METHOD 100.2

DETERMINATION OF ASBESTOS STRUCTURES OVER 10 µm IN LENGTH IN DRINKING WATER

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1.0 SCOPE AND APPLICATION

1.1 This test method is recommended for the determination of the presence and quantitation of asbestos structures in drinking water samples. The method allows for the quantitation of structures greater than 10 µm in length.

1.2 This test method describes the equipment and procedures necessary for the sampling and analysis of drinking water by transmission electron microscopy (TEM).

1.3 The identification of asbestos by TEM is based on (a) morphology, (b) selected area electron diffraction (SAED) and (c) energy dispersive x-ray analysis (EDXA).

1.4 Applicable analytes and Chemical Abstract Service (CAS) Numbers:

Asbestos	CAS Number
Chrysotile	12001-29-5
Crocidolite	12001-28-4
Amosite (Grunerite)	12001-73-5
Anthophyllite	77536-67-5
Tremolite	77536-68-6
Actinolite	77536-66-4

1.5 Data Quality Objectives

Method	Accuracy[a]	Precision[a]	Completeness
TEM	95%	95%	100%

[a.] Confidence coefficient of a confidence interval for a Poisson variable within which count ranges are expected to fall.

1.6 Analytical Sensitivity. A sensitivity of 200,000 fibers per liter (0.2 MFL) is required unless filter loading satisfies the stopping rules in Section 11.31. See TABLE 1.

1.7 Only asbestos structures meeting the definitions set forth in the Chatfield protocol are counted **(1)**.

TABLE 1. Limitation of Analytical Sensitivity by Volume of Water Sample Filtered **(1)**

Volume of Sample Filtered (mL)		Analytical
25 mm Diam. Filter[b]	47 mm Diam. Filter[c]	Sensitivity (f/L)
0.1	0.6	3.0×10^{7}
0.5	2.8	6.0×10^{6}
1.0	5.7	3.0×10^{6}
2.0	11	1.6×10^{6}
5.0	28	6.0×10^{5}
10	57	3.0×10^{5}
15	**85**	**2.0×10^{5}**
25	142	1.2×10^{3}
50	285	6.0×10^{4}
100	570	3.0×10^{4}

[a] Concentration corresponding to 1 structure detected in 10 grid openings of approximately 0.008 mm^2.

[b] Assuming active filter area of 1.99 cm^2.

[c] Assuming active filter area of 11.34 cm^2.

2.0 SUMMARY OF METHOD

Water is collected in a polyethylene or glass container and shipped to the laboratory. Known aliquots of the sample are filtered through a 0.1 to 0.22 µm pore mixed cellulose ester (MCE). A carbon extraction replica is prepared from a portion of the filter and is examined in the TEM at a magnification of 10,000 to 20,000X. Asbestos structures are identified by morphology, selected area electron diffraction (SAED) and energy dispersive x-ray analysis (EDXA). Structures are classified according to the counting rules specified in the Chatfield polycarbonate filter protocol **(1)**. Only asbestos structures greater than 10 µm in length are counted. Some **states** may require identification and measurement of all asbestos fibers,

regardless of size. In this case the use of a 0.1 µm pore-size polycarbonate or MCE filter membrane is necessary to prevent loss of small fibers during filtration.

3.0 DEFINITION OF TERMS

3.1 Analytical Sensitivity -- The waterborne concentration represented by the finding of one asbestos structure in the total area of filter examined. This value will depend on the fraction of the sample filtered and the dilution factor (if applicable).

3.2 Asbestiform -- A specific type of fibrous habit which has greater flexibility and higher tensile strength than other habits of the same mineral.

3.3 Asbestos -- generic term for a group of hydrated mineral silicates.

3.4 Aspect Ratio -- The ratio of the length of a fibrous particulate to its apparent width (equivalent diameter).

3.5 Bundle -- A structure composed of three or more fibers in a parallel arrangement with each fiber lying less than one diameter apart.

3.6 Cluster -- A structure with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group.

3.7 EDXA -- Energy dispersive X-ray analysis.

3.8 Fiber -- For the purposes of this method, a structure having a minimum length of 10 µm and an aspect ratio (length to width) of 3:1 or greater with substantially parallel sides.

3.9 Fibril -- The smallest crystalline fiber that can be separated from a fiber bundle which cannot be subdivided without losing its fibrous properties.

3.10 Grid -- a 3 mm diameter 200-mesh copper lattice used to hold the carbon extraction replica for observation in the TEM.

