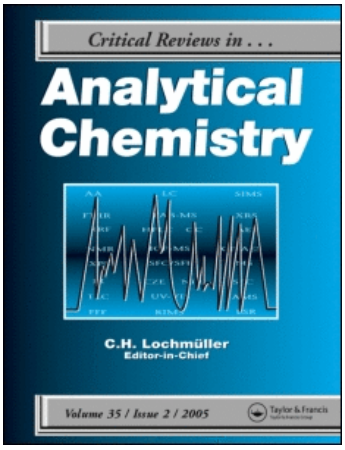

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Electron Microscopic Analysis of Airborne Asbestos Fibers

Patrick N. Breysse[a]
[a] The Johns Hopkins University, Department of Environmental Health Sciences, Division of Environmental Health Engineering, Baltimore, MD

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Electron Microscopic Analysis of Airborne Asbestos Fibers

Patrick N. Breysse, Ph.D.

The Johns Hopkins University, Department of Environmental Health Sciences, Division of Environmental Health Engineering, 615 North Wolfe Street, Baltimore, MD 21205

Referee: Eric Steele, Ph.D., NIST, Center of Analytical Chemistry, Gaithersburg, MD 20899

ABSTRACT: Scanning and transmission electron microscopic (SEM and TEM) methods for airborne asbestos analysis are reviewed. Electron microscopy has been used since the 1950s to evaluate airborne asbestos size distributions. More recently, methods have been developed to evaluate airborne fiber concentrations in occupational and nonoccupational environments. Developments of recent SEM and TEM methods are presented and discussed. This review focuses on filter preparation, fiber counting, and fiber identification issues. Issues discussed include the type of electron microscope to be used, SEM or TEM; the type of filter media to be used, Nuclepore polycarbonate of membrane filter varietes; and the choice of sample transfer technique, direct or indirect. Due to limited analytical capabilities, SEM methods have received limited use. Standardized TEM analytical methods have been developed, resulting in the routine use of TEM analysis for airborne air samples.

KEY WORDS: asbestos, TEM, SEM, electron microscopy.

I. INTRODUCTION

The purpose of this paper is to review current and historical scanning and transmission electron microscopic (SEM and TEM) techniques for evaluation of airborne asbestos fiber exposure. Asbestos is a term used for a group of commercially important fibrous silicate minerals (Table 1). Asbestos minerals can be divided into two broad mineralogical groups, serpentine and amphibole. The majority of asbestos used is of the serpentine variety, chrysotile. Chrysotile has an unusual structure resulting from dimensional mismatch between two crystalline sheets, which results in a scrolled structure referred to as tubular morphology.[1] Under electron microscopic observation, chrysotile fibers appear as long, curved fiber bundles consisting of smaller fibrils. Amphibole asbestos varieties are double-chain silicates with a wide compositional range. Morphologically, amphibole fibers differ from chrysotile fibers in that they are generally shorter and wider.

Inhalation of asbestos fibers has long been associated with a variety of malignant and nonmalignant respiratory diseases. Historically, the major focus of concern has been the exposure to asbestos in occupational environments. In the last 2 decades, however, there has been a shift in focus from manufacturing and production environments to nonoccupational exposures in ambient air and commercial buildings and schools.[2-5] The shift in concern from occupational to nonoccupational exposure to asbestos has resulted in the diminished use of optical microscopy and the development of electron microscopic (EM) techniques for air sample analysis.

II. BACKGROUND

The evolution of methodologies for the determination of airborne fiber concentrations coincided with the discovery that the health effects due to asbestos exposure were related to the fi-

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TABLE 1
Asbestos Mineral Varieties

Serpentine	Amphibole
Chrysotile	Amosite
	Crocidolite
	Anthophyllite
	Tremolite
	Actinolite

brous nature of the dust.[6,7] Historically, exposure evaluation methods utilized optical microscopy and a variety of dust-sampling techniques. Early studies of asbestos exposure utilized optical microscopy to count all particles collected in a midget impinger. This method, originally developed for the evaluation of exposure to dusts in silicosis-producing occupations, provided results in terms of "millions of particles per cubic foot" (mppcf) of air.[8] Although providing an index of general dust exposure, this method was found to be limited for assessing asbestos exposure because, in many environments, even during asbestos mining, much of the collected dust was nonasbestos.

In the 1960s, the techniques for dust sampling changed with the advances in membrane filter technology. Membrane filters have a matted structure consisting of an interwoven matrix, resulting in high filtration efficiencies. They are available in various materials including cellulose nitrate, acetate, or mixed esters, and polyvinyl chloride. These filters can easily be made transparent for light microscopic examination by filling the pores with a liquid of matching refractive index or by collapsing the filters with an appropriate solvent.

In addition to the advent of membrane filter technology, analytical techniques switched from a count of all particles collected to a count of only those particles meeting a given "fiber" definition.[9] A fiber was generally defined as a particle greater than 5 μm in length with an aspect ratio of 3:1.[10] The units of exposure were reported in terms of the number of fibers per cubic centimeter of air, f/cm^3, determined using phase contrast optical microscopy (PCOM). Fibers were counted using a specified set of counting rules. Inclusion of a fiber in the count depended on its physical dimension and its relation to other fibrous and nonfibrous material collected from the air sample. In principle, a fiber should be included in the analysis, i.e., counted, only if it is respirable and capable of causing a pathological response. The counting rules were therefore tuned to some relationship between fiber size and toxicity. The relationship between various counting rules and associated size fractions to asbestos fiber toxicity has been recently reviewed and summarized by Lippman.[11] Current dose-response relationships are based on studies of occupational cohorts where asbestos exposure was determined using optical microscopic count methods. These studies have provided the basis for setting current risk assessment and exposure standards. Exposure standards and guidelines are therefore based on optical microscopic exposure estimates.

A number of well-recognized standard methods for PCOM fiber analysis exist, including the 7400 Method published by the U.S. National Institute for Occupational and Safety and Health (NIOSH)[12] and the European Reference Method.[13] Detailed discussions of the membrane filter method can be found elsewhere.[14] These methods are all similar in that they count fibers on cleared membrane filters using PCOM at approximately 400 × magnification. This provides a resolution of approximately 0.2 μm. Any piece of particulate matter meeting the prescribed definition of a fiber is counted, regardless of identity. Hence, one of the many limitations of PCOM for fiber analysis is the inability of PCOM to ascertain the identity of the observed materials. PCOM provides an adequate index of exposure only if the fibers present in the air sample are, in fact, asbestos. In occupational environments where asbestos is used, the vast majority of fibers collected in an air sample are likely to be asbestos. PCOM is most applicable for this situation. In outdoor air and other nonoccupational environments, however, a broad spectrum of organic and inorganic materials meeting the dimensional fiber criteria will be collected, and few, if any, will be asbestos. A PCOM fiber count in this case will provide little information about the asbestos concentration in the sampled air. In addition, the limit of resolution, approximately 0.2 to 0.4 μm[15], is insufficient to observe all the asbestos fibers

that may be present on the air sample collection filter. Chrysotile asbestos fibrils can be as small as 0.01 μm in diameter. These very thin fibers will not be included in a PCOM fiber count. Hence, PCOM analysis provides an index of exposure only to those fibers optically visible.

For these two reasons (the inability to identify fibers and the limited resolution of PCOM), EM techniques have been developed for the measurement of airborne fibers and used extensively in nonoccupational environments. Modern electron microscopy (SEM and TEM) incorporates the enhanced resolution associated with EM and the ability to identify the different materials present based on morphology and elemental composition information using energy dispersive X-ray analysis (EDXRA). Selected area electron diffraction (SAED) patterns observed using TEM provides crystallographic information which can also be used for asbestos fiber identification.

III. SCANNING ELECTRON MICROSCOPIC METHODS

Scanning electron microscopes produce a focused beam of high energy electrons that systematically scan across the surface of the specimen. The interaction of the electron probe with the specimen produces a number of signals, including low energy secondary and high energy backscattered electrons. High energy backscattered electrons are emitted from the specimen due to elastic interactions. Secondary electrons, resulting from inelastic interactions, have lower energy and can be easily drawn to a positively biased collector system to create an image. Secondary electron detectors are most commonly used for fiber counting. The detector produces a signal which is used to modulate the intensity of a cathode ray tube (CRT). The scanning of the electron beam in the SEM is synchronized with the scanning of the CRT, producing a one-to-one relationship between points on the specimen and points on the CRT.

SEM is generally viewed as an intermediate analytical tool providing increased fiber visibility and moderate analytical capabilities compared with optical microscopy, but generally less fiber visibility and analytical capabilities compared with TEM. Initial investigations using SEM utilized its increased magnification capabilities to evaluate physical parameters of asbestos such as fiber size and shape in samples of known identity. In this capacity, SEM was initially used as a research instrument to supplement fiber count exposure estimates determined using PCOM. Subsequent efforts incorporated EDXRA to aid in the identification of the observed fibers of unknown identity. Although a number of SEM methods exist in the literature, a generally accepted standard method for SEM analysis does not exist. Development of procedures for SEM analysis have focused on the selection of the air sample collection filter type and sample preparation techniques.

In the early 1970s, Beckett[16] proposed that the SEM be used to characterize asbestos dusts in terms of fiber length and diameter distribution. This recommendation was based on the increased fiber visibility of the SEM compared to PCOM and the ease of sample preparation compared to TEM. Three different filter types, mixed cellulose ester (MCE), silver membrane, and Nuclepore polycarbonate (PC), were evaluated by Beckett for suitability in SEM evaluation. The filters were attached directly to SEM stubs using conductive paint and coated with gold to render them electrically conductive. Cellulose ester and silver membrane filters were rejected due to irregular surfaces which tended to obscure fibers, making them difficult to count. Nuclepore PC filters, produced by etching the tracks of high energy particles through a thin film of PC, were found to have a relatively more regular surface, providing a more uniform background for the detection of fibers. SEM photomicrographs of MCE and PC filters are contained in Figures 1 and 2.

