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Comparison of Methods of Assessing Asbestos Fiber Concentrations

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ABSTRACT. Dimensional measurement of the counted fibers has important implications for setting of environmental standards to protect workers' health. Results of fiber concentrations as determined by different methods were compared. Each method was used to determine concentration by examining one sector of membrane filter on which fibrous dust was collected in various workplaces. The concentrations measured by the Asbestos International Association (AIA) method for 35 samples were, on the average, greater than the concentrations determined by the modified British Occupational Hygiene Society—Asbestosis Research Council (BOHS-ARC) method. The AIA and modified BOHS-ARC methods were found to be linearly correlated ($r = 0.82$). The mean of total fiber concentrations obtained by using the indirect transmission electron microscopy (TEM) method was 15.5 times the mean obtained by using the direct TEM method. The difference in concentrations determined by the two TEM methods resulted from disintegration of fiber bundles into single fibers during the ashing and ultrasonifying processes used in the indirect method. At least 83.5% of total fibers observed by TEM escaped detection by phase contrast microscopy.

ASBESTOS FIBER CONCENTRATIONS are measured to: (a) determine compliance with environmental standards, (b) identify sources of asbestos contamination, (c) test the effectiveness of controlling fibrous minerals, and (d) provide information on asbestos exposure levels in relation to health effects. Various methods have been used to determine fiber concentrations in air, water, and mineral samples.

Many countries, including Canada, have adopted the membrane filter method for the routine measurement of airborne asbestos fiber concentrations in industry. With this method, fibers collected on a membrane filter

by drawing dust-laden air through the filter with a portable pump are counted and the number concentration in air is determined by counting fibers on a phase contrast light microscope. Two methods of measurement have been accepted internationally: (1) the British Occupational Hygiene Society (BOHS) method¹ and later modified by the Asbestosis Research Council (ARC)²; and (2) the National Institute for Occupational Safety and Health (NIOSH) method.³ Recently, another method was recommended by the Asbestos International Association (AIA),⁴ which has been recognized as an improved method.

Although it is essential to employ electron microscopy to examine very thin fibers invisible to phase contrast light microscopy, there has been no standard method for routine measurement of asbestos fiber con-

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centrations in workplaces or general environments using electron microscopic methods.

This present report compares the results of determining fiber concentrations using the AIA method and a modified BOHS-ARC method. The results of assessing fiber concentrations by transmission electron microscopy were also compared with those determined by phase contrast microscopy. The purpose of this report is to determine whether, and to what extent, asbestos fiber concentrations in workplaces differ when various methods of measurement are used. The difference, if any, must be accounted for when setting standards to protect workers' health.

MATERIALS AND METHODS

In this study, four methods were chosen for comparison: the AIA, modified BOHS-ARC, and two electron microscopic methods. For electron microscopy, "direct" and "indirect" methods of specimen preparation were used.

Samples. Thirty-five samples of airborne fibrous dust were collected in Quebec, Canada, from a chrysotile mine, a chrysotile mill, a textile plant using chrysotile asbestos, and a ship factory using chrysotile for insulation. All samples were collected on Millipore membrane filters (type AA; $0.8\ \mu$ pore size, 37 mm diameter) using MSA portable pumps. The sampling rate was 2 L/min, with sampling time varying from 6 to 60 min depending on local work and environmental conditions. One-half of each circular membrane filter was cut and prepared for examination by phase contrast microscopy and the other half by electron microscopy.

AIA method. A quarter-section of the membrane filter was used to determine fiber concentration by the AIA method. This method differs from those recommended by BOHS, ARC, and NIOSH in procedure for specimen preparation, graticule, and rules used for counting fibers. In the AIA method, vapor from heated acetone is used to fuse the membrane filter surface before triacetin is conventionally employed to clear the filter materials for examination by phase contrast microscopy. For fiber counting, a recently designed Walton-Beckett eyepiece graticule⁵ is used instead of a conventional Patterson Globe and Circle or a Porton graticule recommended for use by BOHS or NIOSH. The details of the AIA method have been reported by the Asbestos International Association.⁴

