7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring synthetic vitreous fibers, its metabolites, and other biomarkers of exposure and effect to synthetic vitreous fibers. 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, synthetic vitreous fibers are not a single chemical entity, but rather a group of amorphous polysilicates. Because the toxicity of fibrous particles appears to be related primarily to fiber size and chemical durability, modern analytical methods focus on providing information on these parameters, as well as on total number of fibers. 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. A complicating factor in the analysis of synthetic vitreous fibers is distinguishing small fibrous particles from asbestos or other inorganic fibers, and identifying and quantifying the various forms of synthetic vitreous fibers. NIOSH methods for determining fiber concentrations are geared to counting fibers of certain dimensions utilizing detailed rules as to how to count different objects (e.g., objects with split ends or attached particles) (NIOSH 1994a).

Light Microscopic Methods. Phase contrast microscopy (PCM) is most frequently employed to measure the levels of synthetic vitreous fibers. Currently, the standard method for the determination of airborne fibrous particles in the workplace is NIOSH Method 7400, Asbestos and Other Fibers by Phase Contrast Microscopy (NIOSH 1994a). In NIOSH Method 7400, samples are collected on 25 mm cellulose ester filters (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. Different counting rules may be employed when analyzing for synthetic vitreous fibers as compared to asbestos (termed counting rules A and B,

respectively), and these different counting rules often make comparing data from various studies difficult. The details of these counting rules are available in the NIOSH Manual of Analytical Methods (1994). Briefly, when using counting rules B, only fibers <3 μm in diameter and >5 μm in length with aspect ratios of ≥5:1 are counted. The rules further specify the counting of fiber ends only, with sufficient fields to yield at least 200 fiber ends. The counting of fibers in a minimum of 20 fields is also required for statistical validity. When using counting rules A, fibers with diameters >3 μm may be counted as well as fibers possessing an aspect ratio of ≥3:1. A recent study has concluded that using the A counting rules generally gives higher fiber counts than the B counting rules when analyzing the same samples (Breysse et al. 1999). Another recent study recommends using counting rules A in order to decrease the number of non-detects when analyzing for synthetic vitreous fibers on contact surfaces such as desks, floors, bookshelves, etc. inside of office buildings (Vallerino et al. 2003). Fiber concentrations in air are usually reported as fibers/mL or fibers/cc, although fibers/m^3 or fibers/L are occasionally employed. Fiber densities are used to quantify the amount of synthetic vitreous fibers on the surface of objects; these units are usually given in fibers/mm^2 (Vallerino et al. 2003).

Although the PCM method is relatively fast and inexpensive, it does not specifically distinguish between the various forms of synthetic vitreous fibers, and it not sensitive to enough to detect fibers possessing very small diameters. The resolution of a microscope (the ability to distinguish between two very closely spaced objects) is limited by the wavelength of light used for analysis and the numerical aperture of the microscope (Hecht 1989). Because PCM uses visible light of approximately 400–700 nm, the maximum resolution than can be achieved is about 0.25 μm. Frequently, synthetic vitreous fibers can be distinguished from other fibers, like asbestos, based upon their morphology (Switala et al. 1994). Glass fibers have clean, well defined, mostly parallel sides, while other fibers such as asbestos usually have irregular sides or hair-like appendages emanating from the sides (Switala et al. 1994). The ends of glass fibers have three distinctive features that can be useful in their identification: (1) The edges possess a clean break that occurs transverse to the fiber length; (2) the ends exhibit a notch-type break; and (3) the ends will taper similar to that of a sharpened pencil. Other materials will fan or fry, and therefore, the morphology of glass fibers is often distinctive from that of other fiber types. When morphology alone cannot distinguish between synthetic vitreous fibers and other fibers, or greater sensitivity is required, other microscopic techniques may be employed to augment the analysis.

Since synthetic vitreous fibers are amorphous substances, polarized light microscopy is often employed to distinguish synthetic vitreous fibers from natural minerals with a crystalline structure. In this technique,

linear polarized radiation is used to illuminate the sample and a second polarizer placed after the sample is used to analyze the light that is transmitted as the sample is rotated in various spatial directions (NIOSH Method 9002). Amorphous samples like synthetic vitreous fibers have the same refractive index in each direction, while anisotropic materials such as asbestos have optical properties that vary with the orientation of incident light with the crystallographic axes, and demonstrate a range of refractive indices depending both on the propagation direction of light through the substance and on the vibrational plane coordinates.

If improved resolution is required to analyze fibrous samples, transmission electron microscopy (TEM) (NIOSH Method 7402) or scanning electron microscopy (SEM) are often employed. Fibers with diameters as small as 0.05 and 0.005 μm can be counted by SEM and TEM methods, respectively. In the TEM experiment, the wavelength of a 100 KeV electron is 0.0037 nm, which is about 143,000 times smaller than the wavelength of light used in PCM. Hence, using electrons for analysis rather than visible light photons leads to much better resolution than can be achieved with light microscopy. Identification techniques such as energy-dispersive x-ray analysis (EDXA) can be used with SEM and TEM to identify fibers on the basis of elemental composition (Spurny 1994). Because EDXA only identifies the elemental composition of the fibers, it can only distinguish between organic and inorganic fibers, as well as things such as silicate or nonsilicate type fibers. In the TEM experiment, it is also possible to measure the diffraction of electrons by crystalline structures that are traversed by the electron beam. This technique can be useful to distinguish between crystalline structures and amorphous fibers like synthetic vitreous fibers.