3.11 Intersection -- Nonparallel touching or crossing of fibers, with the projection having an aspect ratio $\geq$3:1.

3.12 Matrix -- Fiber or fibers with one free end and the other end embedded in or hidden by a particulate.

3.13 MFL -- million fibers per liter.

3.14 SAED or ED -- Selected area electron diffraction.

3.15 Structure -- A microscopic bundle, cluster, fiber or matrix which may contain asbestos.

3.16 TEM -- transmission electron microscope.

4.0 INTERFERENCES

The minerals listed below can exhibit morphological, chemical or crystal structure similarities to the asbestos minerals. The laboratory QA/QC manual should describe routine techniques to differentiate them from asbestos. In general, these techniques should be the same as those required for accreditation by the National Institute of Standards and Technology/National Voluntary Laboratory Accreditation Program (NIST/NVLAP) for airborne asbestos.

4.1 Antigorite

4.2 Attapulgite (Palygorskite)

4.3 Halloysite

4.4 Horneblende

4.5 Pyroxenes

4.6 Sepiolite

4.7 Vermiculite scrolls

5.0 SAFETY

This test method may involve hazardous materials, operations and equipment, and does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use. Sample filtration take place in a clean HEPA filtered positive pressure hood to avoid possible contamination of the preparation. Collapsing of the filter should be performed in an exhaust hood.

6.0 EQUIPMENT AND SUPPLIES

6.1 Transmission Electron Microscope capable of performing electron diffraction, with a fluorescent screen inscribed with a calibrated measuring scale. The TEM must have EDXA and be able to produce a spot size, at crossover, less than 250 nm in diameter.

6.2 Energy dispersive X-ray analyzer

6.3 High vacuum carbon evaporator with rotating stage

6.4 Positive pressure HEPA filtered hood

6.5 Fume hood

6.6 Table-top low power ultrasonic bath

6.7 Ozone generator capable of generating at least 400g of ozone per day at a concentration of 1% by weight when supplied with dry oxygen.

6.8 Quartz pipets

6.9 Submersible UV lamp (254 µm wavelength)

6.10 Waterproof marker

6.11 Forceps (tweezers)

6.12 Graduated pipettes (1, 5, 10 ml sizes), disposable glass

6.13 25 or 47 mm diameter filter funnel assembly (either glass or disposable plastic)

6.14 1000 mL side arm vacuum filtration flask

6.15 25 or 47 mm diameter mixed cellulose ester (MCE) membrane filters ($\leq$0.22 µm and 5 µm pore size)

6.16 Disposable petri dishes (or suitable equivalent) for storage of filtration membranes

6.17 Glass microscope slides

6.18 Curved scalpel blades

6.19 Low temperature oven or cabinet-type desiccator

6.20 Low temperature plasma asher

6.21 Jaffe washer

6.22 200 mesh copper TEM finder grids

6.23 Carbon rods

6.24 1000 mL glass or polyethylene sample bottles with screw-on caps

7.0 REAGENTS AND STANDARDS

7.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society **(2)**.

7.2 Deionized particle-free water

7.3 Acetone

7.4 Dimethylformamide (DMF)

7.5 Glacial acetic acid

7.6 Chloroform

7.7 1-methyl-2-pyrrolidone also called 1-methyl-2-pyrolidinone or n-methyl-2-pyrrolidone (CAS 872-50-4)

7.8 NIST traceable asbestos standards

7.9 Laboratory standards for the interference minerals listed in Section 4.0.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 The sample container will be an unused, pre-cleaned, screw-capped bottle of glass or low density (conventional) polyethylene of at least 1 liter capacity. It is recommended that the use of polypropylene bottles be avoided since problems of particulate being released into water samples have been observed. Before use, the bottles should be rinsed twice by filling approximately one-third full with fiber-free water and shaking vigorously for thirty seconds. After discarding the rinse water, the bottles should then be filled with fiber-free water and treated in an ultrasonic bath for 15 minutes, followed by several rinses with fiber-free water.

8.2 Blank determinations should be made prior to sample collection. When using polyethylene bottles, one bottle from each batch, or a minimum of one from each 24, should be tested for background level. When using glass bottles, four bottles from each 24 should be tested. Additional blanks may be desirable when sampling waters suspected of containing very low levels of asbestos, or when additional confidence in the bottle blanks are desired. An acceptable bottle blank level is defined as $\leq$0.01 MFL >10 µm.

8.3 SAMPLE COLLECTION-- It is beyond the scope of this procedure to furnish detailed instructions for field sampling; the general principles of obtaining water samples apply. If tests are being made of drinking water in a bulk storage supply, there may be a vertical distribution of particle sizes. If a representative sample of the water supply is required, a carefully designated set of samples should be taken representing the vertical as well as the horizontal distribution and then composited for analysis. Compositing must be done in the laboratory, and not in the field.