Modified BOHS-ARC method. This is a membrane filter method using phase contrast microscopy and has been frequently used in the Environmental Laboratories of the Department of Epidemiology and Health to determine airborne asbestos fiber concentrations in workplaces. This method was a modified version of that recommended by the British Occupational Hygiene Society¹ and by the Asbestosis Research Council.²

To prepare the specimen for phase-contrast microscopic examination, a pipette was used to apply 2 drops of filtered triacetin on a clean microscope slide. Another quarter section of the Millipore membrane filter was cut and placed on top of the triacetin, dust

side up. The sample was then covered with a microscope cover glass. No acetone or dust-fixing solution was used. For counting of fibers by a Zeiss phase contrast microscope at magnification of 500X, a Patterson Globe and Circle graticule was used. The rules used for counting fibers were: (a) an elongated particle was defined as a fiber and counted if its length-to-diameter ratio was greater than 3 : 1; (b) a fiber was counted if it was longer than $5\ \mu$ (however the dimensional criteria of $> 5\ \mu$ in length and $< 3\ \mu$ in diameter were used in the AIA method); (c) a bundle was counted as one fiber if its ends were clearly resolved; (d) when an agglomerate covered more and 1/6 (1/8 for the AIA method) of a microscopic field, this field was rejected; (e) a fiber or bundle was counted if it lay entirely within the counting area defined by the graticule at a magnification of 500X; (f) if only one end of a fiber or bundle was within the counting area it was counted as "one-half" fiber; and (g) at least 20 fields at a magnification of 500X were randomly selected to obtain 100 fibers for each sample (a maximum of 100 fields was necessary if the required number of fibers were not reached. The rules for counting (a), (c), (e), (f) and (g), described above, were also used in the AIA method.

The above modified method, which was simple and practical, was different from the AIA method mainly in sample preparation, eyepiece graticule, and the rules of counting using phase contrast microscopy.

A stage micrometer was used to calibrate the eyepiece graticule at the magnification used.

Electron microscopy. The remaining portion of each Millipore membrane filter used for phase contrast microscopy examination was transferred to electron microscope (EM) grids for examination by transmission electron microscopy (TEM). The procedure of specimen preparation was essentially the one described by Ortiz and Isom.⁶ Some modifications, however, were made: (a) only carbon was used as coating material for transfer of fibers onto EM grids; (b) fusion time varied from 5–20 min depending on sample load; and (c) time for dissolving membrane filters also varied from 20 to 30 min. This method of sample preparation was termed "direct method."

An "indirect method" of specimen preparation^{7,8} for TEM observation was also used in this study. This method has also been used in our Environmental Laboratories to determine fiber mass concentrations and relatively low fiber number concentrations in working or general environments.

With the indirect method of specimen preparation for TEM examination, a $1\ \text{cm}^2$ circular section was cut from the remaining portion of the membrane filter and placed in an upright position in a low-temperature asher (Model LTA-505). The filter was ashed in oxygen plasma at 2 psi pressure for 24 hr. After ashing, the vial was filled with 10 ml of distilled water and placed in an ultrasonic bath for 10 min. The suspension was finally filtered through a Nuclepore polycarbonate filter (47 mm in diameter, $0.1\ \mu$ pore size). After drying at room temperature the filter was coated on the collection side with carbon in vacuum. By using chloroform as a dissolving agent, the fibrous dust sample on the Nucle-

Table 1.—Intra-observer Variation of Fiber Concentrations* for Ten Repeated Fiber Countings of Each of the Eight Samples (AIA Method)

Sample Code	A	B	C	D	E	F	G	H
Mean	0.24	0.26	0.44	0.68	3.29	3.86	9.04	9.48
Range	0.21–0.27	0.20–0.33	0.38–0.59	0.61–0.79	3.0–3.73	3.16–4.25	7.63–10.62	8.21–10.20
SD†	0.03	0.04	0.04	0.06	0.26	0.38	0.91	0.81
SD/mean (%)	12.5	14.2	9.8	8.8	7.9	9.8	10.1	8.6

* Fiber concentrations are expressed as number of fibers > 5 μ in length/ml of air sampled.

