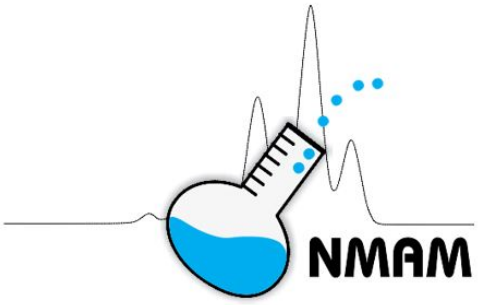

NIOSH Manual of Analytical Methods (NMAM), 5th Edition

Measurement of Fibers

by Paul A. Baron, Ph.D., NIOSH
Adapted from Baron [2001]

The NMAM team gratefully acknowledges Chen Wang, Joe Fernback and Alan Dozier for insightful review of this chapter.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

1 Introduction

Fiber-related disease has provided much of the impetus for fiber research in recent years. Asbestos has been the fiber type most commonly associated with disease. The name "asbestos" is a commercial term applied to the fibrous forms of several minerals that have been used for similar purposes and includes chrysotile, amosite, crocidolite, and the fibrous forms of tremolite, anthophyllite, and actinolite. The three primary diseases associated with asbestos exposure are asbestosis, the result of inflammation and collagen formation in lung tissue; lung cancer; and mesothelioma, an otherwise rare form of cancer associated with the lining surrounding the lungs. A current theory describing the toxicity of fibers indicates that fiber dose, fiber dimension, and fiber durability in lung fluid are the three primary factors determining fiber toxicity [Lippmann 1990].

The dose, or number of fibers deposited in the lungs, is clearly an important factor in determining the likelihood of disease. Both fiber diameter and length are important in the deposition of fibers in the lungs and how long they are likely to remain in the lungs. Figure 1 indicates some of the factors that determine fiber deposition and removal in the lungs. Fiber length is thought to be important because the macrophages that normally remove particles from the lungs cannot engulf fibers having lengths greater than the macrophage diameter.

Thus, longer fibers are more likely to remain in the lungs for an extended period of time. The macrophages die in the process of trying to engulf the fibers and release inflammatory cytokines and other chemicals into the lungs [Blake et al. 1997]. This and other cellular interactions with the fibers appear to trigger the collagen buildup in the lungs known as fibrosis or asbestosis and, over a longer period, produce cancer as well. Fiber diameter is also important because fiber aerodynamic behavior indicates that only small diameter fibers are likely to reach into and deposit in the airways of the lungs. The smaller the fiber diameter, the greater its likelihood of reaching the gas exchange regions. Finally, fibers that dissolve in lung fluid in a matter of weeks or months, such as certain glass fibers, appear to be somewhat less toxic than more insoluble fibers. The surface properties of fibers are also thought to have an effect on toxicity. Asbestos is one of the most widely studied toxic materials and there have been many symposia dedicated to and reviews of its behavior in humans and animals [Selikoff and Lee 1978; Rajhans and Sullivan 1981; WHO 1986; ATSDR 1990; Dement 1990].

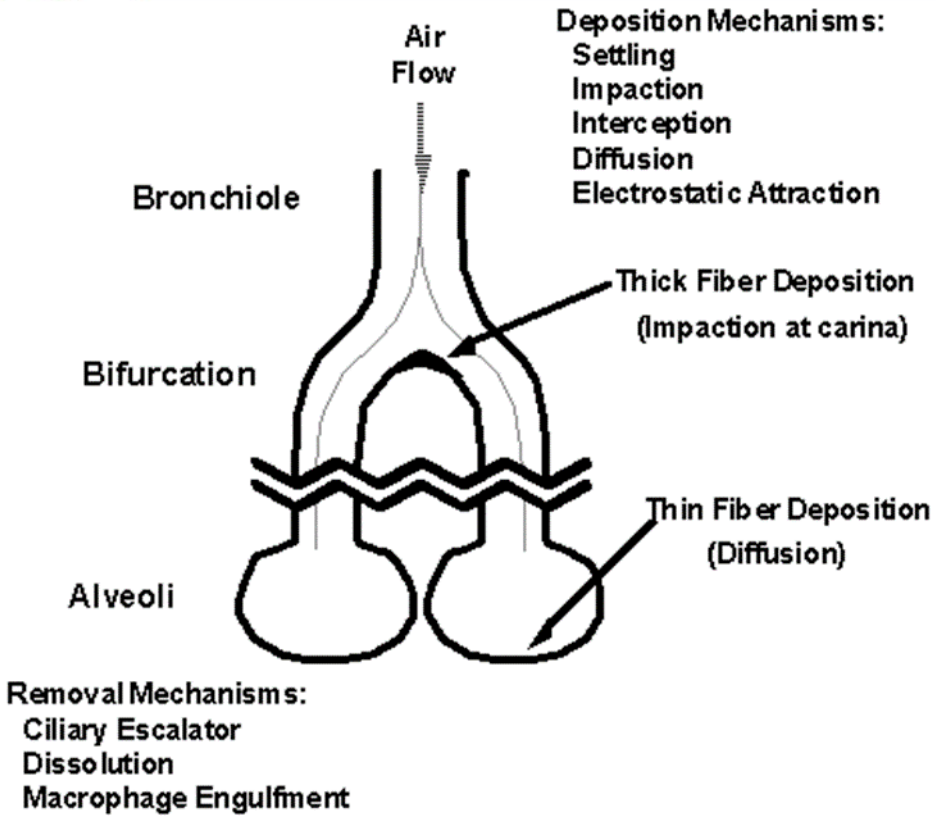

Figure 1. Schematic of mechanisms that affect fiber deposition and retention in the lungs. The deposition depends on all the indicated parameters in a complex fashion. However, larger diameter particles are affected more by gravitational settling, impaction, and interception, resulting in greater deposition further up in the respiratory tract. The saddle points, or carinae, in the branching respiratory tree are often a focal point for deposition of larger diameter fibers. Smaller diameter particles are affected more by diffusion and can collect in the smaller airways and gas exchange region (alveoli). Particle removal from the lungs is primarily effected by the cilia coating the non-gas exchange regions of the lungs; the cilia push mucus produced in the lungs and any particles trapped in the mucus out of the lung and into the gastrointestinal tract in a matter of hours or days. Some fibers are sufficiently soluble in lung fluid that they can disappear in a matter of months. Finally, white blood cells or macrophages roam the gas exchange regions and ingest particles deposited there for removal through the lymph system. Human macrophages are approximately 17 µm in diameter and can only ingest particles smaller than they are. Therefore, thin fibers are likely to deposit in the gas exchange region and, of these, the long insoluble fibers can remain in the lungs indefinitely.

Several techniques were used for asbestos measurement up until the late 1960s [Rajhans and Sullivan 1981]. Earlier than this, it was not widely recognized that the fibrous nature of asbestos was intimately related to its toxicity, so many techniques involved collection of airborne particles and counting all large particles at low magnification by optical microscopy. Thermal precipitators, impactors (konimeters), impingers, and electrostatic precipitators were all used to sample asbestos. Perhaps the primary technique in the United States (US) and the United Kingdom (UK) during this early period was the liquid impinger, in which particles of dust larger than about 1-µm aerodynamic diameter were sampled at 2.7 L/min and impacted into a liquid reservoir [Rajhans and Sullivan 1981]. After sampling, an aliquot of the liquid was placed on a slide in a special cell, particles larger than 5-µm size were counted, and the results were reported in millions of particles per cubic foot. Dissatisfaction with this approach stemmed from lack of correlation between measured particle concentration and disease in the workplace. Various indices of exposure have been developed that attempt to relate a portion of the fiber size distribution to the toxic effects. The appropriate indices for each of the asbestos related diseases as a function of fiber length and diameter (Figure 2) were suggested by Lippmann [Lippmann 1988].

