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Libby, Montana

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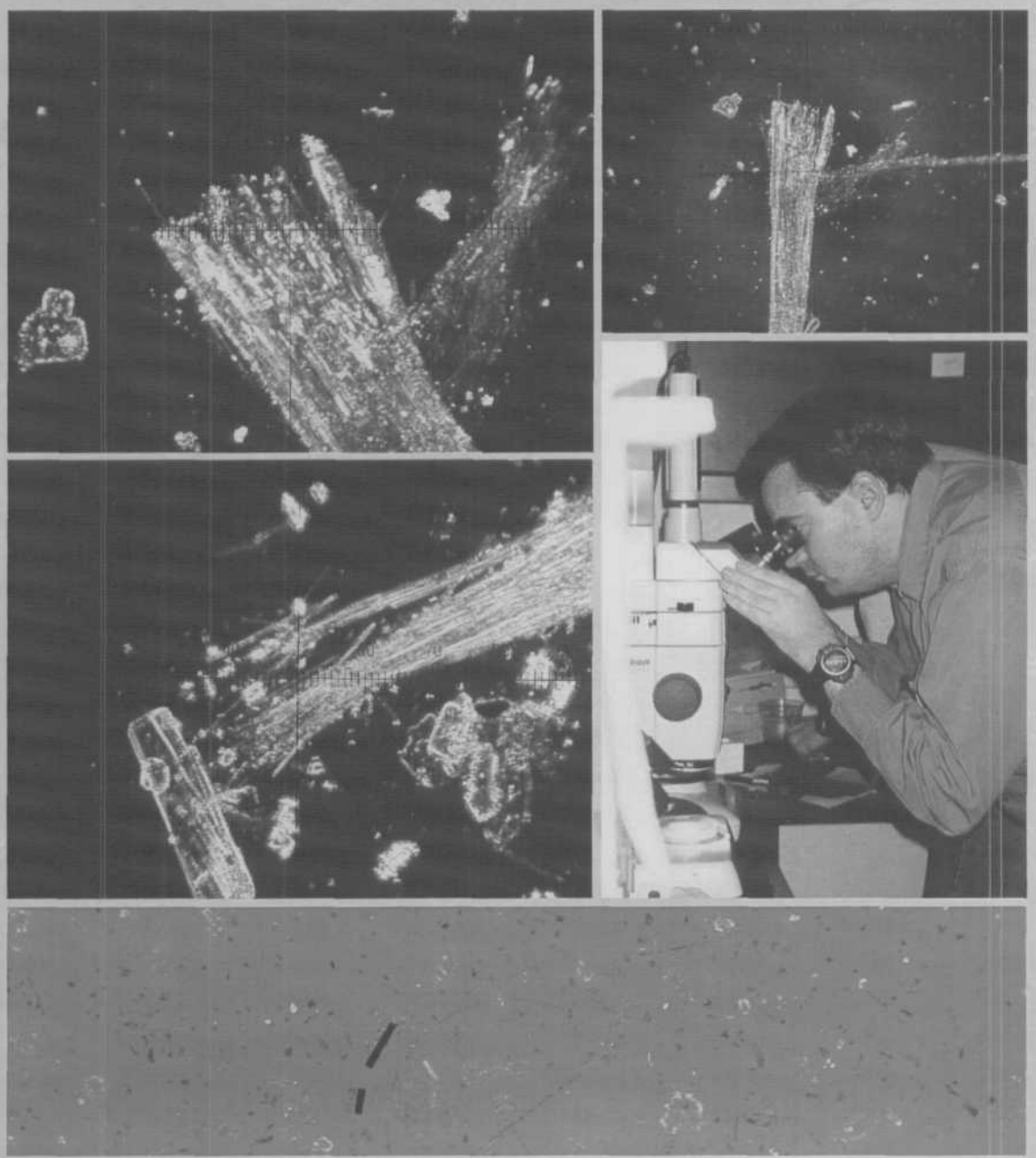

Tabbed Page: Table of Contents

Volume 1: Contents

Section 1

ASTM. 1995. Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Concentrations. ASTM D 5755-95. American Society for Testing and Materials. October 1995.

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Section 10

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Section 11

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Section 12

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Section 13

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Section 14

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Section 16

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Section 17

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Section 18

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Section 19

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Section 20

USEPA. 2000. Asbestos Analysis of Soil by Scanning Microscopy and Energy Dispersive X-Ray Spectroscopy. EPA-LIBBY-01 (Rev. 2). August 28, 2002.

Section 21

USEPA. 2000. Superfund Method for the Determination of Releasable Asbestos in Soils and Bulk Materials (Interim Version). EPA/540/R-97/028. November 2, 2000.

Section 22

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Section 23

USEPA. 2004. Analysis of Asbestos in Soil by TEM Following Water Sedimentation Fractionation. EPA-LIBBY-07 (Rev. 4). August 10, 2004.

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Tabbed Page: 1

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Designation: D 5755 – 95

Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Concentrations[1]

This standard is issued under the fixed designation D 5755; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers a procedure to (*a*) identify asbestos in dust and (*b*) provide an estimate of the concentration of asbestos in the sampled dust reported as the number of asbestos structures per unit area of sampled surface.

1.1.1 If an estimate of the asbestos mass is to be determined, the user is referred to Test Method D 5756.

1.2 This test method describes the equipment and procedures necessary for sampling, by a microvacuum technique, non-airborne dust for levels of asbestos structures. The non-airborne sample is collected inside a standard filter membrane cassette from the sampling of a surface area for dust which may contain asbestos.

1.2.1 This procedure uses a microvacuuming sampling technique. The collection efficiency of this technique is unknown and will vary among substrates. Properties influencing collection efficiency include surface texture, adhesiveness, electrostatic properties and other factors.

1.3 Asbestos identified by transmission electron microscopy (TEM) is based on morphology, selected area electron diffraction (SAED), and energy dispersive X-ray analysis (EDXA). Some information about structure size is also determined.

1.4 This test method is generally applicable for an estimate of the concentration of asbestos structures starting from approximately 1000 asbestos structures per square centimetre.

1.4.1 The procedure outlined in this test method employs an indirect sample preparation technique. It is intended to disperse aggregated asbestos into fundamental fibrils, fiber bundles, clusters, or matrices that can be more accurately quantified by transmission electron microscopy. However, as with all indirect sample preparation techniques, the asbestos observed for quantification may not represent the physical form of the asbestos as sampled. More specifically, the procedure described neither creates nor destroys asbestos, but it may alter the physical form of the mineral fibers.

1.5 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 1193 Specification for Reagent Water[2]

D 1739 Test Method for the Collection and Measurement of Dustfall (Settleable Particulate Matter)[3]

D 3195 Practice for Rotameter Calibration[3]

D 3670 Guide for Determination of Precision and Bias of Methods of Committee D-22[3]

D 5756 Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Mass Concentration[3]

3. Terminology

3.1 *Definitions:*

3.1.1 *asbestiform*—a special type of fibrous habit in which the fibers are separable into thinner fibers and ultimately into fibrils. This habit accounts for greater flexibility and higher tensile strength than other habits of the same mineral. For more information on asbestiform mineralogy, see Refs **(1)**,[4] **(2)** and **(3)**.

3.1.2 *asbestos*—a collective term that describes a group of naturally occurring, inorganic, highly fibrous, silicate dominated minerals, which are easily separated into long, thin, flexible fibers when crushed or processed.

3.1.2.1 *Discussion*—Included in the definition are the asbestiform varieties of: serpentine (chrysotile); riebeckite (crocidolite); grunerite (grunerite asbestos); anthophyllite (anthophyllite asbestos); tremolite (tremolite asbestos); and actinolite (actinolite asbestos). The amphibole mineral compositions are

[1] This test method is under the jurisdiction of ASTM Committee D-22 on Sampling and Analysis of Atmospheresand is the direct responsibility of Subcommittee D22.07on Sampling and Analysis of Asbestos.

Current edition approved August 15, 1995. Published October 1995.

[2] *Annual Book of ASTM Standards*, Vol 11.01.

[3] *Annual Book of ASTM Standards*, Vol 11.03.

[4] The boldface numbers in parentheses refer to the list of references at the end of this test method.

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defined according to nomenclature of the International Mineralogical Association (3).

Asbestos	Chemical Abstract Service No.[5]
Chrysotile	12001-29-5
Crocidolite	12001-28-4
Grunerite Asbestos	12172-73-5
Anthophyllite Asbestos	77536-67-5
Tremolite Asbestos	77536-68-6
Actinolite Asbestos	77536-66-4

3.1.3 *fibril*—a single fiber that cannot be separated into smaller components without losing its fibrous properties or appearance.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *aspect ratio*—the ratio of the length of a fibrous particle to its average width.

3.2.2 *bundle*—a structure composed of three or more fibers in a parallel arrangement with the fibers closer than one fiber diameter to each other.

3.2.3 *cluster*—a structure with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group; groupings of fibers must have more than two points touching.

3.2.4 *debris*—materials that are of an amount and size (particles greater than 1 mm in diameter) that can be visually identified as to their source.

3.2.5 *dust*—any material composed of particles in a size range of ≤1 mm and large enough to settle by virtue of their weight from the ambient air (see definition for settleable particulate matter in Test Method D 1739).

3.2.6 *fiber*—a structure having a minimum length of 0.5 μm, an aspect ratio of 5:1 or greater, and substantially parallel sides (4).

3.2.7 *fibrous*—of a mineral composed of parallel, radiating, or interlaced aggregates of fibers, from which the fibers are sometimes separable. That is, the crystalline aggregate may be referred to as fibrous even if it is not composed of separable fibers, but has that distinct appearance. The term fibrous is used in a general mineralogical way to describe aggregates of grains that crystallize in a needle-like habit and appear to be composed of fibers. Fibrous has a much more general meaning than asbestos. While it is correct that all asbestos minerals are fibrous, not all minerals having fibrous habits are asbestos.

3.2.8 *indirect preparation*—a method in which a sample passes through one or more intermediate steps prior to final filtration.

3.2.9 *matrix*—a structure in which one or more fibers, or fiber bundles that are touching, are attached to, or partially concealed by a single particle or connected group of non-fibrous particles. The exposed fiber must meet the fiber definition (see 3.2.6).

3.2.10 *structures*—a term that is used to categorize all the types of asbestos particles which are recorded during the analysis (such as fibers, bundles, clusters, and matrices). Final results of the test are always expressed in asbestos structures per square centimetre.

[5] The non-asbestiform variations of the minerals indicated in 5.1.3 have different Chemical Abstract Service (CAS) numbers.

4. Summary of Test Method

4.1 The sample is collected by vacuuming a known surface area with a standard 25 or 37 mm air sampling cassette using a plastic tube that is attached to the inlet orifice which acts as a nozzle. The sample is transferred from inside the cassette to an aqueous solution of known volume. Aliquots of the suspension are then filtered through a membrane. A section of the membrane is prepared and transferred to a TEM grid using the direct transfer method. The asbestiform structures are identified, sized, and counted by TEM, using SAED and EDXA at a magnification of 15 000 to 20 000X.

5. Significance and Use

5.1 This microvacuum sampling and indirect analysis method is used for the general testing of non-airborne dust samples for asbestos. It is used to assist in the evaluation of dust that may be found on surfaces in buildings such as ceiling tiles, shelving, electrical components, duct work, carpet, etc. This test method provides an index of the concentration of asbestos structures in the dust per unit area analyzed as derived from a quantitative TEM analysis.

5.1.1 This test method does not describe procedures or techniques required to evaluate the safety or habitability of buildings with asbestos-containing materials, or compliance with federal, state, or local regulations or statutes. It is the user's responsibility to make these determinations.

5.1.2 At present, a single direct relationship between asbestos-containing dust and potential human exposure does not exist. Accordingly, the user should consider these data in relationship to other available information in their evaluation.

5.2 This test method uses the definition, settleable particulate material, found in Test Method D 1739 as the definition of dust. This definition accepts all particles small enough to pass through a 1 mm (No. 18) screen. Thus, a single, large asbestos containing particle(s) (from the large end of the particle size distribution) dispersed during sample preparation may result in anomalously large asbestos concentration results in the TEM analyses of that sample. It is, therefore, recommended that multiple independent samples are secured from the same area, and a minimum of three samples analyzed by the entire procedure.

6. Interferences

6.1 The following minerals have properties (that is, chemical or crystalline structure) which are very similar to asbestos minerals and may interfere with the analysis by causing a false positive to be recorded during the test. Therefore, literature references for these materials must be maintained in the laboratory for comparison to asbestos minerals so that they are not misidentified as asbestos minerals.

6.1.1 *Antigorite.*

6.1.2 *Palygorskite (Attapulgite).*

6.1.3 *Halloysite.*

6.1.4 *Pyroxenes.*

6.1.5 *Sepiolite.*

6.1.6 *Vermiculite scrolls.*

6.1.7 *Fibrous talc.*

6.1.8 Hornblende and other amphiboles other than those listed in 3.1.2.

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6.2 Collecting any dust particles greater than 1 mm in size in this test method may cause an interference and, therefore, must be avoided.

7. Materials and Equipment

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.[6]

7.2 *Transmission Electron Microscope (TEM)*, an 80 to 120 kV TEM, capable of performing electron diffraction, with a fluorescent screen inscribed with calibrated gradations, is required. The TEM must be equipped with energy dispersive X-ray spectroscopy (EDXA) and it must have a scanning transmission electron microscopy (STEM) attachment or be capable of producing a spot size of less than 250 nm in diameter in crossover.

7.3 *Energy Dispersive X-ray System (EDXA).*

7.4 *High Vacuum Carbon Evaporator*, with rotating stage.

7.5 *High Efficiency Particulate Air (HEPA)*, filtered negative flow hood.

7.6 *Exhaust or Fume Hood.*

7.7 *Particle-free Water* (ASTM Type II, see Specification D 1193).

7.8 *Glass Beakers* (50 mL).

7.9 *Glass Sample Containers*, with wide mouth screw cap (200 mL) or equivalent sealable container (height of the glass sample container should be approximately 13 cm high by 6 cm wide).

7.10 *Waterproof Markers.*

7.11 *Forceps* (tweezers).

7.12 *Ultrasonic Bath*, table top model (100 W).

7.13 *Graduated Pipettes* (1, 5, 10 mL sizes), glass or plastic.

7.14 *Filter Funnel*, either 25 mm or 47 mm, glass or disposable. Filter funnel assemblies, either glass or disposable plastic, and using either a 25 mm or 47 mm diameter filter.

7.15 *Side Arm Filter Flask*, 1000 mL.

7.16 *Mixed Cellulose Ester (MCE) Membrane Filters*, 25 or 47 mm diameter, ≤0.22 µm and 5 µm pore size.

7.17 *Polycarbonate (PC) Filters*, 25 or 47 mm diameter, ≤0.2 µm pore size.

7.18 *Storage Containers*, for the 25 or 47 mm filters (for archiving).

7.19 *Glass Slides*, approximately 76 by 25 mm in size.

7.20 *Scalpel Blades*, No. 10, or equivalent.

7.21 *Cabinet-type Desiccator*, or low temperature drying oven.

7.22 *Chloroform*, reagent grade.

7.23 *Acetone*, reagent grade.

7.24 *Dimethylformamide (DMF).*

7.25 *Glacial Acetic Acid.*

7.26 *1-methyl-2-pyrrolidone.*

7.27 *Plasma Asher*, low temperature.

7.28 *pH Paper.*

7.29 *Air Sampling Pump*, low volume personal-type, capable of achieving a flow rate of 1 to 5 L/min.

7.30 *Rotameter.*

7.31 *Air Sampling Cassettes*, 25 mm or 37 mm, containing 0.8µ m or smaller pore size MCE or PC filters.

7.32 *Cork Borer*, 7 mm.

7.33 *Non-Asbestos Mineral*, references as outlined in 6.1.

7.34 *Asbestos Standards*, as outlined in 3.1.2.

7.35 *Tygon*[7] *Tubing*, or equivalent.

7.36 *Small Vacuum Pump*, that can maintain a pressure of 92 kPa.

7.37 *Petri Dishes*, large glass, approximately 90 mm in diameter.

7.38 *Jaffe Washer*, stainless steel or aluminum mesh screen, 30 to 40 mesh, and approximately 75 mm by 50 mm in size.

7.39 *Copper TEM Finder Grids*, 200 mesh.

7.40 *Carbon Evaporator Rods.*

7.41 *Lens Tissue.*

7.42 *Ashless Filter Paper Filters*, 90 mm diameter.

7.43 *Gummed Paper Reinforcement Rings.*

7.44 *Wash Bottles*, plastic.

7.45 *Reagent Alcohol*, HPLC Grade (Fisher A995 or equivalent).

7.46 *Opening Mesh Screen*, plastic, 1.0 by 1.0 mm, (Spectra-Mesh #146410 or equivalent).

7.47 *Diffraction Grating Replica.*

8. Sampling Procedure for Microvacuum Technique

8.1 For sampling asbestos-containing dust in either indoor or outdoor environments, commercially available cassettes must be used. Air monitoring cassettes containing 25 mm or 37 mm diameter mixed cellulose ester (MCE) or polycarbonate (PC) filter membranes with a pore size less than or equal to 0.8 µm are required (7.31). The number of samples collected depends upon the specific circumstances of the study.

8.2 Maintain a log of all pertinent sampling information and sampling locations.

8.3 Sampling pumps and flow indicators shall be calibrated using a certified standard apparatus or assembly (see Practice D 3195 and 7.29).

8.4 Record all calibration information (5).

8.5 Perform a leak check of the sampling system at each sampling site by activating the pump (7.29) with the closed sampling cassette in line. Any air flow shows that a leak is present that must be eliminated before initiating the sampling operation.

8.6 Attach the sampling cassette to the sampling pump at the outlet side of the cassette with plastic tubing (7.35). The plastic tubing must be long enough in that the sample areas can

[6] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

[7] Tygon is a registered trademark of the DuPont Co.

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be reached without interference from the sampling pump. Attach a clean, approximately 25.4 mm long piece of plastic tubing (6.35 mm internal diameter) directly to the inlet orifice. Use this piece of tubing as the sampling nozzle. Cut the sampling end of the tubing at a 45° angle as illustrated in Fig. 1. The exact design of the nozzle is not critical as long as some vacuum break is provided to avoid simply pushing the dust around on the surface with the nozzle rather than vacuuming it into the cassette. The internal diameter of the nozzle and flow rate of the pump may vary as long as the air velocity is 100 (±10) cm/s. This air velocity calculation is based on an internal sampling tube diameter of 6.35 mm at a flow rate of 2 L/min.

8.7 Measure and determine the sample area of interest. A sample area of 100 cm^2 is vacuumed until there is no visible dust or particulates matter remaining. Perform a minimum of two orthogonal passes on the surface within a minimum of 2 min of sampling time. Avoid scraping or abrading the surface being sampled. (Do not sample any debris or dust particles greater than 1 mm in diameter (see 4.2).) Smaller or larger areas can be sampled, if needed. For example, some surfaces of interest may have a smaller area than 100 cm^2. Less dusty surfaces may require vacuuming of larger areas. Unlike air samples, the overloading of the cassettes with dust will not be a problem. As defined in 3.2.5, only dust shall be collected for this analysis.

8.8 At the end of sample collection, invert the cassette so that the nozzle inlet faces up before shutting off the power to the pump. The nozzle is then sealed with a cassette end-plug and the cassette/nozzle taped or appropriately packaged to prevent separation of the nozzle and cassette assembly. A second option is the removal of the nozzle from the cassette, then plugging of the cassette and shipment of the nozzle (also plugged at both ends) sealed in a separate closeable plastic bag. A third option is placing the nozzle inside the cassette for shipment. The nozzle is always saved and rinsed because a significant percentage of the dust drawn from a lightly loaded surface may adhere to the inside walls of the tubing.

8.9 Check that all samples are clearly labeled, that all dust sampling information sheets are completed, and that all pertinent information has been enclosed, in accordance with laboratory quality control practices, before transfer of the samples to the laboratory. Include an unused cassette and nozzle as a field blank.

8.10 Wipe off the exterior surface of the cassettes with disposable wet towels (baby wipes) prior to packaging for shipment.

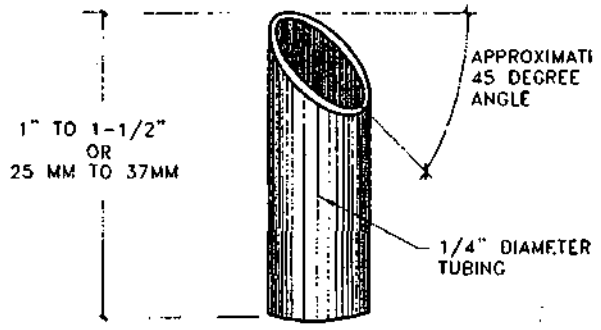

FIG. 1 Example of the Tubing Nozzle

9. Sample Shipment

9.1 Ship dust samples to an analytical laboratory in a sealed container, but separate from any bulk or air samples. The cassettes must be tightly sealed and packed in a material free of fibers or dust to minimize the potential for contamination. Plastic "bubble pack" is probably the most appropriate material for this purpose.

10. Sample Preparation

10.1 Under a negative flow HEPA hood (7.5), carefully wet-wipe the exterior of the cassettes to remove any possible contamination before taking cassettes into a clean preparation area.

10.2 Perform sample preparation in a clean facility that has a separate work area from both the bulk and air sample preparation areas.

10.3 Initial specimen preparation shall take place in a clean HEPA filtered negative pressure hood to avoid any possible contamination of the laboratory or personnel, or both, by the potentially large number of asbestos structures in an asbestos-containing dust sample. Cleanliness of the preparation area hoods is measured by the cumulative process blank concentrations (see Section 11).

