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Identification and Counting of Asbestos Fibers

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A combined analytical electron microscopic/optical count method for the determination of airborne asbestos fibers was tested for precision and bias. A modified phase contrast microscopic count method (NIOSH Method 7400) was used to determine total fiber content. The analytical electron microscope (AEM) procedure was added to identify the fraction of amosite asbestos fibers in airborne, laboratory-generated samples containing amosite and wollastonite fibers. Then this fraction was applied to the routine optical counts of all the samples in the set to estimate the asbestos fiber concentration. The effects of sample to sample, wedge to wedge, within wedge and between counter variability were examined. In addition, the variabilities of the elemental ratio within a fiber and between fibers was also determined to find their possible influence on the ability to identify the fiber as amosite in the presence of other silicate fibers. A precision of 20.1% relative standard deviation (RSD) and a bias of -9.1% for the AEM count method compared with the optical count procedure were found for these mixed fiber samples.

Introduction

During the last twenty years, much of the data presented regarding airborne asbestos fiber concentrations have come from analytical methods that counted the number of fibers collected in impingers or on filters.[1-3] These samples were collected in work areas where asbestos was known to be present, *e.g.*, in asbestos mining, milling, processing or other use. Thus, the data from these optical count methods were reasonable estimates of the airborne concentrations of asbestos fibers.

It was less clear, however, what the fiber concentration represented when samples were collected from a source of mixed or unknown fiber types. Some of the fibers in such samples may indeed have been asbestos, while others were quite likely non-asbestos fibers. To evaluate the levels of asbestos fibers in these settings, the fiber counting methods must be made more specific so that the asbestos fibers can be quantified in samples that contain asbestos as well as non-asbestos fibers.

Reviews of techniques for the specific determination of asbestos in bulk samples by mass and by individual fiber counts in filter samples have been presented elsewhere.[2,4] In general, the determination of asbestos by total mass, *e.g.*, by x-ray diffraction,[5-9] lacks the sensitivity to detect low airborne concentrations (ca. 0.1-1.0 fiber/mL). Although some attempts have been made to correlate fiber mass to fiber count,[10] it is unclear how the results for total mass relate to fiber concentrations for establishing permissible exposure limits.[11] Moreover, mass-determining analytical techniques cannot distinguish between the non-fibrous and fibrous forms of the asbestos minerals. On the other hand, analytical electron microscopy (AEM) has the capability of detecting all fibers, and when supplemented by x-ray analysis, can identify the mineral fiber types. For these reasons AEM techniques were used to determine the feasibility and accuracy of identifying fibers in samples containing both asbestos and non-asbestos fibers.

Others have used AEM techniques for asbestos identification.[4,12,13] However, a direct comparison of fiber counts by this technique to those counts by phase contrast microscopy remains unavailable. Care was taken here to count by AEM only those fibers that were countable by the optical method. This report describes the comparison of the fiber concentrations obtained from the phase contrast microscopic method and the AEM method. Fiber identification was also performed by AEM using selected area electron diffraction (SAED) and energy dispersive x-ray analysis (EDXA). The approach employed was to determine the precision and bias of the AEM counting procedure and to see if fiber count results were equivalent to those obtained by the optical microscopic method. The precision of the combined AEM/optical method was evaluated to see whether AEM/SAED/EDXA could be used to adjust the optical counts by determining the fraction of asbestos fibers. Presumably, the fraction of asbestos fibers determined on a small but representative number of samples could then be applied to the optical fiber counts of the field samples, thereby giving estimates of the airborne asbestos fiber concentration. This procedure minimizes the expense of the AEM analyses and provides positive identification for the asbestos fibers present.[4,14]

Even though it is desirable to use field samples in method comparisons, there is great difficulty in obtaining truly replicate asbestos samples for reliable precision estimates and for determining the fraction of asbestos in the samples. In this study, therefore, replicate filter samples were prepared using laboratory aerosol generation techniques. The samples contained a mixture of fibrous grunerite (amosite - a common amphibole asbestos fiber in non-asbestos mines) and wollastonite (a non-asbestos, calcium silicate fiber).

