7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring asbestos, its metabolites, and other biomarkers of exposure and effect to asbestos. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

As discussed in Chapter 4, asbestos is not a single chemical entity, but is the name for a group of six hydrated fibrous polysilicates. Because the toxicity of asbestos appears to be related primarily to fiber size, modern analytical methods focus on providing information on these parameters, as well as on total number of fibers and mineral type. At present, the number and size distribution of fibers in a sample can only be determined by direct microscopic examination. This may be performed using either light or electron microscopy, as discussed below. It should be noted that OSHA regulations on asbestos refer to the six asbestiform minerals and a fiber is defined as having a minimum length, 5 µm, as aspect ratio of 3:1 (OSHA 1992). NIOSH methods for determining fiber concentrations are geared to counting fibers of these dimensions. In addition, these methods give detailed rules as to how to count different objects (e.g., objects with split ends or attached particles) (NIOSH 1989a, 1989b).

Light Microscopic Method. Phase contrast microscopy (PCM) accurately assesses fiber exposure levels for fibers $5 µm in length and >0.25 µm in diameter. Furthermore, PCM cannot differentiate between asbestos and nonasbestos fibers. Currently, the standard method for the determination of airborne asbestos particles in the workplace is NIOSH Method 7400, Asbestos by Phase Contrast Microscopy (NIOSH 1994a). OSHA considers that sampling and analytical procedures contained in OSHA Method ID-160 and NIOSH Method 7400 are essential for obtaining adequate employee exposure monitoring. Therefore, all employers who are required to conduct monitoring are required to use these or equivalent methods to collect and analyze samples (OSHA 1994). In NIOSH Method 7400, asbestos is collected on a 25 mm cellulose ester filter (cassette-equipped with a 50 mm electrically-conductive cowl). The filter is treated to make it transparent and then is analyzed by microscopy at 400–450x magnification, with phase-

contrast illumination, using a Walton-Beckett graticule. A fiber is defined as any particle with a length >5 µm and a length-to-diameter ratio of $3:1. Although the PCM method is relatively fast and inexpensive, it does not distinguish between asbestos and nonasbestos fibers, and it cannot detect fibers thinner than 0.25 µm. Consequently, this method is most useful for the analysis of samples that are composed mainly of asbestos, but only where a significant fraction of the fibers are large enough to be counted. If samples are grossly contaminated by nonasbestiform fibers, then transmission electron microscopy (NIOSH Method 7402) should be used for positive identification. For fibers greater than 1 µm in diameter, then polarized light microscopy (NIOSH Method 7403) may be useful in identifying polymorphs (NIOSH 1987). Concentrations are reported as fibers/mL or fibers/cm^3. Recent improvements in filter preparation procedures now allow for viewing at higher magnification (1250x), resulting in a several-fold improvement in sensitivity for these fibers (Pang et al. 1989). Polarized light microscopy is frequently used for determining the asbestos content of bulk samples of insulation or other building materials (see, for example, NIOSH Method 9002 [NIOSH 1989c] and OSHA method ID-191 [OSHA 1994]); however, this approach is not used for measuring asbestos in environmental media. Method 9002 also enables one to qualitatively identify asbestos types using fiber morphology, color, and refractive index.

In summary, PCM is a useful tool in assessing occupational exposure to workers engaged in activities that generate airborne asbestos fibers. However, in nonoccupational settings where large proportions of other fibers (e.g., wool, cotton, glass) are present, PCM will overestimate the asbestos fiber concentration. In addition, the sensitivity of PCM is approximately 0.01 f/mL, an asbestos level higher than that generally found in nonoccupational environments.