† SD = Standard deviation.

pore filter was transferred to TEM grids in a way similar to the direct transfer method. The only differences were: (a) no fusing process was needed; (b) in the dissolving process chloroform was used for Nuclepore membrane to replace acetone in dissolving Millipore membrane; and (c) the dissolving process in the indirect transfer method took 12 hr, while the direct transfer method required approximately 30 min.

A Philips Model EM 300 transmission electron microscope was used for counting fibers prepared on the EM grids by the two transfer methods described above. The rules for counting fibers by transmission electron microscopy were similar to those described in the modified BOHS-ARC method. The graticule on the EM fluorescent screen, however, was different from the graticule used in the eyepieces of the light microscope. The EM graticule consisted of three concentric circles of different radii and two sets of radially oriented straight lines of different lengths. These straight lines and circles were used to measure the lengths and diameters of fibers. The TEM examination was performed at a magnification of 21,000X. A ruled diffraction grating with 2160 lines per mm was used for calibration of the EM graticule at the given magnification.

To compare the methods of specimen preparation (i.e., direct and indirect transfer methods) the fibers were counted and their dimensions measured on 100 EM fields on each sample at 21,000X. These randomly sampled EM fields occupied an equivalent area of 6080 μ^2 of the Millipore membrane filter on which the original fibrous dust was collected.

Table 2.—Mean and Range of Fiber Concentrations Determined for 35 Samples by AIA and Modified BOHS-ARC Methods

Method	Mean	Range	SD
AIA			
(Number of fibers > 5 μ in length, f/ml)	2.18	0.03–10.06	2.39
Modified BOHS-ARC			
(Number of fibers > 5 μ in length, f/ml)	1.25	0.07–4.02	1.07

RESULTS

Intra-observer variations. Eight samples were randomly chosen and the observations were repeated (blind) 10 times for each sample using the AIA method by one of the authors (Z.M.W.). The results showed that the variations of fiber count were found to be less than 15% (Table 1). Samples A and B, with lower mean concentrations, have higher percent variations (12.5 and 14.2%, respectively). The repeated sample E, with a mean concentration of 3.29 fibers per ml, showed the lowest variation of 7.9%. Sample H, with the highest mean concentration (9.48 fibers/ml), had a variation of 8.6%.

Concentrations determined by phase contrast microscopy. Mean concentrations of 35 samples obtained by the AIA and modified BOHS-ARC methods are shown in Table 2. The mean concentration determined by the AIA method was found to be significantly higher than that determined by the modified BOHS-ARC method ($P < .01$, two-tailed t test). Of 35 samples examined, 25 had higher concentrations determined by the AIA method compared with that determined by the modified method.

Figure 1 shows the relationship between the concentrations determined for the 34 samples by the AIA and the modified method. One sample was not presented because of an unusually high concentration (10.07 fibers/ml), determined by the AIA method, and unusually low concentration (2.28 fibers/ml), determined by the modified method. The two methods were correlated with a correlation coefficient of $r = 0.82$. The linear regression line (Fig. 1) of the AIA method on the modified method was obtained as

$$y = 1.56x + 0.03$$

where y and x are the number concentrations of fibers longer than 5 μ as determined by the AIA and the modified BOHS-ARC methods, respectively. This equation, therefore, can be used to predict the AIA concentrations from any observed concentrations determined by the modified method.

Concentrations determined by electron microscopy. The fiber concentrations of 25 randomly selected samples (among the 35 samples collected) prepared by the direct and indirect methods and examined by TEM

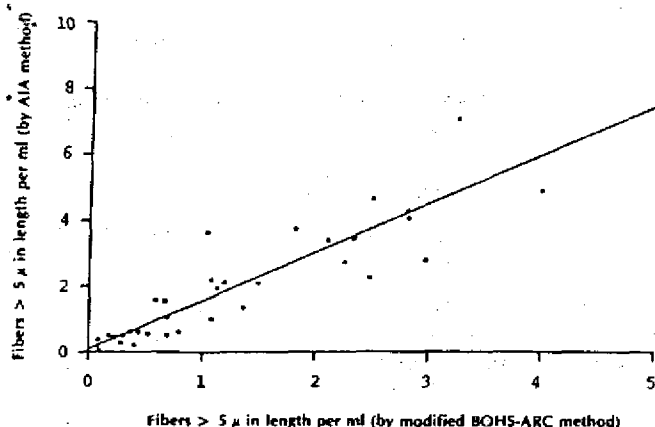


Fig. 1. Relationship between fiber concentrations as determined by the AIA and the modified BOHS-ARC methods.