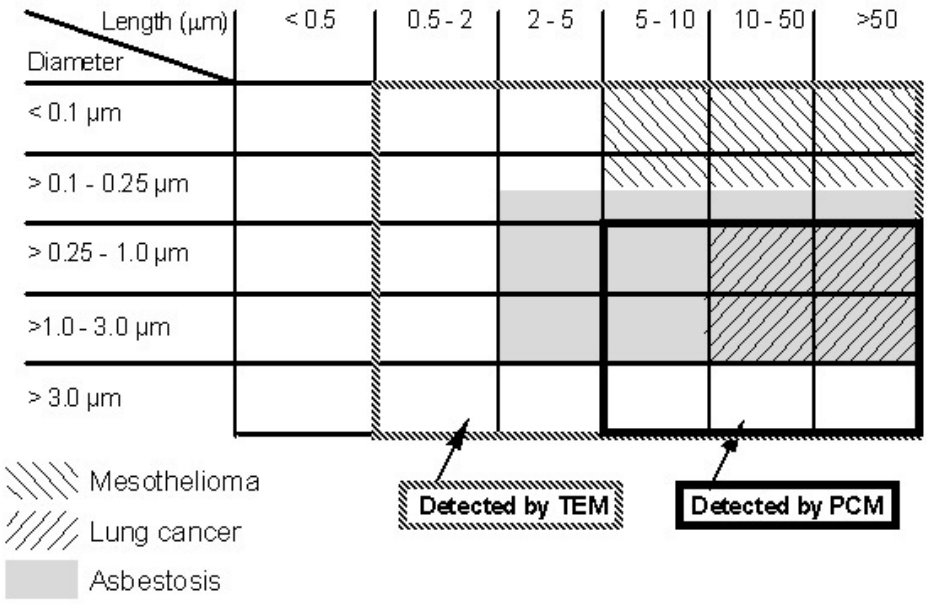

Figure 2. Comparison of proposed size ranges of asbestos fibers causing specific diseases compared with the fiber sizes detected using TEM and PCM techniques. Lung cancer and mesothelioma are more likely to occur at current occupational and environmental levels than asbestosis. PCM can cover only a portion of the total fiber distribution; PCM is used as an indicator of total exposure. TEM can cover the entire size range, but most methods emphasize one size range over another through selection of magnification and counting rules.

2 Fiber dimensions

Fibers are particles that have one dimension significantly larger than the other two. Fibers are often characterized or selected according to their aspect ratio, i.e., the ratio of the large dimension to one of the small dimensions. If no other criteria are used, then materials that might not normally be considered fibrous may contain a fraction of particles that meet the criteria for fibers. The distribution of fiber dimensions in a sample can usually be characterized by assuming a cylindrical geometry (i.e., the two small dimensions are identical) and measuring the length and diameter of individual fibers. The distribution of airborne fiber sizes generated by grinding bulk material or by mechanically releasing particles into the air often results in a two-dimensional (bivariate) lognormal distribution. Such a distribution is characterized by five parameters: the geometric mean length, the geometric mean diameter, the length and diameter geometric standard deviations, and a correlation term that relates length to diameter [Schneider et al. 1983]. In addition, several other parameters that are a function of length and diameter, such as aerodynamic diameter, can also be characterized by a lognormal distribution [Cheng 1986].

Often the discussion of fibers assumes that fibers are straight objects that can be well defined by several parameters as indicated above. However, many real-world particles are not so simple to describe. In fact, the detailed features of many fibers can aid in their identification [McCrone 1980]. Fibers are often curved, have splayed ends, or differ in other ways from a cylindrical shape. Asbestos mineral is composed of fibrils (about 0.03-µm diameter) that are packed together. This fibrillar structure is characteristic of asbestiform minerals. When the mineral is broken apart mechanically, the material separates primarily between fibrils and the resulting fibers are usually bundles of fibrils. The ends of the fibers can be broken apart, with smaller bundles or individual fibrils spread apart, yet still be part of the fiber. Fibers can be contaminated by attachment of other dust particles, creating a complex structure with aerodynamic behavior not matching that of cylindrical fibers. The complexity of fiber shapes affects all of the measurement and separation techniques described below and frequently makes it difficult to compare one method to another.

In addition to asbestos fibers, there are many types of fibrous materials being produced for commercial purposes. These include fibrous glass, mineral wool, refractory ceramic fibers, wood and other plant fibers, and synthetic organic fibers. Most of these fibers tend to have larger diameters than asbestos fibers. On the other hand, carbon nanotubes (CNTs) (<0.005um diameter) have recently been produced in small-scale commercial quantities and because of their high tensile strength, high conductivity, and other special properties, show great promise as a commercial material [Liu et al. 1998]. Unlike asbestos fibers, which have discrete lengths and diameters (i.e., aspect ratios), CNTs occur mainly as entangled particle agglomerates and may contain varying amounts of amorphous carbon and residual catalyst

metal. The complexity and variety of structures makes CNT particle counting a challenge. Measurement techniques must be tailored to the size distribution and physicochemical properties of these materials.

This review primarily relates to measurement of fibers in air. There are several techniques that address concentration of asbestos and other fibers in bulk material and measurement of mass concentration of fibers [Beard and Rook 2001]. One of these bulk methods, polarizing light microscopy, will be discussed below.

3 Phase contrasting light microscope counting (PCM)

As asbestos-induced disease became widely studied in the 1960s, cellulose-based membrane filter sampling was applied to asbestos sampling in combination with high magnification phase contrast light microscopy (PCM) for counting fibers. This technique involved collection of fibers uniformly over the surface of a cellulose ester filter, placing the filter or a segment of the filter on a microscope slide and making it transparent, and observing the fibers in the sample with a high magnification (~450X) phase contrast light microscope. Over the years, many researchers have endeavored to improve and standardize the PCM method. One researcher, Walton, discussed many aspects of this technique in a review [Walton 1982]. The high variability of the analysis results and the method's dependence on operator technique made method improvement and research difficult. The PCM method does not measure all fibers; typically only those >0.25 μm diameter are visible and counted and only those >5 μm length are counted by protocol. Therefore, the PCM method is only an index of exposure and uses the assumption that what is detected is correlated with the fibers actually causing disease (Figure 2). The PCM method does not allow identification of asbestos fibers. This is an important limitation when the method is used in settings where fiber concentrations with a significant non-asbestos fraction may occur. This should be remembered when considering some of the parameters discussed below. The aim of evaluating changes to the PCM technique may depend on whether consistency with other laboratories within a country or throughout the world is more important than making measurements that are more closely related to health effects. A number of factors which influence analysis results have been investigated, including the following:

a. Microscope-related parameters

1.) Microscope magnification

The exact level of microscope magnification depends on microscope design, but most current methods use 450X (±10%) total magnification. Pang and coworkers investigated 1250X magnification to improve fiber detectability, but this has not been adopted in any established methods [Pang et al. 1989]. Pang also investigated the effect of using lower magnification (400X) and found that counts were lower for chrysotile asbestos by 25%, but that amosite fiber counts were unaffected [Pang 2000].

2.) Phase contrast optics

This contrast enhancement technique allows detection of asbestos fibers down to about 0.25 µm diameter for chrysotile and about 0.15 µm for amphiboles. Other techniques such as dark field microscopy may offer improved detectability, but also increase the background from non-fibrous particles.

3.) Test slide to check optics

A test slide was developed to allow a check of proper alignment and magnification in the microscope [LeGuen et al. 1984]. This ensures a reasonable level of uniformity in microscope setup and operation, including the operator's visual perception. Improper setup can reduce detectability of fibers. There have also been cases where the optics were "too good," and results were obtained that were higher than the reference count.

4.) Counting area in microscope field

Some early measurements with the phase contrast microscope were made using a rectangular graticule for defining the counting area, while others were made using the entire microscope viewing area. It was found that larger viewing areas resulted in lower counts, so the Walton-Beckett graticule [Walton and Beckett 1977] was developed that nominally gave a 100-µm diameter counting area (the area is calibrated more precisely for each microscope) and has been incorporated in all current methods.

b. Sample preparation techniques

1.) Filter type

Virtually all measurements are made using 0.8-µm pore size mixed cellulose ester (MCE) filters. Some measurements are made using 1.2-µm pore size filters when sampling low concentrations to allow higher flow rate through the filter. Smaller pore size filters are used to ensure that fibers are deposited as near the surface of the filters as possible. This results in fibers ending up in the same plane so that they can be readily viewed with a minimum change of focus during fiber counting. Pore sizes

smaller than 0.8 µm are only used with line-operated pumps because of limited suction power available with personal sampling pumps.