Methods for preparing biological and environmental samples for microscopy are described below.

7.1 BIOLOGICAL MATERIALS

The analysis of fibers in biological samples involves digesting the biological material in a strong base (e.g., potassium hydroxide) or a powerful oxidant (e.g., hypochlorite). The insoluble residue (including the fibrous portion) 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.

Methods for sampling fibrous particles in biological tissue are not standardized and it is often difficult to compare results of one laboratory with another (WHO 1988). There is also evidence that indicates that digestion of biological materials with potassium hydroxide or sodium hypochlorite can cause substantial loss of synthetic vitreous fibers in the samples (McDonald et al. 1990). A review on the methods of sampling, analysis, and identification of fibers in lung tissue has been published (Davis et al. 1986). It was concluded that low temperature ashing in the presence of nascent oxygen is a superior extraction method than chemical digestion alone (Davis et al. 1986). A method for the sampling and identification of nonrespirable fibers in the eyes of workers frequently exposed to synthetic vitreous fibers has been published (Schneider and Stockholm 1981). In this procedure, mucous threads and dried mucous samples were ashed in a low temperature asher and examined by PCM. A correlation was reported between synthetic vitreous fibers levels in the eyes and total dust samples in the workplace. The techniques used to analyze synthetic vitreous fibers in biological samples are summarized in Table 7-1.

7.2 ENVIRONMENTAL SAMPLES

For the analysis of 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. Two types of filters are commonly employed for air sample collection, cellulose ester membrane filters (MF) and polycarbonate (Nuclepore) filters (NPF) (Spurny 1994). The NPF is a surface filter that retains fibers on the exterior of the filter, while the MF is a spongy depth type filter. The sensitivity of the methods depends on the volume of air drawn through the filter, the filtration procedure, and the microscopic method employed. Filtration studies have shown that an MF with pore size of 0.8 μm and an NPF with a pore size of 0.4 μm are capable of collecting most fibers in the workplace (Spurny 1994). Air samples obtained using NPFs can be applied directly for SEM or EDXA analysis without further preparation; however, specimen preparation for TEM analysis is more complex. In the workplace, where PCM is the standard method, the working range of countable fibers for a short-term sample (15 minutes) is around 0.5–0.04 fiber/cc, but may be reduced to 0.001 fiber/cc using an 8-hour sample because a larger volume of air is collected (NIOSH 1976). Improvements in filter preparation procedures now allow for viewing at higher magnification (1,250x), resulting in a several-fold improvement in sensitivity for these fibers (Pang et al. 1989). Recently, detection limits of $1x10^{-5}$ f/cc have been obtained using a 12-hour sampling procedure (Switala et al. 1994). It is important to keep in mind that only fibers with diameters >0.25 μm can be counted by PCM, while fibers with diameters as small as 0.05 and 0.005 μm can be

Table 7-1. Analytical Methods for Determining Synthetic Vitreous Fibers in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Broncho-alveolar fluid	Digestion with sodium hypochlorite; membrane filter; dry	PCM	No data	No data	Spurny 1994
Urine	Membrane filtration followed by ashing and dispersion in 1% acetic acid followed by nuclepore filtration (0.1 µm)	TEM	No data	No data	Spurny 1994
Eye mucous	Low temperature ashing	PCM	No data	No data	Schneider and Stockholm 1981
Lung tissue	Low temperature ashing in a plasma asher; filtration with 0.2 µm nuclepore	TEM	0.04 µm (diameter) 0.09 µm (length)	No data	McDonald et al. 1990

PCM = phase contrast microscopy; TEM = transmission electron microscopy

counted by SEM and TEM methods, respectively (WHO 1988). The techniques used to analyze synthetic vitreous fibers in air samples are summarized in Table 7-2.

While no methods were located for the analysis of synthetic vitreous fibers in water, TEM-based methods exist that can quantify asbestos concentrations in water samples, and these methods should also be applicable toward the measurement of synthetic vitreous fibers (Brackett et al. 1992; Melton et al. 1978). No methods were located for the analysis of asbestos or synthetic vitreous fibers 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 synthetic vitreous fibers is available. Where adequate information is not available, ATSDR, in conjunction with 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 synthetic vitreous fibers.

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.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Standardized techniques to evaluate the levels of synthetic vitreous fibers in biological tissues do not exist. In general, biological tissues are subject to digestion with a strong base or oxidizing agent, followed by ashing. The tissue is then analyzed by microscopic examination. A method for the sampling and identification of nonrespirable fibers in the eyes of workers frequently exposed to synthetic vitreous fibers has been published (Schneider and Stockholm 1981). A data need exists to develop reliable methods to analyze for synthetic vitreous fibers in human tissue.