10.4 All sample preparation steps 10.4.1-10.4.6 shall take place in the dust preparation area inside a HEPA hood.

10.4.1 Remove the upper plug from the sample cassette and carefully introduce approximately 10 mL solution of a 50/50 mixture of particle-free water and reagent alcohol into the cassette using a plastic wash bottle (7.44). If the plugged nozzle was left attached to the cassette, then remove the plug and introduce the water/alcohol solution into the cassette through the tubing, and then remove the tubing, if it is visibly clean.

10.4.2 Replace the upper plug or the sample cap and lightly shake the dust suspension by hand for 3 s.

10.4.3 Remove the entire cap of the cassette and pour the suspension through a 1.0 by 1.0 mm opening screen (7.46) into a pre-cleaned 200 mL glass specimen bottle (7.9). All visible traces of the sample contained in the cassette shall be rinsed through the screen into the specimen bottle with a plastic wash bottle containing the 50/50 solution of particle-free water and alcohol. Repeat this procedure two additional times for a total of three washings. Next, rinse the nozzle two or three times through the screen into the specimen bottle with the 50/50 mixture of water and alcohol. Typically, the total amount of the 50/50 mixture used in the rinse is 50 to 75 mL. Discard the 1.0 by 1.0 mm screen and bring the volume of solution in the specimen bottle up to the 100 mL mark on the side of the bottle with particle-free water only.

10.4.4 Adjust the pH of the suspension to 3 to 4 using a 10.0 % solution of acetic acid. Use pH paper for testing. Filter the suspension within 24 h to avoid problems associated with bacterial and fungal growth.

10.4.5 Use either a disposable plastic filtration unit or a glass filtering unit (7.14) for filtration of aliquots of the suspension. The ability of an individual filtration unit to

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produce a uniform distribution may be tested by the filtration of a colored particulate suspension such as diluted India ink (suspension of carbon black).

10.4.5.1 If a disposable plastic filtration unit is used, then unwrap a new disposable plastic filter funnel unit (either 25 or 47 mm diameter) and remove the tape around the base of the funnel. Remove the funnel and discard the top filter supplied with the apparatus, retaining the coarse polypropylene support pad in place. Assemble the unit with the adapter and a properly sized neoprene stopper, and attach the funnel to the 1000 mL side-arm vacuum flask (7.15). Place a 5.0 µ m pore size MCE (backing filter) on the support pad. Wet it with a few mL of particle-free water and place an MCE (7.16) or PC filter (≤0.22 µm pore size) (7.17) on top of the backing filter. Apply a vacuum (7.36), ensuring that the filters are centered and pulled flat without air bubbles. Any irregularities on the filter surface requires the discard of that filter. After the filter has been seated properly, replace the funnel and reseal it with the tape. Return the flask to atmospheric pressure.

10.4.5.2 If a glass filtration unit is used, place a 5 µm pore size MCE (backing filter) on the glass frit surface. Wet the filter with particle-free water, and place an MCE or PC filter (≤0.22 µm pore size) on top of the backing filter. Apply a vacuum, ensuring that the filters are centered and pulled flat without air bubbles. Replace the filters if any irregularities are seen on the filter surface. Before filtration of each set of sample aliquots, prepare a blank filter by filtration of 50 mL of particle-free water. If aliquots of the same sample are filtered in order of increasing concentration, the glass filtration unit need not be washed between filtration. After completion of the filtration, do not allow the filtration funnel assembly to dry because contamination is then more difficult to remove. Wash any residual suspension from the filtration assembly by holding it under a flow of water, then rub the surface with a clean paper towel soaked in a detergent solution. Repeat the cleaning operation, and then rinse two times in particle-free water.

10.4.6 With the flask at atmospheric pressure, add 20 mL of particle-free water into the funnel. Cover the filter funnel with its plastic cover if the disposable filtering unit is used.

10.4.7 Briefly hand shake (3 s) the capped bottle with the sample suspension, then place it in a tabletop ultrasonic bath (7.12) and sonicate for 3.0 min. Maintain the water level in the sonicator at the same height as the solution in sample bottle. The ultrasonic bath shall be calibrated as described in 20.5. The ultrasonic bath must be operated at equilibrium temperature. After sonicating, return the sample bottle to the work surface of the HEPA hood. Preparation steps 10.4.8-10.4.14 shall be carried out in this hood.

10.4.8 Shake the suspension lightly by hand for 3 s, then let it rest for 2.0 min to allow large particles to settle to the bottom of the bottle or float to the surface.

10.4.9 Estimate the amount of liquid to be withdrawn to produce an adequate filter preparation. Experience has shown that a light staining of the filter surface will yield a suitable preparation for analysis. Filter at least 1.0 mL, but no more than half the total volume. If after examination in the TEM, the smallest volume measured (1.0 mL) (7.13) yields an overloaded sample, then perform additional serial dilutions of the suspension. If it is estimated that less than 1.0 mL of solution has to be filtered because of the density of the suspension, perform a serial dilution.

10.4.9.1 If serial dilutions are required, repeat step 10.4.8 before the serial dilution portion is taken. Do not re-sonicate the original solution or any serial dilutions. The recommended procedure for a serial dilution is to mix 10 mL of the sample solution with 90 mL of particle-free water in a clean sample bottle to obtain a 1:10 serial dilution. Follow good laboratory practices when performing dilutions.

10.4.10 Insert a new disposable pipette halfway into the sample suspension and withdraw a portion. Avoid pipetting any of the large floating or settled particles. Uncover the filter funnel and dispense the mixture from the pipette into the water in the funnel.

10.4.11 Apply vacuum to the flask and draw the mixture through the filter.

10.4.12 Discard the pipette.

10.4.13 Disassemble the filtering unit and carefully remove the sample filter with fine tweezers (7.11). Place the completed sample filter particle side up, into a precleaned, labeled, disposable, plastic petri dish (7.48) or other similar container.

10.4.14 In order to ensure that an optimally-loaded filter is obtained, it is recommended that filters be prepared from several different aliquots of the dust suspension. For this series of filters, it is recommended that the volume of each aliquot of the original suspension be a factor of five higher than the previous one. If the filters are prepared in order of increasing aliquot volume, all of the filters for one sample can be prepared using one plastic disposable filtration unit, or without cleaning of glass filtration equipment between individual filtration. Before withdrawal of each aliquot from the sample, shake the suspension without additional sonification and allow to rest for 2 min.

10.4.15 There are many practical methods for drying MCE filters. The following are two examples that can be used: (*1*) dry MCE filters for at least 12 h (over desiccant) in an airtight cabinet-type desiccator (7.21); (2) to shorten the drying time (if desired), remove a plug of the damp filter and attach it to a glass slide (7.19) as described in 12.1.2 and 12.1.3. Place the slide with a filter plug or filter plugs (up to eight plugs can be attached to one slide) on a bed of desiccant, in the desiccator for 1 h.

10.4.16 PC filters do not require lengthy drying before preparation, but shall be placed in a desiccator for at least 30 min before preparation.

10.5 Prepare TEM specimens from small sections of each dried filter using the appropriate direct transfer preparation method.

11. Blanks

11.1 Prepare sample blanks that include both a process blank (50 mL of particle-free water) for each set of samples analyzed and one unused filter from each new box of sample filters (MCE or PC) used in the laboratory. If glass filtering units are used, prepare and analyze a process blank each time the filtering unit is cleaned. Blanks will be considered contaminated, if after analysis, they are shown to contain more

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than 53 asbestos structures per square millimetre. This generally corresponds to three or four asbestos structures found in ten grid openings. The source of the contamination must be found before any further analysis can be performed. Reject samples that were processed along with the contaminated blanks and prepare new samples after the source of the contamination is found.

11.2 Prepare field blanks which are included with sample sets in the same manner as the samples, to test for contamination during the sampling, shipping, handling, and preparation steps of the method.

12. TEM Specimen Preparation of Mixed Cellulose Ester (MCE) Filters

NOTE 1—Use of either the acetone or the dimethylformamide-acetic acid method is acceptable.

12.1 *Acetone Fusing Method*:

12.1.1 Remove a section (a plug) from any quadrant of the sample and blank filters. Sections can be removed from the filters using a 7 mm cork borer (7.32). The cork borer must be wet wiped after each time a section is removed.

12.1.2 Place the filter section (particle side up) on a clean microscope slide. Affix the filter section to the slide with a gummed page reinforcement (7.43), or other suitable means. Label the slide with a glass scribing tool or permanent marker (7.10).

12.1.3 Prepare a fusing dish from a glass petri dish (7.37) and a metal screen bridge (7.38) with a pad of five to six ashless paper filters (7.42) and place in the bottom of the petri dish (4). Place the screen bridge on top of the pad and saturate the filter pads with acetone. Place the slide on top of the bridge in the petri dish and cover the dish. Wait approximately 5 min for the sample filter to fuse and clear.

12.2 *Dimethylformamide-Acetic Acid Method*:

12.2.1 Place a drop of clearing solution that consists of 35 % dimethylformamide (DMF), 15 % glacial acetic acid, and 50 % Type II water (v/v) on a clean microscope slide. Gauge the amount used so that the clearing solution just saturates the filter section.

12.2.2 Carefully lay the filter segment, sample surface upward, on top of the solution. Bring the filter and solution together at an angle of about 20° to help exclude air bubbles. Remove any excess clearing solution. Place the slide in an oven or on a hot plate, in a fume hood, at 65 to 70°C for 10 min.

12.3 Plasma etching of the collapsed filter is required.

12.3.1 The microscope slide to which the collapsed filter pieces are attached is placed in a plasma asher (7.27). Because plasma ashers vary greatly in their performance, both from unit to unit and between different positions in the asher chamber, it is difficult to specify the exact conditions that must be used. Insufficient etching will result in a failure to expose embedded fibers, and too much etching may result in the loss of particles from the filter surface. To determine the optimum time for ashing, place an unused 25 mm diameter MCE filter in the center of a glass microscope slide. Position the slide approximately in the center of the asher chamber. Close the chamber and evacuate to a pressure of approximately 40 Pa, while admitting oxygen to the chamber at a rate of 8 to 20 cm^3/min. Adjust the tuning of the system so that the intensity of the plasma is maximized. Determine the time required for complete oxidation of the filter. Adjust the system parameters to achieve complete oxidation of the filter in a period of approximately 15 min. For etching of collapsed filters, use these operating parameters for a period of 8 min. For additional information on calibration, see the *USEPA Asbestos-Containing Materials in Schools* (4) or *NIST/NVLAP Program Handbook for Airborne Asbestos Analysis* (6) documents.

12.3.2 Place the glass slide containing the collapsed filters into the low-temperature plasma asher, and etch the filter.

12.4 Carbon coating of the collapsed and etched filters is required.

12.4.1 Carbon coating must be performed with a high-vacuum coating unit (7.4), capable of less than 10^{-4} torr (13 MPa) pressure. Units that are based on evaporation of carbon filaments in a vacuum generated only by an oil rotary pump have not been evaluated for this application and shall not be used. Carbon rods (7.40) used for evaporators shall be sharpened with a carbon rod sharpener to a neck of about 4 mm in length and 1 mm in diameter. The rods are installed in the evaporator in such a manner that the points are approximately 100 to 120 mm from the surface of the microscope slide held in the rotating device.

12.4.2 Place the glass slide holding the filters on the rotation device, and evacuate the evaporator chamber to a vacuum of at least 13 MPa. Perform the evaporation in very short bursts, separated by 3 to 4 s to allow the electrodes to cool. An alternate method of evaporation is by using a slow continuous applied current. An experienced analyst can judge the thickness of the carbon film to be applied. Conduct tests on unused filters first. If the carbon film is too thin, large particles will be lost from the TEM specimen, and there will be few complete and undamaged grid openings on the specimen.

12.4.2.1 If the coating is too thick, it will lead to a TEM image that is lacking in contrast, and the ability to obtain electron diffraction patterns will be compromised. The carbon film shall be as thin as possible and still remain intact on most of the grid openings of the TEM specimen.

12.5 *Preparation of the Jaffe Washer*— The precise design of the Jaffe washer is not considered important, so any one of the published designs may be used (7, 8). One such washer consists of a simple stainless steel bridge contained in a glass petri dish.

12.5.1 Place several pieces of lens tissue (7.41) on the stainless steel bridge. The pieces of lens tissue shall be large enough to completely drape over the bridge and into the solvent. In a fume hood, fill the petri dish with acetone (or DMF) until the height of the solvent is brought up to contact the underside of the metal bridge as illustrated in Fig. 2.

12.6 *Placing the Specimens into the Jaffe Washer*:

12.6.1 Place the TEM grids (7.39) shiny side up on a piece of lens tissue or filter paper so that individual grids can be easily picked up with tweezers.

12.6.2 Prepare three grids from each sample.

12.6.2.1 Using a curved scalpel blade (7.20), excise at least two square (3 mm by 3 mm) pieces of the carbon-coated MCE filter from the glass slide.

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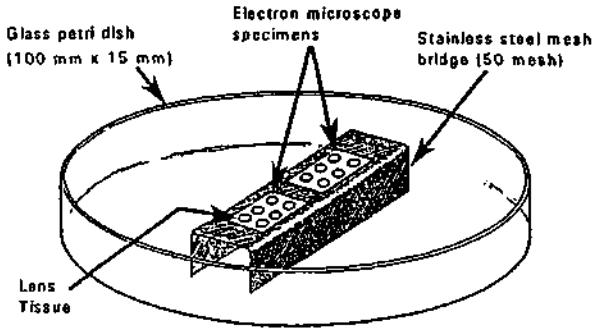

FIG. 2 Example of Design of Solvent Washer (Jaffe Washer)

12.6.2.2 Place the square filter piece carbon-side up on top of a TEM specimen grid.

12.6.2.3 Place the whole assembly (filter/grid) on the saturated lens tissue in the Jaffe washer.

12.6.2.4 Place the three TEM grid sample filter preparations on the same piece of lens tissue in the Jaffe washer.

12.6.2.5 Place the lid on the Jaffe washer and allow the system to stand for several hours.

12.7 Alternately, place the grids on a low level (petri dish filled to the ⅛ mark) DMF Jaffe washer for 60 min. Add enough solution of equal parts DMF/acetone to fill the washer to the screen level. Remove the grids after 30 min if they have cleared, that is, all filter material has been removed from the carbon film, as determined by inspection in the TEM.

12.8 Carefully remove the grids from the Jaffe washer, allowing the grids to dry before placing them in a clean marked grid box.

13. TEM Specimen Preparation of Polycarbonate (PC) Filter

13.1 Cover the surface of a clean microscope slide with two strips of double-sided adhesive tape.

13.2 Cut a strip of filter paper slightly narrower than the width of the slide. Position the filter paper strip on the center of the length of the slide.

13.3 Using a clean, curved scalpel blade, cut a strip of the PC filter approximately 25 by 6 mm. Use a rocking motion of the scalpel blade to avoid tearing the filter. Place the PC strip particle side up on the slide perpendicular to the long axis of the slide. The ends of the PC strip must contact the double sided adhesive tape. Each slide can hold several PC strips. With a glass marker, label each PC strip with the individual sample number.

13.4 Carbon coat the PC filter strips as discussed in 12.4.2. PC filters do not require etching.

NOTE 2—**Caution:** Do not overheat the filter sections while carbon coating.

13.5 Prepare a Jaffe washer as described in 12.5, but fill the washer with chloroform or 1-methyl-2-pyrrolidone to the level of the screen.

13.6 Using a clean curved scalpel blade, excise three, 3-mm square filter pieces from each PC strip. Place the filter squares carbon side up on the shiny side of a TEM grid. Pick up the grid and filter section together and place them on the lens tissue in the Jaffe washer.

13.7 Place the lid on the Jaffe washer and rest the grids in place for at least 4 h. Best results are obtained with longer wicking times, up to 12 h.

13.8 Carefully remove the grids from the Jaffe washer, allowing the grids to dry before placing them in a clean, marked grid box.

14. Grid Opening Measurements

14.1 TEM grids must have a known grid opening area. Determine this area as follows:

14.2 Measure at least 20 grid openings in each of 20 random 75 to 100 μm (200-mesh) copper grids for a total of 400 grid openings for every 1000 grids used, by placing the 20 grids on a glass slide and examining them under the optical microscope. Use a calibrated graticule to measure the average length and width of the 20 openings from each of the individual grids. From the accumulated data, calculate the average grid opening area of the 400 openings.

14.3 Grid area measurements can also be made at the TEM at a calibrated screen magnification of between 15 000 and 20 000X. Typically measure one grid opening for each grid examined. Measure grid openings in both the x and y directions and calculate the area.

14.4 Pre-calibrated TEM grids are also acceptable for this test method.

15. TEM Method

15.1 Microscope settings: 80 to 120 kV, 15 000 to 20 000X screen magnification for analysis (7.2).

15.2 Analyze two grids for each sample. Analyze one-half of the sample area on one sample grid preparation and the remaining half on a second sample grid preparation.

15.3 *Determination of Specimen Suitability*:

15.3.1 Carefully load the TEM grid, carbon side facing up (in the TEM column) with the grid bars oriented parallel/perpendicular to the length of the specimen holder. Use a hand lens or loupe, if necessary. This procedure will line up the grid with the X and y translation directions of the microscope. Insert the specimen holder into the microscope.

15.3.2 Scan the entire grid at low magnification (250X to 1000X) to determine its suitability for high magnification analysis as specified in 15.3.3.

15.3.3 Grids are acceptable for analysis if the following conditions are met:

15.3.3.1 The fraction of grid openings covered by the replica section is at least 50 %.

15.3.3.2 Relative to that section of the grid covered by the carbon replica, the fraction of intact grid openings is greater than 50 %.

15.3.3.3 The fractional area of undissolved filter is less than 10 %.

15.3.3.4 The fraction of grid openings with overlapping or folded replica film is less than 50 %.

15.3.3.5 At least 20 grid openings, that have no overlapping or folded replica, are less than 5 % covered with holes and have less than 5 % opaque area due to incomplete filter dissolution.

15.4 *Determination of Grid Opening Suitability*:

15.4.1 If the grid meets acceptance criteria, choose a grid opening for analysis from various areas of the grid so that the

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entire grid is represented. Determine the suitability of each individual grid opening prior to the analysis.

15.4.2 The individual grid opening must have less than 5 % holes over its area.

15.4.3 Grid openings must be less than 25 % covered with particulate matter.

15.4.4 Grid openings must be uniformly loaded.

15.5 Observe and record the orientation of the grid at 80 to 150X, on a grid map record sheet along with the location of the grid openings that are examined for the analysis. If indexed grids are used, a grid map is not required, but the identifying coordinates of the grid square must be recorded.

16. Recording Data Rules

16.1 Record on the count sheet any continuous grouping of particles in which an asbestos fiber is detected. Classify asbestos structures as fibers, bundles, clusters, or matrices as defined in 5.2.

16.2 Use the criteria for fiber, bundle, cluster, and matrix identification, as described in the *USEPA Asbestos-Containing Materials in Schools* document (4). Record, for each AHERA structure identified, the length and width measurements.

16.3 Record NSD (No Structures Detected) when no structures are detected in the grid opening.

16.4 Identify structures classified as chrysotile identified by either electron diffraction or X-ray analysis (7.3) and recorded on a count sheet. Verify at least one out of every ten chrysotile structures by X-ray analysis.

16.5 Structures classified as amphiboles by X-ray analysis and electron diffraction are recorded on the count sheet. For more information on identification, see Yamate, et al, (7) or Chatfield and Dillon (8).

16.6 Record a typical electron diffraction pattern for each type of asbestos observed for each group of samples (or a minimum of every five samples) analyzed. Record the micrograph number on the count sheet. Record at least one X-ray spectrum for each type of asbestos observed per sample. Attach the print-outs to the back of the count sheet. If the X-ray spectrum is stored, record the file and disk number on the count sheet.

16.7 *Counting Rules*:

16.7.1 At a screen magnification of between 15 000 and 20 000X evaluate the grids for the most concentrated sample loading; reject the sample if it is estimated to contain more than 50 asbestos structures per grid opening. Proceed to the next lower concentrated sample until a set of grids are obtained that have less than 30 asbestos structures per grid opening.

16.8 *Analytical Sensitivity*—An analytical sensitivity of approximately 1000 asbestos structures per square centimetre (calculated for the detection of a single asbestos structure) has been designed for this analysis. This sensitivity can be achieved by increasing the amount of liquid filtered, increasing the number of grid openings analyzed, or decreasing the size of the final filter. Occasionally, due to high particle loadings or high asbestos concentration, this analytical sensitivity cannot be practically achieved and stopping rules apply.

16.9 *Limit of Detection*—The limit of detection for this method is defined as, at a minimum, the counting of four asbestos structures during the TEM analysis. If less than four asbestos structures are counted during the analysis then the analytical result which will be reported will be less than the limit of detection and a "less than" sign (<) will appear before the number. All data shall be provided in the laboratory report.

16.10 *Stopping Rules*:

16.10.1 The analysis is stopped upon the completion of the grid square that achieves an analytical sensitivity of less than 1000 asbestos structures per square centimetre.

16.10.2 If an analytical sensitivity of 1000 asbestos structures per square centimetre cannot be achieved after analyzing ten grid openings then stop on grid opening No. 10 or the grid opening which contains the 100th asbestos structure, whichever comes first. A minimum of four grid squares shall be analyzed for each sample.

16.10.2.1 If the analysis is stopped because of the 100th structure rule, the entire grid square containing the 100th structure must be counted.