2.) Selection of the liquid for making filter transparent

A liquid is placed on the filter that closely matches the filter refractive index, yet has an index that is as far as possible from that of the fibers being detected. Rooker et al. showed that refractive index difference between cleared filter and fibers translated directly into detectability of small diameter fibers [Rooker et al. 1982]. A viscous solution of dimethyl phthalate and diethyl oxalate mixed with cellulose filter material was commonly used in the 1970s and early 1980s. However, it did not result in a permanent sample, with crystallization of the mount and movement of fibers often occurring several days after sample preparation. Permanent slides were needed for quality assurance purposes and the sample preparation technique was also slow and required some skill. A rapid acetone-based filter clearing technique was developed that could be used safely in field situations [Baron and Pickford 1986]. After clearing, filters were coated with triacetin to surround the fibers. This resulted in a longer lasting sample (typically months to years) and is currently specified in most methods. Another technique uses a resin called Euparal to surround the fibers and results in a permanent slide preparation [Ogden et al. 1986].

3.) Filter loading

The number of fibers on the filter is usually specified to be within a certain loading range to ensure consistent counting. Cherrie et al. demonstrated using a serial dilution technique that counting efficiency was a function of concentration of fibers on the filter [Cherrie et al. 1986]. At very low filter loadings (<100 fibers/mm^2) there was a tendency to count high relative to an intermediate range of concentrations (100-1300 fibers/mm^2), where the counts were a linear function of loading. This "overcounting" was apparently due to greater visibility of fibers in a clean visual field. This effect was noted for both human counters and an image analysis system. At high filter loadings (>1300 fibers/mm^2), undercounting occurred due to overlap of fibers with other fibers and with nonfibrous particles. Most published methods indicate that optimum counting occurs within the 100-1300 fibers/mm^2 range, while some restrict the range further to less than 650 fibers/mm^2.

4.) Fiber counting rules

The basic fiber counting rules for most current methods indicate that a countable fiber should be longer than 5 µm, narrower than 3 µm, and have an aspect ratio greater than 3:1. These rules were selected because shorter fibers were difficult to detect by optical microscopy and the 3:1 aspect ratio was used to discriminate between fibrous and non-

fibrous particles in occupational settings. There has been a great deal of controversy over these rules. The use of a longer fiber cutoff, e.g., 15-20 µm, has been suggested, based on two separate arguments: first, that most asbestos fibers are relatively long and thin (with high aspect ratio) and the longer fiber cutoff would discriminate better toward fibers that were truly asbestos fibers according to mineralogical definitions [Wylie 1979]; and second, that fibers that enter the lungs are removed readily by macrophages if they are shorter than about 15 µm [Blake et al. 1997]. Longer fibers cannot readily be engulfed by macrophages, thus staying in the lungs for a long period and causing continuing fibrosis.

The aspect ratio criterion has also been questioned because many non-asbestiform particles have shape distributions that include particles with aspect ratios greater than 3:1. Since asbestos and other minerals often contain single crystal particles not in the asbestiform habit, it has been argued that these single crystals, or cleavage fragments, should not be counted. However, the Occupational Health and Safety Administration (OHSA) has supported the 3:1 minimum aspect ratio through legal precedent. The National Institute for Occupational Safety and Health (NIOSH) has noted that because of the great difficulty in differentiating whether individual high aspect ratio particles are cleavage fragments or asbestiform fibers, all such particles should be counted. These high aspect ratio particles may cause disease whether or not they are asbestiform.

Other aspects of fiber counting have been investigated, including how to count non-standard fiber shapes, overlapping fibers, overlapping compact particles on fibers, and bundles of fibers. Each of these factors can have a noticeable effect on the final count. Cowie and Crawford investigated the effect of some of these factors and estimated most of them made a difference in the final count on the order of 20% [Cowie and Crawford 1982]. Many of the methods currently in use have slight variations in their interpretation of which fibers to count and thus can contribute to variation in results between countries and organizations.

NIOSH Method 7400 contains two sets of counting rules, the A and the B rules. The A rules are used for asbestos and are consistent with counting rules in previous NIOSH methods. The A rules are required for asbestos counting by OSHA because of legal precedent in regard to the 3:1 aspect ratio rule. The A rules do not have an upper diameter limit for fibers to be counted. The B rules were introduced as an alternative to the A rules when Cowie and Crawford found that these rules agreed best with previous PCM counts, yet had improved precision [Cowie and Crawford 1982]. The B rules have been informally adopted for use with fibers other than asbestos because these rules include the upper diameter limit of 3 µm. This upper diameter limit

significantly reduces the counting of typically large-diameter fibers, e.g., glass and cellulose, which are unlikely to deposit in the lungs [Breysse et al. 1999].

c. Quality assurance schemes

1.) Sample recounts

Most methods require individual counters to recount about 10% of the field samples to ensure consistent counting procedures and alert the analyst in the case of problem samples. It is also recommended that counters have samples that are routinely recounted to ensure consistent counting within a laboratory over time.

One of the difficulties in analyzing errors made by analysts during PCM counting is that individual fields are difficult to relocate after the analyst has finished counting a slide. Differences in counts between analysts have often been ascribed to local variations in loading on the filter. Pang's development of a slide coverslip that defines counting areas on the sample solves this problem [Pang 2000]. Areas on the coverslip are vacuum coated with a thin layer of gold and platinum using an electron microscope grid as a mask. This leaves defined areas on the coverslip that can be located by grid index marks. Thus, specific fields in a sample can be readily located. Using this grid mapping approach, the location, orientation and shape of each fiber can be noted and differences in counts can be reconciled on a fiber-by-fiber basis. The coverslips have been used to study fiber counting accuracy by comparing routine counting of specified fields to counts agreed upon by a group of competent counters. It was found that the principal errors for chrysotile fiber samples were due to missing fibers close to the visibility limit, while the principal errors for amosite fiber samples were caused by incorrectly sizing fiber length near the 5-µm limit. The chrysotile samples were therefore typically undercounted (negative bias), while the amosite samples had increased variability with individual counters being biased either high or low. Both these errors can be reduced by training counters with pre-counted reference slides prepared using Pang's coverslips [Pang 2000] (Omega Specialty Instrument Co. Chelmsford MA). In addition, these reference slides can be used on a routine basis to ensure consistency in counting. These coverslips or modified versions show great promise for training analysts and perhaps for improving quality assurance schemes.

2.) Interlaboratory sample exchanges

Crawford et al. found that use of sample exchange programs was more important in ensuring agreement between laboratories than similarity in details of the counting rules [Crawford et al. 1982]. Thus, exchange of field samples between laboratories is commonly performed to improve consistency of counting. A description of several quality assurance techniques for asbestos fiber counting is described by Abell et al.

[Abell et al. 1989]. To fulfill Method 7400 requirements for an interlaboratory sample exchange, Tombes and Calpin have described a simple approach using appropriate statistical tests [Tombes and Calpin 2002].

3.) Quality check samples

In order to get agreement between laboratories within a country or internationally, several programs send out identical samples to participating laboratories to assess their relative performance [Schlecht and Shulman 1986; Kauffer 1989; Crawford et al. 1992; Arroyo and Rojo 1998]. These programs provide feedback, often tied to laboratory accreditation, which provides incentive for laboratories to ensure that their performance is similar to that of other laboratories.

d. Qualitative fiber analysis

In addition to simply counting the fibers, there are techniques available for providing at least tentative identification of fiber type; use of these techniques is commonly called differential counting. Fiber shape can be used to limit the type of fiber counted. For instance, glass fibers tend to be straighter, with smoother sides than chrysotile fibers. Polarizing light techniques can also be used to identify larger diameter (> 1 μm) fibers. These are based on the optical properties of the materials, including refractive index and crystallinity. These techniques can provide quite positive identification for the presence of certain types of fibers, but are limited in application to airborne fibers because they only work for the larger diameter fibers. These techniques are often used in analysis of bulk materials [NIOSH 1994a]. The use of identification techniques is not allowed in reporting fiber counts using Method 7400 so that the results are consistent between laboratories. Considerable confusion has been caused in the past by individual laboratories using some of these identification techniques to change the counting procedure and, hence, the final results.

