestos by morphological features. This may account for the differences observed.

The results within an industry vary as they do between industries. Only a few studies have compared PCM to TEM and scanning electron microscopy (SEM)[9,10,20–27] fiber counts. Only four of these studies involved applying two techniques on the same filter. The study of Winer and Cossette,[9] and also reported by Chatfield,[10] involved 11 filters. The Hwang and Wang[24] study was carried out on 25 filters, while the Marconi et al.[25] study involved 23 filters, and the Breysse et al.[27] study involved 5 filters. The current study is somewhat more comprehensive, involving 65 filters. Table IV summarizes for comparison earlier studies along with the results of this study. It becomes apparent when reviewing the literature that while many studies have been done, a consistent procedure has not been adopted either for analysis of the samples or for presentation of the results. In many of the studies some of the data needed to calculate the ratio between EM_{PCME} fibers and PCM fibers is not available either because light microscope counts were not performed or the data was not presented in the publication.

With all of the variation inherent in the methods, it would seem that a consistent way of presenting data may be more important then the specific method used. The data of comparative studies should be presented in the manner shown in Table II, which would permit derivation of various desired ratios. To obtain such data each fiber has to be sized accurately with respect to its diameter and length using an EM, which admittedly is very time consuming.

In earlier studies PCM counts have been compared to total EM fibers of all diameters but length greater than 5 μm, which produced a very high ratio of about 66 (EM to PCM). The criteria used here is not truly a PCM equivalent, since it will include diameters greater than 3 μm as well as those smaller than 0.3 μm. In other studies the PCM counts have been compared to the EM_{PCME} fibers (i.e., fibers of diameter between 0.3 and 3 μm and lengths greater than 5 μm) and to the EM_{PCME} plus (i.e., fibers of diameter less than 3 μm and length greater than 5 μm).[24,25,27]

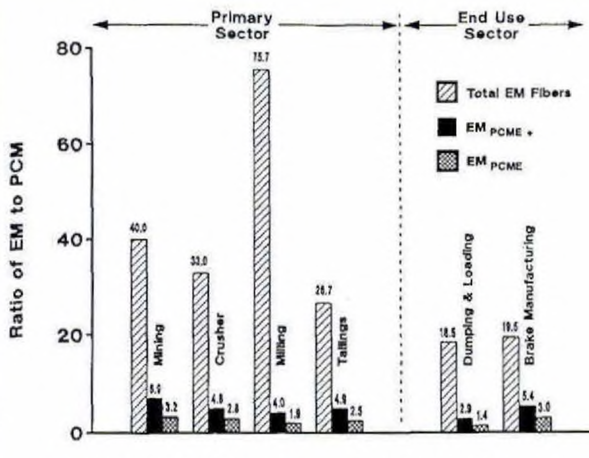

FIGURE 4. EM fiber counts ratioed to PCM counts (EM_{PCME}, fibers with lengths > 5 μm and diameters between 0.3 and 3 μm; EM_{PCME+}, fibers with lengths > 5 μm and diameters < 3 μm)

TABLE IV. EM_{PCME} Ratioed to PCM and as Percentage of Total EM Fibers

Process	Number of Filters	EM_{PCME}: PCM Mean	EM_{PCME}: PCM Std. Dev.	EM_{PCME}[A] Percent of Total[B] Mean	EM_{PCME}[A] Percent of Total[B] Std. Dev.	Source and Notes[C] (Reference Number)
Drying chrysotile	2	*		10		Gibbs and Hwang, 1975[20] 0.5 μm diameter
Bagging chrysotile	2	*		4		
Carding chrysotile	2	*		2.1		
Total = 6						
Various	11	66.3	63.9	4.1	3.4	Winer and Cossette, 1979[9] No diameter specified
Mining	NR	*		0.8		Gibbs and Hwang, 1986[21] No diameter specified
Bagging	NR	*		1.5		
Making brake linings	66 fibers	*		21.2		Rendall and Skikne, 1980[22] 0.4 μm diameter
Milling	139 fibers	*		14.4		
Mining (underground)	249 fibers	*		10.0		
Mining	NR	*		0.4		Hwang and Gibbs in Walton, 1982[4] 0.5 μm diameter
Bagging	NR	*		1.2		
Various	25	4.8		5.8		Hwang and Wang, 1983[24] 0.3 μm diameter
Asbestos cement	6	1.5	0.6	25.7	12.5	Marconi et al., 1984[25] 0.3 μm diameter
Insulation	9	1.1	0.6	29.4	14.7	
Brake and clutch manufacturing	8	1.3	0.4	53.9	19.3	
Total = 23						
Generated standard samples	4	2.2	0.5	11.5	0.8	Pang et al., 1984[26]
Water tank insulation removal	5	D		27.2	10.0	Breysse et al., 1989[27] European Reference Method (ERM) Fibers
Mining	15	3.2	3.3	9.5	6.8	Verma and Clark (this study)
Crusher	10	2.8	1.2	9.0	3.9	
Milling	16	1.9	1.4	4.3	2.5	
Tailings	3	2.5	1.8	11.3	7.4	
Dumping and loading	8	1.4	0.6	8.6	4.1	
Brake manufacturing	13	3.0	2.4	18.2	10.3	
Total = 65						