Air samples collected in a chrysotile textile factory and in an animal exposure chamber containing dispersed Union Internationale Contre le Cancer (UICC) reference amosite asbestos were collected by Beckett, using side-by-side MCE membrane and Nuclepore PC filters to be analyzed using PCOM and SEM respectively. Comparison of asbestos fiber length distributions for fibers longer than 5 μm determined using SEM and PCOM at similar magnifications provided similar results. Beckett concluded that the good

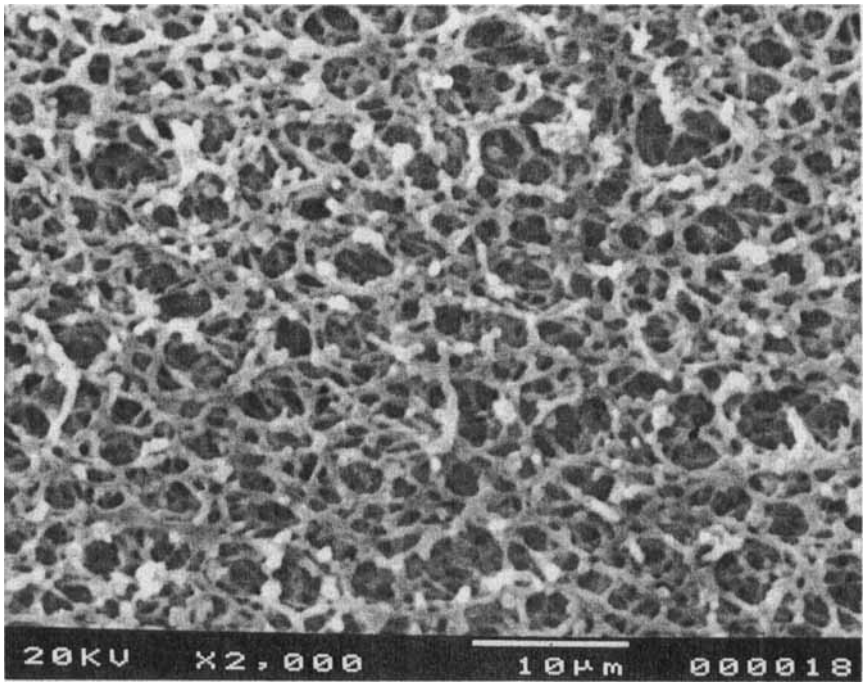

FIGURE 1. SEM photomicrograph of an uncollapsed, gold-coated mixed cellulose ester membrane filter. (Magnification × 2000.)

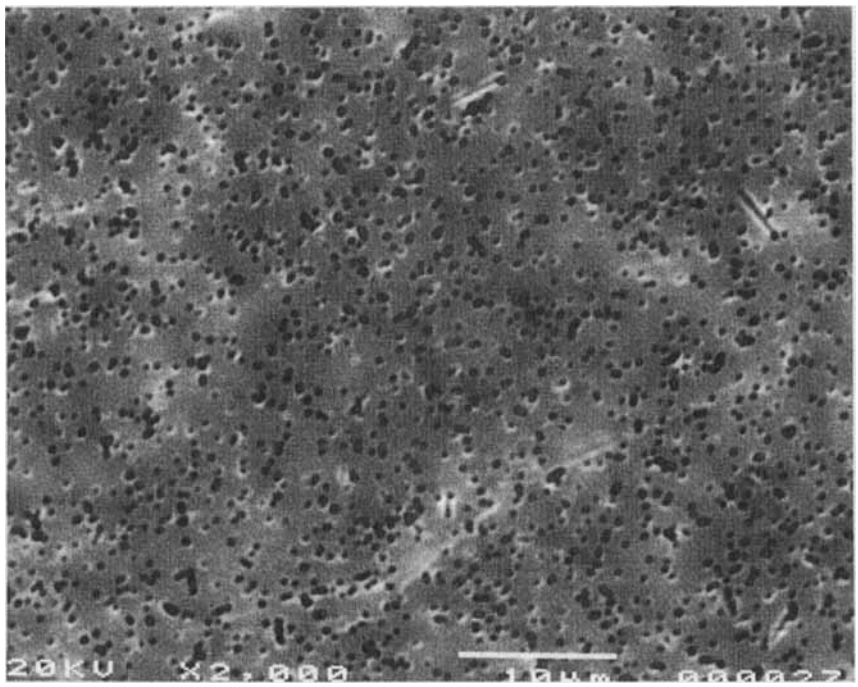

FIGURE 2. SEM photomicrograph of a gold-coated Nuclepore polycarbonate filter. (Magnification × 2000.)

agreement between the results suggested that the two methods of evaluation were comparable. This conclusion, however, was confined to a limited number of samples and to counting only those fibers greater than 5 μm in length. Since large amounts of extraneous nonasbestos dust were not present in the samples, these results are not directly comparable to a heterogeneous air dust sample.

Gibbs and Hwang[17] used SEM to describe the physical parameters of length, aspect ratio, mass, and shape for airborne fibers in a variety of asbestos industries. TEM analysis was not used because the SEM sample preparation required much less laboratory manipulation. Airborne dust samples were collected on Nuclepore PC filters with a 0.4-μm pore size. Samples were prepared for examination similarly to Beckett, by coating a portion of the filter with 150 Å layer of gold-palladium. The Nuclepore PC filter was used because it offered a superior flat surface for investigation, while the gold coat provided electrical conductivity. This technique was also used by Gibbs to investigate fiber release from asbestos garments worn to protect workers in hot environments.[18] The SEM was used by Gibbs to evaluate the concentration of fibers not visible using standard optical analytical techniques. Results indicated, contrary to Beckett, that substantial numbers of fiber ≥5 μm were present in the airborne dust that were not visible by optical methods.

In addition to backscatter and secondary electrons, which are used for SEM image formation, the interaction of the electron beam with a specimen also produces X-ray energy spectra characteristic of the elements present in the sample. Electron probe techniques using EDXRA for analysis of asbestos on a fiber-by-fiber basis were initially developed for the analysis of fiber burden in lungs at autopsy.[19,20] In order to conduct X-ray analysis on a single fiber, the electron beam is focused to a spot and centered on the fiber. The X-rays produced are then processed using an energy dispersive analyzer. Production of X-ray spectra will depend not only on fiber diameter, but also on fiber composition, shape, SEM accelerating voltage, substrate composition and thickness, conductive coating composition and thickness, X-ray detector efficiency, orientation, beam current, spot size, collection time, and detection criteria. Rubin and Maggiore described the use of this technique for the identification of asbestos air samples analyzed using SEM.[21] Examples of typical spectra from different asbestos varieties presented by Rubin and Maggiore are contained in Figure 3. The relative concentration of elements present, estimated from peak height, were used to help identify the asbestos species present. The authors claimed a lower limit of identification for particles in the range of 0.05 μm in diameter. However, based on studies by other investigators,[23] who reported practical limits of identification approximately twice that reported by Rubin and Maggiore, it is questionable whether the Rubin and Maggiore limit can be readily achieved.

An SEM method incorporating EDXRA for determining the total number of asbestos fibers and fiber length distributions for air samples collected on MCE filters was reported by Pattnaik and Meakin.[22] A schematic diagram of the steps involved in membrane filter preparation is contained in Figure 4. The MCE filter is coated with a carbon layer about 100 Å thick. A circular piece of the filter is then placed carbon-side down on a beryllium SEM stub. The filter is collapsed using acetone vapors and completely dissolved by immersing the filter in acetone. All organic components (including the carbon coating) are then eliminated in a low temperature plasma oven, leaving the asbestos fibers atop the beryllium stub. Collapsing and then dissolving the filter followed by low temperature ashing provides a featureless background for fiber observation. The authors rejected Nuclepore PC filters because the PC film distorted under solvent attack, resulting in particles shifting from their original position. It was also noted that this procedure, with minor variations, could be used to prepare samples for TEM analysis.

Fiber losses using this technique were reported to be low. Fiber loss was only qualitatively estimated, however, by observing portions of the filter before ashing, after partial ashing, and after complete ashing. This evaluation would not observe any fiber or particle losses associated with filter collapsing and dissolution. Since this latter source of error was reported to be minor, such losses were not discussed.

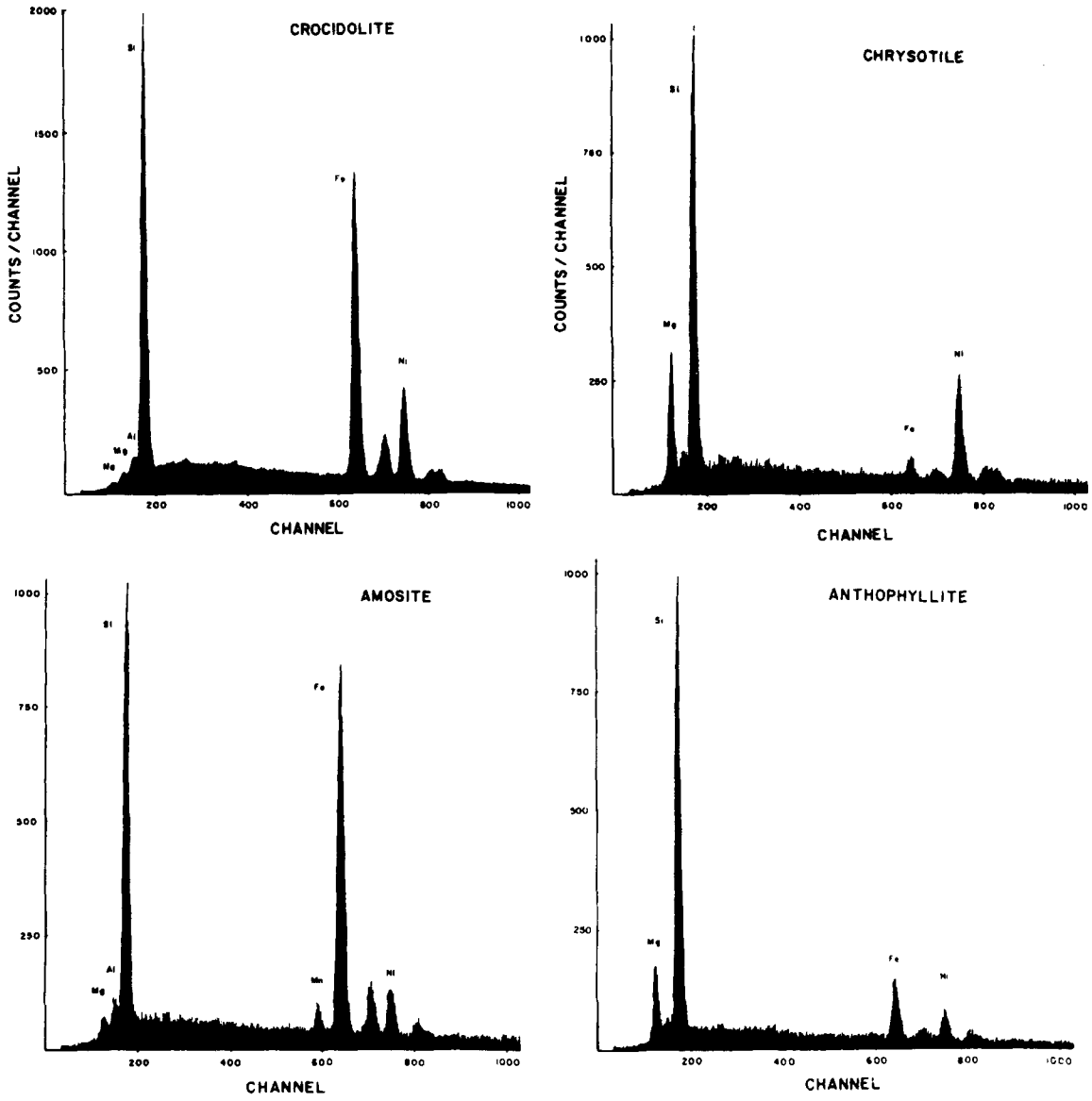

FIGURE 3. Typical energy dispersive X-ray spectra from different types of asbestos. Note the nickel-lines are present due to the specimen support. (Taken from Rubin, I. B. and Maggiore, C. J., *Environ. Health Perspect.*, 9, 81, 1974. With permission.)