Experimental

Sample Generation

Atmospheres of the fibers were generated in a system consisting of a fludized bed and a 1.83-m (6-ft) by 10.2-cm (4-in) diameter plastic cylinder reported elsewhere.[15] The flui-

dized bed generator (TSI Model 3400) was charged with amosite (North American Asbestos Corporation, Chicago, IL) and with wollastonite prepared for an earlier study.[16] Both fibrous minerals were ground and passed through 200-mesh screens. An air flow of 9 Lpm through the generator dispersed the fibers in the exposure chamber. The generator chain drive was turned off since it was found that the chain did not feed the bulk dust to the fluidized bed at a uniform rate. The fluidized bed was charged with fiber dust for each generation period. Clean air entered the chamber through four 6.3-mm (1/4-in) ports in the bottom cover. The clean air mixed with the dispersed fibers and moved past the spoiler and flow straightener to the twelve sampling ports and through a dump filter (Gelman Type A, 5.1-cm [2-in] glass fiber) at the top. Air compressors and vacuum pumps provided the air flow for the fluidized bed generator, dump filter, and dilution air. The air pressure within the exposure chamber was balanced at atmospheric pressure by varying the dump filter flow. The total air flow through the chamber was about 29 Lpm.

Samples were collected on open-faced, 37-mm diameter mixed cellulose ester filters (Millipore AAWP) and were drawn by 1/4- and 1/6-Hp vacuum pumps from two manifolds through calibrated critical orifices at a nominal flow rate of 2 Lpm for each sampler. These pumps maintained a pressure drop across the orifices of greater than 0.53 atm. Exposure chamber fiber concentrations were estimated at one of the sampling ports using a Fibrous Aerosol Monitor (GCA Corp., Model FAM-1) operating in the total fiber mode. The sampling times for a run were adjusted to get a countable fiber density on the sets of filters. Total fiber densities were in the range of 280-660 fibers per square millimeter as determined by the optical counts. Two sets of ten filters each were collected.

Sample Preparation

Each of the twenty filters was cut in half; one-half was used for optical counting and the other half for analytical electron microscopic analysis. For the optical preparations, two filter wedges were cut from the half-filter with a surgical knife using a rocking motion to prevent any tearing during cutting. The filter wedges were placed on glass slides and cleared with acetone vapors from a heated flask.[17] A drop of triacetin was placed on each clean glass cover slip, and the slide with the cleared filter wedge attached, was inverted and gently pressed onto the cover slip. A quick-drying cement (Duco) was used to glue the corners of the cover slip to the slide.

For the AEM preparation, two circular samples were randomly excised from the other half of the filter sample with a No. 5 cork borer and were affixed to acetone-cleaned, glass slides with gummed reinforcements (Dennison, No. 52-102). The filter samples on the glass slides were fused in a Petri dish containing several acetone-soaked Whatman filter papers for 4-5 minutes. Then, the samples were carbon coated in a vacuum evaporator (JEOL, JEE-4B) to hold the fibers in place prior to dissolving the filter substrate. A quarter section of the coated sample was then cut out with a surgical knife, and turned upside down on a Formvar-coated, 200 mesh copper grid that was likewise carbon-coated to provide sample support. These samples were then placed grid side down on top of 10 to 15 acetone-saturated 9-cm filters (Whatman No. 1, Qualitative) in a standard Petri dish. Only enough acetone was added to saturate the filters without forming standing pools. After 16-24 hours, the filter was dissolved by the acetone leaving the fibers affixed to the coated copper grids. The prepared grids were not maintained in a wet dish.