are summarized in Table 3. The corresponding results from phase contrast microscopy are also shown in this Table. The mean concentrations of fibers greater than 5 μ in length and greater than 0.3 μ in diameter observed by TEM were small compared to the mean total fiber concentrations. The concentrations of light-optically visible fibers [i.e., fibers > 5 μ in length and > 0.3 μ in diameter (by TEM-direct)] were correlated to the concentrations of fibers determined by the AIA method ($r = 0.87$) and to the concentrations determined by the modified BOHS-ARC method ($r = 0.87$). The mean concentration determined by the direct TEM method was found to be 4.8 and 7.5 times higher than the mean concentrations determined by the AIA and the modified methods, respectively (Table 3).

The numbers of fibers observed in 100 fields (which was equivalent to 6080 μ^2 of Millipore membrane filter area) examined by TEM were averaged (Table 4). Most of the fibers observed (92.7 and 76.5% for the indirect and direct methods, respectively) were < 5 μ in length and $\leq$ 0.3 μ in diameter. The proportion of light-optically visible fibers (> 0.3 μ in diameter) was higher (16.5%) using the direct method compared to that obtained by the indirect method (1.7%). Unlike the rela-

tionship between the two phase contrast microscopy methods, the direct and indirect methods of TEM specimen preparation correlated poorly ($r = 0.45$). Table 5 shows the correlation coefficients obtained for the concentrations of light-optically visible fibers determined by the four different methods. As judged by the concentrations of fibers visible to light microscopy, the direct TEM method was much better correlated with either the AIA and the modified BOHS-ARC method than was the indirect TEM method.

A linear relationship (Fig. 2) was found when the concentrations of light-optically visible fibers determined by the direct TEM method were plotted (on logarithmic graph paper) against the fiber concentrations determined by either the AIA or the modified method.

DISCUSSION

The comparisons described in this report were made using the procedure in which air samples of fibrous dust collected on Millipore membrane filters were prepared and counted using different methods. The intra-observer variations were found to be from 7.9 to 14.2% and similar to that reported by Ashcroft and Heppleston.⁹

The finding that the recently proposed AIA membrane filter method closely related to the modified BOHS-ARC method implies that both membrane filter methods are equally useful in determining airborne asbestos fiber concentration in industry. The AIA method, however, has consistently higher fiber counts compared to the modified method. This mostly results from the different ways in which dust samples on a membrane filter were transferred onto a microscope slide for fiber counting by phase contrast microscopy. We have found that the different counting rules and eyepiece graticules had less effect on the difference in concentrations determined by the two light microscopic methods. One of the possible reasons why the specimen preparation in the AIA method gives higher fiber concentrations than the modified method is that the fusing process by heated acetone vapor before

Table 3.—Fiber Concentrations Determined for 25 Samples by Transmission Electron Microscopy and Phase Contrast Microscopy

Method		Fiber Concentration (per ml)	Range	Mean	SD
Transmission electron microscopy	Direct	Total number of fibers	5.3-1818.4	180.4	366.3
	Direct	Number of fibers*	1.2-32.8	10.5	8.3
	Indirect	Total number of fibers	394.4-9450.1	2795.7	2421.2
	Indirect	Number of fibers*	0-70.4	7.5	8.8
Phase contrast microscopy	AIA	Number of fibers	0.1-10.0	2.2	2.2
	Modified	Number of fibers	0.1-4.3	1.4	1.2

* Fibers > 5 μ in length and > 0.3 μ but < 3 μ in diameter.