When sampling from a distribution system a commonly used faucet should be chosen. Remove all hoses or fittings from the faucet and allow the water to run to waste for a sufficiently long period to ensure that the sample collected is representative of the fresh water supply. For most buildings this may be indicated by a change in temperature of the water at the faucet. Faucets or valves should not be adjusted until all samples have been collected. Samples should not be taken from hydrants or other faucets at the deadend of a distribution system.

As an additional precaution against contamination, each bottle may be rinsed several times in the source water being tested before the final sample is taken. This procedure is not suitable when taking depth samples from a storage tank, however.

8.4 QUANTITY OF SAMPLE-- Two samples of approximately 800 mL should be collected from each site. No preservatives should be added during sampling. This sample volume will leave an air space to allow efficient redispersal of settled material, by shaking, before analysis. Each bottle should be labeled with the date, time, place, field sample number and sampler's name using a waterproof marker.

8.5 SAMPLE SHIPMENT-- Samples must be transported to the analytical laboratory as soon as possible after collection. The samples should be shipped in a sealed container, separate from any bulk or air samples. Samples should be shipped in a cooler with ice to retard bacterial or algal growth in the samples. Do not freeze the samples. Samples should be received and filtered in the laboratory within 48 hours of collection. Samples must be accompanied by a properly executed chain of custody document.

8.6 SAMPLE PRESERVATION-- Samples should be filtered immediately after arrival at the laboratory or stored in a refrigerator until filtered.

8.7 SAMPLE COMPOSITING-- Up to five samples may be composited after receipt in the laboratory. The composite sample must be prepared from the individual samples within 48 hours of collection, or, if the samples have been stored for more than 48 hours, they must be individually treated with O_3-UV in the original containers. Samples should be sonicated in the original container and equal amounts extracted to make up the composite. It may also be prudent to filter an aliquot of each sample for analysis in case the composite sample exceeds $1/5^{th}$ the MCL (1.4 MFL >10 µm long). If, later, the original samples are to be filtered separately, they must be treated again with O_3-UV in the original containers and re-sonicated.

9.0 QUALITY CONTROL

9.1 The quality control checks required for this method generally follow those specified in the Federal Register for AHERA analysis of air samples **(3)** and the NISTIR document relating to airborne asbestos analysis **(4)**. These requirements are summarized in Table 2. The criterion for acceptability of bottle and process blanks is $\leq$0.01 MFL >10 µm in length.

10.0 CALIBRATION AND STANDARDIZATION

10.1 MAGNIFICATION CALIBRATION-- Magnification calibration must be done at the fluorescent screen and must be performed at the magnification used for fiber counting, generally 10,000 and 20,000X. Calibration is performed using a grating replica (e.g., one containing at least 2,160 lines/mm). Define a field of view on the fluorescent screen either by markings or physical boundaries. The field of view must be measurable or previously inscribed with a scale or concentric circles (all scales should be metric). If the instrument contains a tilting stage (goniometer), the z-axis must be adjusted to ensure that the stage is in the eucentric position prior to performing any measurements. A logbook must be maintained with the dates of the calibration recorded. Frequency of calibration will depend on the service history of the instrument. It is recommended that calibrations be performed monthly to establish the stability of the magnification. Also, the calibration should be checked following any maintenance of the microscope involving adjustment of the lens or high voltage power supplies or the disassembly of the electron optical column apart from filament exchange.

10.2 CAMERA CONSTANT-- The camera length of the TEM in the electron diffraction (ED) mode must be calibrated before ED patterns of unknown samples are observed. This can be achieved by using a carbon-coated grid on which a thin film of gold has

been sputtered or evaporated. A thin film of gold can also be evaporated on the specimen grids to obtain ED patterns superimposed on the ring pattern from the polycrystalline gold film. In practice, it is desirable to optimize the thickness of the gold film so that only one or two sharp rings are obtained on the superimposed ED pattern. Thicker gold films will tend to mask weaker diffraction spots from the fibrous particulates. Since the unknown d-spacings of most interest in asbestos analysis are those which lie closest to the transmitted beam, multiple gold rings from thicker films are unnecessary. Alternatively, a gold standard specimen can be used to obtain an average camera constant on a regular basis for each TEM in the laboratory. The stage must be at the eucentric position for this calibration. The camera constant calculated for that particular instrument can then be used for ED patterns of unknowns taken during the corresponding period.

10.3 SPOT SIZE-- The diameter of the smallest beam spot at crossover must be measured regularly. Photograph the beam at crossover at 20,000 to 25,000X at a short exposure setting (to avoid spreading of the exposed spot on the film). Measure the diameter on the negative and divide by the magnification used.