Several PCM fiber counting methods have been published by national [NHMRC 1976; HSE 1990] and international organizations [Asbestos International Association 1979; WHO 1997]. Most countries have methods very similar to the ones referenced here.

e. Sampling volume for asbestos abatement applications

Sampling for asbestos after abatement requires the selection of a sampling volume so that one can have high confidence that the air meets acceptable concentration standards. The following is an example of how to calculate this sampling volume.

The approach assumes that one wishes to select sampling parameters in order to have a high degree of confidence that a target exposure standard (e.g. NIOSH REL, OSHA PEL, EPA clearance standard) is met.

Several factors need to be established in order to perform this calculation if the target exposure standard involves clearance monitoring. The U.S. Environmental Protection Agency (EPA) authorizes the use of PCM for some clearance monitoring applications and specifies that a level of 0.01 fibers/mL be met. On the method synopsis page, Method 7400 indicates that the limit of detection (LOD) for PCM analysis is 5.5 fibers/100 fields. This is based on intralaboratory variability. A major difference between Method 7400 and other analytical methods in the NIOSH Manual of Analytical Methods (NMAM) is that there is no reference method for Method 7400. Therefore, the consensus mean is the "true" value and the interlaboratory results effectively define the method accuracy. Under the heading "Evaluation of Method, B. Interlaboratory comparability," Method 7400 provides a means of calculating the confidence limits on a single analysis result (Equations 3 and 4). From Equation 3, the interlaboratory variability at the LOD is such that the upper 95% confidence limit on a measured value is 300% greater than (or 4 times) the measured value.

Using the upper confidence limit, the equation in Section 21 in Method 7400 can be used to estimate the sampling volume.

$$\frac{\frac{number\ of\ fibers}{area\ of\ 100\ fields} * total\ filter\ area}{sampling\ volume} = \frac{target\ level}{4}$$

With the appropriate values inserted, the equation becomes

$$\frac{\frac{5.5\ fibers}{0.785\ mm^2} * 385\ mm^2}{sampling\ volume} = \frac{0.01\frac{fiber}{mL}}{4}$$

Solving this equation for sampling volume gives 1080 L. This is the minimum volume that will give a result allowing a single sample to indicate compliance with the 0.01 fiber/mL limit with 95% confidence. It requires that the sample give a result less than or equal to the LOD or 5.5 fibers per 100 fields. A higher fiber count may still indicate that the concentration meets the target level, but not with the same level of confidence. This is likely to be a conservative estimate of concentration and additionally ensure compliance with the standard because the fiber concentration is low and, as indicated above, low fiber

loadings are usually overestimated. However, the background concentration of non-fibrous dust on the filter also must be low to ensure that fibers are not obscured.

f. Other techniques

Since fiber counting by human analysts produces relatively high biases and variability, several researchers have attempted to develop automated counting systems. With the increases in computer power over the last 25 years, it has been tempting to assume that fiber counting is a solvable problem and significant efforts have been made to develop such a system. The most intensive effort to produce a fiber counting system was carried out by Manchester University in collaboration with the Health and Safety Executive in the UK [Kenny 1984]. The Manchester Asbestos Program (MAP) was able to give reasonably good agreement with human counters for certain types of samples. It was used as a reference analyst for the US and UK reference sample programs for several years. Eventually, the MAP was dropped as the reference because it was not sufficiently consistent for all types of samples.

The principle problems with image analysis of asbestos fibers include: the complexity of many fiber shapes, including bundles, agglomerates, and split fibers; the fibers often go in and out of the plane of focus; the background includes many particles and other non-fibrous shapes; the phase contrast optics produces haloes around particles in the sample that can be detected as fibers; and finally, and perhaps most importantly, the contrast between the fibers and background is poor and many fibers are near the detection threshold. An evaluation of the MAP program indicated that a significant fraction of the fibers were misidentified as multiple fibers, not detected at all, and groups of compact particles or edges of large particles were detected as fibers [Baron and Shulman 1987].

Inoue and coworkers have more recently developed image analysis software using a microprocessor-based PC [Inoue et al. 1998]. Initial tests indicate that it works approximately as well as human counters. Inoue also evaluated how well human counters and the image analyzer did in detecting the same fibers in a sample and found that only about 50% of the fibers were consistently counted by all counters, so the image analysis system did approximately as well as the human counters [Inoue et al. 1999]. Further testing of the image analysis system is needed.

In addition to image analysis, optical microscopy can be enhanced using a personal computer to more easily observe the image and to mark and measure fiber dimensions, with automatic recording of the fibers counted [Lundgren et al. 1995]. This does not appear to improve the counting accuracy since the analyst still decides which fibers are to be counted.

4 Polarizing light microscopy (PLM) of bulk materials (Adapted from Baron [1993])

The asbestos fibers in bulk material can be released and become airborne when the bulk material is disturbed. For this reason, it is desirable to measure the asbestos content of bulk samples. PLM is often used to determine the percent asbestos in bulk material. The EPA [Asbestos-containing materials, 1987] has defined asbestos containing material (ACM) as material containing more than 1% asbestos using the PLM method, which effectively estimates concentration by area observed. Some confusion exists regarding the units of asbestos percentage. EPA originally indicated that the limit for ACM was 1% by mass [Asbestos-containing materials, 1987], but because of the difficulties in determining corrections for differences in material density and in determining particle volumes, the limit was changed to 1% by area as determined by the PLM method [EPA 1990b]. OSHA does not specify units for percent asbestos in its regulations [OSHA 1994].

Several PLM techniques are used for identifying fiber type as well as semi-quantifying the percent fibrous material (usually asbestos) in a sample [McCrone et al. 1978; Middleton 1979; Asbestos-containing materials, 1990; Perkins and Harvey 1993; NIOSH 1994a]. These techniques depend on particle shape, the refractive index, and other optical properties of individual particles. Many of these PLM techniques require visual observation of color in the fiber and become less reliable for fibers thinner than about 1 μm [Vaughan et al. 1981].

a. Sampling

Several procedures have been suggested for obtaining representative bulk samples of ACM in a fashion that prevents unnecessary exposure to asbestos aerosol [EPA 1985a,b; Jankovic 1985]. Representative sampling of commercial ACM materials is often problematic; these materials may vary significantly in asbestos concentration between nearby locations and even at different depths at the same location. Sampling from multiple locations and compositing samples helps improve the likelihood of obtaining a representative sample.

The material should be wetted or sealed during sample removal. A small coring device, such as a cork borer, can be used to obtain a sample from the full depth of the material. At least three samples per 1000 ft^2 of ACM should be taken [Asbestos-containing materials, 1987]. The sample should be placed in a well-sealed, rugged container. Finally, the sampled area should be repaired or sealed to minimize further fiber release.

Surface sampling has been proposed by several groups, but there is no relationship between airborne fibers and those found on surfaces [Chatfield 2000]. Therefore, surface sampling for fibers is not recommended.

b. Sample preparation analysis

Sample preparation for a PLM analysis involves grinding the material to the optimum particle size range (1-15-µm diameter) and dispersing the particles in a liquid of known refractive index on a glass slide [Perkins and Harvey 1993]. Particle size uniformity in the prepared sample is extremely important. A few large chunks of material may contain more asbestos than hundreds of much smaller particles. Friable material, i.e., that which is crumbly or can be crushed by hand, may readily release fibers and is considered more hazardous. Friable materials are generally easier to prepare for analysis than some other ACMs, such as vinyl asbestos floor tiles, which may require dissolution or ashing of the matrix material so that the fibers are separated and visible in the microscope. Before and after preparation, the sample is observed with a stereomicroscope at 10-100X magnification to evaluate sample uniformity and observe whether fibrous material is present.

Some materials that interfere with accurate fiber identification either by their similarity or by covering up the fibers can be removed by physical treatment of the sample. For instance, organic materials, such as cellulose fibers or diesel soot can be removed by low temperature, oxygen-plasma ashing [Baron and Platek 1990]. Leather fibers and chrysotile have a similar appearance and refractive index. The leather can be removed by ashing at 400°C [Churchyard and Copeland 1988].