Sample Analysis

All samples prepared for optical microscopic analysis were counted by one experienced counter using a phase contrast microscope (Zeiss Binocular, West Germany) with a green filter over the light source. Samples were analyzed at a total magnification of 400X with a 40X objective of numerical aperture of 0.65, and a standard 10X eyepiece. A calibrated Walton-Beckett graticule (Graticules Ltd., Tonebridge, Kent, England) was used. This graticule has an optical field area of 0.00785 mm^2 (100-μm diameter). The resolution limit of the phase contrast microscope was estimated to be 0.25 μm by observing reference slides, one containing latex spheres of known diameter, and the other having grooved lines of known width scratched into the surface of a test slide developed and calibrated by National Physical Laboratory, United Kingdom (P.T.R. Optics Ltd., England). One filter wedge was counted once and the second wedge counted twice to evaluate between and within wedge variability. The modified counting rules of the United Kingdom Health and Safety Executive Central Reference Scheme were used.[18] The rules are:

1. All fiber ends falling within the graticule area are counted until the 200 ends or 100 fields are counted.
2. For fiber bundles or split fibers, each free end shall be counted as one end. If the number of ends exceeds ten, then the group is counted as ten ends.
3. Fibers in contact with particles and meeting the size criteria below are counted without regard to the sizes of the particles.
4. The resulting number of ends is then divided by two to give the total fiber count.

Only fibers or fiber ends greater than 5 μm in length with a length to diameter aspect ratio 5:1 and diameters between 0.25 and 3 μm were counted in this study. This procedure has been recommended as an International Organization for Standardization (ISO) method by their Working Group 5 (Crystalline SiO_2 and Inorganic Fibers) to Subcommittee 2, Technical Committee 146 because recent studies indicate that the method produces improved interlaboratory precision.[19,20]

The AEM samples were counted by two experienced counters on a scanning transmission electron microscope (JEOL 100B) with an actual magnification on the screen of 8330X at an accelerating voltage of 100 kV. Only fibers having the same aspect ratio and diameter restrictions as the optical counts were recorded. A number of grid openings

were measured, and an average area of 0.00706 mm^2 was calculated. Each grid opening was considered to be a counting field and the optical counting criterion of counting only 20-100 fields was observed. A complete traverse of each grid opening was done. Due to the fiber density on the grids (220-770 fibers/mm^2), the total number of fields never reached 100. As for the optical counts, one grid was counted once and the other counted twice for each filter sample.

Each fiber counted was analyzed to determine if it was amosite or wollastonite by observing its: (a) morphology, size and shape, (b) crystalline structure, and (c) elemental composition. Qualitative interpretations of zone axis SAED patterns showed whether the fiber had a crystalline structure, and the observed diffraction pattern indicated whether it was an amphibole. In addition, the elemental composition was determined using energy dispersive x-ray analysis (EDAX International, Model 707B). A representative set of amosite fibers from these samples were characterized by a Fe:Si intensity ratio of 0.732 (7.2% RSD), and a Mg:Si intensity ratio of 0.091 (10.0% RSD) with only a trace amount of calcium. The wollastonite fibers exhibited a Ca:Si intensity ratio of 1.23 (10.8% RSD) with only a trace amount of iron.

Results and Discussion

Although the two sets of samples were generated on separate days, there was no significant difference in the mean airborne fiber concentrations of the two sets as measured by the optical counts. Therefore, the results were combined into one data set and average counts obtained. The optical counts of the combined set were normally distributed with a mean concentration of 4.06 fibers per milliliter (f/mL) and a relative standard deviation of 15.7%. See Table I. An analysis of variance was used to examine the variability from three sources. These were the variances between samples, between wedges from the same sample, and within wedges. The ANOVA showed that two-thirds of the variation can be explained by sample to sample variability. Although the confidence intervals on the variance components were wide, it appears that, since the interwedge variability was small compared with between-sample variability, the source of the observed imprecision was due more to the inhomogeneity within the lab generation system than to the variations in the actual counting process.

In contrast to the optical counts, the AEM fiber counts using the same counting rules gave a mean concentration of 3.67 fibers/mL (25.5% RSD). This concentration represents the mean of data from both counters. The majority of the variability was attributed to the AEM counting process itself. Since the AEM counts come from a small portion of the collection filter area on the grid while the optical counts come from random fields spread across the entire radius of the filter, we hypothesized that the AEM analysis may be considered a less representative counting procedure and thus subject to greater variability. However, the analysis of variance indicated that, for AEM counts, there is greater variability in the replicate counts on a grid than of counts between separate grids. This suggests that the hypothesis is not valid.