Electron Microscopic Methods. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) methods can detect smaller fibers than PCM and also fiber type, but fiber counting accuracy is unacceptably poor. This is a result of the small area that can be scanned at high magnification. Accuracy is more limited with long (>5 µm) fibers. NIOSH Method 7402, Asbestos by TEM, is used to determine asbestos fibers in the optically visible range and is intended to complement NIOSH Method 7400. Examination of a fiber sample by either TEM or SEM allows the detection of much smaller fibers than light microscopy, and so more thorough data can be collected on fiber length and diameter distribution. Of these two methods, TEM has greater sensitivity for small fibers, and is the most common method for measuring asbestos in ambient air or inside schools or other buildings. SEM analysis usually images fibers that are more than 0.2 µm in diameter because of contrast limitations, while TEM can visualize fibers of all sizes. In addition, most modern transmission electron microscopes are

equipped with instrumentation that allows examination of individual fibers by electron diffraction or energy-dispersive x-ray analysis. This permits determination of the crystalline and elemental composition of the fiber. Thus, reliable distinctions can be made not only between asbestos and nonasbestos fibers, but also between different asbestos mineral classes (NIOSH 1994b). SEM may also incorporate energy-dispersive x-ray analysis devices. Although TEM clearly provides the most information about a fiber sample, TEM methods are relatively slow and costly compared to PCM methods.

Two different procedures are used for preparation of samples for TEM analysis (HEI 1991). Direct transfer methods retain particles in the same relative position during analysis as they were on the original filter with a minimum of change to the airborne particles. Indirect methods involve dispersing the particulate matter from the original filter into a liquid and capturing the suspended particles particulates onto intermediate filters that are used to prepare the TEM specimens. By varying the proportion of liquid, one is able to concentrate or dilute the sample analyzed. In addition, one is able to remove organic and other unwanted particulate matter by ashing or dissolution, thereby selectively concentrating the asbestos. In dispersing the particles in water the sample may be gently sonicated. In the process, fiber bundles may be separated into individual fibrils or fibers broken.

Application of either PCM or TEM methods to the determination of asbestos fibers in biological or environmental media (air or water) requires that the fibers be separated from interfering material and collected on appropriate supports. Methods for preparing biological and environmental samples for microscopy are described below.

7.1 BIOLOGICAL SAMPLES

Asbestos fibers are particularly resistant to chemical and thermal degradation, and this property is used to the advantage in the analysis of biological materials for asbestos. In most cases, the bulk of the biological material is solubilized by digesting the tissue in strong base (e.g., KOH) or a powerful oxidant (e.g., hypochlorite). The insoluble residue (including the asbestos fibers) is collected by ultracentrifugation or filtration, and may be further cleared of biological material by ashing. In some cases, biological material may be removed by ashing without prior digestion. Residual material is then dispersed and transferred to a suitable support for microscopy. Sample handling during sample preparation and dispersal onto a support for microscopy can break fibers or result in the breakup of fiber aggregates. If fiber breakage results in fibers shorter than 5 μm, a lower fiber count would result.

Conversely, if aggregates are separated, a higher fiber count could result. Tissue samples are often embedded in paraffin for sectioning and to preserve the sample for retrospective analysis.

In collecting and preparing samples for fiber analysis by electron microscopy, care must be taken to avoid contamination. Asbestos contamination of laboratory materials, including paraffin (Lee et al 1995), grids (Case 1994; Rogers 1984), and especially cross-contamination by tissues themselves (Case 1994) must be accounted for. While good laboratory practice required that all reagents and materials used in asbestos analysis be tested for the presence of asbestos, paraffin used to embed tissue has generally avoided scrutiny, being viewed by the laboratory as part of the tissue sample, rather than a reagent. Lee et al. (1995) observed that paraffin used to embed tissue of a mesothelioma victim was contaminated with asbestos. Both the surface and portions cut from the washed paraffin blocks contained chrysotile and amphibole fibers. These finding led to an investigation of asbestos structures in raw paraffin and paraffin from tissue blocks from several sources in different parts of the country. Asbestos was present in 24 of 27 cases; of these 24 cases, 11 had levels that could be considered above background and 4 were severely contaminated. While asbestos was observed in some samples of raw paraffin, the highest levels were seen in prepared blocks. Therefore, it is not clear whether contamination was present in the wax or introduced in the reagents used or during the embedding process. These results raises questions about the validity of tissue analyses by electron microscopy for asbestos unless blank control blocks were part of the procedure.