16.11 After analysis, remove the grids from the TEM, and replace them in the appropriate grid storage holder.

17. Sample Storage

17.1 The washed-out sample cassettes can be discarded after use.

17.2 Sample grids and unused filter sections (7.18) must be stored for a minimum of one year.

18. Reporting

18.1 Report the following information for each dust sample analyzed:

18.1.1 Concentration in structures/cm^2.

18.1.2 The analytical sensitivity.

18.1.3 Types of asbestos present.

18.1.4 Number of asbestos structures counted.

18.1.5 Effective filtration area.

18.1.6 Average size of the TEM grid openings that were counted.

18.1.7 Number of grid openings examined.

18.1.8 Sample dilution used.

18.1.9 Area of the surface sampled.

18.1.10 Listing of size data for each structure counted.

18.1.11 A copy of the TEM count sheet or a complete listing of the raw data. An example of a typical count sheet is shown in Appendix X1.

18.2 Determine the amount of asbestos in any accepted sample using the following formula:

$$\frac{EFA \times 100\ \text{mL} \times \#STR}{GO \times GOA \times V \times SPL} = \text{asbestos structures/cm}^2 \qquad (1)$$

where:

#STR = number of asbestos structures counted,
EFA = effective filter area of the final sampling filter, mm^2,
GO = number of grid openings counted,
GOA = average grid opening area, mm^2,
SPL = surface area sampled, cm^2, and
V = volume of sample filtered in step 10.4.9, representing the actual volume taken from the original 100 mL suspension, mL.

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19. Quality Control/Quality Assurance

19.1 In general, the laboratory's quality control checks are used to verify that a system is performing according to specifications regarding accuracy and consistency. In an analytical laboratory, spiked or known quantitative samples are normally used. However, due to the difficulties in preparing known quantitative asbestos samples, routine quality control testing focuses on re-analysis of samples (duplicate recounts).

19.1.1 Re-analyze samples at a rate of 1/10 of the sample sets (one out of every ten samples analyzed not including laboratory blanks). The re-analysis shall consist of a second sample preparation obtained from the final filter.

19.2 In addition, quality assurance programs must follow the criteria shown in the *USEPA Asbestos-Containing Materials in Schools* document (**4**) and in the *NIST/NVLAP Program Handbook for Airborne Asbestos Analysis* document (**6**). These documents describe sample custody, sample preparation, blank checks for contamination, calibration, sample analysis, analyst qualifications, and technical facilities.

20. Calibrations

20.1 Perform calibrations of the instrumentation on a regular basis, and retain these records in the laboratory, in accordance with the laboratory's quality assurance program.

20.2 Record calibrations in a log book along with dates of calibration and the attached backup documentation.

20.3 A calibration list for the instrument is as follows:

20.3.1 *TEM*:

20.3.1.1 Check the alignment and the systems operation. Refer to the TEM manufacturer's operational manual for detailed instructions.

20.3.1.2 Calibrate the camera length of the TEM in electron diffraction (ED) operating mode before ED patterns of unknown samples are observed. Camera length can be measured by using a carbon coated grid on which a thin film of gold has been sputtered or evaporated. A thin film of gold is evaporated on the specimen TEM grid to obtain zone-axis ED patterns superimposed with a 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. Thick gold films will tend to mask weak diffraction spots from the fibrous particles. Since the unknown d-spacings of most interest in asbestos analysis are those which lie closest to the transmitted beam, multiple gold rings from thick films are unnecessary. Alternatively, a gold standard specimen can be used to obtain an average camera constant calculated for that particular instrument and can then be used for ED patterns of unknowns taken during the corresponding period.

20.3.1.3 Perform magnification calibration at the fluorescent screen. This calibration must be performed at the magnification used for structure counting. Calibration is performed with a grating replica (7.47) (for example, one containing at least 2160 lines/mm).

(a) (*a*) Define a field of view on the fluorescent screen. The field of view must be measurable or previously inscribed with a scale or concentric circles (all scales should be metric).

(b) (*b*) Frequency of calibration will depend on the service history of the particular microscope.

(c) (*c*) Check the calibration after any maintenance of the microscope that involves adjustment of the power supply to the lens or the high voltage system or the mechanical disassembly of the electron optical column (apart from filament exchange).

(d) (*d*) The analyst must ensure that the grating replica is placed at the same distance from the objective lens as the specimen.

(e) (*e*) For instruments that incorporate a eucentric tilting specimen stage, all specimens and the grating replica must be placed at the eucentric position.

20.3.1.4 The smallest spot size of the TEM must be checked.

(a) (*a*) At the crossover point, photograph the spot size at a screen magnification of 15 000 to 20 000X. An exposure time of 1 s is usually adequate.

(b) (*b*) The measured spot size must be less than or equal to 250 nm.

20.4 *EDXA*:

20.4.1 The resolution and calibration of the EDXA must be verified.

20.4.1.1 Collect a standard EDXA Cu peak from the Cu grid.

20.4.1.2 Compare the X-ray energy versus channel number for the Cu peak and be certain that readings are within ±10 eV.

20.4.2 Collect a standard EDXA of crocidolite asbestos (NIST SRM 1866).

20.4.2.1 The elemental analysis of the crocidolite must resolve the Na peak.

20.4.3 Collect a standard EDXA of chrysotile asbestos.

20.4.3.1 The elemental analysis of chrysotile must resolve both Si and Mg on a single chrysotile fiber.

20.5 Ultrasonic bath calibration shall be performed as follows:

20.5.1 Fill the bath water to a level equal to the height of suspension in the glass sample container that will be used for the dust analysis. Operate the bath until the water reaches the equilibrium temperature.

20.5.2 Place 100 mL of water (at approximately 20°C) in another 200-mL glass sample container, and record its temperature.

20.5.3 Place the sample container in the water in the ultrasonic bath (with the power turned off). After 60 s, remove the glass container and record its temperature.

20.5.4 Place 100 mL of water (at approximately 20°C) in another 200-mL glass sample container, and record its temperature.

20.5.5 Place the second sample container into the water in the ultrasonic bath (with the power turned on). After 60 s, remove the glass container and record its temperature.

20.5.6 Calculate the rate of energy deposition into the sample container using the following formula:

$$R = 4.185 \times \sigma \times \rho \times \frac{(\theta_2 - \theta_1)}{t} \quad (2)$$

where:

4.185 = Joules/cal,

R = energy deposition, watts/mL,

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θ_1 = temperature rise with the ultrasonic bath not operating, °C,
θ_2 = temperature rise with the ultrasonic bath operating, °C,
t = time in seconds, 60 s (20.5.3 and 20.5.5),
σ = specific heat of the liquid in the glass sample container, 1.0 cal/g, and
ρ = density of the liquid in the glass sample container, 1.0 g/cm^3.

20.5.7 Adjust the operating conditions of the bath so that the rate of energy deposition is in the range of 0.08 to 0.12 MW/m 3, as defined by this procedure.

21. Precision and Bias

21.1 *Precision*—The precision of the procedure in this test method is being determined using round robin data from participating laboratories.

21.2 *Bias*—Since there is no accepted reference material suitable for determining the bias of the procedure in this test method, bias has not been determined (see Specification D 3670).

NOTE 3—Round robin data is under development and will be presented as a research report.

22. Keywords

22.1 asbestos; microvacuuming; settled dust; TEM

APPENDIX

(Nonmandatory Information)

X1. DUST SAMPLE ANALYSIS

X1.1 See Figs. X1.1 and X1.2 for the dust analysis worksheet and the TEM count sheet.

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DUST SAMPLE ANALYSIS

Client: _____
Sample ID: _____
Job Number: _____
Date Sample Analyzed: - -
Number of Openings/Grids Counted: | |
Grid Accepted, 600X: Yes No
Percent Loading: _____ %
Grid Box #1: _____

Accelerating Voltage: _____
Indicated Mag: _____ KX
Screen Mag: _____ KX
Microscope: 1 2 3 4 5
Filter Type: _____
Filter Size: _____
Filter Pore Size (μm): _____
Grid Opening: 1) _____ μm x _____ μm
2) _____ μm x _____ μm

Analyst: _____

Reviewer: _____

Counting Rules: AHERA LEVEL II

Calculation Data:

Effective Filter Area in mm^2:	(EFA)	_____
Number of Grid Openings Counted:	(GO)	_____
Avderage Grid Opening Area in mm^2:	(GOA)	_____
Volume of sample Filtered in ml:	(V)	_____
Surface area Sampled in cm^2:	(SPL)	_____
Number of Asbestos Structures Counted:*	(#STR)	_____

* If the number of asbestos structures counted is less than or equal to 4, enter 4 structures as the limit of detection here.

FORMULA FOR CALCULATION OF ASBESTOS STRUCTURES "DUST" PER CM^2:

$$\frac{EFAX\ X\ 100\ X\ \#STR}{GO\ X\ GOA\ X\ VX\ SPL} = \text{(Asbestos Structures per } cm^2\text{)}$$

Results for Total Asbestos Structures: _____
(Structures per cm^2)

Results for Structures ≥ microns: _____
(Structures per cm^2)

FIG. X1.1 Dust Sample Analysis Work Sheet

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Job Number: ______________________________

Structure #	Grid # Square #	Type	Structure	Length Microns	Width Microns	Confirmation		
						Morph.	SAED	EDS

Note: Keys to Abbreviations Used in Figure:

Type:

C = Chrysotile
AM = Amosite
CR = Crocidolite
AC = Actinolite
TR = Tremolite
AN = Anthophyllite
N = Non Asbestos

Structure:

F = Fiber
B = Bundle
C = Cluster
M = Matrix

Others:

NSD = No Structures Detected
Morph = Morphology
SAED = Selected Area Electron Diffraction
EDS = Energy Dispersive X-Ray Spectroscopy
ER = Inter-Row Spacing
NP = No Pattern

FIG. X1.2 TEM Count Sheet

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Tabbed Page: 2

Designation: D 6620 – 00

Standard Practice for Asbestos Detection Limit Based on Counts[1]

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1. Scope

1.1 This practice presents the procedure for determining the detection limit (DL)[2] for measurements of fibers or structures[3] using microscopy methods.

1.2 This practice applies to samples of air that are analyzed either by phase contrast microscopy (PCM) or transmission electron microscopy (TEM), and samples of dust that are analyzed by TEM.

1.3 The microscopy methods entail counting asbestos structures and reporting the results as structures per cubic centimeter of air (str/cc) or fibers per cubic centimeter of air (f/cc) for air samples and structures per square centimeter of surface area (str/cm^2) for dust samples.

2. Referenced Documents

2.1 *ASTM Standards:*

D 1356 Terminology Relating to Sampling and Analysis of Atmospheres[4]

D 5755 Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Concentrations[4]

D 6281 Test Method for Airborne Asbestos Concentration in Ambient and Indoor Atmospheres as Determined by Transmission Electron Microscopy Direct Transfer (TEM)

D 6480 Test Method for Wipe Sampling of Surfaces, Indirect Preparation, and Analysis of Asbestos Structure Number Concentration by Transmission Electron Microscopy[4]

E 456 Terminology for Relating to Quality and Statistics[5]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *average, n*—the sum of a set of measurements (counts) divided by the number of measurements in the set.

3.1.1.1 *Discussion*—The *average* is distinguished from the *mean*. The average is calculated from data and serves as an estimate of the *mean*. The *mean* (also referred to as the *population mean, expected value,* or *first moment*) is a parameter of the underlying statistical distribution of counts.

3.1.2 *background, n*—a statistical distribution of structures introduced by (*i*) analyst counting errors and (*ii*) contamination on an unused filter or contamination as a consequence of the sample collection and sample preparation steps.

3.1.2.1 *Discussion*—This definition of *background* is specific to this practice. The only counting errors considered in this definition of *background* are errors that result in an over-count (that is, false positives). Analyst counting errors are errors such as, determining the length of structures or fibers and whether, based on length, they should be counted; counting artifacts as fibers; determining the number of structures protruding from a matrix; and interpreting a cluster as one, two, or more structures that should be counted only as zero or one structure. For purposes of developing the DL, assume that background contamination sources have been reduced to their lowest achievable levels.

3.1.3 *blank, n*—a filter that has not been used to collect asbestos from the target environment.

3.1.3.1 *Discussion*—Blanks are used in this practice to determine the degree of asbestos contamination that is reflected in asbestos measurements. Contamination may be on the virgin filter or introduced in handling the filter in the field or when preparing it for inspection with a microscope. The data required to determine the degree of contamination consists, therefore, of measurements of field blanks that have experienced the full preparation process.

3.1.4 *decision value, n*—a numerical value used as a boundary in a statistical test to decide between the null hypothesis and the alternative hypothesis.

3.1.4.1 *Discussion*—In the present context, the decision value is a structure count that defines the boundary between "below detection" (the null hypothesis) and "detection" (the alternative hypothesis). If a structure count were larger than the decision value, then one would conclude that detection has been achieved (that is, the sample is from a distribution other than the background distribution). If the count were less than or

[1] This practice is under the jurisdiction of ASTM Committee D22 on Air Quality and is the direct responsibility of Subcommittee D22.07 on Sampling and Analysis of Asbestos.

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[2] The DL also is referred to in the scientific literature as Limit of Detection (LOD), Method Detection Limit (MDL), and other similar descriptive names.

[3] For purposes of general exposition, the term "structures" will be used in place of "fibers or structures." In the examples in Section 8, the specific term, "fiber" or "structure," is used where appropriate. These terms are defined separately in Section 3.

[4] *Annual Book of ASTM Standards*, Vol 11.03.

[5] *Annual Book of ASTM Standards*, Vol 14.02.

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equal to the decision value, the result would be reported as "below detection," which means that the sample cannot be differentiated from a sample that would have been collected from the background distribution.

3.1.5 *detection limit*—the mean of a structure count population that is sufficiently large so a measurement from this population would have a high probability (for example, 0.95 or larger) of exceeding the decision value that determines detection.

3.1.5.1 *Discussion*—The DL is the value of a parameter, the true mean of a structure count population in the statistical hypothesis testing problem, that underlies the DL concept. Specifically, it is the true mean of the alternative hypothesis that ensures a sufficiently high power for the statistical test that determines detection.

3.1.6 *count*, *n*—the number of fibers or structures identified in a sample.

3.1.7 *fiber*, *n*—any of various discrete entities with essentially parallel sides counted by a particular method that specifies length, width, and aspect ratio.

3.1.7.1 *Discussion*—The definitions of "fiber" and "structure" are similar because the measurement method employed specifies the shape, length, width, and aspect ratio.

3.1.8 *mean*, *n*—the mean value of the number of structures in the population of air or dust sampled.

3.1.8.1 *Discussion*—The *mean* in this definition is intended to be the population mean, expected value, or first moment of a statistical distribution. It is a theoretical parameter of the distribution that may be estimated by forming an average of measurements (refer to Terminology E 456 for definition of population).

3.1.9 *power*, *n*—the probability that a count exceeds the decision value for a sample that was obtained from a population other than the background population.

3.1.9.1 *Discussion*—Power is the probability of selecting, based on a statistical test, the alternative hypothesis when it is true. In the present context, this means the probability of making the correct decision to report a structure concentration for a sample that was collected from a population other than the background population. The *power* of the statistical test equals 1 minus the *type II error rate*.

3.1.10 *replicate*, *n*—a second measurement is a replicate of the initial measurement if the second measurement is obtained from an identical sample and under identical conditions as the initial measurement.

3.1.10.1 *Discussion*—"Identical," as applied to sample, can mean" same subsample preparation," "separate preparation of a distinct subsample," or a distinct sample obtained from the same population as the initial sample. For this practice, "identical" means distinct sample obtained from the same population as the initial sample.

3.1.11 *sample*, *n*—the segment of the filter that is inspected, and thereby, embodies the air or dust that was collected and the subset of structures that were captured on the portion of the filter subjected to microscopic inspection (also, see Terminology D 1356).

3.1.12 *sensitivity*, *n*—the structure concentration corresponding to a count of one structure in the sample.

3.1.13 *structure*, *n*—any of various discrete entities counted by a particular method that specifies shape, length, width, and aspect ratio.

3.1.14 *type I error*, *n*—choosing, based on a statistical test, the alternative hypothesis over the null hypothesis when the null hypothesis is, in fact, true; a false positive outcome of a statistical test.

3.1.14.1 *Discussion*—A type I error would occur if the count for a sample exceeded the decision value, but the sample was, in fact, obtained from the background population. The analyst erroneously would be led by the statistical test to report a structure concentration (that is, choose the alternative hypothesis of the statistical test), where the result should be reported as "below the detection limit" (that is, the null hypothesis of the statistical test is true).

3.1.15 *type II error*, *n*—choosing, based on a statistical test, the null hypothesis over the alternative hypothesis when the alternative hypothesis is, in fact, true; a false negative outcome of a statistical test.

3.1.15.1 *Discussion*—A type II error would occur if the count for a sample does not exceed the decision value, but the sample was, in fact, obtained from a population other than the background population. The analyst would erroneously be led by the statistical test to report a "below the detection limit" result (that is, choose the null hypothesis of the statistical test), where the result should be reported as a structure concentration (that is, the alternative hypothesis of the statistical test is true).

3.1.16 *type I error rate*, *n*—the probability of a type I error (also referred to as the *significance level*, *α-level*, or *p-value* of the statistical test).

3.1.17 *type II error rate*, *n*—the probability of a type II error (also referred to as the β-level of the statistical test).

3.1.18 λ—lambda, the Greek letter used to represent the population mean of a Poisson distribution.

3.1.19 λ_0—the population mean of the Poisson distribution of *background* counts.

3.1.19.1 *Discussion*—λ_0 is the population mean of the Poisson distribution under the null hypothesis in the statistical hypothesis testing problem that defines the DL.

3.1.20 λ_1—the population mean of the Poisson distribution under the alternative hypothesis in the statistical hypothesis testing problem that defines the DL (DL = λ_1).

3.1.21 x_0—decision value for determining detection. If the count in a measurement is not greater than x_0, the measurement is reported as "below detection."

3.1.22 *X*—a Poisson distributed random variable used to denote the number of structures (fibers) counted in a sample.

3.1.23 *A*—the area of the filter inspected to obtain a structure count.

3.1.24 $P(X>x/\lambda, A)$—the Poisson probability of a structure count exceeding x structures (fibers) when the population mean is equal to λ and an area, *A*, of the filter is inspected.

4. Significance and Use

4.1 The DL concept addresses potential measurement interpretation errors. It is used to control the likelihood of reporting a positive finding of asbestos when the measured asbestos level cannot clearly be differentiated from the background contamination level. Specifically, a measurement is reported as being

"below the DL" if the measured level is not statistically different than the background level.

4.2 The DL, along with other measurement characteristics such as bias and precision, is used when selecting a measurement method for a particular application. The DL should be established either at the method development stage or prior to a specific application of the method. The method developer subsequently would advertise the method as having a certain DL. An analyst planning to collect and analyze samples would, if alternative measurement methods were available, want to select a measurement method with a DL that was appropriate for the intended application.[6] The most important use of the DL, therefore, takes place at the planning stage of a study, before samples are collected and analyzed.

5. Descriptive Terms and Procedures

5.1 *Introduction*:

5.1.1 The DL is one of a number of characteristics used to describe the expected performance of a measurement method.[7] The DL concept addresses certain potential measurement interpretation errors. Specifically, a measurement is reported as being "below the DL" if the measured level cannot be distinguished from zero or from the randomly varying background contamination level. Stated differently, the DL provides protection against a false positive finding. When a measured value is less than an appropriately specified decision value, the analyst is instructed to disregard the measured value and report the result only as "below the DL."

5.1.2 The DL concept for asbestos measurements, which are based on microscopy, is simpler than the DL concept for measurement methods that depend, for example, on spectroscopy. For asbestos, the measurement is derived from a direct count of discrete structures using a microscope. For spectroscopy methods, the measurement is indirect requiring a calibration curve, and is subject to interferences and unspecified background signals that could be responsible for measurement values that are false positives.

5.1.3 The sources of false positives for asbestos counts are (*i*) analyst errors (for example, determining the length of structures or fibers and whether, based on length, they should be counted; counting artifacts as fibers; determining the number of structures protruding from a matrix; interpreting a cluster as one, two, or more structures that should be counted only as zero or one), and (*ii*) contamination (for example, virgin filter contamination or contamination introduced during sample collection or sample preparation). Collectively, these sources are referred to subsequently as "background." For purposes of developing the DL, assume that each background source has been reduced to its lowest achievable level.

5.2 *DL—General Discussion*:

5.2.1 DLs often have been misspecified and misinterpreted because the DL concept has not been defined with sufficient clarity for translation into operational terms; however, the DL concept and operational implementation have been presented correctly in the scientific literature by a number of authors.[8] These authors describe the DL as a theoretical value, specifically the true mean concentration of a substance in a sampled medium. This true mean, the DL, must be large enough to ensure a high probability (for example, 0.95 or larger) of concluding based on one or more measurements from a sample of the medium that the true concentration in the medium is, in fact, greater than zero or greater than an appropriately defined background level. The DL, therefore, is a parameter in the statistical decision that determines whether the concentration of a substance in a sample is consistent with the background level, which may be zero, or is greater than the background level.

5.2.2 Determining whether the mean concentration of a substance in a sample is consistent with the background concentration or is greater than the background concentration is a statistical decision problem. Due to statistical variation, replicate measurements of a sample or measurements from replicate samples do not yield identical results; thus, a measurement may exceed the true background mean level even if the sample were collected from the background distribution. Differences in replicate results are characterized as statistical variation. Values of replicate measurements are described by a probability distribution. The decision concerning whether or not a measurement is consistent with the background concentration fits the standard hypothesis testing framework in statistics. The statistical testing problem, therefore, provides the necessary structure for determining a numerical value for the DL, as well as a rule for reporting measurements as "below the DL."