Fiber morphology, i.e. the structure and shape of the fiber, can be used to assist in its identification. Morphology of fibers can give some indication of fiber type. For instance, chrysotile fibers tend to be curly, while amphibole fibers are straight, especially when they are shorter than 50 µm. Asbestos fibers often have frayed or split ends, while glass or mineral wool fibers are typically straight or slightly curved with fractured or bulbous ends. Many plant fibers are flattened and twisted, with diameters between 5-20 µm. Note that it is not recommended to base identification solely on morphology.

Fiber refractive index and other crystalline properties can be used to identify fiber type with reasonable certainty. Several techniques for determining these properties can be used in a polarizing light microscope. When viewed in the microscope with crossed polarizing filters, isotropic (isometric or amorphous) fibers appear consistently bright when rotated, while anisotropic (uni- or biaxial crystal structure) fibers appear bright, but disappear when rotated to their extinction angle, which is a function of crystal structure. Thus,

amorphous materials such as glass or mineral wool fibers can easily be discriminated from asbestos.

During PLM analysis, fibers are immersed in a fluid selected to have a known refractive index. When a fiber has a larger refractive index than the surrounding fluid medium, the bright halo (Becke line) around that fiber appears to move into it as the microscope focus is raised; when the fiber has a smaller refractive index, the Becke line moves out of it. Placing the fibrous material into several different refractive index fluids allows the fiber refractive index to be bracketed.

Dispersion, or refractive index change with wavelength, of a fiber can be used for identification. When particles are placed in a liquid whose dispersion is different from that of the particle, the particle may exhibit a color caused by the refraction of light. This technique requires the use of special "dispersion staining" optics. By using several refractive index liquids in series, the refractive index and the dispersion of the fiber can be established and compared with those of standard materials or published data [McCrone 1980].

Once the sample has been uniformly dispersed on a slide in the appropriate refractive index liquid, specific fiber types, e.g., asbestos, can be identified and the percent fibers estimated. Two approaches are typically used: visual comparison with prepared reference slides or pictures and point counting. When attempting to estimate whether a material is ACM (i.e., > 1% asbestos), the visual comparison technique is adequate when more than about 10% of the particles observed are asbestos. Point counting is used for lower concentration samples to provide higher accuracy [EPA 1990a]. It involves observing 400 or more randomly selected "points" (identified with a reticle crosshair) in the sample. The number of points containing asbestos is divided by the total number of points observed to give the percent asbestos. A combination of these approaches balances the analysis time and accuracy of the results [Webber et al. 1990].

PLM also can be used for qualitative analysis of air sample filters by collapsing the filter and using low temperature plasma etching of the surface to expose the fibers. Various refractive index liquids can then be placed on the etched surface to surround the fibers, allowing techniques noted above to be used [Vaughan et al. 1981]. The smallest fibers that can be identified by this method are about 1-µm diameter.

c. Accuracy

PLM analysis is primarily used for qualitative identification of fiber type. Accurate identification of asbestos and other fibers requires proper training in the crystallographic

properties of particles as well as training and familiarization with the PLM. As with fiber counting, a laboratory quality assurance program is necessary to ensure consistently accurate results. The National Voluntary Laboratory Accreditation Program (NVLAP) operated by the National Institute for Standards and Technology (NIST) inspects laboratories for proper practice as well as providing unknown samples four times a year to check their performance in fiber identification. Under a predecessor to this program, approximately 350 laboratories correctly classified 98.5% of the samples as asbestos and correctly identified the specific asbestos types in approximately 97% of the samples. A blind test of 51 laboratories resulted in 97.5% correct classifications and 79.1% correct identifications [EPA 1986]. The American Industrial Hygiene Association Proficiency Analytical Testing Program provides similar PLM audit samples to laboratories. Some common interferences for bulk analysis by PLM include sepiolite, vermiculite, and cleavage fragments of non-asbestos amphiboles.

PLM has been cast in a quantitative measurement role by the EPA requirement of determining whether a school building material meets the 1% asbestos level defining ACM. Many variables including particle size, density and shape are not adequately controlled or measured in the analysis and contribute to errors in the percent mass estimate. Thus, PLM analysis is at best a semi-quantitative technique.

Chatfield indicated that the accuracy of PLM for low concentrations of asbestos was poor and described a set of procedures that concentrated the asbestos into a weighable fraction [Chatfield 2000]. An EPA report describes, in addition to the PLM and Chatfield's gravimetry methods, a TEM and an x-ray diffraction method for bulk analysis of asbestos [Chatfield 2000]. NIOSH Method 9000 describes an x-ray diffraction method for chrysotile [NIOSH 1994c].

5 Electron microscopy

Scanning electron microscopy (SEM) has not been the focus of as much method development as either light microscopy or transmission electron microscopy (TEM). PCM found favor because of the low equipment cost and lower training level required for analysis. TEM is preferred for environmental and research studies because it offers the highest resolution and the most positive identification capabilities. TEM allows visibility of all asbestos fibers down to the individual fibrils, electron diffraction for crystal structure identification, and energy dispersive x-ray analysis for elemental measurement. SEM has intermediate resolution, with many instruments of this type not able to see all asbestos fibers. However, many modern SEMs have the capability of detecting asbestos fibrils, though contrast with background may be poor for some fiber types, especially if a high contrast substrate is not used. Energy dispersive x-ray analysis is also available for many SEMs, providing some qualitative

information of fiber type. However, since electron diffraction typically cannot be performed by SEM, this often leaves open the question of positive identification of fibers.

6 Scanning electron microscopy (SEM)

Particles are observed in the SEM when a beam of electrons is focused onto the sample surface and scanned over an area. The electrons are scattered from the surface and detected above the surface synchronously with the beam scan rate and an image of the scanned surface is created. Thus, the SEM measures the surface of particles on a substrate. The best image can be obtained on conducting objects deposited on a smooth, conducting substrate. Particles are often deposited on aluminum or carbon planchets that fit directly into the SEM or onto polycarbonate membrane (track-etched, Nuclepore®) filters. The samples are usually coated with gold or carbon to increase conductivity.

There have been some SEM methods developed for fiber counting [Asbestos International Association 1984; WHO 1985; ASTM 1996; ISO 2002]. These methods are primarily used for inorganic man-made fibers that have larger diameter fibers than can occur with asbestos. Thus, all the fibers are potentially visible using the SEM.

7 Transmission electron microscopy (TEM)

The transmission electron microscope (TEM) allows detection of particle shape and structure down to the smallest asbestos fibers (Figure 2) and can be used to determine crystal structure from electron diffraction as well as determining elemental composition from energy dispersive x-ray analysis. Although TEM analysis is potentially very powerful and accurate, the process of sample collection and preparation and details involved in sample analysis can degrade the quantitative accuracy of the technique. Several more specialized techniques, such as electron energy loss spectroscopy and secondary ion mass spectrometry, have been used for analyzing particles and can also be applied to fibers [Fletcher et al. 2001].

Airborne fiber samples for TEM analysis are typically collected onto a filter, usually a polycarbonate membrane or MCE membrane filter. For the latter filter type, the filter is chemically collapsed to form a smooth upper surface on which collected fibers are trapped. Sometimes the surface is etched using a low temperature asher to expose the fibers collected on or near the surface of the original filter. The filter is coated with a carbon film that entraps fibers exposed on the filter surface and the filter material is then dissolved away. The carbon film is transferred to a TEM grid (usually 3-mm diameter) and the sample can be placed in the TEM for analysis.

For Method 7402, the surface is not ashed because some fibers, e.g., cellulose, may be removed and give an inaccurate total fiber count [Baron and Platek 1990]. Ashing can thus affect the measurement of the asbestos fiber fraction.

The above approach to preparing MCE filters for TEM analysis is called the direct-transfer approach, since fibers are transferred to the carbon film with minimum disturbance to the way they were collected. An alternative technique is to dissolve the entire filter in liquid, ultrasonicate the suspension to disperse the particles, and deposit an aliquot of the particle suspension onto a polycarbonate filter for final transfer to the carbon film. This is called the indirect-transfer technique. With the indirect technique, the optimum particle loading of the TEM sample can be obtained and soluble particles can be removed from the sample. However, the suspension process can change the apparent size distribution of the particles and fibers by breaking apart agglomerates or even breaking apart asbestos fibers into smaller fibers or fibrils [Sahle and Laszlo 1996]. The breakup problem can be especially severe for chrysotile, causing a large increase in fiber count. Quality assurance is especially important with TEM analysis of fibers. The NVLAP program provides quality assurance accreditation for laboratories performing TEM analysis using the Environmental Protection Agency's Asbestos Hazard Emergency Response Act (AHERA) method. Note that data provided under the AHERA method, because of significant differences in counting rules, the types of structures counted as asbestos, and the size range of fibers, cannot be directly compared with counts by Methods 7400 or 7402.