According to Pattnaik and Meakin, the air sample transferred to a beryllium stub should be observed at 1000 to 3000 × magnification. They recommended higher magnifications such as 10,000 × when the sample contained mostly chrysotile fibrils. X-ray analysis was conducted by reducing the beam scan to the small square scan mode. Spectra were collected for 5 to 500 s, with the longer times required for smaller fibers. Identification was based upon the ratio of elements present compared to typical asbestos varieties as defined by Langer et al.[23] The smallest fiber diameter than can be reliably identified using this technique was reported to be approximately 0.1 μm.

The authors used this technique to evaluate point source and nonpoint source airborne asbestos levels and concluded that it provided a rapid and reliable measure of asbestos fiber identification, total mass concentration, and fiber size distribution. In addition, fiber count estimates of exposure were obtained by counting all asbestos

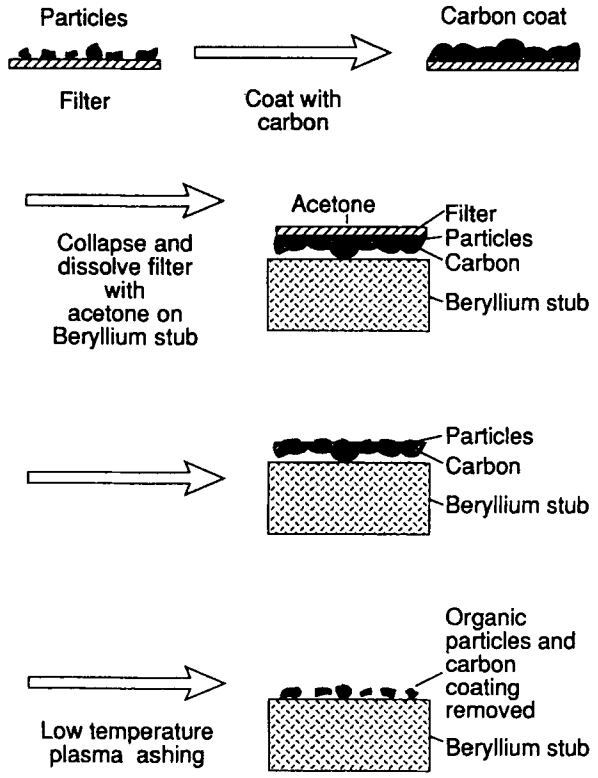

FIGURE 4. SEM mounting technique described by Pattnaik and Meakin.[22]

fibers; however, the relationship between fiber count and mass estimates of exposure was not discussed. The authors recognized that the smallest chrysotile fibrils are typically thinner than the 0.1-μm practical limit of resolution. This was not considered a major limitation because the small fiber contribution to the total mass of asbestos was considered to be minimal. This conclusion would not be valid if a fiber count, rather than mass, was to be determined as an estimate of exposure.

Methods for treating membrane filter varieties making the surface flat and featureless when viewed by SEM are reported by Le Guen et al.[24] The authors reviewed previous approaches to preparing membrane filters, noting that membrane filters were initially rejected because the fibrous matrix of the filter obscured particles from view. Methods developed by Pattnaik and Meakin which dissolved the entire filter were rejected by Le Guen et al. because of the potential for fiber loss. Limitations of Nuclepore PC filters for air sample collection reported by others[25-27] were also discussed. These limitations include particles that appear to change position easily on the filter after sampling, particles that can be lost during filter handling, and particle counts from air samples collected on Nuclepore PC filters that are consistently less than paired membrane filter counts. For these reasons, Nuclepore PC filters were rejected for sample collection.

Three types of membrane filters were evaluated by Le Guen et al.: MCE, Gelman DM450 (copolymer of polyvinyl chloride, 0.45-μm pore size) and DM800 (copolymer of acrylonitrile, 0.8-μm pore size). MCE filters were quickly rejected because they showed very rapid electron beam damage. However, the Gelman DM filters showed no signs of damage even after prolonged exposure to high accelerating voltages and beam currents.

The DM filter preparation technique reported by Le Guen et al. is diagrammed in Figure 5. In this technique, the DM filters are collapsed using 60 to 80 μl of a solution of 33% dioxane and 67% cyclohexanone and etched in a low temperature plasma oven. The etching rate and time were established by trial and error and were adjusted to leave the fibers clear of the collapsed filter matrix and to expose smaller particles potentially trapped in the upper layers of the filter. The filter can then be coated with gold or carbon to render it electrically conductive. This method was also discussed by Vaughn et al.[28] Fiber loss using this technique was reported to be low, but no data were given. Using a similar preparation for TEM analytical techniques, however, Burdett and Rood[29] reported minimal fiber loss.

The Le Guen et al. method for sample preparation represented a significant advance in the use of SEM for asbestos analysis. Prior to this, SEM methods were limited to the use of Nuclepore PC filters, which resulted in handling losses, or to the use of membrane filter mounting techniques which did not provide a smooth background for fiber observation. Dissolution of the entire membrane filter as described by Pattnaik and Meakin, although producing a flat background, would likely result in significant fiber losses. Le Guen et al. described a method which allowed the use of a membrane filter variety for air sample collection, but provided a relatively smooth analytical surface with minimal potential

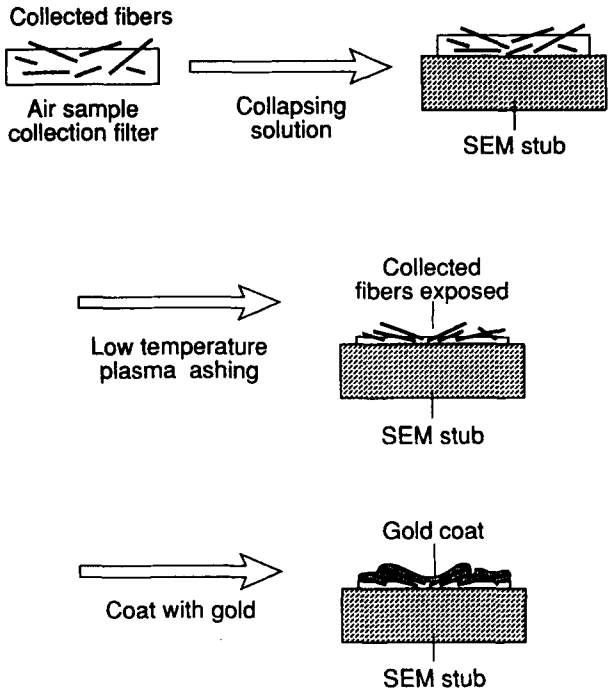

FIGURE 5. SEM mounting technique described by Le Guen et al.[24]

fiber loss. Although this method represented a significant improvement over other SEM methods, it has failed to gain widespread utilization because the limitations of SEM for asbestos analysis in general (discussed in the next section) have led to the development and acceptance of TEM methods.

The Asbestos International Association (AIA)[30] published an SEM method for determination of airborne asbestos fibers and other inorganic fibers. The purpose of the AIA method is to provide an index of exposure for fibers with aspect ratios greater than 3:1, lengths greater than 5 μm, and diameters less than 3 μm, down to the limit of SEM visibility and identification (approximately 0.1 μm). Exposure estimates determined using this method are designed to coincide with optical microscopic estimates. This method is not intended to be an absolute reference method, but rather an intermediate option not encumbered with the time and expense requirements associated with TEM.

According to the AIA method, air samples are collected using Nuclepore PC filters precoated with gold. Although no reason is provided by AIA, it is believed that the gold precoating will help to minimize fiber loss associated with Nuclepore PC filters. After sampling, a section of the filter is attached to a carbon stub using carbon glue. Carbon stubs are recommended because the more common aluminum stubs may produce an aluminum peak which can interfere with EDXRA evaluation. The filter is then ashed to remove any organic material which may have been collected, and is examined under the SEM. If the precoating is sufficient, it is typically not necessary to coat the filter after the ashing step. An additional carbon coating may be applied if sample charging is observed.

Fiber visibility on the SEM will be affected by a number of parameters including the composition and conductivity of the substrate, the composition and thickness of the conductive coating, the raster rate, the beam current (at specimen), various detector parameters, accelerating voltage, magnification, working distance, spot size, stigmation, contrast, and brightness. Large differences in fiber visibility may result in considerable variability in fiber count results. This situation is similar to that which existed for PCOM analysis prior to the use of visibility test slides. PCOM visibility test slides were developed in order to standardize fiber detection limits so that different microscopes and operators can achieve comparable results.[31] Since standardization of measurement depends to a large degree on maintaining a uniform level of visibility, the AIA SEM method provides detailed information of microscope set-up and operation. The AIA method specifies that the microscope must be equipped with a secondary electron detector and be able to detect fibers of 0.2 μm in diameter or less at 2000 × magnification according to specified fiber visibility requirements. Visibility is evaluated by locating a series of asbestos fibers barely visible at 2000 × magnification. The average diameter of these fibers, measured at 20,000 to 40,000 × magnification, should be less than or equal to 0.2 μm for the microscope to be used for fiber counting. In addition, factors affecting SEM image quality and fiber visibility such as accelerating voltage, stage tilt angle, working distance, beam control, focusing, and resolution of viewing screen are discussed, and guidelines

are provided. This level of specificity is needed to provide consistent and reproducible fiber visibility.

Fiber identity, according to the AIA method, is established based on EDXRA results and fiber morphology. Identification guidelines are provided, including X-ray spectra of UICC asbestos varieties on gold-coated Nuclepore filters. These spectra are similar to those presented in Figure 3, except that the gold coating used by the AIA method creates a large gold peak to the right of the silicon peak.

The AIA and other similar methods of SEM analysis have been routinely used in some European countries for evaluation of fibrous dusts. The AIA method was used by Marconi et al.[32] to report airborne mineral fiber concentrations in an Italian urban area near an asbestos-cement plant. Rodelsperger et al.[33] have reported asbestos concentrations using SEM analysis according to a German method which is similar to the AIA method, using precoated Nuclepore filters which are plasma etched after sampling and evaluated at 2000 × magnification. Cherrie and Addison, using a method similar to the AIA method, collected air samples on Nuclepore PC filters and gold coated prior to analysis.[34,35]

The AIA method and other methods which used Nuclepore PC filters are limited due to the potential for fiber losses discussed earlier during shipping and handling. The fiber losses associated with the use of these filters has not been clearly evaluated. In the absence of this information, it is not possible to evaluate the magnitude of this bias. With the advent of methods for the preparation of membrane filter varieties, the use of Nuclepore PC filters for airborne fiber sampling has subsequently declined.

A. Limitations of SEM for Asbestos Analysis

Limitations of SEM analysis for airborne asbestos have been discussed elsewhere.[36-37] The two major limitations deal with the limited SEM resolution and associated fiber visibility issues and the inability of EDXRA to adequately identify the fibers present. These two issues are addressed next.

1. Fiber Visibility and Detection Limitations of SEM

A major limitation of SEM for asbestos fiber counting is the inability to detect submicrometer fibers. The diameter of the thinnest asbestos fiber, a chrysotile fibril, is approximately 0.03 μm.[37] This is well below the limit of visibility for optical microscopy, 0.3 μm, reported by Le Guen et al.[24] Modern SEM, however, can achieve a visibility of about 5 nm. This capability can be realized only on photomicrographs of idealized samples, and in routine practice it is much less.