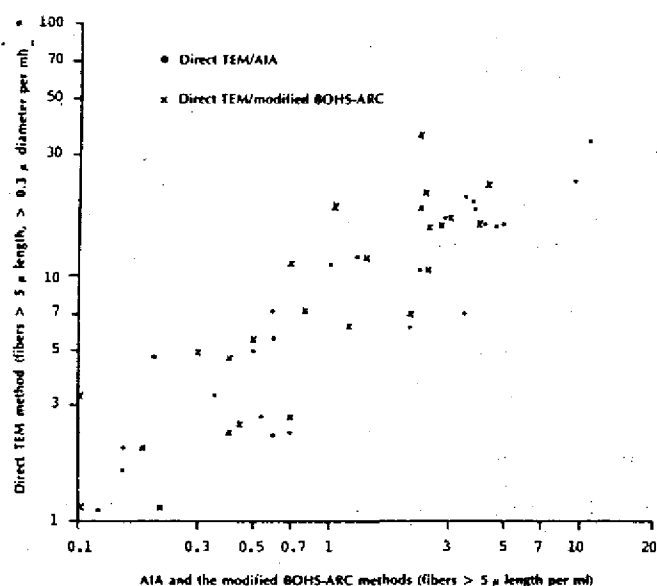


Fig. 2. Relationship between fiber concentrations determined by phase contrast microscopy (AIA or the modified BOHS-ARC method) and concentrations of light-optically visible fibers determined by the direct TEM method.

distribution.¹⁰ These processes probably accounted for the finding that the number concentrations determined by the direct TEM method were not correlated with those determined by the indirect TEM method. The indirect TEM method, however, is useful in determining mass concentrations of fibers in air.

The direct TEM method examines fibers on the membrane filter without redistributing them during the process of specimen preparation. This method has a limitation which requires a suitable number of fibers (i.e., 1 to 6 fibers in $60.8 \mu^2$) on a Millipore membrane filter for reliable fiber counting. Conversely, the indirect TEM method can be used to obtain specimens from the Millipore membrane filters loaded with various densities of fibers by cutting various portions of the membrane filter for ashing.

The concentrations of fibers $> 5 \mu$ in length and $> 0.3 \mu$ in diameter as determined by using direct TEM methods, were found to be highly correlated to the concentrations determined by the two phase contrast microscopy methods. This suggests that, compared to the indirect EM method, the direct EM method determines airborne fiber concentrations closer to the concentrations determined by conventional phase contrast

Table 4.—Mean Number of Fibers on 100 Fields Examined by Transmission Electron Microscopy for 25 Samples

Method of Specimen Preparation	Mean of Total Number of Fibers Observed	$\leq 5 \mu$ length $\leq 0.3 \mu$ diameter		$\leq 5 \mu$ length $> 0.3 \mu$ diameter		$> 5 \mu$ length $\leq 0.3 \mu$ diameter		$> 5 \mu$ length $> 0.3 \mu$ diameter	
		Number of Fibers Observed	% of Total Number Fibers Observed	Number of Fibers Observed	% of Total Number Fibers Observed	Number of Fibers Observed	% of Total Number Fibers Observed	Number of Fibers Observed	% of Total Number Fibers Observed
Indirect	288	267	92.7	3.0	1.0	16.0	5.6	2.0	0.7
Direct	85	65	76.5	6.0	7.1	6.0	7.0	8.0	9.4

clearing by triacetin makes the membrane filter thin and smooth so that fibers lie on the same image plane. The Walton-Becket eyepiece graticule used in the AIA method has advantages over conventional graticules in determining the length, diameter, and aspect ratio (length-to-diameter ratio) of fibers.⁵

For determining fiber concentration by electron microscopy, the identical counting procedure was used to examine the EM specimens prepared by the direct and indirect methods. Very high counts obtained by TEM on the EM specimens prepared according to the indirect method, compared with the counts on the specimens prepared by the direct method, probably result from the fact that large fibers or bundles disintegrate during ashing and ultrasonifying processes (Fig. 3). It has, therefore, been found that the number concentrations determined by the indirect TEM method are much higher than the actual number concentration. Timbrell has reported that techniques used to prepare samples for electron microscopic observation might cause alterations in fiber size (length or diameter)

microscopy.