TABLE 2. SUMMARY OF LABORATORY DATA QUALITY OBJECTIVES

Unit Operation	QC Check	Frequency Expectation	Conformance
Sample Receiving	Review chain-of-custody and sampling data	Each sample	95% complete
Sample Prep	Supplies and reagents	On Receipt	Meet specs.
	Grid opening size	20/20 grids/ lot of 1000 or 1/sample	100%
	Clean area monitoring	After service	Meet specs.
	Lab blank	1/series or 10%	Meet specs.
	Etcher blank	1/20 samples	75%
	≥3 grids/sample	Each sample	≥70% intact openings
Sample Analysis	System check	Each day	Each day
	Alignment check	Each day	Each day
	Mag calibration	Monthly or after service	95%
	Camera constant	Weekly	95%
	EDS Cu K" line	Each day	95%
Performance	Lab blank	1/prep series or 10%	Meet specs.
	Replicate count	≥1/100 samples	1.5XPoisson std dev.
	Duplicate count	≥1/100 samples	2XPoisson std. dev.
	Analysis of standards	Training and comparison with unknowns	100%
	Analysis of SRM	1 per analyst per year	1.5XPoisson std dev.
	Data entry	Each sample	95%
	Record and verify SAED patterns	1/5 samples	80% accuracy
Calculations	Hand calculation of automated data reductions or 2nd analyst check of manual calcs.	1/100 samples	85%

The resulting figure must be <250 nm.

10.4 EDXA SYSTEM-- The resolution and calibration of the EDXA must be checked at least monthly and after service. Initially, the system is calibrated by using two reference elements to calibrate the energy scale of the instrument according to the manufacturer's instructions. This can easily be done using a carbon-coated copper grid upon which a thin film of aluminum has been evaporated. The Al and Cu K" peaks should be centered at 1.48 KeV and 8.04 KeV respectively. The deviation from these energies should be no more than ±10eV. The ability of the system to resolve the sodium K" line from the copper L line should be confirmed by obtaining a spectrum from a standard crocidolite sample on a copper grid. Additional resolution checks are usually found in the manufacturer's instruction manual. K-factors relative to silicon should be calculated for Na, Mg, Al, Si, Ca and Fe using NIST SRM 2063. The k factor for Mg to Fe must be calculated; a value of 1.5 or less is required. EDXA spectra should be obtained from NIST traceable standards and kept on file in the laboratory for comparison with published standard spectra and unknown spectra.

11.0 PREPARATION AND ANALYSIS

11.1 If the water samples are suspected to contain high levels of organic contaminants, or have been stored for periods longer than 48 hours, oxidation of the organics by the ozone-ultraviolet technique **(1)** may be necessary. Details for this procedure appear in Appendix 1.

11.2 Wet-wipe the exterior of the sample bottle before entering the clean area used for specimen preparation. The sample prep area should be separate from the areas used for bulk sample or air sample preparation to avoid possible cross-contamination. Sample filtration should take place in a positive pressure HEPA filtered hood.

11.3 The use of vertical sided 25 or 47 mm diameter glass filtration systems with a sintered glass frit support is recommended to avoid loss of fibers by settling on tapered sides of the reservoir. Disposable plastic funnel assemblies may be substituted for glass apparatus. A few precautions must be taken with re-usable glassware to ensure optimum sample preparations. All glassware should be carefully washed in a detergent solution with a brush before each use and rinsed several times in fiber-free water. Any glassware that has contained asbestos in solution should be placed in soapy water and scrubbed before it has had the opportunity to dry. Sonication in a detergent solution is also recommended. Frequent blanks should be run with fiber-free water to check cleanliness of the apparatus.

11.4 Unwrap an unused disposable plastic filter funnel unit and remove the tape around the base of the funnel. Remove the funnel and discard the top filter membrane supplied with the

unit. Do not remove the coarse polypropylene support pad. Assemble the unit with the adapter and a properly sized neoprene one-hole stopper, and attach the assembly to the 1000 mL vacuum flask.

11.5 Whether using a glass or plastic filtration unit, care must be taken to ensure that the filter support and the mating surface of the filtration base not be damp when the backing filter is placed on the support. If this should occur, the vacuum across the filtration surface will be uneven, resulting in uneven distribution of the filtered particulates. Either the filtration base must be thoroughly dried before use, or completely wetted so that the backing filter and filtration membrane are uniformly wet before filtration is started.

11.6 DISPOSABLE FILTER UNIT-- Wet the support pad with distilled water, if desired, and place a 5 µm pore-size MCE backing filter on the pad. Place a ≤0.22 µm pore-size MCE filter membrane on the backing filter. Ensure that both filter membranes are completely wet, or dry depending upon the technique preferred. Apply a vacuum to the flask and ensure that the filters are centered and pulled flat with no entrapped air bubbles between the membranes. If any irregularities are seen on the filter surface, discard the filters and try again. Replace the funnel and seal the assembly with tape.