5.2.3 The DL is determined by formulating the statistical testing problem as follows.

5.2.3.1 Consider a statistical test, based on one measurement, of the null hypothesis that the true mean concentration, λ, of substance in a sample is equal to the background mean, λ_0, versus the alternative hypothesis that λ is greater than λ_0. The typical decision rule leads to a choice of $\lambda > \lambda_0$ over $\lambda = \lambda_0$ if a standardized measurement[9] is larger than a specified decision value for the statistical test. The decision value is chosen to control the Type I error rate (also referred to here as the false positive rate) of the statistical test. The false positive rate is the probability that a measurement will exceed the

[6] For example, the purpose of the measurements might be to assess differences in the levels of a substance between two sources. If it were anticipated that the levels associated with each source are likely to be less than the DL of a particular measurement method, that method would not be appropriate for the intended application.

[7] Other characteristics are precision, bias, and for asbestos measurements, sensitivity.

[8] Clayton, C. A., Hines, J. W., and Elkins, P. D., "Detection Limits with Specified Assurance Probabilities," *Analytical Chem.* 59, 1987, 2506–2514; Currie, L. A., "Limits of Qualitative Detection and Quantitative Determination: Application to Radiochemistry," *Analytical Chem.*, Vol 40, 1968, 586–593; Currie, L. A., "Lower Limit of Detection: Definition and Elaboration of a Proposed Position for Radiological Effluent and Environmental Measurements," National Bureau of Standards Report, 1984; Fowler, D. P., "Definition of Lower Limits for Airborne Particle Analyses Based on Counts and Recommended Reporting Conventions," *Ann. Occup Hyg.*, Vol 41 Supplement 1, 1997, 203–209.

[9] In this statistical context, a standardized measurement is calculated as the measurement minus the background mean divided by the standard deviation of the background distribution.

chosen decision value, leading to acceptance of $\lambda>\lambda_0$, when the true mean concentration is, in fact, λ_0.[10]

5.2.3.2 The DL concept, although providing protection against false positives in measurement systems, also requires consideration of probabilities associated with true positives. A high degree of confidence (that is, a high probability) is required that decision in favor of $\lambda>\lambda_0$ over $\lambda=\lambda_0$ is correct. In statistical hypothesis testing terminology, this probability is referred to as the "power of the statistical test."

5.2.3.3 The power of a statistical test is the probability that a measurement exceeds the decision value (that is, the probability that the measurement leads to the choice, $\lambda>\lambda_0$) when the true mean concentration is a value larger than λ_0. The power of the test is an increasing function of the true mean, λ. The DL is the value of λ that makes the power sufficiently large. EPA definitions of the DL indicate that power, the probability of a true positive result, should be 0.95 or greater.

5.2.4 Based on the structure outlined in 5.2.3.3 reporting measurements subject to DL considerations would be implemented as follows:

5.2.4.1 Determine the decision value in the statistical test for determining if a measurement is large enough to conclude that $\lambda>\lambda_0$ is correct and determine the value of λ, say λ_1, to achieve sufficient power. λ_1 is the DL.

5.2.4.2 If the measured value exceeds the decision value, report the measured value. If the measured value is less than or equal to the decision value, report that the measurement is "below the DL."

6. Application to Air Samples

6.1 The statistical hypothesis testing formulation described above and the Poisson distribution are employed to define and calculate DLs for measurements of airborne structure concentrations.

6.2 For the DL concept to have meaning there must be a background distribution of structure measurements. The background distribution consists of sources of structures that are not the measurement targets of interest but cannot be eliminated or further reduced.

6.2.1 The background distribution for airborne structure measurements is a combination of (*i*) analyst error and (*ii*) contamination (filter or laboratory).

6.2.1.1 Analyst errors are errors such as: determining the length of structures or fibers and whether, based on length, they should be counted; counting artifacts as fibers; determining the number of structures protruding from a matrix; interpreting a cluster as one, two, or more structures that should be counted only as zero or one.[11]

6.2.1.2 Filters may become contaminated from impurities that are inherent in their production or in the laboratory during filter preparation for analysis in the laboratory. Filter contamination should be minimized by laboratory QA/QC procedures.[12]

6.2.2 All background sources should be reduced to their lowest achievable levels. From an empirical perspective, it is neither practical nor necessary to quantify the background sources separately. The background level may be determined by analyzing blanks without attempting to differentiate among sources.[13]

6.3 *Characterization of Sampling and Analysis to Measure Airborne Asbestos*—As an aid in the subsequent discussion, a simplified characterization of air sampling and analysis for measuring airborne asbestos concentrations is used. Although this characterization of the measurement process may lack important details from a microscopist's perspective, it is adequate for describing how to calculate a DL (refer to D 6281 and NIOSH 7400[14] for additional details).

6.3.1 Air sampling is accomplished by drawing air through a filter at a specified rate for a specified period of time. Airborne particles consisting of asbestos and other matter are deposited on the filter. After air sampling has been completed, a section of the filter is prepared for inspection by microscopy. A specified number of fields of view of known size (that is, graticule fields for PCM and grid openings for TEM), are randomly selected and inspected microscopically. The particles found in each field of view are classified as fibers for PCM or asbestos structures for TEM and a count is recorded. The count obtained from the fields that were inspected is increased by an appropriate factor to produce an estimated count for the total filter. This estimate is divided by the volume of air collected during sampling. The resulting measurement is interpreted as an estimate of the asbestos concentration in the air, and is reported in units of fibers/cc of air (f/cc) for PCM or structures/cc of air (str/cc) for TEM.

6.3.2 The information described in 6.3.1 that is needed to address DLs can be summarized as a single number-measurement "sensitivity." Sensitivity is a characteristic that applies to individual measurements.[15] Sensitivity is defined as the structure concentration corresponding to a count of one structure in the sample. Sensitivity, therefore, depends on air volume and the fraction (a proportion) of the filter that is inspected. The fraction depends on the size of the effective filter collection area, the size of the fields of view, and the number of fields of view that are inspected.

$$\text{Sensitivity } (S) = [(EFA/(FOV*FOVA)]/V \quad (1)$$

where:

[10] This probability also is referred to as the significance level or *p*-value of the test and typically is selected to be 0.05, but could be larger or smaller to reflect the gravity of the consequences of a false positive.

[11] Misclassification of a nonasbestos structure as an asbestos structure is not treated as a false positive in the present discussion of DLs. For purposes of defining a DL, consider only the background sources described above as contributing to false positives.

[12] QA/QC procedures include: testing a sample of filters from a new supply before the new supply is used in the field; and diligently eliminating sources of asbestos contamination from the laboratory.

[13] Background estimation methods are described in 6.4.2.

[14] Asbestos and Other Fibers by PCM, "NIOSH Manual of Analytical Methods, Fourth Edition, 8/15/94.

[15] The sensitivity concept also may be applied to averages of multiple measurements in situations where "a measurement" always means the average of a specified number of independent replicate measurements. This application of sensitivity is not discussed here.

EFA = the effective filter collection area in square millimeters (mm^2);
FOV = the number of fields of view;
FOVA = the average field of view area in mm^2; and,
V = air volume in cubic centimeters (cc).

6.3.3 Given any value as a requirement for sensitivity, the air volume, field of view size, and number of fields of view may be varied to achieve the required value.

NOTE 1—Typical *EFA*s are 385 mm^2 for a filter with a 25-mm diameter and 855-mm^2 for a filter with a 37-mm diameter.

6.3.4 From the definition of sensitivity, it follows that the structure concentration measurement for a sample is the number of structures counted multiplied by sensitivity:

$$str/cc = (\#\ \text{structures})*S \quad (2)$$

6.4 Based on the usual assumption that the structure count from an air sample is described by the Poisson probability distribution, equations were developed for calculating DLs. The DL is stated as a mean structure count. The mean structure count may subsequently be translated to concentration units (str/cc) through multiplication by the sensitivity of the measurement as shown in Eq 2.

6.4.1 *Background Mean Known*[16]—Let X represent the number of structures counted in a sample based on inspection of a filter area equal to A ($A = \Sigma FOV_i \cdot FOVA_i$ where FOV_i is the number of grid openings with area $FOVA_i$). Let λ be the true average structure count. To establish the DL, set up a statistical test of the hypothesis H_0: $\lambda=\lambda_0$ versus the alternative H_1: $\lambda>\lambda_0$ as described in 5.2.3. λ_0 is the true mean count of structures for the background distribution when an area, A, of the filter is inspected.[17] The decision value, x_0, is defined as the solution to $P\ (X>x_0|\lambda=\lambda_0, A)=\alpha$ (α is the significance level or Type I error rate of the statistical test). The power of the statistical test is calculated as $P\ (X>x_0|\lambda=\lambda_1, A)=1-\beta$. β is the Type II error rate of the test and $1-\beta$ is the value specified as the power of the test. The DL is the value of λ_1 that satisfies the equation for the power of the test.

6.4.1.1 The equations for calculating the DL are as follows:
Solve

$$P(X>x_0|\lambda=\lambda_0, A) = \alpha \quad (3)$$

to determine the decision value, x_0.
Then solve

$$P(X>x_0|\lambda=\lambda_1, A) = 1-\beta \quad (4)$$

for λ_1, which is the DL.

6.4.1.2 Calculate the probabilities indicated in Eq 3 and 4 using the following:

$$P(X>x\,|\,\lambda, A) = 1 - \Sigma\lambda^t \cdot e^{-\lambda}/t! \quad (5)$$

where the index t in the sum takes the values 0, 1, 2, ..., x.

6.4.1.3 *Numerical Examples of DLs for Airborne Asbestos*—Based on assumptions about the true value of the underlying background mean, decision values and DLs have been determined and are recorded in Tables 1-4. The examples in Tables 1 and 2 have been developed for a statistical test of $\lambda=\lambda_0$ versus $\lambda>\lambda_0$ with the nominal significance level of $\alpha = 0.05$ and nominal powers equal to 0.95 and 0.99, respectively. Because of the discrete nature of structure counts and the discrete nature of the Poisson distribution, it is not possible to achieve the nominal value of $\alpha = 0.05$ exactly. For each case in Tables 1 and 2, x_0 was chosen to correspond to the largest value of α that is less than or equal to 0.05. The actual values of α are shown in the tables.

TABLE 1 Detection Limits for Different Background Means Measurement Unit Equals Number of Structures (Nominal α = 0.05; Power = 0.95)

NOTE 1—"Structures" applies both to structures and fibers depending on the measurement protocol.

Background Mean (λ_0)	Decision Value x_0	Actual Type I Error Rate (α)	Detection Limit (λ_1)
0.00–0.05	0	0.000–0.048	3.00
0.05–0.35	1	0.002–0.049	4.74
0.35–0.81	2	0.006–0.049	6.30
0.81–1.36	3	0.010–0.049	7.75
1.36–1.97	4	0.013–0.050	9.15
1.97–2.61	5	0.016–0.050	10.51

6.4.1.4 Fig. 1 displays an example of the two Poisson distributions that determine the detection limit. This example is taken from Table 1. The background mean, λ_0, is 0.81, the decision value is 2, and the detection limit is 6.30.

6.4.1.5 It is extremely important to recognize that the background mean, λ_0, and therefore, the DL depend on the area of the filter that is inspected to produce a measurement. For example, if λ_0 equals 0.60 for measurements based on inspecting A mm^2 of the filter, λ_0, would be expected to be 1.20 for measurements based on inspecting $2{\cdot}A$ mm^2 of the filter. The corresponding DLs would be 6.30 and 7.75, respectively, (Table 1) or 8.41 and 10.05 respectively (Table 2).

6.4.1.6 The corresponding DLs may be stated in concentration units by multiplying the values in Tables 1 and 2 by measurement sensitivity. One example for PCM and one example for TEM are provided. For PCM, the results are displayed in Tables 3 and 4 for a measurement sensitivity of 0.0005 f/cc.[18] For TEM, the DLs stated in concentration units (str/cc) are displayed in Tables 5 and 6 for sensitivity, S, equal to 0.0064 str/cc.[19]

6.4.2 *Background Mean Unknown*—It is unlikely that the background mean would be known with certainty. The background mean may be estimated from data collected by analyzing blank filters. The estimate would have statistical error associated with it that must be accounted for in the DL determination. The magnitude of statistical error in the estimate of the background mean varies inversely with the number

[16] One may assume that the background mean, λ_0, has a known value based on a long history in the laboratory of consistent results obtained for blank filters. It is more likely that the mean would be estimated from a recent, fixed number of blank filter analyses. The latter case is discussed in 6.4.2.

[17] λ_0 is an increasing function of the filter area, A, that is inspected.

[18] A PCM measurement based on an 8-h sample at 2 L/min, a filter with effective collection area of 385 mm^2, and inspection of 100 graticule fields of size 0.00785/mm^2 has sensitivity equal to 0.0005 f/cc.

[19] A TEM measurement based on ten grid openings each 0.006-mm^2 on a filter with effective collection area equal to 385 mm^2 (that is, a 25-mm diameter MCE filter), and 1000 L of air would, by Eq 1 have S = 0.0064 str/cc.

TABLE 2 Detection Limits for Different Background Means Measurement Unit Equals Number of Structures (Nominal α = 0.05; Power = 0.99)

NOTE 1—"Structures" applies both to structures and fibers depending on the measurement protocol.

Background Mean (λ_0)	Decision Value x_0	Actual Type I Error Rate (α)	Detection Limit (λ_1)
0.00–0.05	0	0.000–0.048	4.61
0.05–0.35	1	0.002–0.049	6.64
0.35–0.81	2	0.006–0.049	8.41
0.81–1.36	3	0.010–0.049	10.05
1.36–1.97	4	0.013–0.050	11.61
1.97–2.61	5	0.016–0.050	13.11

TABLE 3 Detection Limits for PCM Measurement Units—f/cc (Sensitivity = 0.0005; Nominal α = 0.05; Power = 0.95)

Background Mean (f/cc)	Decision Value (f/cc)	Actual Type I Error Rate (α)	Detection Limit (f/cc)
$0–2.5\times10^{-5}$	0	0.000–0.048	1.5×10^{-3}
$2.5\times10^{-5}–1.8\times10^{-4}$	0.0005	0.002–0.049	2.4×10^{-3}
$1.8\times10^{-4}–4.0\times10^{-4}$	0.0010	0.006–0.049	3.2×10^{-3}
$4.0\times10^{-4}–6.8\times10^{-4}$	0.0015	0.010–0.049	3.8×10^{-3}
$6.8\times10^{-4}–9.9\times10^{-4}$	0.0020	0.013–0.050	4.6×10^{-3}
$9.9\times10^{-4}–1.3\times10^{-3}$	0.0025	0.016–0.050	5.3×10^{-3}

TABLE 4 Detection Limits for PCM Measurement Units—f/cc (Sensitivity = 0.0005; Nominal α = 0.05; Power = 0.99)

Background Mean (f/cc)	Decision Value (f/cc)	Actual Type I Error Rate (α)	Detection Limit (f/cc)
$0–2.5\times10^{-5}$	0	0.000–0.048	2.3×10^{-3}
$2.5\times10^{-5}–1.8\times10^{-4}$	0.0005	0.002–0.049	3.3×10^{-3}
$1.8\times10^{-4}–4.0\times10^{-4}$	0.0010	0.006–0.049	4.2×10^{-3}
$4.0\times10^{-4}–6.8\times10^{-4}$	0.0015	0.010–0.049	5.0×10^{-3}
$6.8\times10^{-4}–9.9\times10^{-4}$	0.0020	0.013–0.050	5.8×10^{-3}
$9.9\times10^{-4}–1.3\times10^{-3}$	0.0025	0.016–0.050	6.6×10^{-3}

of blank filters analyzed to form the estimate. If the number of blank filters employed is large enough to render the statistical error negligible, the DL would be obtained from Tables 1 and 2 by using the estimate as if it were the true value of λ_0. Otherwise, the magnitude of the DL varies directly with the statistical error in the estimate of the background mean, and therefore, inversely with the number of blank filters used to estimate the background mean. In 6.4.2.1, this relationship is discussed and guidance is provided for the number of blank filters that should be analyzed. After estimating the background mean, a quality assurance program including standard quality control measures should be employed to maintain the lowest achievable level of filter contamination.[20]

6.4.2.1 *Method*—The correct value for λ_0, depends on the true value λ_0. Analysis results for blank filters may be used to estimate λ_0, which, in turn, leads to a value for x_0. The estimate of λ_0 is an interim calculation on the way to determining x_0; therefore, the method for determining x_0 presented here is based directly on the blank filter analysis results and does not require that the estimate of λ_0 be calculated. The method should have a high probability of determining the correct value of x_0 and a low probability of indicating a wrong value of x_0. As the number of blank filters that are analyzed to determine x_0 is increased, the probability of a correct determination approaches 1.0 (100 %). There is, however, a cost-accuracy tradeoff between the number of blank filters analyzed to determine x_0 and the degree of error that can be tolerated in x_0.

6.4.2.2 Use 100 blank filter analyses to determine the value of x_0.[21] The 100 blank filter measurements for a particular laboratory may be selected from recent historical blank analysis results obtained in that laboratory. The rule for determining x_0 based on n = 100 blank filter analyses is shown in Table 7.

6.4.2.3 Using a value of x_0 from Table 7, refer to Tables 1 and 2 for the DL (examples are provided in Section 8).

7. Application to Dust Samples

7.1 The development of a DL for dust measurements is similar to the development for air measurements. The DL for dust measurements is the mean value of the alternative in the statistical hypothesis testing formulation that was described in Section 6 for air measurements. Differences in the sample collection and preparation methods may affect the magnitude of the background mean, which, in turn, affects the magnitude of the DL. Also, the calculation of sensitivity for dust measurements is different than for air measurements because of different process steps.

7.2 *Dust Measurement Characterization*—Dust is collected from a surface using either a microvac or a wipe (see Test Methods D 5755 and D 6480). Sample preparation involves various steps including suspension of particles in liquid and filtration. Structures are counted by TEM.

7.2.1 *Sensitivity*—The initial liquid volume and the volume deposited on the filter affect the sensitivity of the measurement. Sensitivity is calculated as follows:

$$S = [EFA/(GO \cdot GOA)]\cdot(100/V)/SPL \quad (6)$$

where:

EFA = effective filter area for the secondary filter (mm^2);
GO = number of grid openings counted;
GOA = average grid opening area (mm^2);
V = volume of sample filtered representing the actual volume taken from the original 100-mL suspension (mL); and,
SPL = the area of the surface vacuumed or wiped.

It follows that the asbestos structure concentration in dust, *STR*/cm^2, is:

$$STR/cm^2 = \#STR \cdot S \quad (7)$$

where:

#STR = number of asbestos structures counted in the sample.

7.3 *Calculating the DL:*

[20] The blank filters under discussion here are those used to establish the background mean. They are not the blanks, typically one for every field batch of filters, that are part of the ongoing QC program. The blanks used in the QC program are intended to flag gross contamination or identify a change in the previously established background mean.

[21] Rules for determining a value for x_0 based on analyzing n = 10, 25, 50, 100, and 200 blank filters have been developed and evaluated. The rules are discussed in the appendix to this practice.