[A] EM_{PCME} fibers are asbestos fibers greater than 5 μm in length
[B] Total fibers include non-asbestos fibers
[C] NOTE: Results have been derived from data in the referenced publications
[D] Data not available

As can be seen from Table III, the ratio of EM/PCM is significantly different for various fractions. For example, for Driller it is 75.6 (σ = 68) for total EM fibers; 17.6 (σ = 17.5) for EM_{PCME+} and 10.4 (σ = 9.4) for EM_{PCME}. It is thus important that criteria selected for comparison be clearly stated and understood. This is the main reason for the widely different ratios being reported in the literature. From a health hazard point of view it may be more suitable to use the EM_{PCME+} to PCM ratio. The total EM/PCM ratio is not really relevant. Previous studies generally have data related to EM_{PCME}, and that is the reason in Table IV the EM_{PCME} ratio of earlier studies has been listed, wherever it was possible to determine from published information, along with this data.

In Figure 4 grouped data from this study is graphically shown as EM to PCM ratio for total EM fibers, EM_{PCME+}, and EM_{PCME}.

Provided that the lengths and diameters of the fibers are measured accurately, and the criterion used for expressing the data (EM_{PCME} or EM_{PCME+}) is clearly established, it may be possible to pool results from the studies for extrapolation of the dose-response curve for assessment of health effects related to low-level asbestos exposure.

A single conversion ratio of 10 has been recommended between EM_{PCME} and PCM counts for asbestos (presumably for all asbestos types and operations).[2] The results of this study as well as a few previous studies, listed in Table IV do not support this relationship. Since the relationship depends on several factors as discussed earlier, it would be prudent to use the relationship from industry and operations that are similar to the one in question. The majority of ratios between EM_{PCME} and PCM seems to range between 1 to 3. Most of the previous studies were conducted generally on a small number of samples and only four of these involved analysis by two techniques on the same filter. This study, conducted on a reasonably large number of samples (65 filters and 10 318 fibers), shows the ratios to be within this range (see Table IV). The authors realize that this study was limited to chrysotile asbestos only.

Further studies, similar to this, should be conducted involving different asbestos fiber types (amosite and crocidolite) on occupationally exposed samples. This may be somewhat difficult now in North America, where occupational exposure to asbestos generally is being phased out. It is important, however, that such studies be conducted, so that conversion factors can be derived for use in the risk estimate of nonoccupational asbestos exposure.

If one excludes tailing samples because of the small sample size, the data in Figure 3 shows a trend of the proportion of long thin fibers increasing as the asbestos processing moves from primary sector (mining) to the end-use sector (manufacturing). This is important in terms of health effects such as occurrence of lung cancer, which has generally been much higher in the end-use sector (e.g., among insulators and manufacturing workers) than in the primary sector (such as mining).

CONCLUSIONS

The following conclusions were reached:

(1) To quantify the health effects of low-level asbestos exposure, relationships between the PCM and TEM counts in occupational settings must be established. The data should be presented in these PCM/TEM comparison studies in a consistent manner so that results can be pooled from different studies. It is also important to include the number of samples and/or fibers analyzed to determine whether or not statistical significance can be placed on the results.

(2) This study indicates that the ratio of EM_{PCME} to PCM is likely to be in the range of 1 to 3.

(3) There appears to be a trend of the proportion of long thin fibers increasing as asbestos processing moves from primary sector (mining) to the end-use sector (manufacturing).

(4) Further work of a similar nature on occupationally exposed samples involving asbestos fibers of different types is needed.

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