11.7 REUSABLE GLASS FILTER UNIT

a. (Dry Filter) Apply vacuum to the flask and leave the vacuum on until the filtration process is completed. Place a ≤5 µm pore-size MCE filter on the glass frit to serve as a backing filter. Be sure that the filter is not creased during installation. If the filter appears to absorb water, it should be discarded and the frit rinsed with methanol to speed drying. Place a ≤0.22 µm pore-size MCE filter membrane on the backing filter. Make sure that the mating surface of the filter reservoir is dry, then place it on the filters and clamp in place.

b. (Wet Filter) Wet the glass frit and place a 5 µm pore-size MCE filter on the frit to serve as a backing filter. Place a ≤0.22 µm pore-size MCE filter membrane on the backing filter. Make sure that both membranes are thoroughly soaked with water. Install the filter reservoir and clamp in place.

11.8 A process blank sample consisting of fiber-free water should be run before the first field sample. The quantity of water should be ≥10 mL for a 25 mm diameter filter and ≥50 mL for a 47 mm diameter filter.

11.9 Shake the capped sample bottle vigorously by hand and place in a low power ultrasonic bath for 15 minutes. Shake the sample by hand again before removal of any aliquots.

11.10 Remove the desired aliquot from the original sample. Large volumes may be measured with a graduated cylinder, smaller volumes should be taken with disposable glass pipettes. Samples taken by pipette should be taken from the vertical center of the original sample. No aliquot less than 1 mL should be taken from the original sample. The minimum volume that should be filtered is 10 mL for a 25 mm diameter filter, or 50 mL for a 47 mm diameter filter. If it is necessary to filter aliquots less than these volumes, the aliquots should be brought up to these levels with fiber-free water and shaken vigorously before filtration. Obtaining a filter with the proper loading is a matter of trial and error. It is best to filter several volumes of the sample. Samples with high particulate or asbestos content may require volumes less than 1 mL to be filtered. In this case, preparation of serial dilutions must be performed.

11.11 Agitate the aliquot and pour into the filter reservoir. Apply vacuum to the flask. When volumes larger than the capacity of the reservoir have to be filtered, the additional solution should be carefully added while the reservoir is over half full to avoid disturbing the particulates already deposited on the filter. **Do not rinse the sides of the reservoir.**

11.12 If a re-usable glass reservoir is used, **immediately** remove the reservoir and place in soapy water.

11.13 Disassemble the filtration apparatus and remove the filters with clean forceps. Carefully separate the working filter from the backing filter and discard the backing filter. Place the working filter in a pre-cleaned disposable petri dish and cover.

11.14 Allow filter to dry. Drying may take place in a HEPA filtered hood, an asbestos-free oven, or a cabinet type desiccator. The cover of the petri dish should be opened slightly to allow water vapor to escape.

11.15 Using a clean scalpel remove a portion of the dry filters for preparation of TEM grids by the direct transfer technique. Be sure to avoid the outer ring of the filter that was covered by the mating surface of the reservoir. Transfer the removed portion to an unused petri dish.

11.16 A portion of an unused filter should also be prepared as a lot blank.

11.17 MCE filters must be collapsed prior to low temperature etching. Use of either the DMF-Acetic acid method or the acetone

method is acceptable. Samples should be transferred to an exhaust hood for this step.

a. DMF-ACETIC ACID METHOD-- Place a drop of the clearing solution (35% dimethylformamide [DMF], 15% glacial acetic acid and 50% water [V/V] on a clean microscope slide. Use just enough solution to saturate the filter. DMF is a relatively toxic solvent and should be used in a fume hood. Carefully lay the filter segment, sample surface upward, on top of the solution, bringing the filter and solution together at an angle of about 20° to help exclude air bubbles. Remove any solution not absorbed by the filter with lens paper. Label the slide with a glass scribing tool or a permanent marker. Place the slide into an oven, or on the surface of a hot plate, at 65-70°C for 10 to 30 minutes.

b. ACETONE METHOD-- Place the filter section on a clean microscope slide. Affix the filter section to the slide with tape around the edges. Label the slide with a glass scribing tool or permanent marker. Place the slide in a petri dish containing several paper filters soaked with acetone. Cover the dish and wait for the sample to fuse and clear (approximately 5 minutes).

c. Plasma etching of the collapsed filter is required if 0.22 µm pore-size membrane filters are used. Plasma etching is optional (but recommended) with 0.1 µm pore-size filters. The microscope slide to which the collapsed filter pieces are attached is placed in a low temperature plasma asher. Because plasma ashers vary greatly in their performance, both from unit to unit and between different positions in the asher barrel, it is difficult to specify the conditions that should be used. Insufficient etching will result in a failure to expose embedded fibrils, and too much etching may result in loss of particulate from the surface. It is recommended that conditions be used which will remove about 10% of the filter mass. Additional information about calibration of the plasma asher can be found in the AHERA **(3)** and NISTIR **(4)** documents.