The finding that the counts of light-optically visible fibers by this direct TEM method were higher than the counts by the AIA and the modified BOHS-ARC methods indicates that certain portions of long, thin fibers (e.g., $\leq 0.3 \mu$ in diameter and $> 5 \mu$ in length) were not observed by the phase contrast microscopy methods. The fiber detection limit of the phase contrast microscopy has been reported to be 0.36μ by Ashcroft and Heppleston,⁹ and 0.21μ by Hwang and Gibbs.¹¹

Table 5.—Correlation Coefficient for the Concentrations of Light-Optically Visible Fibers as Determined by Various Methods

	AIA Method	Modified Method
Direct TEM Method	0.87	0.87
Indirect TEM Method	0.39	0.48

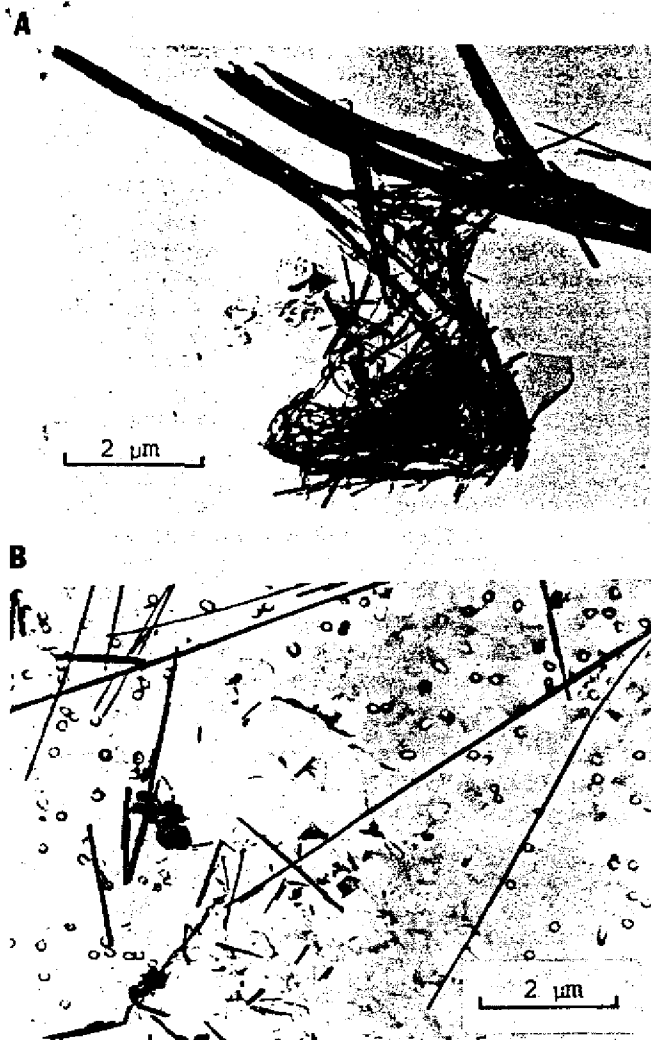


Fig. 3. Electron micrograph (A) shows bundles and agglomerates on specimen prepared by the direct method. Electron micrograph (B) shows fibers and fibrils on specimen prepared by the indirect method. The bundles and agglomerates are rarely observed on specimens prepared by the indirect method.

Experimental studies using animals have shown that both asbestos and non-asbestos fibers, which are long but thin, when placed on the pleura can produce mesothelial tumors.¹²⁻¹⁴ If long, thin fibers, such as those $> 5 \mu$ in length and $\leq 0.3 \mu$ in diameter, present in work environments were responsible for the adverse effects on the workers' health (mesothelioma, in particular), then the standards based on the widely used membrane filter (light optical) methods would not be able to adequately protect the workers' health, unless it could be proven that the concentrations of such long, thin fibers correlate with those determined by the light optical methods.

The findings described in this report have implications not only for the workers' health but also on setting of environmental standards. For a given workplace, concentrations vary if different methods are used for assessing fiber concentration and thus it is not possible to set one threshold limit for exposure to asbestos fibers for the different methods. Furthermore,

in the research on the health effects of asbestos fiber exposure, the exposure dose based on long-term exposure to asbestos varies according to the method chosen for assessing fiber concentrations to which an individual worker has been exposed. This implies that any threshold limit value for asbestos fibers in work environments derived from such research must be completely specified by the method of determining fiber concentrations.

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