Middleton compared the mode of image formation and fiber visibility obtainable for SEM and TEM in order to provide an indication of the comparability of observations made by the two techniques.[38] The results of fiber visibililty experiments reported by Middleton, Figure 6, indicate that TEM provides superior resolution (well below 0.01 μm), and that observation of fibers less than 0.1 μm in diameter using SEM requires operation near the practical limit of detection for such fibers on a filter. These data may not be directly applicable to fiber-counting procedures, however, since the analyst is searching for fibers previously located at a high magnification. When counting fibers collected on filter paper, fibers are assumed to be randomly distributed, and the analyst does not know where to locate any given fiber. In this case, fiber visibility may be less than that reported by Middleton. Fiber visibility

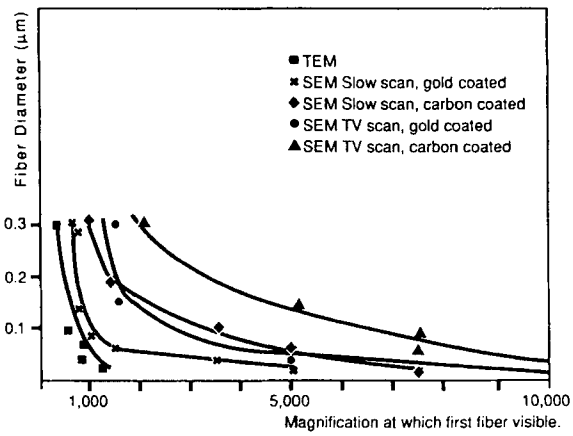

FIGURE 6. Fiber visibility on electron microscope screen presented by Middleton.[38]

is maximized, according to Middleton, by using high magnifications, slow scan speeds and gold rather than carbon for specimen coating. However, slow scan speeds are not practical for fiber counting. Fast scan speeds or TV rates are more commonly used in order to provide quicker analyses.

The SEM-EDXRA method of Pattnaik and Meakin is reported to provide rapid and positive identification for fibers ≥ 0.1 μm in diameter. This limit is in agreement with Middleton. No evidence to support this visibility limit is provided by Pattnaik and Meakin. In the discussion section following their paper, however, the authors stated that 0.5-μm diameter fibers were visible on micrographs using 10,000 × magnification. This is not practical since counting fibers from micrographs would require too much time.

The visibility of chrysotile in SEM analysis was also evaluated by Small.[39] He noted that the visibility of chrysotile fibers, because of their small size and low electron scattering coefficient, was limited by the contrast of the sample rather than the resolution of the SEM. Visibility of fibers mounted on thick and thin substrates was compared. Increased fiber visibility was observed when the samples were prepared on a thin film of carbon, using a TEM mounting technique. Small recommended that the sample be mounted over a hole in the SEM stub so that the beam of electrons passing through the sample are not available for secondary electron excitation and that the sample by analyzed at a high acceleration potential. In addition, Small noted that fiber visibility can be operator dependent and that any standard SEM method should include detailed information on SEM adjustments affecting contrast and brightness to provide consistent results.

2. Limitations of Fiber Identification Using SEM

Using SEM techniques, fiber identification is based on morphology and characteristic X-ray spectra. The quality of the X-ray spectra produced, to a large degree, is a function of the diameter of the fiber being analyzed. Due to high background excitation, the spectra produced from fibers with diameters less than 0.1 μm are difficult to evaluate. Gold coating nuclepore filters, as suggested in the AIA method and others, is also problematic for X-ray analysis.[36,38] The gold peak overlaps with the silicon peak, which may cause additional uncertainty for small diameter fibers. Furthermore, using EDXRA, it is not possible to completely distinguish between different asbestiform and nonasbestiform mineral varieties. Information on the crystalline structure of the fibers, provided in TEM analysis, is needed to identify more completely the mineral species. In addition, the tubular morphology characteristic of chrysotile, which is highly suggestive, can be observed only using TEM. Fiber identification based solely on morphology and EDXRA, therefore, cannot be considered completely confirmatory.

IV. TRANSMISSION ELECTRON MICROSCOPIC METHODS

Due to practical fiber visibility limitations and identification limits for SEM analysis, asbestos fiber count estimates can provide an index of exposure to asbestos fibers greater than approximately 0.1 μm in diameter. Many investigators have therefore concluded that limitations of SEM analysis preclude its use for airborne asbestos determination. TEM methods have been developed to this end.

A TEM is similar to a light microscope with the substitution of an electron source for the lamp illuminator and electromagnetic lenses for optical lenses. In a TEM, the electron source emits a beam of electrons which is focused by an electromagnetic condenser lens onto the specimen plane. The electron beam is partially absorbed and/or scattered during its transmission through the specimen due to differences in mass thickness. Objective and projector lenses enlarge the image of the specimen, projecting it onto a screen which fluoresces when bombarded by electrons passing through the sample. TEMs operate at greater electron beam accelerating voltages than SEMs. Accelerating voltages of around 100 kV are typically used for asbestos analysis.

Early methods for TEM evaluation of asbestos air samples were developed to evaluate fiber

size distributions from air samples collected using PCOM in occupational environments. More recently, TEM methods have been developed for routine asbestos air sample analysis. Widely accepted standard methods for TEM analysis exist and are incorporated into recent government regulations.[5]

In general, two types of filter preparation techniques, direct and indirect transfer, have been used for airborne asbestos analysis. Direct transfer methods attempt to transfer the particulate matter exactly as collected on the air sample filter to an EM grid for observation. The observed asbestos fibers are summed and fiber count exposure indices calculated. Sample preparation using indirect methods provide exposure data in terms of mass, ng/m^3, rather than the more traditional fiber count estimate, f/cm^3. Indirect transfer techniques do not attempt to preserve the particulate matter as collected. Using this method, the dust collected in an air sample is removed or separated from the filter, dispersed in a liquid, filtered, and transferred to an EM grid for analysis. Indirect methods were developed in order to analyze large volume ambient air samples where asbestos is a small fraction of the dust collected. Using this technique, it is possible to select the amount of material to be filtered to achieve the desired loading for optimal investigation.

A. Direct Transfer TEM Methods

1. Early Membrane Filter Methods

The utility of TEM for the examination of airborne particulate matter was recognized in the early 1950s. Fraser described a membrane filter TEM method for the determination of airborne particle size distributions.[40] According to this method, a piece of membrane filter was placed face down on EM screens covered with a thin film of Formvar (polyvinyl formal plastic). The EM screens were then placed in a petri dish with ethyl acetate slowly added until the filter squares were wetted by capillary action. The ethyl acetate dissolved the filter material in approximately 15 min. The particulate matter, freed from the membrane filter, settled on the undissolved Formvar film and was then examined under the TEM. Using this technique, Fraser demonstrated that particle sizing using optical microscopy provided a truncated distribution since particles less than approximately 0.4 μm in diameter were not observed.

The method described by Fraser was used by Lynch et al.[41] to compare PCOM and TEM-based airborne asbestos exposure indices. Samples collected for this investigation were from asbestos textile and various mixing and grinding operations. The authors noted that PCOM counts of fibers >5 μm in length include between 1 and 4% of the total number of fibers present. A number of disadvantages of TEM analysis were noted by Lynch et al., including long preparation time, equipment cost, and availability. The problem of interpreting clumps of fibers observed on the TEM was also noted. The authors concluded that technical difficulties of EM make its routine use infeasible and that, when used, the results are heavily biased in favor of very small fibers.

Oritz and Ibsom reported a transfer technique for mounting asbestos and manmade mineral fiber membrane filter air samples using a technique similar to that of Fraser.[42] In this method, diagrammed in Figure 7, a small portion of the filter was attached to a glass slide and exposed to acetone vapor in a petri dish. The filter, collapsed by the acetone vapor which destroys the microporous structure, is then placed in a vacuum evaporator and successively coated with chromium and carbon. Using a petri dish as a reservoir, polyurethane strips were 90% immersed in acetone and blank EM grids were placed on top. Small pieces of the filter material were cut from the filter and placed on the EM grids. The dissolution of the filter matrix by the acetone left the particulate matter adhering to the metallic coating.

Particle losses due to this transfer technique were reported by Oritz and Ibsom to be less than 10 and 3% for asbestos and manmade mineral fibers, respectively. The observed losses were not reported to be fiber size dependent, but rather related to the pore size of the original filter matrix. Methods used for assessment of particle loss are not reported and the reliability of the reported particle loss estimates therefore cannot be evaluated.

Harwood et al. used TEM to evaluate as-

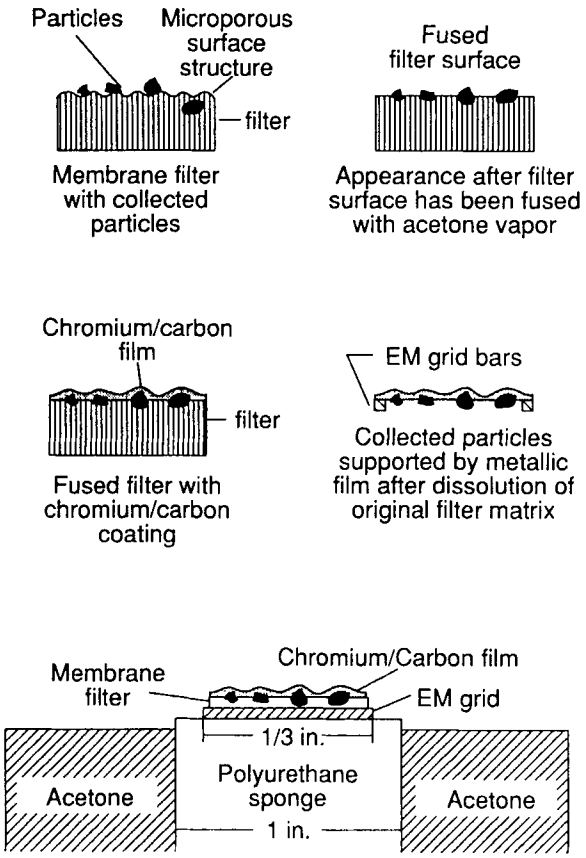

FIGURE 7. TEM specimen preparation technique developed by Oritz and Ibsom.[42]

bestos emissions from baghouse controlled sources.[43] Samples, collected on membrane filters, were prepared for TEM analysis by placing a piece of the filter material onto a carbon-coated grid and placing it in a condensation washer containing acetone as the solvent. The filter medium was washed away by the acetone, depositing the fibers on the carbon substrate of the grid.

Membrane filter techniques discussed earlier focused on the transfer of filter samples for TEM investigation. The utility of TEM analysis was based on the increased resolution associated with electron optics. Samples analyzed were typically from occupational environments where fiber identity was not a concern. The next step in the evolution of TEM analytical techniques was to extend analysis to environmental samples where fiber identification is a major concern.

Holt and Young used a transfer technique similar to Fraser to document the presence of asbestos fibers in urban air.[44] The filters were transferred to EM grids by coating the collection surface with carbon, placing small pieces of the filter collection-side down on EM grids and then dissolving the filter using acetone. Chrysotile, the most commonly found asbestos variety, was identified by observing the characteristic tubular morphology. SAED pattern analysis was also used to confirm the chrysotile identification. Some amphibole asbestos was also identified but no identification criteria were provided.