11.18 CARBON-COATING FILTER SEGEMENTS

a. Coating must be performed with a high-vacuum evaporation unit equipped with a rotating tilting stage. Units based on evaporation of carbon filaments in a vacuum generated only by an oil rotary pump have not been evaluated for this application and must not be used. The carbon rods should be sharpened by a carbon rod sharpener to necks of about 4 mm long and 1 mm in diameter. The rods are installed in the evaporator in such a manner that the points are approximately 10 cm from the surface of the microscope slide.

b. Affix the glass slide to the rotating tilting table and evacuate the chamber to a pressure of ≤0.013 Pa. The evaporation must be performed in very short bursts, separated by some seconds to allow the electrodes to cool to avoid overheating the surface of the filter. An experienced analyst can judge the thickness of the carbon film to be applied, and some tests should be made first on unused filters. If the film is too thin, there will be few complete, undamaged grid openings on the specimen and large particles may be lost. If the coating is too thick, the TEM image will lack contrast and the ability to obtain selected area electron diffraction (SAED) patterns will be compromised. A carbon film that appears similar to a 15% gray scale is usually adequate.

11.19 Prepare the Jaffe washer. The precise design of the Jaffe washer is not important, so any one of the published designs may be used **(3,5)**. Place the washer in a fume hood and fill with DMF, acetone or 1-methyl-2-pyrrolidone to the level of the screen on which the samples will be placed.

11.20 Place calibrated TEM grids in the Jaffe washer. Indexed ("Finder") grids or grids with a unique center mark should be used. The area of the grid square openings must be determined either by using the TEM at a calibrated magnification low enough to measure the sides of the opening, or with phase contrast microscopy at a calibrated magnification (usually 400X). If the measurements are made by TEM, one grid opening per grid is measured. For measurement by phase contrast, 20 grid openings are measured on each of 20 grids and the average size calculated. The TEM grids are first placed on a piece of lens tissue or filter paper so that individual grids can be picked up with forceps. Grids should be placed on pieces of filter paper or on individual screens and placed in the Jaffe washer. Three or more grids should be prepared from each sample.

11.21 Using a clean curved scalpel blade, cut 3 mm square pieces of the carbon coated filter from the glass slide. The point of the scalpel should be placed on the filter and a rocking motion used to cut the 3 mm square segments. Squares should be selected from the center of the filter and at two points between the outer periphery of the active surface and the center. The excised filter segments are placed carbon-side up on the grids. A map of the Jaffe washer should be drawn to keep track of the samples. Place the lid on the Jaffe washer and allow to stand until the filter is adequately dissolved (several hours).

11.22 Remove the grids from the Jaffe washer and allow to dry thoroughly before placing them in marked grid storage boxes (or other suitable containers).

11.23 Analyze the samples by TEM at an accelerating voltage of 80 to 120 kV and a screen magnification of 10,000 to 20,000X.

a. Use at least three grids from each filter to obtain the necessary number of grid openings or structures to reach the required analytical sensitivity.

b. Carefully load the grid into the sample holder. Orient the grid so that the grid bars are parallel and perpendicular to the long axis of the holder. This orientation will align the grid bars with the X and Y axes of the specimen translation controls.

c. Scan the grid at a magnification of 250 to 1000X to determine its suitability for analysis. Reject the grid if:

1. Less than 70% of the grid openings covered by the replica are intact.

2. The replica is folded or doubled.

3. The replica is too dark or has obviously visible filter structure because of incomplete dissolution.

4. The grid is too heavily loaded to obtain an accurate count.

5. The distribution of structures on the grid is obviously not uniform.

d. Reject individual grid openings with greater than 5% of their areas occupied by holes or tears in the carbon film. Reject openings with ≥25% covered by particulates.

11.24 Examine the grid openings at 10-20,000X magnification.

11.25 Record the length and width of any grouping of particles in which an asbestos fiber with an aspect ratio ≥3:1 and a length greater than 10 µm is detected.