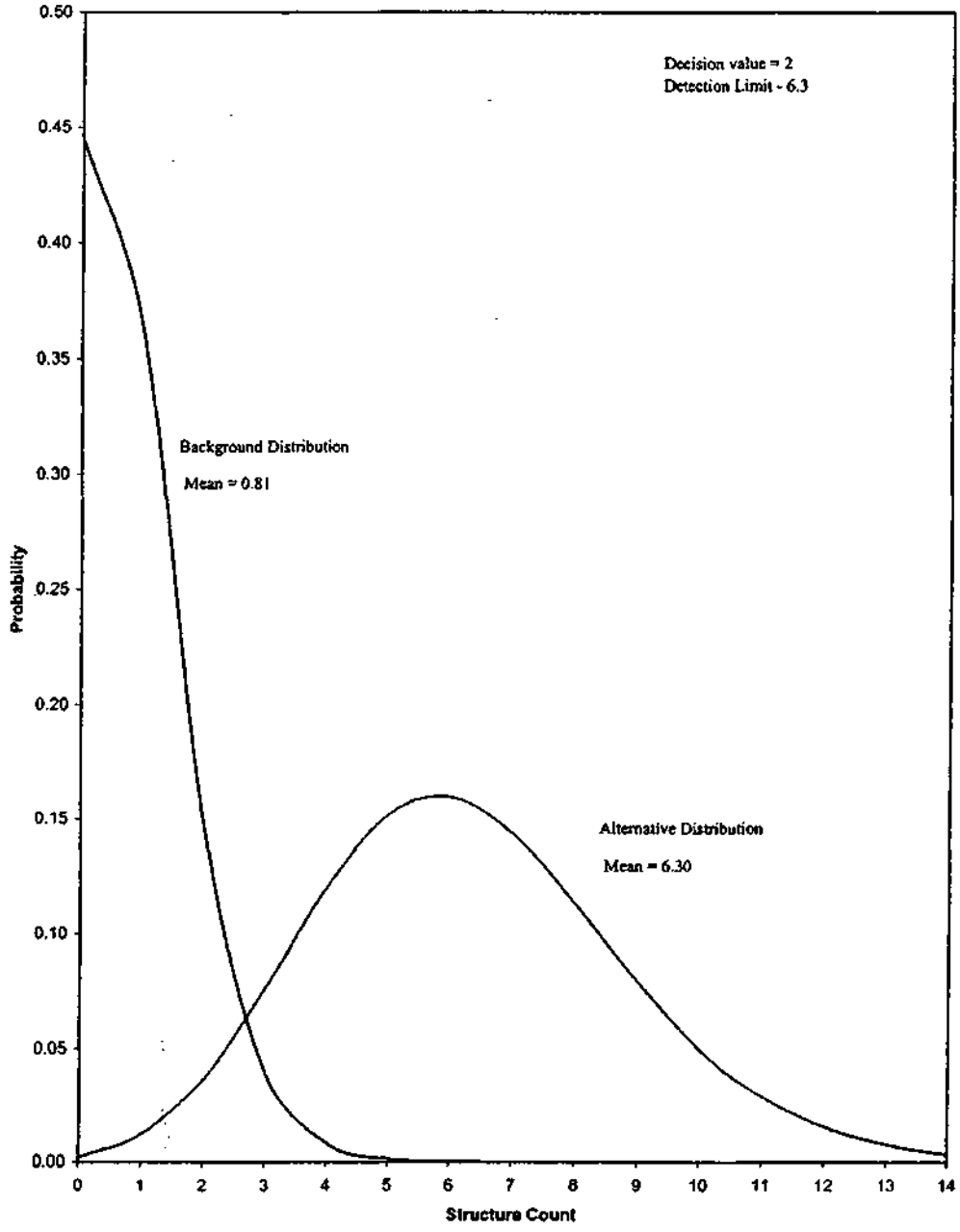

FIG. 1 Background Distribution, Decision Value, and Detection Limit

TABLE 5 Detection Limits for TEM (Direct Preparation) Measurement Units—str/cc (Sensitivity = 0.0064; Nominal α = 0.05; Power = 0.95)

Background Mean (str/cc)	Decision Value (str/cc)	Actual Type I Error Rate (α)	Detection Limit (str/cc)
$0–3.2\times10^{-4}$	0	0.000–0.048	1.9×10^{-2}
$3.2\times10^{-4}–2.4\times10^{-3}$	0.0064	0.002–0.049	3.0×10^{-2}
$2.4\times10^{-3}–5.2\times10^{-3}$	0.0128	0.006–0.049	4.0×10^{-2}
$5.2\times10^{-3}–8.7\times10^{-3}$	0.0196	0.010–0.049	4.9×10^{-2}
$8.7\times10^{-3}–1.3\times10^{-2}$	0.0256	0.013–0.050	5.8×10^{-2}
$1.3\times10^{-2}–1.7\times10^{-2}$	0.0320	0.016–0.050	6.7×10^{-2}

TABLE 6 Detection Limits for TEM (Direct Preparation) Measurement Units—str/cc (Sensitivity = 0.0064; Nominal α = 0.05; Power = 0.99)

Background Mean (str/cc)	Decision Value (str/cc)	Actual Type I Error Rate (α)	Detection Limit (str/cc)
$0–3.2\times10^{-4}$	0	0.000–0.048	3.0×10^{-2}
$3.2\times10^{-4}–2.4\times10^{-3}$	0.0064	0.002–0.049	4.2×10^{-2}
$2.4\times10^{-3}–5.2\times10^{-3}$	0.0128	0.006–0.049	5.4×10^{-2}
$5.2\times10^{-3}–8.7\times10^{-3}$	0.0196	0.010–0.049	6.4×10^{-2}
$8.7\times10^{-3}–1.3\times10^{-2}$	0.0256	0.013–0.050	7.4×10^{-2}
$1.3\times10^{-2}–1.7\times10^{-2}$	0.0320	0.016–0.050	8.4×10^{-2}

TABLE 7 Rule for Selecting x_0 Based on Measurements from 100 Blank Filters

x_0	Total Structure Count in 100 Blank Filter Measurements
0	0–5
1	6–34
2	35–78
3	79–132
4	133–194
5	195–269

7.3.1 *Background Mean Known*—The background sources for dust measurements are the same general sources identified as sources for air measurements, that is, analyst error and laboratory error. The laboratory component for dust measurements may be larger than the laboratory component for air measurements due to additional steps in the preparation process (refer to Test Methods D 5755 and D 6480). For purposes of demonstrating the DL for dust measurements, assume the same range of background means used for air measurements applies.

7.3.1.1 *Sensitivity*—A typical value of sensitivity discussed for dust sampling and analysis methods is 1000 str/cm^2.[22] Using values for λ_0 and λ_1 from Tables 1 and 2, Tables 8 and 9 display DLs in units of str/cm^2 for dust measurements associated with various background means.

7.3.2 *Background Mean Unknown*—Follow the procedure described in 6.4.2 for air samples. In addition, blank filters used for estimating the background level should go through the entire applicable preparation process.

8. Examples

AIR MEASUREMENTS

8.1 *PCM*:

8.1.1 *Estimate Background Mean, Decision Value, and DL*—Compile the measurement results for 100 blank MCE 25 mm diameter filters with EFA 385 mm^2. The count for each filter should reflect the number of fibers with a 3:1 aspect ratio and length of 5 μm or longer in 100 graticule fields each with area 0.00785 mm^2. Use NIOSH 7400 counting rules. Tabulate the total number of fibers counted across the 100 filters.

8.1.2 *Example 1*—Analysis of 100 blank filters yields 150 fibers. Based on Table 7, the decision value, x_0, for detection is four, and from Table 1 the detection limit is 9.15.[23]

8.1.2.1 *Sampling and Analysis*—Using a 25-mm diameter MCE filter, collect a 960-L air sample. Prepare the filter and count the number of fibers in 100 fields each with an area of 0.00785 mm^2. Sensitivity for this measurement is 0.0005 f/cc. The DL, stated in f/cc units, is 9.15·0.0005 = 0.0046 f/cc. If the number of fibers counted in the sample is larger than 4, multiply the count by 0.0005 and report the result as f/cc. If the number of fibers counted in the sample is less than or equal to four, report the measurement as "below the detection limit of 0.0046 f/cc (<0.0046 f/cc)."

TABLE 8 Detection Limits for Dust Measurements by TEM Measurement Unit Equals STR/cm^2 (Nominal α = 0.05; Power = 0.95)

Background Mean (μ_0 in STR/cm^2)	Decision Value y_0	Actual Type I Error Rate (α)	Detection Limit μ_1 in STR/cm^2
0–50	0	0.000–0.048	3000
50–350	1000	0.002–0.049	4740
350–810	2000	0.006–0.049	6300
810–1360	3000	0.010–0.049	7750
1360–1970	4000	0.013–0.050	9150
1970–2610	5000	0.016–0.050	10510

TABLE 9 Detection Limits for Dust Measurements by TEM Measurement Unit Equals STR/cm^2 (Nominal α = 0.05; Power = 0.99)

Background Mean (μ_0 in STR/cm^2)	Decision Value y_0	Actual Type I Error Rate (α)	Detection Limit μ_1 in STR/cm^2
0–50	0	0.000–0.048	4610
50–350	1000	0.002–0.049	6640
350–810	2000	0.006–0.049	8410
810–1360	3000	0.010–0.049	10050
1360–1970	4000	0.013–0.050	11610
1970–2610	5000	0.016–0.050	13110

8.1.2.2 Assume five fibers are counted. The airborne fiber concentration estimate is reported as 0.0025 f/cc. This value is an estimate of the mean concentration for the environment that is sampled. The statistical uncertainty associated with this estimate of the mean can be assessed by calculating the 95 % upper confidence limit (95 % UCL) for this result. The 95 % UCL corresponding to a count of 5 is 10.51, and the 95 % UCL for the concentration is 0.0053 f/cc (see Table 10). If the number of fibers counted in the sample were less than or equal to the decision value of 4, the measurement would be reported as "below detection." For example, if three fibers are counted, the result would be reported as "below the detection limit of 0.0046 f/cc" or "<0.0046 f/cc." If the estimated f/cc value for this sample were calculated, it would be 3·0.0005=0.0015 f/cc with a 95 % UCL of 0.0038 f/cc; however, the 0.0015 f/cc estimate will not be reported. Record the result only as "below the detection limit of 0.0046 f/cc" because the count does not exceed the decision value.

[22] See Test Method D 5755. If 100 cm^2 were vacuumed, EFA = 1320; GOA = 0.01; and 4 mL out of 100 mL were deposited on the filter, then inspecting 30 GOs would yield a sensitivity of approximately 1000 STR/cm^2.

[23] Recall that the DL is the population mean of a fiber count distribution, and, therefore, is unlikely to be an integer value. The DL of 9.15, which is found in Table 1, corresponds to a 0.95 probability that a measurement larger than the decision value of four fibers does not belong to the background distribution. If a probability of 0.99 would be a more acceptable representation of measurement uncertainty relative to the background distribution, the DL would be 11.61 (Table 2).

TABLE 10 Upper Confidence Limits for the Poisson Distribution[A]

Count	95 % UCL	99 % UCL
0	2.996	4.605
1	4.744	6.638
2	6.296	8.406
3	7.754	10.045
4	9.154	11.605
5	10.513	13.108
6	11.842	14.571
7	13.148	16.000
8	14.435	17.403
9	15.705	18.783
10	16.962	20.145
11	18.208	21.490
12	19.443	22.821
13	20.669	24.139
14	21.886	25.446
15	23.097	26.743
16	24.301	28.030
17	25.499	29.310
18	26.692	30.581
19	27.879	31.845
20	29.062	33.103
21	30.240	34.355
22	31.415	35.601
23	32.585	36.841
24	33.752	38.077
25	34.916	39.308
26	36.077	40.534
27	37.234	41.757
28	38.389	42.975
29	39.541	44.190
30	40.691	45.401

[A]Calculations based on formulas in *Biometrika Tables for Statisticians*, Vol 1, Cambridge University Press 1954.

8.1.2.3 A full accounting of the uncertainty in the two measurement results described above is displayed in Table 11[24].

8.1.3 *Example 2*—Analysis of 100 blank filters yields 50 fibers. Based on Table 7, the decision value for detection is two and the detection limit is 6.30 fibers (Table 1).[25]

8.1.3.1 *Sampling and Analysis*—Using a 25-mm diameter MCE filters, collect a 960-L air sample. Prepare the filter and count the number of fibers in 100 fields, each with area 0.00785 mm^2. Sensitivity for this measurement is 0.0005 f/cc. The DL, stated in f/cc units is 6.30·0.0005 = 0.0032 f/cc. If the number of fibers counted in the sample is larger than two, multiply the count by 0.0005 and report the result as f/cc. If the number of fibers counted in the sample is less than or equal to two, report the measurement as "below the detection limit of 0.0032 f/cc (<0.0032 f/cc)."

TABLE 11 Example Demonstrating Application and Interpretation of Detection Limits

		Calculated Results		Reported Results	
Sample ID	Fiber Count	Concentration Estimate (f/cc)	95 % UCL (f/cc)	Concentration Estimate (f/cc)	95 % UCL (f/cc)
1	5	0.0025	0.0053	0.0025	0.0053
2	3	0.0015	0.0039	<0.0046	NA

8.2 *TEM*:

8.2.1 *Estimate Background Mean, Decision Value, and DL*—Compile the measurement results for 100 blank MCE 25 mm diameter filters with EFA 385 mm^2. (The count for each filter should reflect the number of asbestos structures with a 5:1 aspect ratio and length of 0.5 µm or longer in 10 grid openings each with area 0.01 mm^2. Use EPA AHERA counting rules. Tabulate the total number of asbestos structures counted across the 100 filters.

8.2.2 *Example 1*—Analysis of 100 blank filters yields seven structures. Based on Table 7, the decision value for detection is one and the detection limit 4.74 structures (Table 1).

8.2.2.1 *Sampling and Analysis*—Using a 25 mm diameter MCE filters, collect a 2400-L air sample. Prepare the filter and count the number of asbestos structures in 10 grid openings, each with area 0.01 mm^2. Sensitivity for this measurement is 0.0016 str/cc. The DL stated in str/cc is 4.74 × 0.0016 = 0.0076 str/cc. If the number of structures counted in the sample is larger than one, multiply the count by 0.0016 and report the result as str/cc. If the number of structures counted in the sample is either zero or one, report the measurement as "below the detection limit of 0.0076 str/cc (<0.0076 str/cc)."

8.2.3 *Example 2*—Analysis of 100 blank filters yields five structures. Based on Tables 5 and 6, the decision value for detection is zero and the detection limit 3.00 structures (Table 1).

8.2.3.1 *Sampling and Analysis*—Using a 25-mm diameter MCE filter, collect a 2400 liter air sample. Prepare the filter and count the number of asbestos structures in ten grid openings, each with area 0.01 mm^2. Sensitivity for this measurement is 0.0016 str/cc. The DL, stated in str/cc units is 3.00 × 0.0016 = 0.0048 str/cc. If the number of structures counted in the sample is larger than 0, multiply the count by 0.0016 and report the result as str/cc. If the structure count in the sample is 0, report the measurement as "below the detection limit of 0.0016 str/cc (<0.0016 str/cc)."

8.3 *Dust Measurements*:

8.3.1 *Estimate Background Mean, Decision Value, and DL*—Following the procedure described in Test Method D 5755, prepare 100 blank polycarbonate (PC) 47 mm diameter filters for evaluation with a TEM. Count the number of asbestos structures (5:1 aspect ratio; length of 0.5 µm or longer) in 30 grid openings each with area 0.01 mm^2. Use Test Method D 5755 counting rules.

8.3.2 *Example 1*—Analysis of 100 blank filters yields seven structures. Based on Table 7, the decision value for detection is one and the detection limit 4.74 structures (Table 1).

8.3.2.1 *Sampling and Analysis*—Vacuum dust in a 100-cm^2 area. Prepare the sample, which is redeposited on 47 mm diameter polycarbonate filter. Count the number of asbestos

[24] The "reported" concentration estimates in Column 5 of Table 11 might be viewed as nonintuitive or inconsistent because a fiber count of 5 leads to a concentration estimate (0.0025) that is smaller than the estimate for a fiber count of 3 (<0.0046). These "reported results," nevertheless, are correct. The ordering of the concentrations for "calculated results" shows what would be expected; however, the sample with the fiber count of 3 does not satisfy the test for detection. Therefore, the result for that sample is an upper bound, namely the DL of 0.0046 f/cc. The concentration for that sample can be reported, at best, as a range of uncertainty between 0 f/cc and 0.0046 f/cc. The sample with the count equal to 5 satisfies the test for detection. It has a point estimate concentration of 0.0025 f/cc and a range of uncertainty that includes, as an upper bound, 0.0053 f/cc with 95 % confidence.

[25] The DL of 6.30, which is found in Table 1, corresponds to a 0.95 probability that a measurement larger than the decision value of 4 fibers does not belong to the background distribution. If a probability of 0.99 would be a more acceptable representation of measurement uncertainty relative to the background distribution, the DL would be 8.45 (Table 2).

structures in 30 grid openings of area 0.01 mm^2. Sensitivity for this measurement is approximately 1000 str/cm^2. The DL, stated in str/cm^2 units, is 4.74 × 1000 = 4740 str/cm^2. If the number of structures counted in the sample is larger than 1, multiply the count by 1000 and report the result as str/cm^2. If the number of structures counted in the sample is either zero or one, report the measurement as "below the detection limit of 4740 str/cm^2 (<4740 str/cm^2)."

8.3.3 *Example 2*—Analysis of 100 blank filters yields five structures. Based on Table 7, the decision value for detection is zero and the detection limit 3.00 structures (Table 1).

8.3.3.1 *Sampling and Analysis*—Vacuum dust in a 100-cm^2 area. Prepare the sample, which is redeposited on 47-mm diameter polycarbonate filter. Count the number of asbestos structures in 30 grid openings of area 0.01 mm^2. Sensitivity for this measurement is approximately 1000 str/cm^2. The DL, stated in str/cm^2 units, is 3.00 × 1000 = 3000 str/cm^2. If the number of structures counted in the sample is larger than 0, multiply the count by 1000 and report the result as str/cm^2. If the number of structures counted in the sample is zero, report the measurement as "below the detection limit of 3000 str/cm^2 (<3000 str/cm^2)."

9. Keywords

9.1 asbestos; detection limit; fiber count

APPENDIX

(Nonmandatory Information)

X1. ESTIMATING THE BACKGROUND MEAN, λ_0, AND THE DECISION VALUE, x_0

X1.1 If the value of the background mean, λ_0, is known, the decision value, x_0, is uniquely determined (refer to 6.4); however, it is unlikely that the background mean would be known with certainty. Analysis results for blank filters may be used to estimate λ_0, which, in turn, leads to a value for x_0. The estimate of λ_0 is an interim calculation that is not needed to determine x_0; therefore, the method for determining x_0 presented here is based directly on the blank filter analysis results and does not require calculation of the interim estimate of λ_0.

X1.2 The method for determining x_0 should have a high probability of indicating the correct value of x_0 and a low probability of indicating a wrong value of x_0. As the number of blank filters that are analyzed to determine x_0 is increased, the probability of a correct determination approaches 1.0 (100 %). There is, however, a cost-accuracy tradeoff between the number of blank filters analyzed to determine x_0 and the degree of error that can be tolerated in the estimate of x_0.

X1.3 The rules for determining x_0 based on n = 10, 25, 50, 100, and 200 blank filters have been evaluated. With the exception of n = 100 and 200, the probabilities of incorrectly determining the value of x_0 are unacceptably large. The rules for determining the value of x_0 are displayed in Table X1.1 for n = 100 and n = 200.

X1.4 The probabilities of correctly and incorrectly determining x_0 are displayed in Table X1.2. To interpret these entries, consider as an example the case where the correct value of x_0 is two and apply the rule based on data for n = 100 blank filters. The ideal rule for determining x_0 would have high probabilities for correctly determining x_0 and low probabilities for incorrect determinations of x_0. Due to the discrete nature of the Poisson distribution, any improvement of the probability in one cell in Table X1.2 degrades the probability in another cell. When true value of x_0 is 2, the probability that x_0 would be incorrectly determined as 1 is 0.05 (5 %); the probability that x_0 would be incorrectly determined as three is 0.11 (11 %). The probability that x_0 is determined correctly as two is 0.85 (85 %). These probabilities do not add to 1.00 exactly due to rounding errors.

X1.5 On balance, the rules for n = 100 and n = 200 are both reasonable and not substantially different. This follows because the two types of errors in determining x_0, selecting a value too small or selecting a value too large, are not equally important. The DL concept is intended to provide protection against false positive errors. Choosing a value for x_0 that is larger than the correct value will provide additional protection against false positives, and, therefore, is the less significant of the two errors. Upon reinspection of the entries in Table X1.2, note that the probabilities of selecting a value for x_0 that is too small is no greater than 0.05 (5 %) both for the rule based on n = 100 and for the rule based on n = 200. From this assessment, the rule based on n = 100 is adequate.

TABLE X1.1 Rule for Selecting x_0 Based on Blank Filter Measurements

x_0	Total Structure Count in 100 Blank Filter Measurements	Total Structure Count in 200 Blank Filter Measurements
0	0–5	0–12
1	6–34	13–71
2	35–78	72–161
3	79–132	162–270
4	133–194	271–394
5	195–269	395–529

TABLE X1.2 Probabilities of Determining Values for x_0

Correct Value of x_0	Rule	Data for Blanks Indicates x_0= 0	Data for Blanks Indicates x_0= 1	Data for Blanks Indicates x_0= 2	Data for Blanks Indicates x_0= 3	Data for Blanks Indicates x_0= 4	Data for Blanks Indicates x_0= 5	Data for Blanks Indicates x_0= 6
0	n = 100	0.88	0.12					
	n = 200	0.95	0.05					
1	n = 100	0.04	0.87	0.09				
	n = 200	0.04	0.91	0.05				
2	n = 100		0.05	0.85	0.11			
	n = 200		0.04	0.90	0.06			
3	n = 100			0.04	0.84	0.12		
	n = 200			0.04	0.89	0.07		
4	n = 100				0.05	0.84	0.11	
	n = 200				0.05	0.89	0.06	
5	n = 100					0.07	0.88	0.05
	n = 200					0.06	0.89	0.05

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Tabbed Page: 3

INTERNATIONAL STANDARD

ISO 10312

First edition
1995-05-01

Ambient air — Determination of asbestos fibres — Direct-transfer transmission electron microscopy method

Air ambiant — Détermination des fibres d'amiante — Méthode de microscopie électronique à transmission directe

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Contents

Page

1 Scope 1

2 Normative references 2

3 Definitions 2

4 Principle 3

5 Symbols of units and abbreviations 4

6 Reagents 5

7 Apparatus 5

8 Air sample collection 10

9 Procedure for analysis 11

10 Performance characteristics 18

11 Test report 19

Annexes

A Determination of operating conditions for plasma asher 22

B Calibration procedures 23

C Structure counting criteria 25

D Fibre identification procedure 33

E Determination of the concentrations of asbestos fibres and bundles longer than 5 µm, and PCM equivalent asbestos fibres 42

F Calculation of results 43

G Strategies for collection of air samples 47

H Methods for removal of gypsum fibres 48

J Bibliography 49

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 10312 was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 3, *Ambient atmospheres*.

Annexes A, B, C, D, E and F form an integral part of this International Standard. Annexes G, H and J are for information only.