Alste et al. used the Holt and Young transfer technique to determine asbestos concentrations in the vicinity of an Australian freeway.[45] The identification of asbestos in this investigation was based on morphology and electron diffraction studies. Alste et al. compared diffraction patterns to samples of fresh and worn brake linings and to indexed chrysotile diffraction patterns.[46] Alste et al. concluded that SAED pattern analysis is a method of great sensitivity for the identification of asbestos particles in low concentration atmospheric air samples. However, the authors used only SAED pattern analysis for the identification of chrysotile asbestos. The utility of SAED pattern analysis for amphibole samples was not discussed.

A lesson learned from a review of the development of PCOM analytical methods indicates that utilization of the same general analytical procedure does not guarantee comparable analytical results.[7] Very detailed standardization is required in order to achieve comparability of results within a stated statistical confidence. Methods discussed earlier provide details of sample preparation but fall short of the overall specificity required of a standard method.

2. EPA Provisional TEM Methodologies

Samudra et al. developed a detailed provisional standard method for TEM analysis of airborne asbestos for the U.S. Environmental Protection Agency (EPA).[47] This method provided the basis for the Swiss Institute for Occupational Health and Industrial Hygiene Reference Method.[48] The EPA Provisional Method was designed to be a relatively rapid, cost-effective screening tool using Nuclepore PC filters and a direct transfer technique. Similar to SEM meth-

ods, the Nuclepore PC filter was selected due to its more regular flat surface and cylindrical pore structure to promote fiber visibility.[49] However, some care must be taken since the area around the pores may be highlighted in the secondary electron image, which may interfere with the visibility of small fibers. Fiber losses using this technique were assumed to be negligible due to the absence of particle void regions in the carbon film. However, this observation would not identify fiber losses due to handling prior to carbon coating.

The steps in the sample preparation are similar to those discussed previously for the membrane filter preparation method except that chloroform, instead of acetone, is used to dissolve the PC filter material. The dissolution step is performed using a Jaffe-type washer, Figure 8.[50] A 60- or 100-mesh stainless steel grid is placed on top of a stack of paper filters in a petri dish. Chloroform is poured into the petri dish until the level just reaches the stainless steel mesh. A small portion of carbon-coated Nuclepore PC filter is placed particle-side down on an EM grid, which is placed on top of the stainless steel mesh. A 5-μl drop of chloroform is then placed on top of the filter material. Dissolution will require 24 to 48 h.

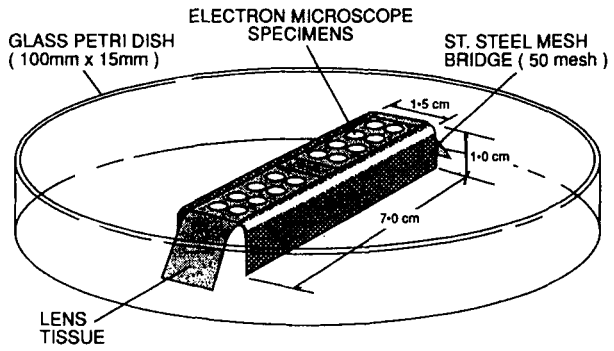

FIGURE 8. Design of the Jaffe washer used for TEM preparation. (Taken from Chatfield, E. J., *A Workshop on Asbestos Fiber Measurements in Building Atmospheres*, Chatfield, E. J., Ed., Ontario Research Foundation, Ontario, Canada, 1985, 115. With permission.)

According to the EPA Provisional Method, the EM grid should be scanned at approximately 20,000 × magnification, recording the length and width of all fibers that have an aspect ratio greater than 3:1 and parallel sides. Tightly bound bundles of fibers are counted as a single fiber. Each fiber is inspected for tubular morphology of chrysotile. SAED pattern analysis, at 0° stage tilt, is then used to classify fibers as either chrysotile or amphibole. According to the EPA Provisional Method, SAED patterns obtained from standard asbestos samples should be used as guides to fiber identification. According to this method, fibers are classified as chrysotile, amphibole, ambiguous, nonasbestos, or unknown.

Samudra et al. recognized that morphology and electron diffraction pattern analysis do not provide irrefutable evidence that a fiber is asbestos. For example, they noted that other particles with fibrous morphology can give layer patterns similar to amphiboles. Quantitative indexing of diffraction patterns, they concluded, was time consuming, complex, and not feasible for routine analysis. In addition, they noted that inspection of the diffraction patterns for some fibers is not always possible, even when they are known to be asbestos. This may be due to several reasons, including interference from nearby particles, small size of a fiber, great thickness of a fiber, and nonsuitable fiber orientation. In addition, some chrysotile fibers may be destroyed in the electron beam, resulting in patterns which fade away within seconds of being formed.

Yamate and Beard reviewed limitations and suggested refinements to the EPA Provisional Method.[27] The suggested refinements resulted in a draft revision of the EPA Provisional Method prepared by Yamate et al.[51] The sample preparation procedure of the revised method was modified to include an additional gold-coating step to aid in the interpretation of the SAED patterns. After the collected particulates are transferred to the EM grid, a thin gold coating is deposited in a vacuum evaporator. The thin gold coat provides an internal standard for SAED analysis to assist in the visual interpretation of fibrous amphibole species. The EM grids are observed using a TEM at a high magnification (20,000 ×).

In the provisional methodology, a fiber is defined as a particle with an aspect ratio ≥3:1. In order to incorporate fibers in various arrangements with other particulate matter and with each other, the revised method defines asbestos struc-

tures. An asbestos structure is defined according to the following categories: single fibers, bundles, clusters, and matrices. Bundles are fibers in parallel arrangement within a fiber diameter of each other. Clusters are random arrangements of fibers such that no fiber stands alone from the group. A matrix is a fiber(s) with one end free and the other end embedded in or hidden by a particle.

Three levels of analysis, summarized in Table 2, are incorporated into the revised provisional method. Each level of analysis provides increasing information and requires greater expertise, training, time, and cost. Level I analysis is the same as the original EPA Provisional Method. Structures are identified as chrysotile, amphibole, ambiguous, or no identity. SAED patterns, using 0° tilt, are used to classify asbestos into two groups, chrysotile or amphibole. The chrysotile pattern is identified by observation of characteristic streaks on layer lines other than the central line with a repeat distance between layer lines of 0.53 nm. Amphibole minerals are qualitatively identified based on layer lines formed by closely spaced dots and layer line repeat distances of 0.53 nm. According to the revised EPA method, micrographs and SAED patterns for standard asbestos samples should be referred to as guides for fiber identification.

TABLE 2
Levels of Analysis for Airborne Asbestos Measurement Using the Revised EPA Provisional Methodology[51]

Level of analysis	Fiber identification criterion	Applicability
Level I	Morphology; visual inspection of SAED pattern	Screening samples
Level II	Morphology; visual SAED; and elemental analysis using EDXRA	Regulatory action
Level III	Morphology; visual SAED; measurement of zone axis SAED from micrographs; and elemental analysis using EDXRA	Confirmatory analysis of controversial samples

In addition to morphological and visual SAED pattern observations, Level II analysis incorporates EDXRA to aid in fiber identification. The profile of the X-ray spectrum is compared with profiles from asbestos standards in a semiquantitative manner. The ratio of peak heights is used along with morphology and SAED patterns to classify mineral fibers. EDXRA is primarily used to identify the presence of amphibole asbestos varieties since chrysotile identification based on morphology and SAED pattern analysis is not as ambiguous as amphibole identification. Similar to Level I analysis, Level II analysis classifies fibers as chrysotile, amphibole, ambiguous, or no identity.

Level III analysis is used when positive identification of the amphibole species is required. SAED patterns obtained in two near exact zone axis orientations are measured from micrographs on 20% of the fibers examined in Level II analysis. Lattice spacings are compared with standard amphibole asbestos minerals. Fibers declared ambiguous or amphibole should be more often included in this analysis. The EM grids used are required to be finder grids so the fiber analyzed can be relocated if necessary.

Fiber count estimates in terms of structures (S/cm^3) rather than individual fibers are determined by scanning until 100 structures are counted or until 10 grid openings (200 mesh grids) are scanned. Asbestos mass estimates can also be determined using this method by sizing all observed structures and converting structure volume to mass using the density.

3. Current TEM Methodologies

Middleton and Jackson reported a sample preparation scheme for MCE membrane filters.[26] This method involved first collapsing the filter using acetone vapor followed by ashing the collapsed filter to expose fibers at the surface. The ashed filter is subsequently carbon coated and the remaining filter removed in a Jaffe-type washer.

Burdett and Rood developed a method for TEM analysis similar to Middleton and Jackson using MCE filters.[29] Nuclepore filters, used in the EPA provisional and revised provisional methods, were rejected due to shortcomings for air sampling as discussed earlier. This method, diagrammed in Figure 9, was an extension of the

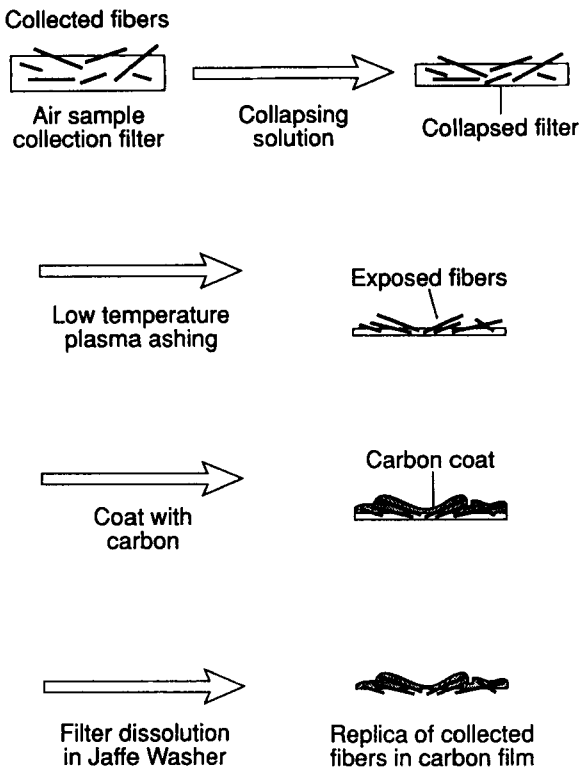

FIGURE 9. Burdett and Rood TEM membrane filter preparation technique.[29]

method originally developed by Oritz and Ibsom[42] with the addition of a low-temperature ashing step. According to this procedure, the filter is first collapsed to about 15% of its original thickness using a clearing solution of 35% dimethylformamide, 15% glacial acetic acid, and 50% water. The filter may now be observed using PCOM, if desired. The filter is then etched in a low-temperature oven, coated with carbon and the remaining filter material dissolved in a Jaffe-type washer. The important feature of the Middleton and Jackson and Burdett and Rood methods which distinguishes them from earlier methods is the ashing step. Ashing exposes fibers on the filter surface so they can be readily trapped in the carbon layer.