11.26 Asbestos structures will be classified as fibers according to the following rules.

a. Fiber. A structure having a minimum length greater than 10 µm and an aspect ratio (length to width) of 3:1 or greater and substantially parallel sides without rounded ends.

b. Count an asbestos bundle >10 µm long as a single fiber. Assign a length equal to the maximum length of any fiber within the bundle. If the bundle has stepped sides assign a width equal to an estimate of the mean width of the bundle.

c. Count a matrix as a single fiber if it contains a fiber or fibers, meeting the length and aspect ratio requirements, with one free end and the other end embedded in or hidden by a

particulate. If two ends are visible which appear to be the ends of a single fiber, the distance between the two ends is measured. If only one end of the fiber is visible, the fiber will be assigned a length equal to twice its visible length, except where this would place the concealed end outside of the particle. In this case, the length will be recorded as the visible length plus the diameter of the portion of the particle at the point of the fiber intersection. If the structure is too complex to be dealt with in this manner, record the overall dimensions of the structure but do not include it in the fiber count.

d. Count and record as single fibers the individual fibers visible with a cluster as long as they meet the fiber definition. If the aggregate is too complex, record the overall dimensions but do not include it in the fiber count.

e. Fibers which intersect the top and left sides of the grid opening are counted and recorded as twice their visible length. Fibers intersecting the bottom and right sides of the grid opening are not recorded.

f. Count only one end of the fiber to avoid the possibility of counting a single fiber more than once.

11.27 Structures classified as chrysotile must first be examined by SAED. If the characteristic chrysotile ED pattern is observed, the fiber will be counted. If no pattern is observed, or the pattern is not distinctive, the fiber must be examined by quantitative EDXA. If EDXA is characteristic of chrysotile, the fiber will be counted. Chrysotile fibers identified by morphology alone can be recorded but <u>not</u> counted towards the regulatory limit. The analysts's count sheet must indicate the method used to verify identity. A modified version of the AHERA count sheet may be used, which has columns to check off the method of identification.

11.28 Structures which are suspected to be an amphibole must first be examined by SAED. If a random orientation ED pattern with a 0.53 nm layer spacing is obtained, the fiber should be analyzed by EDXA. If the elements and peak ratios of the spectra correspond to those of a known amphibole, the fiber will be counted. If the random orientation ED pattern cannot be obtained, is incomplete or is not recognizable as a **non**-amphibole pattern, but an EDXA spectrum corresponding to a known amphibole is obtained, the fiber will be counted. Only fibers classified in this manner or by single or double zone axis SAED can be included in the regulated fiber count. The count sheet should indicate method used for identification.

11.29 Record both a typical SAED pattern and x-ray spectrum for each type of asbestos observed for each set of samples from the same source, or a minimum of every fifth sample analyzed.

Record the micrograph number on the count sheet and attach the x-ray spectrum to the back of the count sheet. If the x-ray spectrum is stored on disk, record the file and disk number on the count sheet.

11.30 Record NSD when no structures are detected in the grid opening.

11.31 Stopping Rules. Counting can be stopped at the completion of the grid opening in which an analytical sensitivity of 0.2 MFL is reached, or at the completion of the grid opening which contains the 100^{th} structure, whichever occurs first. A minimum of 4 grid openings must be analyzed even if this results in the counting of more than 100 asbestos fibers over 10 µm in length.

11.32 The grid openings examined must be drawn approximately equally from the three grids used in the analysis.

11.33 After completion of analysis, remove the grid from the microscope and replace in the labelled specimen storage box. Sample grids must be stored for a minimum of three years from the date of analysis. Sample filters may also be archived if desired.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Calculation of results. The concentration of asbestos in a given sample is calculated using the following formula:

$$\frac{no\ str \times efa \times R_D}{GO \times GOA \times V \times 1000}$$

where--
no str = number of asbestos fibers counted

efa = effective filter area of the sampling filter in mm^2

R_D = dilution ratio of original sample (if applicable)

GO = number of grid openings counted

GOA = area of grid openings in um^2

V = original volume of sample filtered in ml.

12.2 The following information must be reported for each sample analyzed:

a. Mean concentration of asbestos in million fibers per liter.

b. Upper and lower 95% confidence limits on the mean concentration.

c. Aliquot used for analysis and dilution factor (if any).

d. Effective filter area.

e. Total area of filter examined.

f. Number of asbestos structures counted.

g. Analytical sensitivity.

h. Copies of the TEM count sheet, if requested.

i. Number of structures which were too complex to classify, and number of suspected chrysotile and amphibole fibers which could not be positively identified.

13.0 METHOD PERFORMANCE

13.1 Limitations of accuracy can result from errors in identification of asbestos structures. Complete identification of every fiber is not possible due to both instrumental limitations and the nature of some of the fibers.

a. The requirement for a calibrated SAED pattern eliminates the possibility of an incorrect identification of chrysotile fibers. However, it is not always possible to obtain a satisfactory diffraction pattern from every fiber. The only significant possibilities of misidentification occur with halloysite, vermiculite scrolls or palygorskite, all of which can be discriminated from chrysotile by the use of EDXA and by observation of the 0.73 nm (002) reflection of chrysotile in the SAED pattern **(1)**.

b. Complete identification of all amphibole fibers is not practical due to instrumental factors, the nature of some of the fibers, and limitations of time and cost. Particles of a number of other minerals having compositions similar to those of some amphiboles could be mistakenly classified as amphibole when zone axis SAED is not used. However, quantitative EDXA measurements on all fibers as support for the random orientation SAED patterns makes misidentification unlikely. The possibility of

misidentification is further reduced with increasing aspect ratio, since many of the minerals with which amphibole may be confused do not display its prominent cleavage parallel to the c-axis **(1)**.