The process of sample collection and preparation is a complex one that can introduce biases into the final measurement. Since only small portions of the filter are measured during TEM analysis, sampled fibers that deposit non-uniformly onto the filter due to inertial, gravitational, and electrostatic effects will be measured inaccurately [Chen and Baron 1996]. Fibers that penetrate the filter surface and are not transferred to the carbon film will be lost. If the filter is incompletely dissolved away from the carbon film, the sample will be difficult to analyze.

Many of the sources of bias and variability noted in sampling and counting by PCM also apply to TEM analysis. Fiber counting in a TEM can also introduce biases and variability in the final result. There is a tendency to use the high magnification of the TEM to look for the smallest fibers, while ignoring some of the larger ones. Even so, fibers shorter than 0.5 μm tend to be missed because they are difficult to see in the background clutter of the sample [Steel and Small 1985]. Taylor et al. found that TEM counting gave poorer precision than counting the same sample by PCM and recommended that the fraction of asbestos fibers counted by TEM be applied to the PCM count as indicated in Method 7402 [Taylor et al. 1984]. This combined PCM/TEM approach gave better precision than counting by TEM alone.

In addition to recognizing fibrous shape and structure of the several asbestos minerals, qualitative analysis of fibers by TEM primarily involves two techniques, energy dispersive x-ray analysis and electron diffraction. X-ray analysis produces responses for each of the elements (typically atomic number > 6, but is instrument dependent) present in a particle; the responses occur as peaks in an energy spectrum. Specific asbestos minerals can be identified using peak intensity ratios observed in standard samples and as specified in the method.

The crystal structure of individual fibers is evaluated using electron diffraction. Focusing the TEM electron beam on a single fiber produces a diffraction pattern consisting of a number of spots. The spot locations depend not only on the particle crystal structure, but also on the geometry of the electron beam optics and other instrumental parameters. The diffraction spot locations relative to one another give a very specific identification of crystal structure. For easily recognized minerals, such as chrysotile, the visual identification of the diffraction pattern is often sufficient. However, to identify fibers not fitting the x-ray analysis pattern for standard asbestos minerals, careful measurement, or indexing, of the diffraction spots is important.

The combination of x-ray analysis and electron diffraction gives a highly definitive identification of specific minerals. However, as with any analytical methods, there are exceptions that require greater expertise to recognize potential interferences. Some minerals that are difficult to differentiate from regulated asbestos minerals include non-regulated amphiboles and fibrous talcs. There are several established methods for analyzing fibers, especially asbestos fibers, by TEM [Asbestos-containing materials, 1987; NIOSH 1994b; ISO 1995, 1999; ASTM 1998].

8 Optical detection (light scattering)

Two types of light scattering detectors are commonly used for measuring airborne dust concentrations: the optical particle counter (OPC), which detects and counts individual particles, and the photometer (sometimes called a nephelometer), which detects the scattering from all particles in a defined detection volume. A standard OPC was used to detect asbestos concentrations in a workplace where the aerosol was primarily fibrous and good correlation with fiber counts was obtained [Rickards 1978]. A nephelometer may also be used, but may have an even greater interference from non-fibrous dusts.

The fibrous aerosol monitor (Model FM-7400, MIE, Inc. Bedford MA) used an electrostatic alignment technique by applying a field that aligns and rotates individual fibers in a laser beam. The light scattered from the fibers uniquely identified the presence of individual fibers. This allowed specific detection of fibers [Lilienfeld et al. 1979] and was even used to measure fiber length [Marijnissen et al. 1996].

Several field tests have indicated that the fibrous aerosol monitor agrees reasonably well with field measurements of fibers by phase contrast microscopy, though mostly at concentrations above ambient levels. It has been used at abatement sites to provide rapid feedback and ensure acceptable containment of airborne fibers during asbestos removal.

9 Fiber classification

Several devices have been used to measure or separate fibers by diameter. A spiral centrifuge was used to separate fibers and reference spherical particles to estimate fiber aerodynamic diameter [Stöber 1972]. It was found that the aerodynamic diameter was directly proportional to physical diameter, proportional to the square root of the fiber density, and proportional to fiber length to the $1/6^{th}$ power. For mineral fibers having a density of about 3 g/cm^3, the aerodynamic diameter was approximately three to five times the physical diameter of the fiber. Behavior of glass fibers in a cascade impactor was investigated by Burke and Esmen [Burke and Esmen 1978]. A small correction to the aerodynamic diameter was developed to take into account interception of longer fibers with the impaction surface. An inertial spectrometer was used to measure fiber aerodynamic diameter and good diameter separation was achieved [Morigi et al. 1999]. Baron and Deye developed a technique for separating fibers by length using dielectrophoresis [Baron et al. 1994; Deye et al. 1999]. This technique was also shown to be useful for measuring fiber length and diameter distributions [Baron et al. 2000].

As with most airborne dusts, fiber settling will reduce the number of larger diameter fibers in a distribution as the distance from the source of the dust increases. Esmen et al. showed that average fiber concentration in workplaces decreased exponentially with an increase of fiber diameter, indicating that the larger diameter fibers settled out more quickly than smaller diameter fibers [Esmen et al. 1979]. Cyclones, impactors and porous foam classifiers were evaluated for efficiency of removing airborne fibers not likely to deposit in the lungs [Maynard 1996].

The aerodynamic diameter of fibers is dependent primarily on fiber physical diameter and fiber density, with a minor dependence on fiber length [Baron 1996]. The diseases caused by asbestos fibers are lung diseases and so it makes sense to measure only fibers that can enter the lungs, i.e., thoracic fibers. Identical conventions for thoracic samplers have been published by ISO, ACGIH [ACGIH 2002], and CEN. Baron [Baron 1996] showed that sampling fibers with a thoracic sampler was approximately equivalent to counting only mineral fibers with a physical diameter smaller than 3 µm. Jones et al. [Jones et al. 2001] reported that there appeared to be no impediment to using a thoracic sampler for fiber sampling; they found that several samplers matched the thoracic convention, the sample collected by these samplers could be analyzed by standard methods, and that field studies indicated equivalence to the

current method. Maynard [Maynard 1999] also found that there appeared to be no variation in penetration through these samplers as a function of fiber length. The advantage to using a thoracic sampler, apart from adhering to conventional sampling practice, is that it would remove larger compact particles and fibers from the sample and result in a cleaner sample. Although current US practice does not use an upper diameter limit for asbestos fibers, such a limit is commonly used for man-made fibers. Except for the United States, all national and international organization methods use an upper diameter limit of 3 µm for fiber counting of asbestos fibers.

It is likely that thoracic sampling will eventually be in routine use for measurement of asbestos and other fibers. This approach has several advantages. It places the fiber method in line with other dust sampling conventions. It removes some of the larger particles in the sample, resulting in a cleaner sample for the analyst. It removes the need for determining fiber diameter during counting and it is consistent with previous practice of using an upper diameter limit of 3 µm for fiber counting in some methods. Thoracic sampling has the disadvantage of requiring the flow rate for a specific sampler to be fixed. This reduces the flexibility to target the loading of the filter by adjusting the flow rate. However, several classifiers can be designed to operate at selected flow rates to allow some flexibility in sampling.

10 Conclusions

The capability for measurement of fiber size distributions is available through microscopy and, to a much lesser extent, through direct-reading instrumentation. Because of differences in counting rules, resolution capability, and ability to distinguish asbestos from interfering particles or other fibers, PCM, PLM, SEM, and TEM methods often do not produce results which are directly comparable. The traditional methods of microscopy are relatively inaccurate when compared to chemical analysis methods for most other analytes because of the many sources of error in the sampling and analysis procedure. To improve laboratory-to-laboratory agreement, counter training and quality control, including the exchange of samples among laboratories and proficiency testing, are important. Implementation of training through the use of Pang's coverslips allows investigation of counting errors and potential improvement of PCM counting accuracy. Thoracic sampling could eliminate interfering particles and thereby improve measurement methods in the future.