A recent report (Rogers et al. 1999) has demonstrated that *in situ* confocal laser scanning microscopy (CLSM) can provide three dimensional views of fibers retained, undisturbed, in lung tissue tens or hundreds of microns below the surface of the specimen. This allows the three-dimensional location of fibers relative to cells and surrounding tissue to be studied and understood. Tissue samples prepared for asbestos by analysis by TEM are generally digested and ashed. While TEM has been used to image fibers within lung tissue, the process of obtaining 60–70 nm thick tissue sections would be expected to cut apart asbestos fibers and introduce artifacts. While SEM permits intact fibers to be studied, images show primarily the surface of fibers and tissue closest to the observer. There are no standard methods for the analysis of asbestos in biological materials. Table 7-1 summarizes several methods that have been applied for analyzing asbestos fibers in a variety of biological materials.

Table 7-1. Analytical Methods for Determining Asbestos in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Bronchoalveolar fluid	Mix with sodium hypochlorite; membrane filter; dry	PCM	1 AB/mL	No data	Spurny 1994
Urine	Mix with hydrogen peroxide; digest for 20 hours; collect residue on filter	TEM	$0.1\text{-}0.3x10^{-6}$ f/L	No data	Boatman et al. 1983
Urine	Filter through polycarbonate filter; ash filter; wash; collect residue on second filter	TEM	$5x0^{3}$ f/mL	No data	Finn and Hallenbeck 1984
Feces	Dry, ash, dissolve residue in hydrochloric acid; filter; ash filter; transfer residue to grid	TEM	$0.15x10^{6}$ f/g	85.5	Cunningham et al. 1976
Lung tissue	Dry to constant weight;digest with sodium hyroxide (90 EC); ash residue; collect on nucleopore filter	TEM	$0.1x10^{6}$ f/g	No data	Wagner et al. 1982a
Lung tissue	Digest wet tissue in potassium hydroxide; wash residue with water; transfer residue to slide	PCM	5,000 f/g	No data	Whitwell et al. 1977
Tissue sections	Ash on slide; transfer	TEM	No data	No data	Pooley 1976
Tissue specimens	Predigest in 10% potassium hydroxide; collect residue by ultra-centrifugation; ash residue; transfer to carbon grids	TEM	$0.2x10^{5}$ f/g	13–70	Carter and Taylor 1980

f/g = fibers per gram; f/L = fibers per liter; f/mL = fibers per milliliter; PCM = phase contrast microscopy; TEM = transmission electron microscopy

7.2 ENVIRONMENTAL SAMPLES

For the analysis of asbestos fibers in air, a sample of air is drawn through a filter by a vacuum pump (usually at a flow-rate of around 1–2 L/minute), and the fibers retained on the filters are examined microscopically. The sensitivity of the methods depends on the volume of air drawn through the filter and the microscopic method employed. In the workplace, where PCM is the standard method, the theoretical detection limit for a short-term sample (15 minutes) is around 0.04 PCM f/mL, but may be reduced to 0.001 f/mL using an 8-hour sample (NIOSH 1976). In practice, such low detection limits are not readily achievable, and measured values below 0.1 PCM f/mL should not usually be considered reliable (ASTM 1988). Sensitivity of TEM methods for ambient or indoor air are usually around 0.1–1 ng/m^3.

A similar approach is used for measuring asbestos in water. A known volume (generally, at least 1 L) is drawn through a filter, and the filter is then prepared for examination, usually by TEM. Table 7-2 summarizes several representative methods for the analysis of asbestos in air and water. No methods were located for the analysis of asbestos in soil.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of asbestos is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of asbestos.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

Table 7-2. Analytical Methods for Determining Asbestos in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Pump air through filter membrane; convert to optically transparent gel	PCM	<0.5 f/mL	±35	ASTM 1988
Air	Filter	NIOSH 7400; PCM	<0.01 f/mL	No data	NIOSH 1994a
Air	Filter; mount on	NIOSH 7402; TEM	<0.01 f/mL	No data	NIOSH 1994b
Air	Measured volume of air collected on 25 mm diameter, 0.45 Fm MCE filter, Both direct and indirect specimen preparation	Superfund Method. TEM at 20,000X, EXDA, Separate examination of structures of all sizes ($0.5 Fm) and those with a length $5 Fm. Structures have mean aspect ratios $5:1.	Sensitivity >0.5 s/L and $0.02 s/L for all structures and those	No Data	EPA 1990c, 1990d
Water (drinking)	Filter, carbon coat and wash	APHA Method 2570-B; TEM	No data	No data	EMMIWIN 1997
Water	Filter; mount on carbon	TEM at 20,000X	0.01 MFL	100±35	Anderson and Long 1980
Water	Extract into isooctane from water containing anionic surfactant	Microscope or color spot test	0.1 MFL	No data	Melton et al. 1978