13.2 Limitations of accuracy can also result from the overlapping of structures by other (nonasbestos) particulates.

13.3 Inadequate dispersion of fibers can occur if enough organic contaminants were present in the original sample to cause adhesion of the fibers to the container walls or each other.

13.4 Contamination of the aliquots by asbestos during preparation in the laboratory can lead to inaccuracy of results. This is a particular problem with chrysotile, and should be carefully monitored by preparation of blank samples.

13.5 This method has not yet been subjected to an inter-laboratory test round. Precision measurements for EPA intra-laboratory comparisons of results from multiple operators using three TEMs produced a relative standard deviation (RSD) of 26.5% for MCE filters and 25.5% for PC filters for fibers over 10µm in length in standard samples. For similar methods, precision measurements for intra-laboratory comparisons have been found to have an RSD of 13 to 22 percent for standard and environmental water samples, with an RSD of 8.4 to 29 percent for inter-laboratory comparisons **(1)**. Statistical formulae for the establishment of confidence limits on the laboratory results can be found in Chatfield and Dillon **(1)**. An earlier study found an inter-laboratory reproducibility of 25 to 50 percent in standard samples **(6)**. Accuracy measurements from inter- and intra-laboratory studies have demonstrated an RSD of 17 percent for standard chrysotile suspensions and an RSD of 16 percent for standard crocidolite suspensions **(1)**.

13.6 The detection limit will depend upon the concentration of asbestos in the original sample and the constraints of time and cost of analysis. The detection limit can be improved by increasing the amount of water filtered, increasing the number of grid openings counted or decreasing the size of the filter used (when practical). Samples containing a high level of particulates will necessarily have a higher detection limit.

14.0 POLLUTION PREVENTION

14.1 It is the laboratory's responsibility to comply with all federal, state and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations. For further

information on waste management, consult "The Waste Management Manual for Laboratory Personnel", available from the American Chemical Society.

15.0 REFERENCES

1. Chatfield, E. J. and M. J. Dillon. Analytical Method for the Determination of Asbestos in Water. EPA No. 600/4-83-043.

2. "Reagent Chemicals, American Chemical Society Specifications". American Chemical Society, Washington, D.C.

3. USEPA, Asbestos-Containing Materials in Schools: Final Rule and Notice. Federal Register, 40 CFR Part 763, Appendix A to Subpart E., October 30, 1987.

4. NIST/NVLAP Program Handbook for Airborne Asbestos Analysis. NISTIR 89-4137, August, 1989.

5. Burdett, G. J. and A. P. Rood. Membrane-Filter Direct-Transfer Technique for the Analysis of Asbestos Fibers or Other Inorganic Particles by Transmission Electron Microscopy. Environ. Sci. Technol. 17:643-648, 1983.

6. Chopra, K. S. Interlaboratory Measurements of Amphibole and Chrysotile Fiber Concentrations in Water. In: National Bureau of Standards Special Publication 506, Proceedings of the Workshop on Asbestos: Definitions and Measurement Methods, 1977.

APPENDIX 1

OZONE-ULTRAVIOLET OXIDATION TECHNIQUE

1. Assemble and operate the oxidation apparatus under an exhaust hood.

2. Place each sample bottle in the ultrasonic bath for 15 minutes.

3. Mark the level of liquid in each sample bottle using a waterproof marker.

4. Wash a quartz pipet thoroughly and attach to the ozone supply.

5. Suspend the pipet in the sample so that the tip is close to the bottom of the bottle.

6. Wash the UV lamp and immerse in the sample prior to turning the power supply on.

7. At an ozone concentration of 4% in oxygen, adjust flow to approximately 1 liter per minute. Treat for 3 hours. At other ozone concentrations, adjust the oxidation time so that each sample receives about 10g of ozone. Gas flow should be sufficient to produce a mixing action without splashing sample out of the bottle.

8. When oxidation is complete, remove the UV lamp and quartz pipet, recap the bottle and sonicate for 15 minutes.

9. If the water level has fallen, add known fiber-free water to bring it back to the original level marked on the bottle. Sonicate for 15 minutes.

10. Proceed with removal of aliquot and filtration immediately.