Disclaimer

Mention of any company or product does not constitute endorsement by NIOSH. In addition, citations to websites external to NIOSH do not constitute NIOSH endorsement of the sponsoring organizations or their programs or products. Furthermore, NIOSH is not responsible for the content of these websites. All web addresses referenced in this document were accessible as of the publication date.

11 References

Abell MT, Shulman SA, Baron PA [1989]. The quality of fiber count data. Appl Ind Hyg *4*:273-285.

ACGIH [2002]. Appendix D: particle size-selective sampling criteria for airborne particulate matter. In: 2002 TLVs and BEIs based on the documentation of the Threshold Limit Values for chemical substances and physical agents and Biological Exposure Indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Arroyo MC, Rojo JM [1998]. National versus international asbestos fibre counting schemes: comparison between the Spanish Interlaboratory Quality Control Program (PICC-FA) and the Asbestos Fibre Regular Informal Counting Arrangement (AFRICA). Ann Occup Hyg *42*:97-104.

Asbestos-containing materials in schools. 40 CFR Part 763 Subpart E (1987).

Asbestos-containing materials in schools. 40 CFR Part 763 Subpart E Appendix E (1990).

Asbestos International Association [1979]. Reference method for the determination of airborne asbestos fibre concentrations at workplaces by light microscopy (membrane filter method). Recommended technical method 1 (RTM1). London: Asbestos Information Association.

Asbestos International Association [1984]. Method for the determination of airborne asbestos fibres and other inorganic fibres by scanning electron microscopy. Recommended technical method 2 (RTM2). London: Asbestos Information Association.

ASTM [1996]. D6059-96 Standard test method for determining concentration of airborne single-crystal ceramic whiskers in the workplace environment by scanning electron microscopy. Philadelphia, PA: American Society for Testing and Materials.

ASTM [1998]. D6281-98 Standard test method for airborne asbestos concentration in ambient and indoor atmospheres as determined by transmission electron microscopy direct transfer (TEM). Philadelphia, PA: American Society for Testing and Materials.

ATSDR [1990]. Toxicological profile for asbestos. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, TP-90-04.

Baron PA [1993]. Measurement of asbestos and other fibers. In: Willeke K, Baron PA, eds. Aerosol measurement: principles, techniques, and applications. New York: Van Nostrand Reinhold.

Baron PA [1996]. Application of the thoracic sampling definition to fiber measurement. Am Ind Hyg Assoc J *57*:820-824.

Baron PA [2001]. Measurement of airborne fibers: a review. Ind Health *39*:39-50.

Baron PA, Pickford GC [1986]. An asbestos sample filter clearing procedure. Appl Ind Hyg *1*:169-171.

Baron PA, Platek SF [1990]. NIOSH method 7402-asbestos fibers (revision #1)--low temperature ashing of filter samples. Am Ind Hyg Assoc J *51*:A730-A731.

Baron PA, Shulman SA [1987]. Evaluation of the Magiscan image analyzer for asbestos fiber counting. Am Ind Hyg Assoc J *48*:39-46.

Baron PA, Deye GJ, Fernback J [1994]. Length separation of fibers. Aerosol Sci Technol *21*:179-192.

Baron PA, Deye GJ, Fernback JE, Jones WG [2000]. Direct-reading measurement of fiber length/diameter distributions. In: Beard ME, Rook HL, eds. Advances in environmental measurement methods for asbestos. Philadelphia, PA: American Society for Testing Materials.

Beard ME, Rook HL, eds. [2001]. Advances in environmental measurement methods for asbestos. STP 1342. Philadelphia, PA: American Society for Testing Materials.

Blake T, Castranova V, Schwegler-Berry D, Deye GJ, Baron P, Li C, Jones W [1997]. Effect of fiber length on glass microfiber cytotoxicity. J Tox Environ Health *54A*:243-259.

Breysse PN, Lees PSJ, Rooney BC [1999]. Comparison of NIOSH method 7400 A and B counting rules for assessing synthetic vitreous fiber exposures. Am Ind Hyg Assoc J *60*:526-532.

Burke WA, Esmen NA [1978]. The inertial behavior of fibers. Am Ind Hyg Assoc J *39*:400-405.

Chatfield EJ [2000a]. A validated method for gravimetric determination of low concentrations of asbestos in bulk materials. In: Beard ME, Rook HL, eds. Advances in environmental measurement methods for asbestos. Philadelphia, PA: American Society for Testing and Materials.

Chatfield EJ [2000b]. Correlated measurements of airborne asbestos containing particles and surface dust. In: Beard ME, Rook HL, eds. Advances in environmental measurement methods for asbestos. Philadelphia, PA: American Society for Testing Materials.

Chen C-C, Baron PA [1996]. Aspiration efficiency and wall deposition in the fiber sampling cassette. Am Ind Hyg Assoc J *52*:142-152.

Cheng Y-S [1986]. Bivariate lognormal distribution for characterizing asbestos fiber aerosols. Aerosol Sci Technol *5*:359-368.

Cherrie J, Jones AD, Johnston AM [1986]. The influence of fiber density on the assessment of fiber concentration using the membrane filter method. Am Ind Hyg Assoc J *47*:465-474.

Churchyard MP, Copeland GKE [1988]. Is it really chrysotile? Ann Occup Hyg *32*:545-547.

Cowie AJ, Crawford NP [1982]. 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. TM/82/23. Edinburgh, Scotland: Institute of Occupational Medicine.

Crawford NP, Brown P, Cowie AJ [1992]. The RICE and AFRICA schemes for asbestos fibre counting. Ann Occup Hyg *36*:59-69.

Crawford NP, Thorpe HL, Alexander W [1982]. 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. TM/82/23. Edinburgh, Scotland: Institute of Occupational Medicine.

Dement JM [1990]. Overview: workshop on fiber toxicology research needs. Environ Health Perspec *88*:261-268.

Deye GJ, Gao P, Baron PA, Fernback JE [1999]. Performance evaluation of a fiber length classifier. Aerosol Sci Technol *30*:420-437.

EPA [1985a]. Asbestos in buildings: simplified sampling scheme for friable surfacing materials. Washington, DC: U.S. Environmental Protection Agency, EPA 560/5-85-030a.

EPA [1985b]. Guidance for controlling asbestos-containing materials in buildings. Washington, DC: U.S. Environmental Protection Agency, EPA 560/5-85-024.

EPA [1986]. Asbestos containing materials in school buildings: bulk sample analysis quality assurance program. Bulk sample rounds 12, 13 and blind round III. Washington, DC: U.S. Environmental Protection Agency, EPA/600/4-86/028.

EPA [1990a]. 40 CFR Part 61. National emission standards for hazardous air pollutants; asbestos. NESHAP revision, final rule. Nov. 20. Washington DC: U.S. Environmental Protection Agency, http://www.epa.gov.

EPA [1990b]. 40 CFR Part 61.141 Subpart M. National emission standard for asbestos. Washington DC: U.S. Environmental Protection Agency, http://www.epa.gov.

Esmen N, Corn M, Hammad Y, Whittier D, Kotsko N [1979]. Summary of measurements of employee exposure to airborne dust and fiber in sixteen facilities producing man-made mineral fibers. Am Ind Hyg Assoc J *40*:108-117.

Fletcher RA, Small JA, Scott JHJ [2001]. Analysis of individual collected particles. In: Baron P, Willeke K, eds. Aerosol measurement: principles, techniques and applications. New York: John Wiley and Sons, Inc.

HSE [1990]. MDHS 39/3 Asbestos fibres in air. June (revised). London: Health and Safety Executive.

Inoue Y, Kaga A, Yamaguchi K [1999]. Cross-check between automatic counting system and visual counting facilities of asbestos fibers. J Aerosol Res Jpn *14*:129-137.

Inoue Y, Kaga A, Yamaguchi K, Kamoi S [1998]. Development of an automatic system for counting asbestos fibers using image processing. Particul Sci Technol *16*:263-279.