Introduction

This International Standard is applicable to the determination of airborne asbestos in a wide range of ambient air situations, including the interior atmospheres of buildings, and for detailed evaluation of any atmosphere in which asbestos structures are likely to be present. Because the best available medical evidence indicates that the numerical fibre concentration and the fibre sizes are the relevant parameters for evaluation of the inhalation hazards, a fibre counting technique is the only logical approach. Most fibres in ambient atmospheres are not asbestos, and therefore there is a requirement for fibres to be identified. Many airborne asbestos fibres in ambient atmospheres have diameters below the resolution limit of the optical microscope. This International Standard is based on transmission electron microscopy, which has adequate resolution to allow detection of small fibres and is currently the only technique capable of unequivocal identification of the majority of individual fibres of asbestos. Asbestos is often found, not as single fibres, but as very complex, aggregated structures which may or may not be also aggregated with other particles. The fibres found suspended in an ambient atmosphere can often be identified unequivocally, if a sufficient measurement effort is expended. However, if each fibre were to be identified in this way, the analysis would become prohibitively expensive. Because of instrumental deficiencies or because of the nature of the particulate, some fibres cannot be positively identified as asbestos, even though the measurements all indicate that they could be asbestos. Subjective factors therefore contribute to this measurement, and consequently a very precise definition of the procedure for identification and enumeration of asbestos fibres is required. The method specified in this International Standard is designed to provide the best description possible of the nature, numerical concentration, and sizes of asbestos-containing particles found in an air sample. This International Standard is necessarily complex, because the instrumental techniques used are complex, and also because a very detailed and logical procedure must be specified to reduce the subjective aspects of the measurement. The method of data recording specified in this International Standard is designed to allow re-evaluation of the structure counting data as new medical evidence becomes available. All of the feasible specimen preparation techniques result in some modification of the airborne particulate. Even the collection of particles from a three-dimensional airborne dispersion onto a two-dimensional filter surface can be considered a modification of the particulate, and some of the particles in most samples are modified by the specimen preparation procedures. However, the procedures specified in this International Standard are designed to minimize the disturbance of the collected particulate material, and the effect of those disturbances which do occur can be evaluated.

This International Standard describes the method of analysis for a single air filter. However, one of the largest potential errors in characterizing asbestos in ambient atmospheres is associated with the variability between filter samples. For this reason, it is necessary to design a replicate sampling scheme in order to determine this International Standard's accuracy and precision.

Ambient air — Determination of asbestos fibres — Direct-transfer transmission electron microscopy method

1 Scope

1.1 Substance determined

This International Standard specifies a reference method using transmission electron microscopy for the determination of the concentration of asbestos structures in ambient atmospheres and includes measurement of the lengths, widths and aspect ratios of the asbestos structures. The method allows determination of the type(s) of asbestos fibres present. The method cannot discriminate between individual fibres of the asbestos and non-asbestos analogues of the same amphibole mineral.

1.2 Type of sample

The method is defined for polycarbonate capillary-pore filters or cellulose ester (either mixed esters of cellulose or cellulose nitrate) filters through which a known volume of air has been drawn. The method is suitable for determination of asbestos in both exterior and building atmospheres.

1.3 Measuring range

The range of concentration which can be determined is 50 structures/mm^2 to 7 000 structures/mm^2 on the filter. The air concentrations represented by these values are a function of the volume of air sampled. There is no lower limit to the dimensions of asbestos fibres which can be detected. In practice, microscopists vary in their ability to detect very small asbestos fibres. Therefore, a minimum length of 0,5 µm has been defined as the shortest fibre to be incorporated in the reported results.

1.4 Limit of detection

The limit of detection theoretically can be lowered indefinitely by filtration of progressively larger volumes of air and by extending the examination of the specimens in the electron microscope. In practice, the lowest achievable limit of detection for a particular area of TEM specimen examined is controlled by the total suspended particulate concentration.

For total suspended particulate concentrations of approximately 10 µg/m^3, corresponding to clean, rural atmospheres, and assuming filtration of 4 000 litres of air, an analytical sensitivity of 0,5 structure/l can be obtained, equivalent to a limit of detection of 1,8 structure/l, if an area of 0,195 mm^2 of the TEM specimens is examined. If higher total suspended particulate concentrations are present, the volume of air filtered must be reduced in order to maintain an acceptable particulate loading on the filter, leading to a proportionate increase in the analytical sensitivity.

Where this is the case, lower limits of detection can be achieved by increasing the area of the TEM specimens that is examined. In order to achieve lower limits of detection for fibres and bundles longer than 5 µm, and for PCM equivalent fibres, lower magnifications are specified which permit more rapid examination of larger areas of the TEM specimens when the examination is limited to these dimensions of fibre. The direct analytical method cannot be used if the general particulate loading of the sample collection filter exceeds approximately 10 µg/cm^2 of filter surface, which corresponds to approximately 10 % coverage of the collection filter by particulate. If the total suspended particulate is largely organic material, the limit of detection can be lowered significantly by using an indirect preparation method.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 4225:1994, *Air quality — General aspects — Vocabulary.*

ISO 4226:1993, *Air quality — General aspects — Units of measurement.*

ISO Standard Handbook No. 2:1993, *Quantities and units.*

ISO Standard Handbook No. 3:1989, *Statistical Methods.*

3 Definitions

For the purposes of this International Standard, the following definitions apply (see also ISO 4225).

3.1 acicular: The shape of an extremely slender crystal with cross-sectional dimensions which are small relative to its length, i.e. needle-like.

3.2 amphibole: A group of rock-forming ferromagnesium silicate minerals, closely related in crystal form and composition, with the nominal formula:

$$A_0 \text{ or } {}_1B_2C_5T_8O_{22}(OH,F,Cl)_2$$

where

A = K, Na

B = Fe^{2+}, Mn, Mg, Ca, Na

C = Al, Cr, Ti, Fe^{3+}, Mg, Fe^{2+}

T = Si, Al, Cr, Fe^{3+}, Ti

In some varieties of amphibole, these elements can be partially substituted by Li, Pb or Zn. Amphibole is characterized by a cross-linked double chain of Si-O tetrahedra with a silicon:oxygen ratio of 4:11, by columnar or fibrous prismatic crystals and by good prismatic cleavage in two directions parallel to the crystal faces and intersecting at angles of about 56° and 124°.

3.3 amphibole asbestos: Amphibole in an asbestiform habit.

3.4 analytical sensitivity: The calculated airborne asbestos structure concentration in asbestos structures/litre, equivalent to counting of one asbestos structure in the analysis. The method in this International Standard does not specify an analytical sensitivity.

3.5 asbestiform: A specific type of mineral fibrosity in which the fibres and fibrils possess high tensile strength and flexibility.

3.6 asbestos: A term applied to a group of silicate minerals belonging to the serpentine and amphibole groups which have crystallized in the asbestiform habit, causing them to be easily separated into long, thin, strong fibres when crushed or processed. The Chemical Abstracts Service Registry Numbers of the most common asbestos varieties are: chrysotile (12001-29-5), crocidolite (12001-28-4), grünerite asbestos (amosite) (12172-73-5), anthophyllite asbestos (77536-67-5), tremolite asbestos (77536-68-6) and actinolite asbestos (77536-66-4).

3.7 asbestos structure: A term applied to any connected or overlapping grouping of asbestos fibres or bundles, with or without other particles.

3.8 aspect ratio: The ratio of length to width of a particle.

3.9 blank: A structure count made on TEM specimens prepared from an unused filter, to determine the background measurement.

3.10 camera length: The equivalent projection length between the specimen and its electron diffraction pattern, in the absence of lens action.

3.11 chrysotile: A fibrous mineral of the serpentine group which has the nominal composition

$$Mg_3Si_2O_5(OH)_4$$

Most natural chrysotile deviates little from this nominal composition. In some varieties of chrysotile, minor substitution of silicon by Al^{3+} may occur. Minor substitution of magnesium by Al^{3+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Mn^{2+} and Co^{2+} may also be present. Chrysotile is the most prevalent type of asbestos.

3.12 cleavage: The breaking of a mineral along one of its crystallographic directions.

3.13 cleavage fragment: A fragment of a crystal that is bounded by cleavage faces.

3.14 cluster: A structure in which two or more fibres, or fibre bundles, are randomly oriented in a connected grouping.

3.15 d-spacing: The distance between identical adjacent and parallel planes of atoms in a crystal.

3.16 electron diffraction: A technique in electron microscopy by which the crystal structure of a specimen is examined.

3.17 electron scattering power: The extent to which a thin layer of substance scatters electrons from their original directions.

3.18 energy dispersive X-ray analysis: Measurement of the energies and intensities of X-rays by use of a solid state detector and multichannel analyser system.

3.19 eucentric: The condition when the area of interest of an object is placed on a tilting axis at the intersection of the electron beam with that axis and is in the plane of focus.

3.20 field blank: A filter cassette which has been taken to the sampling site, opened, and then closed. Such a filter is used to determine the background structure count for the measurement.

3.21 fibril: A single fibre of asbestos, which cannot be further separated longitudinally into smaller components without losing its fibrous properties or appearances.

3.22 fibre: An elongated particle which has parallel or stepped sides. For the purposes of this International Standard, a fibre is defined to have an aspect ratio equal to or greater than 5:1 and a minimum length of 0,5 µm.

3.23 fibre bundle: A structure composed of parallel, smaller diameter fibres attached along their lengths. A fibre bundle may exhibit diverging fibres at one or both ends.

3.24 fibrous structure: A fibre, or connected grouping of fibres, with or without other particles.

3.25 habit: The characteristic crystal growth form, (or combination of these forms), of a mineral, including characteristic irregularities.

3.26 limit of detection: The calculated airborne asbestos structure concentration in structures per litre, equivalent to counting 2,99 asbestos structures in the analysis.

3.27 matrix: A structure in which one or more fibres, or fibre bundles, touch, are attached to, or partially concealed by, a single particle or connected group of nonfibrous particles.

3.28 Miller index: A set of either three or four integer numbers used to specify the orientation of a crystallographic plane in relation to the crystal axes.

3.29 PCM equivalent fibre: A fibre of aspect ratio greater than or equal to 3:1, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm.

3.30 PCM equivalent structure: A fibrous structure of aspect ratio greater than or equal to 3:1, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm.

3.31 primary structure: A fibrous structure that is a separate entity in the TEM image.

3.32 replication: A procedure in electron microscopy specimen preparation in which a thin copy, or replica, of a surface is made.

3.33 selected area electron diffraction: A technique in electron microscopy in which the crystal structure of a small area of a sample is examined.

3.34 serpentine: A group of common rock-forming minerals having the nominal formula

$$Mg_3Si_2O_5(OH)_4$$

3.35 structure: A single fibre, fibre bundle, cluster or matrix.

3.36 twinning: The occurrence of crystals of the same species joined together at a particular mutual orientation, such that the relative orientations are related by a definite law.

3.37 unopened fibre: An asbestos fibre bundle of large diameter which has not been separated into its constituent fibrils or fibres.

3.38 zone-axis: The line or crystallographic direction through the centre of a crystal which is parallel to the intersection edges of the crystal faces defining the crystal zone.

4 Principle

A sample of airborne particulate is collected by drawing a measured volume of air through either a

capillary-pore polycarbonate membrane filter of maximum pore size 0,4 µm or a cellulose ester (either mixed esters of cellulose or cellulose nitrate) membrane filter of maximum pore size 0,45 µm by means of a battery-powered or mains-powered pump. TEM specimens are prepared from polycarbonate filters by applying a thin film of carbon to the filter surface by vacuum evaporation. Small areas are cut from the carbon-coated filter, supported on TEM specimen grids, and the filter medium is dissolved away by a solvent extraction procedure. This procedure leaves a thin film of carbon which bridges the openings in the TEM specimen grid, and which supports each particle from the original filter in its original position. Cellulose ester filters are chemically treated to collapse the pore structure of the filter, and the surface of the collapsed filter is then etched in an oxygen plasma to ensure that all particles are exposed. A thin film of carbon is evaporated onto the filter surface and small areas are cut from the filter. These sections are supported on TEM specimen grids and the filter medium is dissolved away by a solvent extraction procedure.

The TEM specimen grids from either preparation method are examined at both low and high magnifications to check that they are suitable for analysis before carrying out a quantitative structure count on randomly-selected grid openings. In the TEM analysis, electron diffraction (ED) is used to examine the crystal structure of a fibre, and its elemental composition is determined by energy dispersive X-ray analysis (EDXA). For a number of reasons, it is not possible to identify each fibre unequivocally, and fibres are classified according to the techniques which have been used to identify them. A simple code is used to record, for each fibre, the manner in which it was classified. The fibre classification procedure is based on successive inspection of the morphology, the electron diffraction pattern for a selected area, and the qualitative and quantitative energy dispersive X-ray analyses. Confirmation of the identification of chrysotile is done only by quantitative ED, and confirmation of amphibole is done only by quantitative EDXA and quantitative zone axis ED.

In addition to isolated fibres, ambient air samples often contain more complex aggregates of fibres, with or without other particles. Some particles are composites of asbestos fibres with other materials. Individual fibres and structures that are more complex are referred to as "asbestos structures". A coding system is used to record the type of fibrous structure, and to provide the optimum description of each of these complex structures. The two codes remove the requirement to interpret the structure counting data from the microscopist, and allow this evaluation to be made later without the requirement for re-examination of the TEM specimens. Several levels of analysis are specified, the higher levels providing a more rigorous approach to the identification of fibres. The procedure permits a minimum required fibre identification criterion to be defined on the basis of previous knowledge, or lack of it, about the particular sample. Attempts are then made to achieve this minimum criterion for each fibre, and the degree of success is recorded for each fibre. The lengths and widths of all classified structures and fibres are recorded. The number of asbestos structures found on a known area of the microscope sample, together with the equivalent volume of air filtered through this area, is used to calculate the airborne concentration in asbestos structures/litre of air.

5 Symbols of units and abbreviations

5.1 Symbols of units (see also ISO 4226 and ISO No. 2)

eV = electron volt

kV = kilovolt

l/min = litres per minute

µg = microgram (10^{-6} gram)

µm = micrometre (10^{-6} metre)

nm = nanometre (10^{-9} metre)

W = watt

5.2 Abbreviations

DMF	Dimethylformamide
DE	Electron diffraction
EDXA	Energy dispersive X-ray analysis
FWHM	Full width, half maximum
HEPA	High efficiency particle absolute
MEC	Mixed esters of cellulose
PC	Polycarbonate
PCM	Phase contrast optical microscopy
SAED	Selected area electron diffraction
SEM	Scanning electron microscope
STEM	Scanning transmission electron microscope
TEM	Transmission electron microscope

UICC Union Internationale Contre le Cancer

6 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and water (6.1).

WARNING — Use the reagents in accordance with the appropriate health and safety regulations.

6.1 water, fibre-free.

A supply of freshly distilled, fibre-free water, or another source of fibre-free, pyrogen-free water shall be used.

6.2 Chloroform, analytical grade, distilled in glass, preserved with 1 % (*V/V*) ethanol.

6.3 1-Methyl-2-pyrrolidone.

6.4 Dimethylformamide.

6.5 Glacial acetic acid.

6.6 Acetone.

7 Apparatus

7.1 Air sampling — Equipment and consumable supplies

7.1.1 Filter cassette

Field monitors, comprising 25 mm to 50 mm diameter three-piece cassettes, with cowls which project less than 2 cm in front of the filter surface shall be used for sample collection. The cassette shall be loaded with either a capillary pore polycarbonate filter of maximum pore size 0,4 µm or an MEC or cellulose nitrate filter of maximum pore size 0,45 µm. Either type of filter shall be backed by a 5 µm pore size MEC or cellulose nitrate filter, and supported by a cellulose back-up pad. When the filters are in position, an elastic cellulose band or adhesive tape shall be applied to prevent air leakage. Suitable precautions shall be taken to ensure that the filters are tightly clamped in the assembly, so that significant air leakage around the filter cannot occur.

Representative filters from the filter lot shall be analysed as specified in 9.7 for the presence of asbestos structures before any are used for air sample collection.

7.1.2 Sampling pump

The sampling pump shall be capable of a flow-rate sufficient to achieve the desired analytical sensitivity. The face velocity through the filter shall be between 4,0 cm/s and 25,0 cm/s. The sampling pump used shall provide a non-fluctuating airflow through the filter, and shall maintain the initial volume flow-rate to within ± 10 % throughout the sampling period. A constant flow or critical orifice controlled pump meets these requirements. Flexible tubing shall be used to connect the filter cassette to the sampling pump. A means for calibration of the flow-rate of each pump is also required.

7.1.3 Stand

A stand shall be used to hold the filter cassette at the desired height for sampling, and shall be isolated from the vibrations of the pump (7.1.2).

7.1.4 Variable area flowmeter

A calibrated variable are a flowmeter with a range of approximately 1 l/min to 10 l/min is required for calibration of the air sampling system.

The variable area flowmeter shall be cleaned before use to avoid transfer of asbestos contamination from the flowmeter to the sample being collected.

7.2 Specimen preparation laboratory

Asbestos, particularly chrysotile, is present in varying quantities in many laboratory reagents. Many building materials also contain significant amounts of asbestos or other mineral fibres which may interfere with the analysis if they are inadvertently introduced during preparation of specimens. It is most important to ensure that, during preparation, contamination of TEM specimens by any extraneous asbestos fibres is minimized. All specimen preparation steps shall therefore be performed in an environment where contamination of the sample is minimized. The primary requirement of the sample preparation laboratory is that a blank determination shall yield a result which will meet the requirements specified in 9.7. A minimum facility considered suitable for preparation of TEM specimens is a laminar flow hood with positive pressure. However, it has been established that work practices in specimen preparation appear to be more important than the tape of clean handling facilities in use. Preparation of samples shall be carried out only after acceptable blank values have been demonstrated.

NOTE 1 It is recommended that activities involving manipulation of bulk asbestos samples not be performed in the

same area as TEM specimen preparation, because of the possibilities of contaminating the TEM specimens.

7.3 Equipment for analysis

7.3.1 Transmission electron microscope

A TEM operating at an accelerating potential of 80 kV to 120 kV, with a resolution better than 1,0 nm, and a magnification range of approximately × 300 to × 100 000 shall be used. The ability to obtain a direct screen magnification of about × 100 000 is necessary for inspection of fibre morphology; this magnification may be obtained by supplementary optical enlargement of the screen image by use of a binocular if it cannot be obtained directly. It is also required that the viewing screen of the microscope be calibrated such that the lengths and widths of fibre images down to 1 mm width can be measured in increments of 1 mm, regardless of image orientation. This requirement is often fulfilled through the use of a fluorescent screen with calibrated gradations in the form of circles, as shown in figure 1.

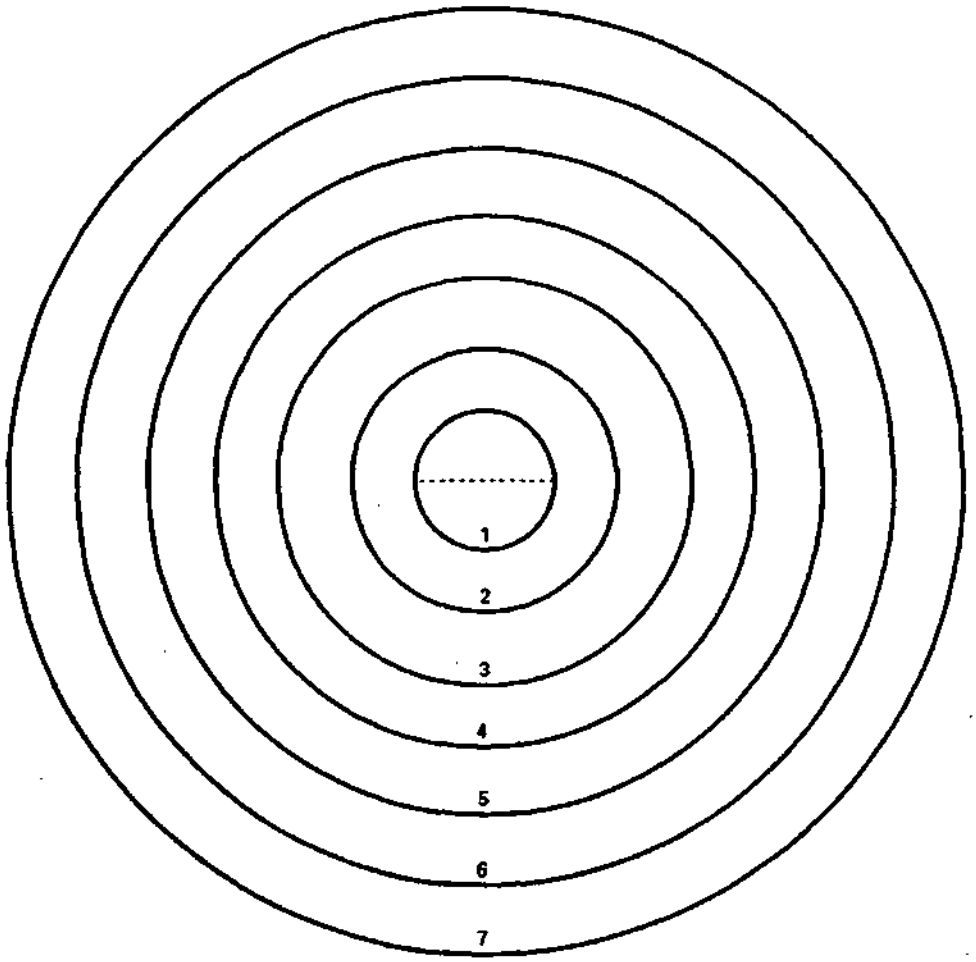


Figure 1 — Example of calibration markings on TEM viewing screen

For Bragg angles less than 0,01 rad, the TEM shall be capable of performing ED from an area of 0,6 μm^2 or less, selected from an in-focus image at a screen magnification of × 20 000. This performance requirement defines the minimum separation between particles at which independent ED patterns can be obtained from each particle. If SAED is used, the performance of a particular instrument may normally be calculated using the following equation

$$A = 0{,}785\ 4 \times \left(\frac{D}{M} + 2\ 000 C_s \theta^3 \right)^2$$

where

A is the effective SAED area, in square micrometres;

D is the diameter, in micrometres, of the SAED aperture;

M is the magnification of the objective lens;

C_s is the spherical aberration coefficient, in millimetres, of the objective lens;

θ is the maximum required Bragg angle, in radians.

It is not possible to reduce the effective SAED area indefinitely by the use of progressively smaller SAED apertures, because there is a fundamental limitation imposed by the spherical aberration coefficient of the objective lens.