The depth to which fibers penetrate membrane filters will depend on the nominal pore size, the size of the fibers, and the viscosity and flow rate of the filtered medium. Fraser reported that particles can penetrate 0.8-μm pore size membrane filters to a depth of 15 to 20 μm.[40] Some fibers are therefore likely to be completely engulfed in the membrane filter when it is collapsed and will not be trapped in a carbon layer. These fibers will be lost when the filter material is dissolved away. The ashing step removes the top layer of the filter material exposing these fibers so they can be held in the carbon layer and not washed away during the final dissolution step. This problem was recognized by Oritz and Ibsom, who noted that if the membrane filter is collapsed for too long a period, the membrane will flow around the collected particulate matter and encapsulate them in the filter matrix. Paying careful attention to the collapsing time, Oritz and Ibsom reported losses less than 3% for fibrous particles. This observation cannot be evaluated because no method for loss evaluation was provided by the authors.

Fiber loss due to penetration into the filter matrix was evaluated by Burdett and Rood by etching filters for increasing periods of time and counting the observed fibers. These data, presented in Table 3, indicate that fibers are sufficiently buried to avoid being trapped in the carbon layer, and that if no ashing were done losses of up to 50% may occur. After 6 min of etching, no further increase in fiber counts were observed. Fiber loss due to over-etching was evaluated by photographing the same field under phase contrast illumination after successive periods of ashing. No loss or movement of fibers was observed for ashing times up to 45 min. Since PCOM was used, this technique would not be able to evaluate the loss, if any, of small fibers, <0.1 μm in diameter. Over-ashing may therefore be a source of fiber loss for small diameter fibers. Based on these data and observations, Burdett and Rood concluded that fiber losses will be negligible provided that the surface of the collapsed filter is etched for 6 min in an oxygen plasma.

Middleton and Jackson also evaluated fiber loss due to low temperature ashing of MCE filters by direct observation of the same area of the filters by PCOM (after collapsing), by SEM (after ashing), and by TEM (after final dissolution). These observations, similar to Burdett and Rood, indicated that neither etching or dissolution resulted in fiber loss or breakage.

According to Yamate and Beard, Nuclepore PC filters were selected for air sample collection in the EPA provisional and revised provisional methods based on the conclusion that they pro-

TABLE 3
TEM Fiber Counts as a Function of Time of Ashing for Airborne Fiber Samples

Time of ashing in oxygen plasma, (min)	Mean fiber count per grid opening: Chrysotile on a 0.8-μm pore size Gelman filter	Chrysotile on a 1.2-μm pore size Millipore filter	Microquartz on a 0.8-μm pore size Gelman filter
0	12.4	7.7	17.8
3	11.3	10.9	23.6
6	13.4	14.6	27.0
12	7.7	12.5	27.4

Note: Mean fiber size: chrysotile, 0.06 μm diameter, 1.7 μm length; microquartz, 0.32 μm diameter, 7.1 μm length.

Taken from Burdett, G. J. and Rood, A. P., *Environ. Sci. Technol.*, 17(11), 643, 1983. With permission.

vide a substrate yielding minimum losses during transfer to the EM grid.[27] It should be noted that this conclusion was based on evaluation of MCE transfer methods developed prior to Burdett and Rood. Yamate and Beard noted that, based on X-ray fluorescence data, Nuclepore PC filters may be subject to large losses, up to 40% of the mass, due to handling during transport. They also noted that this was likely due to the loss of large particulate matter and that, for very small particles, shipping the Nuclepore filters through the mail resulted in negligible loss. No data or references are provided to evaluate these observations.

Burdett and Rood performed a fiber count comparison between 0.1-μm pore size membrane and Nuclepore PC filters with equal loadings of chrysotile fibrils. These data are presented in Table 4. Both filters were prepared using direct transfer techniques, with MCE filters prepared by ashing prior to carbon coating. Based on a comparison of mean count per grid opening for five different samples covering a range of loadings, the Nuclepore filters gave consistently low fiber counts which were statistically significant at the 99% level. In addition, the fiber size distribution showed that the mean fiber size was smaller on the membrane filters, which is consistent with smaller fibrils being lost from the Nuclepore filters. In contrast to these findings, Chatfield found little difference between Nuclepore PC and MCE filter direct transfer tech-

TABLE 4
Fiber Count Comparisons between 0.1-μm Pore Size Millipore and Nuclepore PC Filters with Equal Loadings of Chrysotile Fibrils

Millipore[a]			
Mean count per grid opening	SD	95% Confidence limit: Upper	Lower
11	3	13	8
42	3	44	40
82	8	92	72
172	10	179	165
353	32	376	330

[a] Measured fibril size: mean diameter 0.046μm, mean length 1.07 μm.

Nuclepore[b]			
Mean count per grid opening	SD	95% Confidence limit: Upper	Lower
8	2	11	6
24	4	26	21
72	8	77	67
121	7	126	116
241	20	255	227

[b] Measured fibril size: mean diameter 0.06 μm, mean length 1.37 μm.

Taken from Burdett, G. J. and Rood, A. P., *Environ. Sci. Technol.*, 17(11), 643, 1983. With permission.

niques.[52] Using 0.2-μm Nuclepore PC and 0.8-μm MCE filters, Chatfield sampled two different chrysotile aerosols, single fibril and highly aggregated aerosol. MCE filters were prepared using the Burdett and Rood method. According to Chatfield, the results for single fibril and highly aggregated samples showed that the two filter types provide equivalent estimates. These results were based on comparing only one sample for each filter and aerosol type and are therefore considered limited.

The Burdett and Rood sample preparation procedure has been extensively used to evaluate airborne levels of asbestos in the U. K.[53-57] Two levels of analysis are recommended by Burdett.[54] In the first level, the TEM is operated at 1000 × magnification to count fibers >5 μm in length. The morphology and elemental composition using EDXRA are then determined at 10,000 × magnification. The first level of analysis is designed as a screening tool for quick and inexpensive results. In the second level of analysis, complete asbestos fiber counts, mass concentrations, and size distributions are determined by scanning the sample grid at approximately 20,000 × magnification and identifying each fiber with an aspect ratio of 3:1 and parallel sides. Each fiber is analyzed using EDXRA and SAED as required to establish the identity.

In response to the Asbestos Hazard Emergency Response Act (AHERA), the Environmental Protection Agency promulgated the Asbestos-Containing Materials in Schools Regulations.[5] Appendix A to this regulation specifies two TEM methods, mandatory and nonmandatory, to be used to determine when asbestos abatement jobs are complete. Samples can be collected using either Nuclepore PC or MCE filters and prepared according to direct transfer methods. The low temperature ashing technique developed by Burdett and Rood is specified for MCE filter preparation. The original EPA Provisional methodology is specified for Nuclepore PC filter preparation.

The mandatory method contains the minimum requirements for TEM analysis, while the nonmandatory method is intended as a supplement by including additional steps to improve the analysis. These methods specify a high screen magnification (15,000 to 20,000 ×) for analysis. Any contiguous grouping of particles in which an asbestos fiber with an aspect ratio ≥5:1 and a length ≥0.5 μm is detected should be counted. This differed from earlier EPA methods which specified a 3:1 aspect ratio. The EPA-AHERA Method changed the aspect ratio definition based on the opinion of a panel of microscopists, who observed that asbestos structures have aspect ratios ≥5:1 whereas nonasbestos structures, e.g., gypsum particles, have aspect ratios <5:1. Fiber identification is based upon visual observation of SAED pattern and EDXRA. The nonmandatory method provides more detail for SAED pattern analysis and specifies the classification of fiber structures into two size classes, greater than and less than 5 μm in length. This size classification scheme works well for individual fibers but not for other structures, such as clumps and matrices, which may contain more than one fiber with different lengths.

In addition to the EPA methods, NIOSH has published a recommended TEM procedure, Method 7402, for asbestos analysis.[58] This method, originally published in 1987 and revised in 1989, is designed to be used in occupational environments, in conjunction with phase contrast analysis according to NIOSH Method 7400. The original Method 7402 specifies MCE filters and direct transfer preparation similar to the Burdett and Rood procedure.[59] The Revised Method 7402, however, does not incorporate the ashing step recommended by Burdett and Rood. Sample preparation is therefore similar to that originally published by Oritz and Ibsom. Although no explanation for the deletion of the ashing step is provided, it is likely due to the fact that the purpose of this method appears to have shifted.

The original Method 7402 specified that all particles <3 μm in diameter with an aspect ratio of 3:1 be counted and sized. The identity of each fiber was to be established based on morphology, SAED pattern analysis, and EDXRA. Using this method, the total asbestos fiber concentration can be calculated. In addition, the fraction of asbestos not included in the PCOM analysis can be determined by including only those fibers shorter than 5 and <0.25 μm in diameter in the calculation.

By comparison, the revised NIOSH Method 7402 counts only those fibers meeting the opti-

cally visible definition discussed previously to obtain the fraction of optically visible fibers which are asbestos. This fraction is applied to fiber counts obtained by PCOM analysis to obtain a PCOM asbestos fiber count. The ashing step, which was deleted from the revised method, would destroy any organic fibers present which would be counted using PCOM analysis. Destruction of these fibers may, therefore, result in an overestimation of the asbestos fiber fraction in an air sample.

According to both NIOSH procedures, asbestos is identified based on qualitative EDXRA and SAED pattern analysis by comparison to standard asbestos materials. Based on SAED pattern analysis, fibers are classified as chrysotile, amphibole, ambiguous, or other. EDXRA is used to classify the various amphibole minerals.

4. TEM Fiber Classification

Early investigators paid little attention to problems of mineral fiber identification, providing minimal information on fiber classification. If the source of fiber contamination is well characterized, such as occupational environments or ambient environments proximate to asbestos mines or mills, then fiber identification is less troublesome. However, air samples from the general environment may contain fibers whose origin is unknown, making identification problematic.

Many investigators have reported detailed discussions of mineral fiber identification.[1,37,60-65] Methods of SAED and EDXRA identification and their practical limitations for asbestos analysis were reviewed by Lee, who noted that the classification of chrysotile is relatively straightforward compared with amphibole varieties of asbestos.[64] Conclusions reached by Lee are (1) visual interpretation of the SAED pattern works well with chrysotile but will result in highly variable results when amphibole asbestos varieties are being identified from unknown sources; and (2) the use of EDXRA is an improvement over simple observation of the SAED pattern but attempting to identify amphiboles from unknown sources will still be subject to uncertainty. Positive identification of mineral species, according to Lee, requires two indexed SAED patterns, or in some cases, one pattern and EDXRA for positive identification of minerals. The procedure recommended by Lee for analyzing fibrous mineral of unknown origin is to analyze a large number of fibers by EDXRA and assign tentative mineral species based on relative peak intensities. About 10% of these particles should then be identified by quantitative SAED pattern analysis. This procedure will establish the homogeneity of the mineral classification and allow the estimation of uncertainty of identification. This recommendation is similar to the Level III analysis of the revised EPA Provisional Method.