There was no significant difference between the two AEM counters. But the t-test used here would have indicated statistical significance only if one of the counter's data were, on the average, more than about 20% greater than the other counter's data. Thus, we are stressing that the results on the interchangeability of counters was inconclusive. Others, however, have shown much greater differences when counts were compared among several laboratories.[21,22]

The AEM fiber counts in this study averaged 0.37 f/mL lower than the optical counts. The average was obtained by first adjusting for differences from sample to sample, and then using the residual variation to compute the difference between the AEM and optical counts. This procedure was equivalent to computing the average difference sample by sample and computing the mean (0.37 f/mL) of these differences over all samples. The indication was that on the average, the AEM counts were 9.1% lower than the optical counts.

The total fiber count difference between the AEM and optical methods was statistically significant but should be explored further at lower filter loading levels (ca. 30-50 fibers/mm^2). Sample preparation was one possible source of the AEM bias since there are differences in sample preparation for the AEM and the optical methods. However, unless some fibers were lost during the AEM sample preparation, one would not expect sample analysis by AEM to have a negative bias. Some authors have noted larger biases with AEM on water samples of amosite and chrysotile fibers.[12,23] These studies did not state the independent counting method, or apparently, restrict the AEM counting rules on fiber size. The AEM counts here were restricted to those larger fibers which would be counted optically. In any event, unless the bias were to increase greatly at lower fiber densities, this

TABLE I
Airborne Fiber Concentrations

Method	Mean (f/mL)[A]	Precision (%RSD)
Phase Contrast Microscopy	4.06	15.7
Analytical Electron Microscopy		
Total	3.67	25.5
Amosite	2.41	27.5
Amosite Fraction (0.657)		11.0
Combined PCM/AEM	2.74	20.1

[A]60 counts on 20 samples

factor should not significantly affect the accuracy of AEM counts compared with optical counts.

In addition to the overall bias and precision exhibited by the AEM counting method, two other sources of error were investigated in this study. These were the variability of the fraction of fibers determined to be asbestos, and the variability of the calculated asbestos fiber concentration using the optical count method. In practice, it should be possible to determine the fraction of total fibers that are asbestos and use this fraction to adjust the fiber concentration determined by optical microscopy. The mean asbestos fiber fraction for the twenty filter samples was 0.657 with a relative standard deviation of only 11.0%. Multiplying this fraction by the mean optical counts gave an estimated asbestos count by the combined AEM/ optical methods of 2.67 f/ mL. In comparison, the direct AEM count of only asbestos fibers gave a concentration of 2.41 f/ mL (27.5% RSD). The 9.1% bias between the AEM and optical counting methods was represented here (2.41 vs. 2.67 f/ mL) since it was derived from the prior bias. But what is perhaps more important was the variability of the calculated concentration of asbestos fibers when the AEM-determined fraction was applied to the optical counts. The asbestos concentration was determined by multiplying this fraction by each optical count in the following equation.

$$C = (855\ F\ D)/V\ ,$$

where

- C = estimated asbestos air concentration (f/mL);
- 855 = effective collection area of 37-mm filter (mm^2);
- F = fraction of fibers that are asbestos as determined by AEM;
- D = deposition of fibers on filter determined by optical count (f/mm^2); and
- V = volume of air sampled (mL).

The mean asbestos concentration found with the formula was 2.74 f/ mL (20.1% RSD). Most of the variance was attributable to the sample to sample imprecision and not the counting process.

The asbestos identification process provided other opportunities for inaccuracy. In the AEM analysis, each fiber was counted and classified as either amosite, wollastonite or unidentified by SAED and EDXA. The identification of amosite was based upon the presence of the amphibole diffraction pattern and the elemental composition. The amosite fibers could be identified by their SAED pattern and elemental composition. The compositional identification of a fiber as amosite asbestos was based upon the ratios of iron and magnesium to silicon and whether other significant elements are present as judged by comparison with reference spectra. Misidentification of fibers may occur in environmental samples unless particular attention is given to a rigorous analysis of two or more SAED patterns.