ISO [1995]. ISO 10312 Ambient air-determination of asbestos fibres-direct transfer transmission electron microscopy method. Geneva, Switzerland: International Organization for Standardization.
ISO [1999]. ISO 13794 Ambient air-determination of asbestos fibres-indirect transfer transmission electron microscopy method. Geneva, Switzerland: International Organization for Standardization.

ISO [2002]. ISO 14966 Determination of numerical concentration of inorganic fibrous particles-scanning electron microscopy method. Geneva, Switzerland: International Organization for Standardization.

Jankovic JT [1985]. Asbestos bulk sampling procedure. Am Ind Hyg Assoc J *46*:B8-B10.

Jones AD, Aitken RJ, Armbruster L, Byrne P, Fabries JF, Kauffer E, Liden G, Lumens M, Maynard A, Riediger G, Sahle W [2001]. Thoracic sampling of fibres. Contact research report 349/2001. Norwich, UK: Health and Safety Executive.

Kauffer E [1989]. The French asbestos quality control scheme. In: Sixth International Colloquium on Dust Measurement Technique and Strategy. Jersey, Channel Islands: Asbestos International Association.

Kenny LC [1984]. Asbestos fibre counting by image analysis-the performance of the Manchester asbestos program on Magiscan. Ann Occup Hyg *28*:401-415.

LeGuen JMM, Ogden TL, Shenton-Taylor T, Verrill JF [1984]. The HSE/NPL phase-contrast test slide. Ann Occup Hyg *28*:237-247.

Lilienfeld P, Elterman P, Baron P [1979]. Development of a prototype fibrous aerosol monitor. Am Ind Hyg Assoc J *40*:270-282.

Lippmann M [1988]. Asbestos exposure indices. Environ Res *46*:86-106.

Lippmann M [1990]. Effects of fiber characteristics on lung deposition, retention, and disease. Environ Health Perspec *88*:311-317.

Liu J, Rinzler AG, Dai H, Hafner JH, Bradley RK, Boul PJ, Lu A, Iverson T, Shelimov K, Huffman CB, Rodriguez-Macias F, Shon Y-S, Lee TR, Colbert DT, Smalley RE [1998]. Fullerene pipes. Science *280*:1253-1256.

Lundgren L, Lundström S, Laszlo I, Westling B [1995]. Modern fibre counting-a technique with the phase-contrast microscope on-line to a Macintosh computer. Ann Occup Hyg *39*:455-467.

Marijnissen J, Lilienfeld P, Zhou Y [1996]. A laser monitor for the fiber deposition in a lung model. J Aerosol Sci *27*:S523-S524.

Maynard AD [1996]. The development of samplers for size selective sampling of fibres: phase 2 report: selection and development of personal thoracic samplers. IR/A/96/11. Sheffield, UK: Health and Safety Laboratory.

Maynard AD [1999]. Measurement of aerosol penetration through six personal thoracic samplers under calm air conditions. J Aerosol Sci *30*:1227-1242.

McCrone WC [1980]. The asbestos particle atlas. Ann Arbor, MI: Ann Arbor Science Publishers, Inc.

McCrone W, McCrone L, Delly J [1978]. Polarized light microscopy. Ann Arbor, MI: Ann Arbor Science Publishers, Inc.

Middleton AP [1979]. The identification of asbestos in solid materials. In: Michaels L, Chissick SS, eds. Asbestos: properties applications and hazards. Chichester, UK: John Wiley and Sons, Inc.

Morigi MP, Giacomelli GM, Prodi V [1999]. Mineral fibre sampling and size selection. Ann Occup Hyg *43*:117-124.

NHMRC [1976]. Membrane filter method for estimating airborne asbestos dust. Woden, Australia: National Health and Medical Research Council.

NIOSH [1994a]. Asbestos (Bulk) by PLM: Method 9002. In: Eller PM, Cassinelli ME, eds. NIOSH manual of analytical methods. 4th ed. Cincinnati OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 94-113.

NIOSH [1994b]. Asbestos by TEM: Method 7402. In: Eller PM, Cassinelli ME, eds. NIOSH manual of analytical methods. 4th ed. Cincinnati OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 94-113.

NIOSH [1994c]. Asbestos, chrysotile by XRD: Method 9000 In: Eller PM, Cassinelli ME, eds. NIOSH manual of analytical methods. 4th ed. Cincinnati OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No.94-113.
Ogden TL, Thomson DJ, Ellwood PA [1986]. Euparal and its use in measurement of asbestos. Microscope *34*:173-179.

OSHA [1994]. 29 CFR 1910.1001b Occupational safety and health standards: detailed procedure for asbestos sampling and analysis - Non-Mandatory. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration.

Pang TWS [2000]. Precision and accuracy of asbestos fiber counting by phase contrast microscopy. Am Ind Hyg Assoc J *61*:529-538.

Pang TWS, Schonfeld-Starr FA, Patel K [1989]. An improved membrane filter technique for evaluation of asbestos fibers. Am Ind Hyg Assoc J *50*:174-180.

Perkins RL and Harvey BW [1993]. Method for the determination of asbestos in bulk building materials. Washington, DC: Environmental Protection Agency, EPA/600/R- 93/116.

Rajhans GS, Sullivan JL [1981]. Asbestos sampling and analysis. Ann Arbor, MI: Ann Arbor Science Publishers, Inc.

Rickards AL [1978]. The routine monitoring of airborne asbestos in an occupational environment. Ann Occup Hyg *21*:315-322.

Rooker SJ, Vaughan NP, LeGuen JM [1982]. On the visibility of fibers by phase contrast microscopy. Am Ind Hyg Assoc J *43*:505-515.

Sahle W, Laszlo I [1996]. Airborne inorganic fibre level monitoring by transmission electron microscope (TEM): comparison of direct and indirect sample transfer methods. Ann Occup Hyg *40*:29-44.

Schlecht PC, Shulman SA [1986]. Performance of asbestos fiber counting laboratories in the NIOSH Proficiency Analytical Testing (PAT) Program. Am Ind Hyg Assoc J *47*:259-269.

Schneider T, Holst E, Skotte J [1983]. Size distribution of airborne fibres generated from man-made mineral fiber products. Ann Occup Hyg *27*:157-171.

Selikoff IJ, Lee DHKE [1978]. Asbestos and disease. New York: Academic Press, Inc.

Steel EB, Small JA [1985]. Accuracy of transmission electron microscopy for the analysis of asbestos in ambient environments. Anal Chem *57*:209-213.

Stöber W [1972]. Dynamic shape factors of nonspherical aerosol particles. In: Mercer TT, Morrow PE, Stöber W, eds. Assessment of airborne particles. Springfield, IL: Charles C. Thomas.

Taylor DG, Baron PA, Shulman SA, Carter JW [1984]. Identification and counting of asbestos fibers. Am Ind Hyg Assoc J *45*:84-88.

Tombes, C, Calpin JA [2002]. A simple quality control system for evaluation of interlaboratory differences in fiber counting in accordance with NIOSH Method 7400. Am Ind Hyg Assoc J *49*:A695-A697.

Vaughan NP, Rooker SJ, LeGuen JM [1981]. In situ identification of asbestos fibres collected on membrane filters for counting. Ann Occup Hyg *24*:281-290.

Walton WH [1982]. The nature, hazards, and assessment of occupational exposure to airborne asbestos dust: a review. Ann Occup Hyg *25*:115-247.

Walton WH, Beckett ST [1977]. A microscope eyepiece graticule for the evaluation of fibrous dusts. Ann Occup Hyg *20*:19-23.

Webber JS, Janulis RJ, Carhart LJ, Gillespie MB [1990]. Quantitating asbestos content in friable bulk sample: development of a stratified point-counting method. Am Ind Hyg Assoc J *51*:447-452.

WHO [1985]. Reference methods for measuring airborne man-made mineral fibres (MMMF). Geneva, Switzerland: World Health Organization.

WHO [1986]. Asbestos and other natural mineral fibres. Geneva, Switzerland: World Health Organization.

WHO [1997]. Determination of airborne fibre number concentration: a recommended method by phase contrast optical microscopy (membrane filter method). Geneva, Switzerland: World Health Organization.

Wylie AG [1979]. Fiber length and aspect ratio of some selected asbestos samples. In: Selikoff IG, Hammond EC, eds. Health hazards of asbestos exposure. New York: Annals of the New York Academy of Science.