Chatfield reviewed techniques for asbestos fiber identification and proposed a detailed scheme for mineral fiber identification.[49,66] Chatfield notes, similar to Lee, that chrysotile asbestos is more readily identified than amphibole varieties. He observed, however, that other mineral species, such as vermiculite, can exhibit a scrolled structure that can be confused with chrysotile tubular morphology. Quantitative SAED pattern analysis is needed to accurately distinguish these minerals. The Chatfield classification scheme for fibers with and without tubular morphology is presented in Tables 5 and 6. According to this scheme, it is important to establish the desired level of classification and then record for each fiber the level of classification actually achieved. This classification differs from other schemes in that Chatfield recommended the use of quantitative EDXRA data rather than the more commonly used qualitative approach of observing relative peak heights. Chatfield describes a technique used by Cliff and Lorimer to perform quantitative analysis of elemental composition (within ap-

TABLE 5
Classification of Fibers with Tubular Morphology[52]

TM — Tubular morphology not sufficiently characteristic for classification as chrysotile
CM — Characteristic chrysotile morphology
CD — Chrysotile SAED pattern
CQ — Chrysotile composition by quantitative EDXA
CMQ — Chrysotile morphology and composition by quantitative EDXA
CDQ — Chrysotile SAED pattern and composition by quantitative EDXA
NAM — Nonasbestos mineral

TABLE 6
Classifications of Fibers without Tubular Morphology[52]

UF — Unidentified fiber
AD — Amphibole by random orientation SAED (shows layer pattern of 0.53-nm spacing)
AX — Amphibole by qualitative EDXA; spectrum has elemental components consistent with amphibole
ADX — Amphibole by random orientation SAED and qualitative EDXA
AQ — Amphibole by quantitative EDXA
AZ — Amphibole by one zone axis SAED
ADQ — Amphibole by random orientation SAED and quantitative EDXA
AZQ — Amphibole by one zone axis SAED pattern and quantitative EDXA
AZZ — Amphibole by two zone axis SAED patterns with consistent inter-axial angle
AZZQ — Amphibole by two zone axis SAED patterns, consistent inter-axial angle and quantitative EDXA
NAM — Nonasbestos mineral

proximately 10%) using X-rays generated from a thin specimen.[67]

Focusing on the difficulties of amphibole identification, Chatfield defined four different levels of analysis, listed in Table 7. For analysis of unknown samples, Level III analysis is required in order to confirm the presence of amphiboles. For this level of analysis, the target level of classification is based on random orientation SAED pattern analysis and quantitative EDXRA. In addition, it is recommended that at least one fiber from each suspected amphibole be examined by zone axis SAED to confirm identification.

B. Indirect Transfer Methods

Indirect transfer methods were developed primarily for situations where the airborne asbestos concentration was either too dilute or too concentrated for accurate analysis. In addition, indirect transfer methods were initially selected because the direct transfer methods had not yet been adequately developed. Indirect transfer techniques for sample preparation are reviewed by Nicholson.[68]

One indirect method of filter preparation, referred to as the rub-out technique, was reported by Selikoff et al.[69] and used to evaluate ambient asbestos air pollution.[70,71] In these studies, air samples were collected on membrane filters and completely ashed, removing the filter material and any organic matter collected. The ashed material was ground on a glass slide in a nitrocellulose film to break up any chrysotile fibers into

TABLE 7
Levels of Analysis for Amphibole Identification[52]

Level of analysis	Application	Target classification for all fibers	Required classification for confirmation of amphibole in a proportion of the fibers
1	Routine monitoring of known and well-characterized sources for one mineral fiber type	ADX	Not applicable
2	Routine monitoring of known and well-characterized sources where discrimination between two or more amphibole fiber types is required	ADQ	Not applicable
3	Routine samples from uncharacterized sources in which presence or absence of amphibole is to be confirmed	ADQ	AZZ, AZQ, or AZZO solutions must include only amphiboles
4	Samples where precise identification of all amphibole fibers is an important issue	AZQ	AZZQ solutions must include only amphiboles

fibrils and then transferred to EM grids. Grids were scanned at 25,000 × or more magnification and mass concentration calculated by measuring the asbestos fiber volume and multiplying by the appropriate density. Mass concentration estimates were chosen over fiber counts, according to the authors, due to the uncertainty of the relationship between biological effect and fiber size and because mass represents a more conservative concentration estimate since the biological effect of the sample dominated by large fibers may be overestimated.

A similar indirect method was used by Rickards to determine airborne chrysotile mass estimates at several urban and rural sites.[72] According to this method, the dust collected from a large volume ambient air sample was filtered onto a cellulose nitrate membrane filter and subsequently ashed at 450°C for 2 h. The authors noted that prior to ashing, the filter had to be wetted with dibutyl phthalate to prevent explosive combustion of the filter and resulting loss of collected material. Fiber loss during the ashing step was estimated to be minimal based on evaluation of X-ray diffraction traces before and after ignition.

Subsequent to ashing, the samples were placed in an ultrasonic water bath operating at 45 kHz and 100 W for a period of 4 h. The samples were then diluted to an appropriate concentration, depending on the amount of material originally collected, and sonicated for an additional 8 h. The purpose of the sonication was to break up any chrysotile present into individual fibrils and to homogenize the suspension. The suspension was then mounted for TEM examination by placing a drop on a carbon-coated EM grid and evaluated at 1000 × magnification. Chrysotile fibrils were identified using electron diffraction patterns and fiber length determined from photomicrographs. Fiber volume, calculated assuming that chrysotile fibrils have a mean diameter of 340 Å, was converted to mass using an assumed theoretical density. The analytical time, not including sample preparation, was reported to be 5 h.

Rickards determined mass estimates in order to compare to an X-ray diffraction method developed earlier. The X-ray diffraction method was not sensitive enough for low level environmental concentrations of chrysotile encountered in these samples. The TEM method reportedly lowered the analytical sensitivity, by a factor of 1000, to 0.1 ng/m^3. However, the author did not attempt to relate mass estimates to more common fiber count estimates of exposure.

A similar indirect transfer technique for sample preparation was used by Nicholson et al. to evaluate airborne asbestos concentrations in schools with damaged asbestos-containing surface treatments.[73]

Sebastien et al. utilized an indirect transfer preparation technique similar to that published by both Rickards and Nicholson.[74,75] Air samples, collected on membrane filters, were completely ashed in a low temperature asher. After ashing, water was added to the residue and the mixture was treated ultrasonically for 2 h in a 150-W ultrasonic bath. The sonicated mixture was filtered through a 0.2-μm pore size Nuclepore PC filter previously coated with a layer of carbon. After filtration, an additional layer of carbon was deposited on the filter, embedding the collected particulate matter between layers of carbon. Small sections of this filter were transferred to a EM grid and the filter material was dissolved using chloroform.

Randomly chosen grid squares were examined at 33,000 × magnification. Chrysotile fibers were identified based on morphology and SAED pattern analysis. Characteristic X-ray spectra were used to identify the various amphibole species. Exposure results are reported in mass terms, ng/m^3.

Airborne asbestos levels in nonoccupational environments in Japan were evaluated by Kohyaman of the Japanese National Institute of Industrial Health.[76] TEM analysis was conducted according to a modified indirect transfer technique. The air sample collection filter was made to adhere to a glass slide using a couple of drops of acetone and ashed in a low temperature plasma asher. The ashed material was then wetted with a few drops of isopropanol and shaved off the slide with a blade. Both the blade holding the shaved residue and the glass slide were placed in a flask containing isopropanol and dispersed ultrasonically for a short period of time. The exact time of sonication and the power level of the sonicator were not provided by the author. After sonication, the suspension was filtered through

a Nuclepore PC filter. The filter was then coated with carbon and transferred to an EM grid in a Jaffe washer with chloroform to dissolve the filter material. The grids were examined and asbestos fibers counted and sized. No information on the identification criteria was provided by the author. The mass concentration of asbestos was calculated from the fiber concentration and the average fiber length and diameter.

C. Comparison of Indirect and Direct Transfer Procedures

Indirect transfer methods were initially developed in order to provide mass estimates of airborne asbestos concentration. To this end, procedures which altered the airborne fiber size distribution were not considered problematic. The mass of a large bundle of asbestos should be the same as the sum of the masses of the individual fibers comprising the bundle. Epidemiological data used for risk assessment purposes, however, are based upon fiber count estimates of exposure. Exposure estimates based on mass are therefore difficult to interpret in terms of direct health risk, leading Doll to the conclusion that fiber count estimates are more appropriate.[77]

The major advantage of indirect transfer methods, i.e., that large volumes of air and associated heavy particle loadings can be sampled and analyzed, has led a number of investigators to evaluate the degree to which indirect methods of preparation disturb the fiber distribution and bias the associated fiber count.

The effect of sonication on the fiber size distribution of amphibole fibers was evaluated by Bishop et al.[78] A portion of a Nuclepore filter was prepared using direct preparation by carbon coating and dissolution while another portion was rinsed into water and sonicated prior to mounting for TEM analysis. No significant differences were found between the fiber counts produced by the two methods of preparation. However, this conclusion is of limited application to chrysotile analysis because, unlike amphiboles, chrysotile, a serpentine mineral, exists in bundles of fibrils which are more likely to be broken up during sonication.

Bishop et al. also investigated the potential for losses associated with filter ashing. Losses were evaluated by ashing a filter loaded with ^{59}Fe-labeled amosite. Gamma activity measured before and after ashing indicated that losses associated with ashing were minor.

Direct and indirect sample preparation techniques were also compared by Hwang and Wang.[79] Direct preparation of chrysotile-loaded membrane filter samples was conducted according to Oritz and Ibsom.[42] Portions of the same filters were mounted using an indirect method which included ashing with a 10-min sonication prior to refiltration and mounting for TEM analysis. The indirect preparation was found to more than triple the total number of fibers observed. These results are difficult to interpret, however, since the Oritz and Ibsom method lacks a low temperature ashing step. The discrepancy between the indirect and direct methods may be due in part to the inability to see fibers which may have been trapped in the filter matrix using the Oritz and Ibsom method.

Burdett compared TEM fiber count and mass estimates using direct and indirect preparation procedures for samples collected while investigating asbestos release from damaged amosite insulation.[80] One quarter of each sample was analyzed using the Burdett and Rood method discussed earlier.[28] Another quarter of the filter was prepared using an indirect method by ashing the filter and sonicating the residue dispersed in distilled water for 5 min at 75-W power. The suspension was then filtered through another membrane filter and prepared using the above direct transfer technique. Although he evaluated only three samples, Burdett concluded that the changes in number concentration and size distribution resulting from the indirect method were sufficient to limit its use.

Chatfield compared direct and indirect methods of preparation for both Nuclepore PC and MCE filter samples by sampling laboratory-generated single fibril and highly aggregated chrysotile aerosols.[52] The Burdett and Rood direct transfer technique was used for the MCE filters. Nuclepore PC filters were prepared according to the direct transfer procedure specified in Yamate Revised EPA Provisional Method.[51] Indirect preparation for the Nuclepore filter involved removing the deposited material by hand shaking

in water and filtration onto a Nuclepore PC filter. The MCE filter indirect preparation involved ashing and ultrasonic redispersal followed by filtration onto a Nuclepore PC filter. No details on the power or length of sonication were provided. For the aggregate aerosol, the indirect method resulted in an increase in the number of fibers counted. This increase was greatest for short fibers, less than 2.5 μm in length. Chatfield concluded therefore that for analyses of fibers >5 μm in length the direct and indirect methods provide comparable fiber count results.