Water	Filter; mount on carbon film	TEM	No data	No data	WHO 1986

Table 7-2. Analytical Methods for Determining Asbestos in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Place in ultrasonic bath (15 minutes); filter; dry and collapse filter; plasma etch; mount on carbon film	TEM	No data	No data	Brackett et al. 1992

f/mL = fibers per milliliter; MCE=mixed cellulose ester; MFL = million fibers per liter; PCM = phase contrast microscopy; TEM = transmission electron microscopy; EXDA=energy dispersive x-ray analysis

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Reliable methods exist for measuring asbestos fibers in biological tissues and fluids (Boatman et al. 1983; Carter and Taylor 1980; Wagner et al. 1982b). These methods (based on microscopic examination of fibers remaining after ashing and digestion) are sufficiently sensitive to quantify fiber burden in samples from both control (background) and exposed populations. However, there is considerable variability in the details of sample preparation, and this makes inter-study comparisons difficult. For this reason, it would be helpful to develop a standardized method or group of methods for analysis of asbestos in biological materials, similar to the standardized methods for asbestos in air and water. A major limitation to current methods is that lung retained fibers can only be measured by examining excised lung tissue (see Section 3.8.1). Concentrations of fibers or asbestos bodies in broncho-alveolar lavage or sputum samples may provide indications of exposure to asbestos fibers. Consequently, it is not possible to estimate retained fiber in lung tissue of living persons except by fiber analysis of these samples that are, to various degrees, invasively obtained. Development of some noninvasive method that would permit accurate estimation of asbestos content *in vivo* would be especially valuable.

Effect. There are no chemical analytical methods recognized for measuring asbestos-induced health effects in humans. Clinical methods (x-ray, spirometry) for evaluating effects are discussed in Chapter 3. Development of sensitive and specific chemical or biochemical tests for asbestos-induced effects would be very valuable, especially if preclinical changes could be detected.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Standardized methods have been established in the United States for measurement of asbestos in air by PCM and TEM, the media most likely to lead to human exposure (NIOSH 1989a, 1989b), Standard TEM methods are also available for measuring asbestos in water (WHO 1986). These methods are sufficiently sensitive to quantify asbestos both at background levels and at levels of health concern. There are variations in both sampling conditions and counting rules in PCM methods used in other countries that lead to significant differences in results (Dion and Perrault 1994). Improved comparability would be achieved if an international consensus could be reached to resolve these differences. However, the electron microscopic techniques that give the greatest amount of useful data are also the slowest and most costly. TEM equipment allows fiber type to be identified and finer fibers to be counted. Fiber size,

shape, and mineralogy are important factors for assessing risk. Improved analytical methods for screening samples and determining the chemical structure of asbestos fibers would be useful. Further efforts to reduce the time and cost per analysis would also be helpful.

7.3.2 Ongoing Studies

Given the need and financial incentives for improved, faster asbestos analysis, studies are ongoing to improve these areas. Intense activity is underway in the areas of automation and computerization, especially with TEM and analytical electron microscopy. Another area of investigation is to identify the fiber types and sizes most closely identified with risk of lung cancer and mesothelioma and develop methodology that will give results that are most closely correlated with risk (Berman et al. 1995).

A major area of concern is the possibility that asbestos fibers adsorb carcinogens in smoke, such as benzidine, N,N-dimethylanaline, and benzo(a)pyrene, and carry them to cells. Investigations are being carried out to detect such chemical impurities on asbestos fiber surfaces by a technique known as laser microprobe mass analysis (Warner 1988).

Reliability of asbestos analysis should be improved by new regulations requiring accreditation of asbestos-testing laboratories. The National Institute of Science and Technology (formerly the National Bureau of Standards) is conducting programs for accreditation of polarized light microscopy and TEM laboratories.