Table 7-2. Analytical Methods for Determining Synthetic Vitreous Fibers in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit[a]	Percent recovery	Reference
Air	Pump air through filter membrane; convert to optically transparent gel	PCM	0.01 f/cc	±35	ASTM 1988
Air	Filter with 0.45–1.2 µm cellulose ester membrane filter	NIOSH 7400; PCM	0.003 f/cc	No data	Jacob et al. 1993; NIOSH 1994a
Air	Filter with 0.45–1.2 µm cellulose ester membrane filter	PCM; polarized light microscopy	$1x10^{-5}$ f/cc	No data	Switala et al. 1994
Air	Filter with 0.45–1.2 µm cellulose ester membrane filter	NIOSH 7402; TEM	0.001 f/cc	No data	NIOSH 1994a; Spurny 1994
Surfaces	Collect samples using 12.56 or 50.24 mm^2 finger-print lifters; transfer to microscope slide	NIOSH 7400	0.06–0.24 f/mm^2	No data	Vallarino et al. 2003

[a]Sample detection limits in air are a function of the volume of air collected and thus the sampling time. Detection limits on the surface of an object are a function of the number of fibers collected per unit area.

f/cc = fibers per cubic centimeter; NIOSH = National Institute for Occupational Safety and Health; PCM = phase contrast microscopy; TEM = transmission electron microscopy

Exposure. Uncoated or coated fibers in bronchoalveolar lavage fluid samples or in autopsied or surgically resected lung tissue samples are the principal biomarkers of exposure to biopersistent asbestos fibers (Agency for Toxic Substances and Disease Registry 2001).[1] However, similar biomarkers to identify or quantify human exposure to synthetic vitreous fibers, which are less biopersistent than asbestos fibers, have not been developed for routine clinical use. Nevertheless, aluminum-silicate fibers with chemical compositions consistent with synthetic vitreous fibers have been detected in human lung tissues (McDonald et al. 1990; Roggli 1989; Sébastien et al. 1994) and in bronchoalveolar lavage samples (Dumortier et al. 2001).

Among 1,800 bronchoalveolar samples submitted to a Belgium hospital between 1992 and 1997 for fiber analysis, pseudoasbestos bodies were detected in samples from nine patients (0.5%) (Dumortier et al. 2001). In samples from these nine patients (all of whom had occupational experience with furnaces or welding), fibers of composition consistent with refractory ceramic fiber composition were detected in 42% of core fibers analyzed (Dumortier et al. 2001). Other nonasbestos fibers and asbestos fibers accounted for 28% and 30% of the core fibers analyzed in these samples.

Effect. Epidemiological studies of synthetic vitreous fiber manufacturing workers have not found consistent evidence for increased risks of malignant or nonmalignant respiratory or pleural effects, but results from animal experiments indicate that repeated inhalation exposure to synthetic vitreous fibers may result in pulmonary or pleural fibrosis, lung cancer, or mesothelioma, depending on fiber dimensions, fiber durability in the lung, duration of exposure, and exposure levels.

The chest x-ray is the most common means of detecting the onset of pleural or pulmonary changes that may precede or accompany fibrosis (i.e., irreversible scarring of lung or pleural tissue that can lead to restricted breathing). The International Labour Office (ILO) established a classification system for profusion of opacities in chest x-rays that includes four categories of increasing severity, each with three subcategories: 0 (0/-, 0/0, 0/1); 1 (1/0, 1/1, 1/2); 2 (2/1, 2/2, 2/3); and 3 (3/2, 3/3, 3/4) (ILO 1989). The American Thoracic Society (1986) recommends that chest x-rays be scored for pleural and pulmonary changes separately because of the experience with asbestos-exposed workers indicating that pleural and pulmonary fibrosis have differences in "epidemiology, clinical features, and prognosis." Lung function

[1] Particles or fibers that are deposited in the lung and are too large to be phagocytized by alveolar macrophages may become coated with an iron-rich protein coat. The generic term for these structures is ferruginous bodies. When the core fiber is asbestos, the resultant structure is termed an asbestos body (Agency for Toxic Substances and Disease Registry 2001). Ferruginous bodies having the appearance of asbestos bodies under light microscopy and a nonasbestos core fiber have been termed pseudoasbestos bodies (Dumortier et al. 2001).

tests are also useful to characterize the development of pulmonary or pleural fibrosis; forced vital capacity is diminished with increasing severity of pulmonary or pleural fibrosis.

Development of sensitive and specific chemical or biochemical tests for synthetic vitreous fibers effects would be useful.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods are available to measure synthetic vitreous fibers in air (Jacob et al. 1993; NIOSH 1994a; Spurny 1994; Switala et al. 1994). These methods are precise, and are sensitive enough to detect levels that are frequently encountered in both occupational and non-occupational settings. No specific methods of measuring the levels of synthetic vitreous fibers in other environmental media such as soil, water, and sediment exist. Since exposure to synthetic vitreous fibers primarily occurs through inhaling air, no data need has been identified at this time.

7.3.2 Ongoing Studies

No data were found regarding ongoing studies involving analytical methods for the detection of synthetic vitreous fibers.