In sets of samples from a given work site, the elemental ratios for a given fiber type should be reasonably constant. Table II presents the elemental concentration ratios and their variability within a single fiber determined in this study. The constituents are determined relatively precisely in the fiber as shown by the precision values. It is not necessary to analyze them more than once for routine determinations. On the other hand, the ratios do vary some between fibers of different diameter. Table III shows the trend of slight increases in the Fe:Si ratio for fibers of progressively greater diameter. These measurements are in agreement with results elsewhere.[12,23]

TABLE II
Elemental Concentration Ratios

Fiber	Fe:Si[A]	Mg:Si[B]	Ca:Si[C]
Amosite[D]			
Mean	0.93	0.14	
Range	0.85-1.18	0.04-0.19	
Precision (% RSD)	7.2	10.0	
Wollastonite[E]			
Mean			1.23
Range			1.13-1.53
Precision (% RSD)			10.8

[A]Intensity Ratio × 1.27 (From Reference 12); n = 36
[B]Intensity Ratio × 1.5 (From Reference 12); n = 36
[C]Intensity Ratio × 1.0 (From Reference 12); n = 11
[D]Diameters 0.3 - 0.7 μm
[E]Diameters 0.3 - 2.3 μm

Although there is variability in the elemental ratios, it is not likely that misidentifications of asbestos would be common when morphology, crystalline structure, and elemental composition are all taken into consideration.

Finally, as a practical consideration, the combined AEM/ optical count method is more labor intensive than the optical count alone. It is estimated that about eight hours per sample will be necessary to determine the fraction of asbestos fibers in four representative samples of a 20-sample set. This ratio can then be applied to the less labor intensive optical count analysis (40 min for each of 20 samples) when positive asbestos identification is required.

TABLE III
Elemental Intensity Ratios

Fiber	Diameter (μm)	Fe:Si	Mg:Si	Ca:Si
Amosite	0.3	0.748	0.092	
	0.4	0.721	0.086	
	0.5	0.726	0.079	
	0.6	0.840	0.074	
	0.8	0.802	0.082	
	1.0	0.844	0.081	
Wollastonite	0.3			1.210
	1.0			1.155
	1.1			1.130
	1.4			1.157
	1.5			1.123
	1.6			1.132
	1.8			1.144
	1.8			1.344
	1.8			1.381
	2.3			1.264
	2.3			1.531

Mean of 2-4 fibers analysed at given diameter

Conclusions

This paper has explored a number of variables that may contribute to errors in determining the concentration of airborne asbestos fibers. The method used was the phase contrast count procedure corrected for non-asbestos fiber count using AEM analysis on samples containing asbestos and non-asbestos fibers. The AEM analysis determined the fraction of asbestos fibers on samples from the same set. It was found that sources of error in the combined AEM/optical analysis process included those associated with optical counting such as sample to sample, within sample, and between counter variations, and also included errors associated with the AEM asbestos identification process, *e.g.*, variations in the fiber elemental ratios of identifying amphibole or serpentine diffraction patterns. Although an overall precision of 20.1% RSD with a bias of -9.1% for total AEM count relative to the phase contrast technique was achieved, the time required for these analyses on a routine basis was large. The present work did not include a study of the effects of varying non-fiber particulate loading, fiber loading or asbestos types. These additional variables should be the subject of future research.