If zone-axis ED analyses are to be performed, the TEM shall incorporate a goniometer stage which permits the TEM specimen to be either

a) rotated through 360°, combined with tilting through at least + 30° to − 30° about an axis in the plane of the specimen;

b) tilted through at least + 30° to − 30° about two perpendicular axes in the plane of the specimen.

The analysis is greatly facilitated if the goniometer permits eucentric tilting, although this is not essential. If EDXA and zone-axis ED are required on the same fibre, the goniometer shall be of a type which permits tilting of the specimen and acquisition of EDXA spectra without changing the specimen holder.

The TEM shall have an illumination and condenser lens system capable of forming an electron probe of diameter less than 250 nm.

NOTE 2 Use of an anti-contamination trap around the specimen is recommended if the required instrumental performance is to be obtained.

7.3.2 Energy dispersive X-ray analyser

The TEM shall be equipped with an energy dispersive X-ray analyser capable of achieving a resolution better than 180 eV (FWHM) on the MnKα. Since the performance of individual combinations of TEM and EDXA equipment is dependent on a number of geometrical factors, the required performance of the combination of the TEM and X-ray analyser is specified in terms of the measured X-ray intensity obtained from a fibre of small diameter, using a known electron beam diameter. Solid state X-ray detectors are least sensitive in the low energy region, and so measurement of sodium in crocidolite shall be the performance criterion. The combination of electron microscope and X-ray analyser shall yield, under routine analytical conditions, a background-subtracted NaKα integrated peak count rate of more than 1 count per second (cps) from a fibre of UICC crocidolite, 50 nm in diameter or smaller, when irradiated by an electron probe of 250 nm diameter or smaller at an accelerating potential of 80 kV. The peak/background ratio for this performance test shall exceed 1,0.

The EDXA unit shall provide the means for subtraction of the background, identification of elemental peaks, and calculation of background-subtracted peak areas.

7.3.3 Computer

Many repetitive numerical calculations are necessary, and these may be performed conveniently by relatively simple computer programmes. For analyses of zone-axis ED pattern measurements, a computer with adequate memory is required to accommodate the more complex programmes involved.

7.3.4 Plasma asher

For preparation of TEM specimens from MEC filters, a plasma asher, with a radio frequency power rating of 50 W or higher, shall be used to etch the surface of collapsed MEC filters. The asher shall be supplied with a controlled oxygen flow, and shall be modified, if necessary, to provide a valve to control the speed of air admission so that rapid air admission does not disturb particulates from the surface of the filter after the etching step.

NOTE 3 It is recommended that filters be fitted to the oxygen supply and the air admission line.

7.3.5 Vacuum coating unit

A vacuum coating unit capable of producing a vacuum better than 0,013 Pa shall be used for vacuum deposition of carbon on the membrane filters. A sample

holder is required which will allow a glass microscope slide to be continuously rotated during the coating procedure.

NOTE 4 A mechanism which also allows the rotating slide to be tilted through an angle of approximately 45° during the coating procedure is recommended. A liquid nitrogen cold trap above the diffusion pump may be used to minimize the possibility of contamination of the filter surfaces by oil from the pumping system. The vacuum coating unit may also be used for deposition of the thin film of gold, or other calibration material, when it is required on TEM specimens as an internal calibration of ED patterns.

7.3.6 Sputter coater

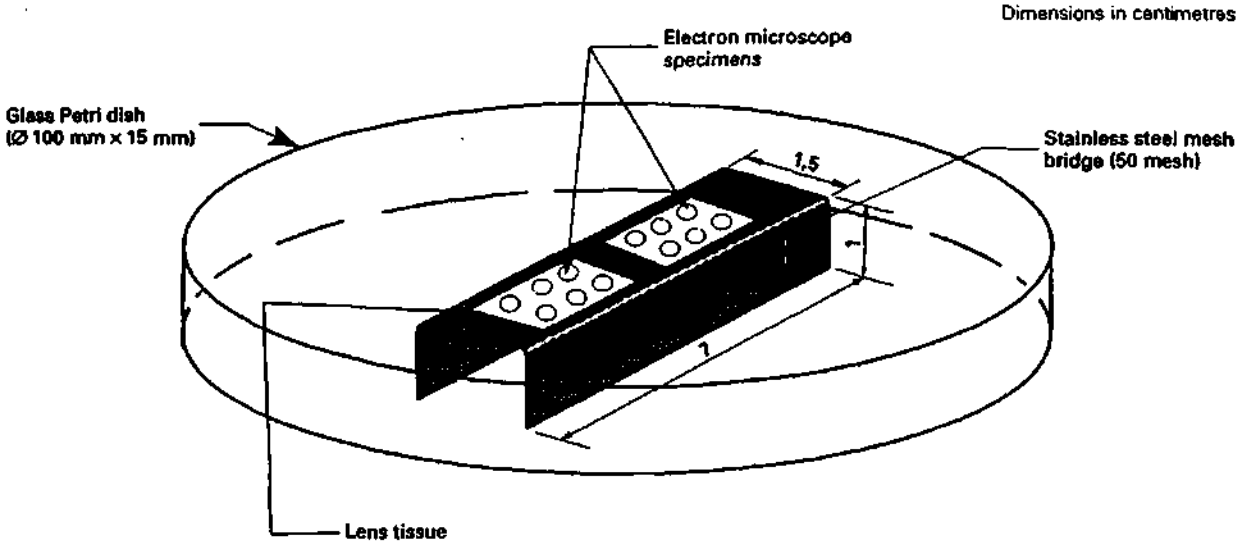
A sputter coater with a gold target may be used for deposition of gold onto TEM specimens as an integral calibration of ED patterns. Other calibration materials are acceptable. Experience has shown that a sputter coater allows better control of the thickness of the calibration material.

7.3.7 Solvent washer (Jaffe washer)

The purpose of the Jaffe washer is to allow dissolution of the filter polymer while leaving an intact evaporated carbon film supporting the fibres and other particles from the filter surface. One design of a washer which has been found satisfactory for various solvents and filter media is shown in figure 2. In general, either chloroform or 1-methyl-2-pyrrolidone has been used for dissolving polycarbonate filters and dimethylformamide or acetone has been used for dissolving MEC or cellulose nitrate filters. The higher evaporation rates of chloroform and acetone require that a reservoir of 10 ml to 50 ml of solvent be used, which may need replenishment during the procedure. Dimethylformamide and 1-methyl-2-pyrrolidone have lower vapour pressures and much smaller volumes of solvent may be used. It is recommended that all washers be used in a fume hood, and when specimens are not being inserted or removed, the Petri dish lid shall be in place during the solvent dissolution. The washer shall be cleaned before it is used for each batch of specimens.

7.3.8 Condensation washer

For more rapid dissolution of the filter polymer, or if difficulties are experienced in dissolving the filter polymer, use a condensation washer, consisting of a flask, condenser and cold finger assembly, with a heating mantle and means for controlling the temperature. A suitable assembly is shown in figure 3, using either acetone or chloroform as the solvent, depending on the type of filter.



NOTE — Solvent is added until the meniscus contacts the underside of the stainless steel mesh bridge.

Figure 2 — Example of design of solvent washer (Jaffe washer)

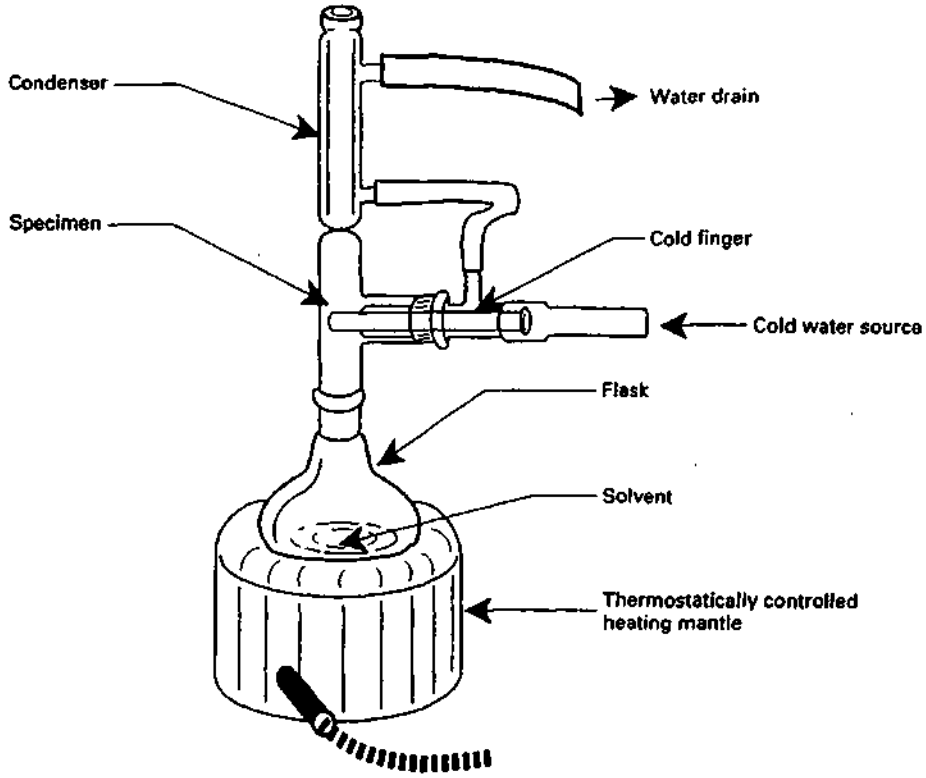


Figure 3 — Example of design of condensation washer

7.3.9 Slide warmer or oven

Use either a slide warmer or an oven for heating slides during the preparation of TEM specimens from MEC or cellulose nitrate filters. It is required to maintain a temperature of 65 °C to 70 °C.

7.3.10 Ultrasonic bath

An ultrasonic bath is necessary for cleaning the apparatus used for TEM specimen preparation.

7.3.11 Carbon grating replica

A carbon grating replica with about 2 000 parallel lines per millimetre shall be used to calibrate the magnification of the TEM.

7.3.12 Calibration specimen grids for EDXA

TEM specimen grids prepared from dispersions of calibration minerals are required for calibration of the EDXA system. Some suitable calibration minerals are riebeckite, chrysotile, halloysite, phlogopite, wollastonite and bustamite. The mineral used for calibration of the EDXA system for sodium shall be prepared using a gold TEM grid.

7.3.13 Carbon rod sharpener

The use of necked carbon rods, or equivalent, allows the carbon to be evaporated onto the filters with a minimum of heating.

7.3.14 Disposable tip micropipettes

A disposable tip micropipette, capable of transferring a volume of approximately 30 µl, is necessary for the preparation of TEM specimen grids from MEC filters.

7.4 Consumable supplies

7.4.1 Copper electron microscope grids

Copper TEM grids with 200 mesh are recommended. Grids which have grid openings of uniform size such that they meet the requirement specified in 9.6.2 shall be chosen. To facilitate the relocation of individual grid openings for quality assurance purposes, the use of grids with numerical or alphabetical indexing of individual grid openings is recommended.

7.4.2 Gold electron microscope grids

Gold TEM grids with 200 mesh are recommended to mount TEM specimens when sodium measurements are required in the fibre identification procedure. Grids which have grid openings of uniform size such that they meet the requirement specified in 9.6.2 shall be chosen. To facilitate the relocation of individual grid openings for quality assurance purposes, the use of grids with numerical or alphabetical indexing of individual grid openings is recommended.

7.4.3 Carbon rod electrodes

Spectrochemically pure carbon rods, shall be used in the vacuum evaporator (7.3.5) during carbon coating of filters.

7.4.4 Routine electron microscopy tools and supplies

Fine-point tweezers, scalpel holders and blades, microscope slides, double-coated adhesive tape, lens tissue, gold wire, tungsten filaments and other routine supplies are required.

7.4.5 Reference asbestos samples

Asbestos samples, shall be for preparation of reference TEM specimens of the primary asbestos minerals. The UICC set of minerals is suitable for this purpose.

8 Air sample collection

The desired analytical sensitivity is a parameter that shall be established for the analysis prior to sample collection. It is defined as the structure concentration corresponding to the detection of one structure in the analysis. For direct transfer methods of TEM specimen preparation, the analytical sensitivity is a function of the volume of air sampled, the active area of the collection filter, and the area of the TEM specimen over which structures are counted. If total airborne dust levels are high, it may be necessary to terminate sampling before the required volume has been sampled. If this happens, the analytical sensitivity required can be achieved only by counting structures on more grid openings, or by selective concentration of asbestos structures using an indirect TEM specimen preparation technique. Select the sampling rate and the period of sampling to yield the required analytical sensitivity, as detailed in table 1. Before air samples are collected, unused filters shall be analysed as described in 9.7 to determine the mean asbestos structure count for blank filters.

Air samples shall be collected using filter cassettes (7.1.1). During sampling, the cassette shall be supported on a stand (7.1.3) which is isolated from the vibrations of the pump (7.1.2). The cassette shall be held facing vertically downwards at a height of approximately 1,5 m to 2,0 m above ground/floor level, and shall be connected to the pump with a flexible tube.

Measure the sampling flow-rate at the front end of the cassette, both at the beginning and end of the sampling period, using a calibrated variable area flowmeter (7.1.4) temporarily attached to the inlet of the cassette. The mean value of these two measurements shall be used to calculate the total air volume sampled.

Basic strategies for monitoring environmental sources of airborne asbestos are described in annex G. After sampling, a cap shall be placed over the open end of the cassette, and the cassette packed with the filter face-upwards for return to the laboratory. Field blank filters shall also be included, as specified in 9.7, and submitted to the remaining analytical procedures along with the samples.

NOTES

5 In table 1 a collection filter area of 385 mm^2 is assumed, and the TEM grid openings are assumed to be 85 μm^2 square. The limit of detection is defined as the upper 95 % confidence limit of the Poisson distribution for a count of 0 structures. In the absence of background, this is equal to 2,99 times the analytical sensitivity. Backgrounds that are different from 0 observed during analysis of blank filters will degrade the limit of detection.

6 The analytical sensitivity S, expressed in number of structures per litre, is calculated using the following equation:

$$S = \frac{A_f}{kA_g V}$$

where

A_f is the active area, in square millimetres, of sample collection filter;

A_g is the mean area, in square millimetres, of grid openings examined;

k is the number of grid openings examined;

V is the volume of air sampled, in litres.

Table 1 — Examples of the minimum number of grid openings required to achieve a particular analytical sensitivity and limit of detection

Analytical sensitivity	Limit of detection	Volume of air sampled (litres)					
structures/l	structures/l	500	1 000	2 000	3 000	4 000	5 000
0,1	0,30	1 066	533	267	178	134	107
0,2	0,60	533	267	134	89	67	54
0,3	0,90	356	178	89	60	45	36
0,4	1,2	267	134	67	45	34	27
0,5	1,5	214	107	54	36	27	22
0,7	2,1	153	77	39	26	20	16
1,0	3,0	107	54	27	18	14	11
2,0	6,0	54	27	14	9	7	6
3,0	9,0	36	18	9	6	5	4
4,0	12	27	14	7	5	4	4
5,0	15	22	11	6	4	4	4
7,0	21	16	8	4	4	4	4
10	30	11	6	4	4	4	4

9 Procedure for analysis

9.1 General

The techniques used to prepare TEM specimens are different for polycarbonate and cellulose ester filters. The preparation method to be used shall be either 9.3 or 9.4, depending on the type of membrane filter used for air sampling. Cleaning of the sample cassettes before they are opened, preparation of the carbon evaporator, criteria for acceptable specimen grids, and the requirement for blank determinations are identical for the two preparation techniques. TEM examination, structure counting, fibre identification and reporting of results are independent of the type of filter or preparation technique used.

The ability to meet the blank sample criteria is dependent on the cleanliness of equipment and supplies. Consider all supplies such as microscope slides and glassware as potential sources of asbestos contamination. It is necessary to wash all glassware before it is used. Wash any tools or glassware which come into contact with the air sampling filters or TEM specimen preparations both before use and between handling of individual samples. Where possible, disposable supplies should be used.

9.2 Cleaning of sample cassettes

Asbestos fibres can adher to the exterior surfaces of air sampling cassettes, and these fibres can be inadvertently transferred to the sample during handling. To prevent this possibility of contamination, and after ensuring that the cassette is tightly sealed, wipe the exterior surfaces of each sampling cassette before it is placed in the clean facility or laminar flow hood.

9.3 Direct preparation of TEM specimens from polycarbonate filters

9.3.1 Selection of filter area for carbon coating

Use a cleaned microscope slide to support representative portions of polycarbonate filter during the carbon evaporation. Double-coated adhesive tape is used to attach the filter portions to the glass slide. Take care not to stretch the polycarbonate filters during handling. Using freshly cleaned tweezers, remove the polycarbonate filter from the sampling cassette, and place it on to a second cleaned glass microscope slide which is used as a cutting surface. Using a freshly cleaned curved scalpel blade, cut the filter by rocking the blade from the point, pressing it into contact with the filter. Repeat the process as necessary. Several such portions may be mounted on the same microscope slide. The scalpel blade and tweezers shall be washed and dried between the handling of each filter. Identify the filter portions by writing on the glass slide.

9.3.2 Carbon coating of filter portions

Place the glass slide holding the filter portions on the rotation-tilting device, approximately 10 cm to 12 cm

from the evaporation source, and evacuate the evaporator chamber (7.3.5) to a vacuum better than 0,013 Pa. The evaporation of carbon shall be performed in very short bursts, separated by a few seconds to allow the electrodes to cool. If evaporation of carbon is too rapid, the strips of polycarbonate filter will begin to curl, and cross-linking of the surface will occur. This cross-linking procedures a layer of polymer which is relatively insoluble in organic solvents, and it will not be possible to prepare satisfactory TEM specimens. The thickness of carbon required is dependent on the size of particles on the filter, and approximately 30 nm to 50 nm has been found to be satisfactory. If the carbon film is too thin, large particles will break out of the film during the later stages of preparation, and there will be few complete and undamaged grid openings on the specimen. Too thick a carbon film will lead to a TEM image which is lacking in contrast, and the ability to obtain ED patterns will be compromised. The carbon film thickness should be the minimum possible, while retaining most of the grid openings of the TEM specimen intact.

9.3.3 Preparation of the Jaffe washer

Place several pieces of lens tissue, as shown in figure 2, on the stainless steel bridge (7.1.3) and fill the washer (see 7.3.7) with chloroform (6.2) or 1-methyl-2-pyrrolidone (6.3) to a level where the meniscus contacts the underside of the mesh, resulting in saturation of the lens tissue.

9.3.4 Placing of specimens in the Jaffe washer

Using a curved scalpel blade, cut three 3 mm square pieces of carbon-coated polycarbonate filter form the carbon-coated filter portion. Select three squares to represent the centre and the periphery of the active surface of the filter. Place each square of filter, carbon side up, on a TEM specimen grid, and place the grid and filter on the saturated lens tissue in the Jaffe washer. Place the three specimen grids from one sample on the same piece of lens tissue. Any number of separate pieces of lens tissue may be placed in the same Jaffe washer. Cover the Jaffe washer with the lid, and allow the washer to stand for at least 8 h.

NOTE 7 It has been found that some polycarbonate filters will not completely dissolve in the Jaffe washer, even after exposure to chloroform for as long as 3 d. This problem is more severe if the surface of the filter was overheated during the carbon evaporation. It has been found that the problem of residual undissolved filter polymer can be overcome in several ways:

a) condensation washing of the grids, using chloroform as the solvent, after the initial Jaffe washer treatment, can often remove much of the residual filter medium in a period of approximately 30 min. To carry out this procedure, transfer the piece of lens tissue supporting the specimen grids to the cold finger of the condensation washer (7.3.8), which has achieved stable operating conditions. Operate the washer for approximately 30 min after inserting the grids;

b) used in a Jaffe washer, 1-methyl-2-pyrrolidone has been found to be a more effective solvent than chloroform for polycarbonate filters. This solvent is more effective if the lens paper is not used and grids are placed directly on the stainless steel mesh of the Jaffe washer. A dissolution period of 2 h to 6 h has been found to be satisfactory. After dissolution is complete, remove the stainless steel mesh from the Jaffe washer and allow the grids to dry. 1-methyl-2-pyrrolidone evaporates very slowly. If it is required to dry the grids more rapidly, transfer the stainless steel bridge into another Petri dish, and add water (6.1) until the meniscus contacts the underside of the mesh. After approximately 15 min, remove the mesh and allow the grids to dry. If it is desired to retain water-soluble particle species on the TEM grids, ethanol may be used instead of water (6.1) for the second wash;

c) a mixture of 20 % 1,2-diaminoethane [ethylenediamine] and 80 % 1-methyl-2-pyrrolidone, used in a Jaffe washer, completely dissolves polycarbonate filters in 15 min, even if the surface of the filter has been overheated. To use this solvent, place the grids directly on the stainless steel mesh of the Jaffe washer, do not use the lens paper. After a period of 15 min, transfer the stainless steel bridge into another Petri dish, and add water (6.1) until the meniscus contacts the underside of the mesh. After approximately 15 min, remove the mesh and allow the grids to dry. If it is desired to retain water-soluble particle species on the TEM grids, ethanol may be used instead of water (6.1) for the second wash.

9.3.5 Rapid preparation of TEM specimens from PC filters

TEM specimens can be prepared rapidly from PC filters, if desired, by washing for approximately 1 h in a Jaffe washer, followed by washing for 30 min in a condensation washer using chloroform as the solvent. The alternative filter dissolution procedures described in note 7 may also be used.

9.4 Direct preparation of TEM specimens from cellulose ester filters

9.4.1 Selection of area of filter for preparation

Using clean tweezers, remove the filter from the filter cassette, and place it on a cleaned microscope slide. Using a clean, curved scalpel blade, cut out a portion of the filter.