Direct and indirect preparation techniques for MCE filters were compared by Sebastien.[75] Direct preparation followed the method of collapsing and ashing developed by Middleton and Jackson.[26] The indirect method consisted of ashing the entire filter and sonicating the filter residue in an ultrasonic bath for periods of time ranging from 1 to 120 min. The material was then refiltered onto a Nuclepore filter and prepared for TEM examination by carbon coating and filter dissolution. Sebastien reported a low success rate for the direct preparation technique, with only 6 out of 17 filters successfully prepared. In addition, these preparations resulted in low fiber loadings which were therefore difficult to evaluate. By comparison, the indirect method produced acceptable preparations, whose loadings could be adjusted for better microscopic analysis. Sonication time was reported to have a marginal effect on the numerical fiber concentrations; however, no data were provided to support this conclusion.

A major difference between direct and indirect transfer preparation techniques is the redispersion procedure followed by refiltration. Redispersion most often involves sonication for some period of time. It is evident, based on data generated by Burdett and Chatfield, that sonication can, in some cases, alter the existing size distribution of the collected aerosol. More research is needed to define the conditions under which sonication will preserve the original size distribution before it will be an accepted approach.

V. COMPARISON OF TEM AND SEM METHODS FOR ASBESTOS ANALYSIS

The superiority of TEM analysis, compared with SEM, for airborne asbestos quantification has been extensively recognized and discussed. SEM is not widely used because the contrast-limited resolution restricts analysis to fibers >0.1 to 0.2 μm in diameter, and the inability to analyze the crystalline structure of fibers results in uncertain identification. However, recent publications suggest that the analytical superiority of TEM may be less important when the tremendous variability associated with electron microscopic fiber counting is considered.

Cherrie et al. compared fiber counts from SEM, TEM, and PCOM for three different types of samples: laboratory prepared samples of mixed fiber type, chrysotile asbestos textile factory samples, and nonoccupational samples from sites where asbestos might be found.[81] SEM air samples were collected on PC filters and analyzed using the AIA method. Total fibers and fibers >5 μm in length were counted at 10,000 × and 2000 × magnification, respectively. TEM samples were collected on Gelman DM800 filters and prepared according to the Burdett and Rood direct transfer technique. In order to compare results, the counting rules used for TEM analysis were the same as those used for SEM analysis. TEM analysis produced total fiber counts which were greater than SEM, which, in turn were greater than the PCOM counts. When the analysis was limited to fibers longer than 5 μm, however, SEM and TEM provided similar results. These results indicate that TEM analysis is advantageous only when counting or sizing smaller fibers (<5 μm in length).

A similar conclusion was reported by Breysse et al., who reported results of TEM and SEM analysis for side-by-side samples collected during removal of chrysotile-insulated water tanks.[82] SEM samples were collected on PC filters and gold coated for analysis. MCE filters were prepared for TEM analysis using the Burdett and Rood method. Fiber identification was based on morphology and EDXRA. SAED pattern analysis was not used for TEM evaluation because morphology and elemental analysis were sufficient to distinguish the chrysotile fibers from the other manmade mineral fibers also present in the samples. Size-selective fiber counts were conducted according to the counting rules specified in European Reference Method (ERM) for

PCOM analysis.[13] ERM fiber counts are restricted to fibers >5 μm in length and <3 μm in diameter.

Comparison of ERM size-selective asbestos fiber counts presented by Breysse et al. is contained in Figure 10. Error bars are based on 95% confidence limits calculated on the basis of Poisson error only. These confidence intervals are expected to underestimate the true variability which should include counter interpretation errors.[83] In seven of the sample pairs, there was no difference (based on 95% confidence intervals) between the ERM asbestos fiber concentrations determined by SEM at 10,000 × magnification and by TEM at 2200 × magnification. In three of the samples, the SEM concentration was significantly higher and in two of the samples the TEM concentration was higher. Five of the TEM samples were also analyzed using TEM at 17,000 × magnification. This magnification produced the highest count in every case but one. In two of the cases, the high magnification TEM fiber concentration was significantly different from the SEM concentration and in three of the cases significantly different from the low magnification TEM concentration. This variability was attributed to the large error associated with fiber counting, particularly in interpreting clumps and bundles of fibers. The author concluded that SEM and TEM fiber exposure estimates of fibers >5 μm in length may be indistinguishable.

The data presented by Cherrie et al. and Breysse et al. suggest that TEM and SEM fiber count estimates of fibers >5 μm in length provide equivalent results. Furthermore, a recent review of asbestos exposure indices by Lippman concluded that asbestos-induced cancers may be more closely associated with the inhalation of

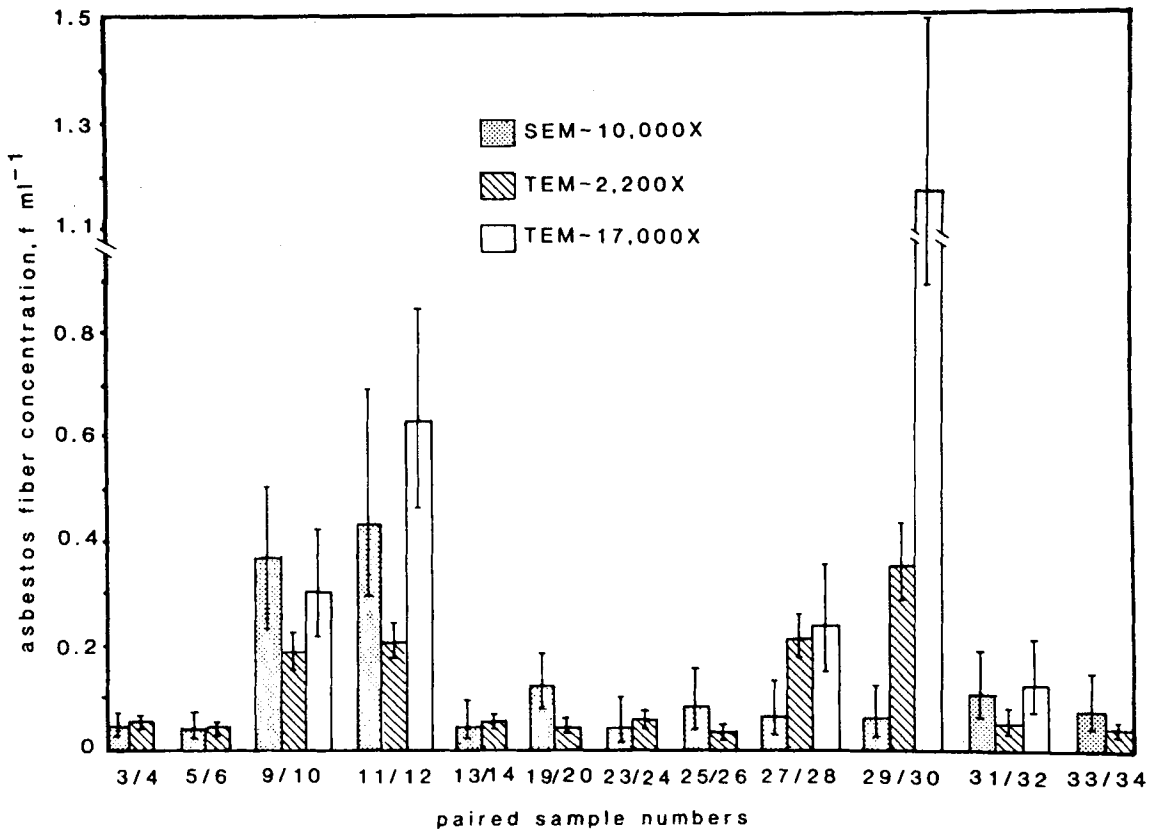

FIGURE 10. Comparison of European Reference Method asbestos fiber concentration determined using SEM (magnification × 10,000), TEM (magnification × 2200), and TEM (magnification × 17,000 and × 2200). (Taken from Breysse, P. N., Cherrie, J. W., Addison, J., and Dodgson, J., *Ann. Occup. Hyg.*, 33(2), 243, 1989. With permission.)

longer fibers (>5 to 10 μm).[11] If this were true, the ability of the TEM to see the very smallest asbestos fibers in air sampling analysis may be immaterial to risk assessment and management strategies. Such an SEM analysis would be significantly cheaper than TEM, and hence, would greatly reduce the analytical cost over that of TEM asbestos air sample analysis. However, the results presented by Cherrie et al. and Breysse et al. are limited and need to be corroborated for different types of asbestos environments before SEM analysis is exclusively adopted over TEM analysis for this purpose.

For the detection and identification of fibers <5 μm, the costs of attaining comparable SEM and TEM analyses are more equivalent. The cost of SEM analysis is generally about half that of TEM analysis. The longer time needed to produce an SEM analysis for fibers < 5 μm that is comparable in quality to a TEM analysis can make the cost of the former similar to or somewhat higher than the latter. The increased time for SEM analysis is due to several factors: the increased time needed on each field of view due to raster timing, the increased counting time needed to attain the same sensitivity as the TEM (due to the reduced peak to background for the X-ray field of view on the SEM), and the increased time in calibration of fiber visibility in SEM to match TEM fiber visibility.

VI. CONCLUSIONS

The literature on electron microscopic methods for airborne asbestos analysis is extensive, beginning in the early 1950s. Early electron microscopic methods were developed as research tools to evaluate airborne fiber size characteristics where exposures were routinely evaluated using optical microscopic methods. With the expansion of the health concerns to include nonoccupational exposures, electron microscopic methods were developed to be used for routine exposure assessment. Research into method development expanded at this time. Method development issues included the type of electron microscope to be used, SEM or TEM; the type of filter paper to be used, Nuclepore PC or membrane filter varieties; and the choice of transfer techniques, direct or indirect.

Initial research included investigations into both SEM and TEM methods. The need to completely resolve and accurately identify the population of fibers potentially present in an air sample led to the widespread recognition of TEM methods as superior to SEM analysis. However, recent research suggests that when indices of exposure to fibers >5 μm in length are evaluated, TEM and SEM methods may be comparable.

Le Guen et al. described a method for SEM analysis using collapsed and ashed membrane filters. This method provided a relatively smooth analytical surface with minimal potential fiber loss and represented a significant improvement over other SEM methods using Nuclepore PC filters or collapsed unashed membrane filters. It has failed to gain widespread utilization, however, due to the development and acceptance of standardized TEM methods.

Early investigations into environmental asbestos pollution resulted in mass estimates of exposure using indirect transfer methods. These methods were developed in part because no reliable direct transfer method was available. The uncertainty surrounding particle losses from Nuclepore PC filters was a major limitation of early TEM methods. Additionally, fiber mass exposure estimates were found to be of limited utility due to the inability to relate them to the more common fiber count estimates. TEM procedures developed by Middleton and Jackson and Burdett and Rood have led to the widespread use of direct transfer mounting procedures that allow the use of membrane filters for air sample collection. With the advent of acceptable direct transfer procedures, the indirect methods and associated mass estimates of exposure have become less prevalent.

TEM analysis for samples collected on membrane filters and prepared using direct transfer techniques is now a readily accepted analytical tool for evaluation of low level environmental nonoccupational exposure to asbestos.

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