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References

1. **Lynch, J.R. and H.E. Ayer:** Measurement of Dust Exposure in the Asbestos Textile Industry. *Am. Ind. Hyg. Assoc. J. 27*:431-437 (1966).
2. **Revised Recommended Asbestos Standard:** DHEW (NIOSH) Publication No. 77-169 (1976).
3. **Irwig, L.M., *et al.*:** Risk of Asbestosis in Crocidolite and Amosite Mines in South Africa, in Health Hazards of Asbestos Exposure. *Ann. N.Y. Acad. Sci. 330*:35-52 (1979).
4. **Grasserbauer, M. and C. Malami:** Characterization of Individual Airborne Particles-Methods Used and Their Limitation. *Trends in Anal. Chem. 1*:98-104 (1981).
5. **Crable, J.V. and M.J. Knott:** Quantitative X-ray Diffraction Analysis of Crocidolite and Amosite in Bulk or Settled Dust Samples. *Am. Ind. Hyg. Assoc. J. 27*:449-453 (1966).
6. **Crable, J.V.:** Quantitative Determination of Chrysotile, Amosite, and Crocidolite by X-ray Diffraction. *Am. Ind. Hyg. Assoc. J. 27*:293-298 (1966).
7. **Lange, B.A. and J.C. Haartz:** Determination of Microgram Quantities of Asbestos by X-Ray Diffraction: Chrysotile in Thin Dust Layers of Matrix Material. *Anal. Chem. 15*:520-525 (1979).
8. **Taylor, M.:** Methods for the Quantitative Determination of Asbestos and Quartz in Bulk Samples Using X-ray Diffraction. *The Analyst 103*:1009-1020 (1978).
9. **Richards, A.L.:** Estimation of Trace Amounts of Chrysotile Asbestos by X-ray Diffraction. *Anal. Chem. 99*:1872-1873 (1972).
10. **Lee, R.J., J.F. Kelley, and J.S. Walker:** Considerations in the Analysis and Definition of Asbestos Using Electron Microscopy, *NBS Special Publication 619*:132-137 (1982).
11. *Threshold Limit Values for Chemical Substances in Work Air Adopted by ACGIH for 1982,* ACGIH Inc., Cincinnati, OH (1982).
12. **Beaman, D.R. and H.J. Walker:** Mineral Fiber Identification Using the Analytical Transmission Electron Microscope, in *Proceedings of Workshop on Asbestos: Definitions and Measurement Methods,* C.C. Gravatt, *et al.*, eds., *NBS Special Publication 506*:249-269 (1978).
13. **Clark, R.L.:** MSHA Standard Method for Fiber Identification by Electron Microscopy, *NBS Special Publication 619*:207-210 (1982).
14. **Steel, E.B., J.A. Small, and P. Sheridan:** Analytical Errors in Asbestos Analysis by Analytical Electron Microscopy, *NBS Special Publication 619*:162-168 (1982).
15. **Cassinelli, M.E. and D.G. Taylor:** Monitoring for Airborne Inorganic Acids, pp. 137-152, in *Chemical Hazards in the Workplace,* G. Choudhary, ed., ACS Symposium Series, American Chemical Society (1981).
16. **Talvitie, N.A.:** Determination of Quartz in Presence of Silicates Using Phosphoric Acid, *Anal. Chem. 23*:623-626 (1951).
17. Asbestos International Association, Reference Method for the Determination of Airborne Asbestos Fibre Concentration at Workplaces by Light Microscopy (Membrane Filter Method), Appendix A, London (1981).
18. **Ogden, T.:** United Kingdom Health and Safety Executive, Private Communication (1981).
19. **Crawford, N. and A.J. Cowie:** A Comparison of the Effects of Different Counting Rules and Aspect Ratios on the Level and Reproducibility of Asbestos Fibre Counts. Part I: Effects on Level, Report No. TM/82/23 Institute of Occupational Medicine, Edinburgh (1982).
20. **Crawford, N.P., H.L. Thorpe and W. Alexander:** A Comparison of the Effects of Different Counting Rules and Aspect Ratios on the Level and Reproducibility of Asbestos Fibre Counts. Part II: Effects on Reproducibility, Report No. TM/82/84, Institute of Occupational Medicine, Edinburgh (1982).
21. **Beckett, S.T. and M.D. Attfield:** Inter-laboratory Comparisons of the Counting of Asbestos Fibers Sampled on Membrane Filters, *Ann. Occup. Hyg. 17*:85-96 (1974).
22. **Schlecht, P.C. and S. Shulman:** A Statistical and Graphical Analysis of NIOSH Proficiency Analytical Testing, Presented at American Industrial Hygiene Conference, Philadelphia, PA (1983).
23. **Beaman, D.R. and D.M. File:** Quantitative Determination of Asbestos Fiber Concentrations, *Anal. Chem. 48*:101-110 (1976).

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