9.4.2 Preparation of solution for collapsing cellulose ester filters

Mix 35 ml of dimethylformamide (6.4), and 15 ml of glacial acetic acid (6.5) with 50 ml of water (6.1). Store this mixture in a clean bottle, The mixture is stable and suitable for use for up to 3 months after preparation.

9.4.3 Filter collapsing procedure

Using a micropipette with a disposable tip (7.3.14), place 15 $\mu l/cm^2$ to 25 $\mu l/cm^2$ of the solution prepared in 9.4.2 on a cleaned microscope slide, and using the end of the pipette tip, spread the liquid over the area to be occupied by the filter portion. Place the filter portion, active surface upwards, on top of the solution, lowering the edge of the filter at an angle of about 20° so that air bubbles are not created. Remove any solution not absorbed by the filter by allowing a paper tissue to contact the liquid at the edge of the filter. More than one filter portion may be placed on one slide. Place the slide either on a thermostatically controlled slide warmer (7.3.9) at a temperature of 65 °C to 70 °C, or in an oven (7.3.9) at this temperature, for 10 min. The filter collapses slowly to about 15 % of its original thickness. The procedure leaves a thin, transparent polymer film, with particles and fibres embedded in the upper surface.

9.4.4 Plasma etching of the filter surface

The optimum conditions and time for plasma etching (see 7.3.4) have been determined experimentally from the recovery of fine chrysotile fibrils on 0,8 µm pore size MEC filters. The conditions required in a particular plasma asher shall be established using the procedure specified in annex A. Place the microscope slide holding the collapsed filter portions in the plasma asher, and etch for the time and under the conditions determined. Take care to ensure that the correct conditions are respected. After etching, admit air slowly to the chamber and remove the microscope slide.

Adjust the air admission valve of the plasma asher such that the time taken for the chamber to reach atmospheric pressure exceeds 2 min. Rapid air admission may disturb particulates on the surface of the etched filter.

9.4.5 Carbon coating

Coat the microscope slide holding the collapsed filter portions with carbon as specified in 9.3.2.

9.4.6 Preparation of the Jaffe washer

Place several pieces of lens tissue on the stainless steel bridge, and fill the washer with dimethylformamide (6.4) or acetone (6.6) to a level where the meniscus contacts the underside of the mesh, resulting in saturation of the lens tissue.

9.4.7 Placing of specimens in the Jaffe washer

Place the specimens in the Jaffe washer as specified in 9.3.4. Specimens are normally cleared after approximately 4 h.

9.4.8 Rapid preparation of TEM specimens from cellulose ester filters

An alternative washing procedure may be used to prepare TEM specimens from cellulose ester filters more rapidly than can be achieved by the Jaffe washing procedure. After the specimens have been washed in a Jaffe washer for approximately 1 h, transfer the piece of lens tissue supporting the specimens to the cold finger of a condensation washer (7.3.8) operating with acetone as the solvent because dimethylformamide shall not be used in a condensation washer. Operate the condensation washer for approximately 30 min. This treatment removes all the remaining filter polymer.

9.5 Criteria for acceptable TEM specimen grids

Valid data cannot be obtained unless the TEM specimens meet specified quality criteria. Examine the TEM specimen grid in the electron microscope at a sufficiently low magnification (× 300 to × 1 000) for complete grid openings to be inspected. Reject the grid if

a) the TEM specimen has not been cleared of filter medium by the filter dissolution step. If the TEM specimen exhibits areas of undissolved filter medium, and if at least two of the three specimen grids are not cleared, either additional washing with solvent shall be carried out, or new specimens shall be prepared from the filter;

b) the sample is overloaded with particulate. If the specimen grid exhibits more than approximately 10 % obscuration on the majority of the grid openings, the specimen shall be designated as overloaded. This filter cannot be alanysed satisfactorily using the direct preparation methods because the grid is too heavily loaded with debris to allow separate examination of individual particles by ED and EDXA, and obscuration of fibres by

other particulates may lead to underestimation of the asbestos structure count;

c) the particulate deposits on the specimen are not uniformly distributed from one grid opening to the next. If the particulate deposits on the specimen are obviously not uniform from one grid opening to the next, the specimen shall be designated as non-uniform. This condition is a function either of the air sampling conditions or of the fundamental nature of the airborne particulate. Satisfactory analysis of this filter may not be possible unless a large number of grid openings is examined;

d) the TEM grid is too heavily loaded with fibrous structures to make an accurate count. Accurate counts cannot be made if the grid has more than approximately 7 000 structures/mm^2; or

e) more than approximately 25 % of the grid openings have broken carbon film over the whole grid opening. Since the breakage of carbon film is usually more frequent in areas of heavy deposit, counting of the intact openings can lead to an underestimate of the asbestos structure count.

NOTE 8 If the specimens are rejected because unacceptable numbers of grid openings exhibit broken carbon replica, an additional carbon coating may be applied to the carbon coated filter, and new specimen grids prepared. The larger particles can often be supported by using a thicker carbon film. If this action does not produce acceptable specimen grids, this filter cannot be analysed using the direct preparation methods.

If one or more of the conditions described in b), c), d) or e) exists, it may not be possible to analyse the sample by this method.

9.6 Procedure for structure counting by TEM

9.6.1 General

The examination consists of a count of asbestos structures which are present on a specified number of grid openings. Fibres are classified into groups on the basis of morphological observations, ED patterns and EDXA spectra. The total number of structures to be counted depends on the statistical precision desired. In the absence of asbestos structures, the area of the TEM specimen grids which must be examined depends on the analytical sensitivity required. The precision of the structure count depends not only on the total number of structures counted, but also on their uniformity from one grid opening to the next. Additional structure counting will be necessary if greater precision is required.

In order that the estimate of the structure density on the sampling filter shall not be based on the small area represented by one specimen grid, grid openings shall be examined on two of the three specimen grids prepared. Then combine the results in the calculation of the structure density. Structure counts shall be made at a magnification of approximately × 20 000, and shall be terminated at the end of the examination of the grid opening on which the 100th asbestos structure is observed, except that the count shall be continued until a minimum of 4 grid openings have been examined. Otherwise, the structure count shall continue to that number of grid openings at which the specified analytical sensitivity has been achieved.

NOTE 9 The normal range for the number of grid openings which should be examined is from 4 to 20. If insufficient air has been sampled through the filter, the calculation in 9.6.4 can indicate that an impractically large number of grid openings should be examined. When this situation occurs, a larger value of analytical sensitivity may have to be accepted.

9.6.2 Measurement of mean grid opening area

The mean grid opening area shall be measured for the type of TEM specimen grids in use. The standard deviation of the mean of 10 openings selected from 10 grids should be less than 5 %. As an optional procedure, or if the 5 % standard deviation criterion cannot be demonstrated, the dimensions of each grid opening examined in the TEM shall be measured at a calibrated magnification.

9.6.3 TEM alignment and calibration procedures

Before structure counting is performed, align the TEM according to instrumental specifications. Calibrate the TEM and EDXA system according to the procedures specified in annex B.

9.6.4 Determination of stopping point

Before structure counting is begun, calculate the area of specimen to be examined in order to achieve the selected analytical sensitivity. Calculate the maximum number of grid openings to be examined using the following equation:

$$k = \frac{A_f}{A_g V S}$$

where

k is the number of grid openings to be examined, rounded upwards to the next highest integer;

A_f is the area, in square millimetres, of sample filter;

A_g is the area, in square millimetres, of TEM specimen grid opening;

V is the volume of air sampled, in litres;

S is the required analytical sensitivity, expressed in number of structures per litre.

9.6.5 General procedure for structure counting and size analysis

Use at least two specimen grids prepared from the filter in the structure count. Select at random several grid openings from each grid, and combine the data in the calculation of the results.

Use a form similar to that shown in figure 4 to record the data. Insert the first specimen grid into the TEM.

NOTE 10 In order to facilitate quality assurance measurements which require re-examination of the same grid opening by different microscopists, the grid should be inserted into the specimen holder in a standard orientation with the grid bars parallel and perpendicular to the axis of the specimen holder. This will provide scan directions parallel to the edges of the grid opening. It should be ensured that all microscopists begin scanning at the same starting point on the grid opening, and that they use similar scan patterns. This procedure permits rapid relocation of fibrous structures for further examination if necessary.

Select a typical grid opening and set the screen magnification to the calibrated value (approximately × 20 000). Adjust the sample height until the features in the centre of the TEM viewing screen are at the eucentric point. Set the goniometer tilt angle to zero. In column 1 of the data recording form, record the number or letter used to identify the grid. In column 2, record the identification of the particular grid opening. Position the specimen so that the grid opening is positioned with one corner visible on the screen. Move the image by adjustment of only one translation control, carefully examining the sample for fibres, until the opposite side of the grid opening is encountered. Move the image by a predetermined distance less than one screen diameter, using the other translation control, and scan the image in the reverse direction. Continue the procedure in this manner until the entire grid opening has been inspected in a pattern similar to that shown in figure 5. When a fibrous structure is detected, assign a sequential number to the primary structure in column 3, perform the identification procedures required as detailed in annex E, and enter the appropriate compositional classification on the structure counting form in column 5. Assign a morphological classification to the structure according to the procedures specified in annex D, and record this in column 6. Measure on the TEM viewing screen the length and width of the image of the primary structure, in millimetres, and record these measurements in columns 7 and 8. For a disperse cluster or matrix, assign a compositional classification and a morphological classification to each structure component, measure the length and width, and enter the data in columns 4 to 8. Use column 4 of the data recording form to tabulate the sequential number of total structures taking into account structure components. If non-asbestos fibres are observed, note their presence and type, if known. After a fibrous structure has been examined and measured, relocate the original field of view accurately before continuing scanning of the specimen. Failure to do this may cause structures to be overlooked or counted twice. Continue the examination until the completion of the grid opening on which the 100th asbestos structure has been recorded, or until the number of grid openings required to achieve the specified analytical sensitivity, calculated according to 9.6.4, have been examined whichever occurs first. The data shall be drawn approximately equally from a minimum of two grids. Regardless of the value calculated according to 9.6.4, fibrous structures on a minimum of four openings shall be counted.

9.6.6 Measurement of concentration for asbestos fibres and bundles longer than 5 µm

Consider improving the statistical validity for measurement of asbestos fibres and bundles longer than 5 µm by additional examination at a lower magnification, taking account only of the longer fibres and bundles. Perform this extended examination for fibres and bundles longer than 5 µm in accordance with the procedures specified in annex E. Use a magnification of approximately × 10 000 for counting all asbestos fibres and bundles longer than 5 µm, or approximately × 5 000 if only fibres and bundles within the diameter range 0,2 µm to 3,0 µm are to be counted. Continue the count until completion of the grid opening on which 100 fibres and bundles have been recorded, or until a sufficient area of the specimen has been examined to achieve the desired analytical sensitivity. Only those structures which are identified as, or are suspected to be, either chrysotile or one of the amphibole minerals will be reported in either the original or the extended TEM examination. Other materials, such as gypsum, cellulose fibres, and filter artifacts such as undissolved filter strands, will not be included in the fibre count. This restriction is intended to ensure that the best statistical validity is obtained for the materials of interest.

TEM asbestos structure count (page of)

Report number: .....
Sample number: .....
File name: .....
Sample description: .....
.....
.....
Preparation date: ..... By: .....
Analysis date: ..... By: .....
Computer entry date: ..... By: .....

Air volume: ..... litres
Sample filter area: ..... mm^2
Magnification: .....
Grid opening dimension: ..... µm
Level of analysis (C): .....
(A): .....

Grid	Grid opening	Number of structures		Class	Type of structure	Length	Width	Comments
		primary	total			mm	mm	

Figure 4 — Example of structure counting form

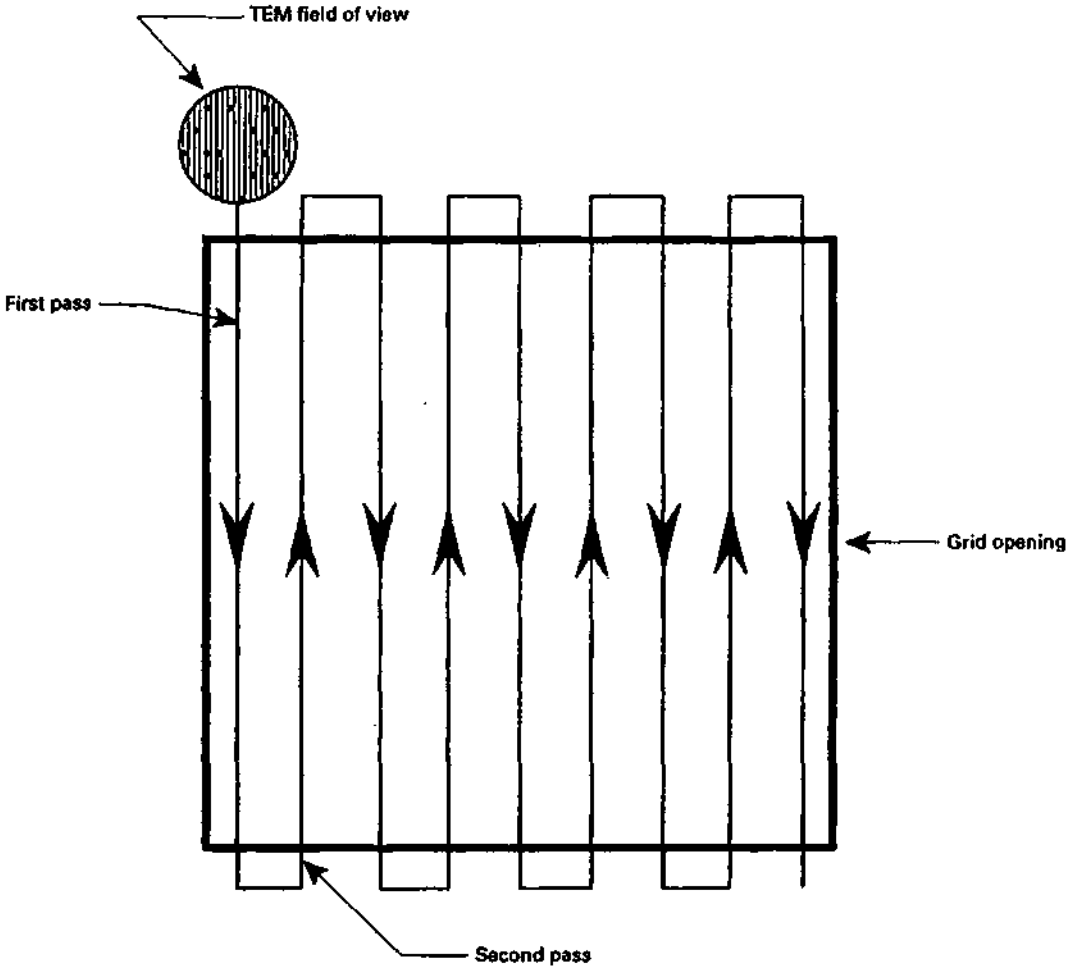


Figure 5 — Example of scanning procedure for TEM specimen examination

9.7 Blank and quality control determinations

Before air samples are collected, a minimum of two unused filters from each filter lot of 100 filters shall be analysed to determine the mean asbestos structure count. If the mean count for all types of asbestos structures is found to be more than 10 structures/mm^2, or if the mean fibre count for asbestos fibres and bundles longer than 5 µm is more than 0,1 fibre/mm^2, reject the filter lot.

To ensure that contamination by extraneous asbestos fibres during specimen preparation is insignificant compared with the results reported on samples, establish a continuous programme of blank measurements. At least one field blank shall be processed along with each batch of samples. In addition, at least one unused filter shall be included with every group of samples prepared on one microscope slide.

Initially, and also at intervals afterwards, ensure that samples of known asbestos concentrations can be analysed satisfactorily. Since there is a subjective component in the structure counting procedure, it is necessary that recounts of some specimens be made by different microscopists, in order to minimize the subjective effects. Such recounts provide a means of maintaining comparability between counts made by different microscopists. Variability between and within microscopists and between laboratories shall be characterized. These quality assurance measurements shall constitute approximately 10 % of the analyses. Repeat results should not differ at the 5 % significance level.

9.8 Calculation of results

Calculate the results using the procedures detailed in annex F. Prior to the TEM examination of the specimens, the level of analysis was specified. Before the results are calculated, the compositional and morphological classifications to be included in the result shall be specified. The chi-squared uniformity test shall be conducted using the number of primary asbestos structures found on each grid opening, prior to the application of the cluster and matrix counting criteria. The concentration result shall be calculated using the numbers of asbestos structures reported after the application of the cluster and matrix counting criteria.

10 Performance characteristics

10.1 General

It is important to use this analytical method in conjunction with a continuous quality control programme. The quality control programme should include use of standard samples, blank samples, and both interlaboratory and intralaboratory analyses.

10.2 Interferences and limitations of fibre identification

Unequivocal identification of every chrysotile fibre is not possible, due to both instrumental limitations and the nature of some of the fibres. The requirement for a calibrated ED pattern eliminates the possibility of an incorrect identification of the fibre selected. However, there is a possibility of misidentification of fibres for which both the morphologies and the ED patterns are reported on the basis of visual inspection only. 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 ED pattern.

As in the case of chrysotile fibres, complete identification of every amphibole fibre is not possible due to instrumental limitations and the nature of some of the fibres. Moreover, complete identification of every amphibole fibre is not practical due to the limitations of both time and cost. Particles of a number of other minerals with compositions similar to those of some amphiboles could be erroneously classified as amphibole when the classification criteria do not include zone-axis ED techniques. However, the requirement for quantitative EDXA measurements on all fibres as support for the random orientation ED technique makes misidentification very unlikely, particularly when other similar fibres in the same sample have been identified as amphibole by zone-axis methods. The possibility of misidentification is further reduced with increasing aspect ratio, since it is rare for the minerals with which amphibole may be confused to display an asbestiform habit.

10.3 Precision and accuracy (see ISO Standard Handbook No. 3)

10.3.1 Precision

The analytical precision that can be obtained is dependent upon the number of structures counted, and also on the uniformity of the particulate deposit on the original filter. Assuming that the structures are randomly deposited on the filter, if 100 structures are counted and the loading is at least 3,5 structures/grid opening, computer modelling of the counting procedure shows that a coefficient of variation of about 10 % can be expected. As the number of structures counted decreases, the precision will also decrease approximately as $\sqrt{N}$, where N is the number of structures counted. In practice, particulate deposits obtained by filtration of ambient air samples are rarely ideally distributed, and it is found that the precision is correspondingly reduced. Degradation of precision is a consequence of several factors, such as:

a) non-uniformity of the filtered particulate deposit;

b) distorsion of the fibre distribution by application of the structure counting criteria;

c) variation between microscopists in their interpretation of the fibrous structures;

d) variation between microscopists in their ability to detect and identify fibres.

The 95 % confidence interval about the mean for a single structure concentration measurement using this analytical method should be approximately ± 25 % when 100 structures are counted over 10 grid openings.

10.3.2 Accuracy

There is no independent method available to determine the accuracy.

NOTE 11 It has been demonstrated that, after polycarbonate membrane filters have been coated with carbon, particulate material is transferred to the TEM specimens without measurable losses. However, if the filters are heavily loaded by particulate material, some of this may be lost before they are coated with carbon. Good comparability between the capillary-pore polycarbonate pro-

cedure and the cellulose ester filter procedure has been demonstrated for laboratory-generated aerosols of chrysotile asbestos.

10.3.3 Interlaboratory and intralaboratory analyses

Interlaboratory and intralaboratory analyses are required in order to monitor systematic errors that may develop among microscopists when using this method. These analyses should be designed to test both the overall method and the performance of individual microscopists. Repeating preparation of TEM grids from different sectors of a filter, followed by examination of the grids by a different microscopist, is a test for the reproducibility of the whole method. However, non-uniformity of the particulate deposit on the filter may lead to differences which are not related to the performance of the microscopists. Verified fibre counting (counting of asbestos structures on the same grid opening of a TEM grid by two or more operators, followed by resolution of any discrepancies) may be used both as a training aid and to determine the performance of different microscopists. The use of indexed TEM grids as described in 7.4.1 and 7.4.2 is recommended in order to facilitate relocation of specific grid openings.

10.4 Limit of detection

The limit of detection of the method can be varied by choice of the area of the collection filter, the volume of air sampled and the area of the specimen examined in the TEM. It is also a function of the background of asbestos structures on unused filters. A limit of detection shall be quoted for each sample analysis.

NOTE 12 In practice, the lowest limit of detection is frequently determined by the total suspended particulate concentration, since each particle on the filter must be separated from adjacent ones by a distance large enough for the particle to be identified without interference. Particulate loadings on sampling filters greater than 25 $\mu g/cm^2$ usually preclude preparation of TEM specimens by the direct methods. If the analysis is to be performed with an acceptable expenditure of time, the area of the specimen examined in the TEM for structures of all sizes is limited in most cases to between 10 and 20 grid openings. In typical ambient or building atmospheres, it has been found that an analytical sensitivity of 1 structure/l can be achieved. In some circumstances, where the atmosphere is exceptionally clean, this can be reduced to 0,1 structure/l or lower. For fibres and bundles longer than 5 µm, the reduced magnifications specified permit larger areas of the TEM specimens to be examined with an acceptable expenditure of time, resulting in proportionately lower limits of detection. If no structures are found in the analysis, the upper 95 % confidence limit can be quoted as the upper boundary of the concentration, corresponding to 2,99 times the analytical sensitivity if a Poisson distribution of structures on the filter is assumed. This 95 % confidence limit for 0 structures counted is taken as the detection limit. Since there is sometimes contamination of unused samples filters by asbestos structures, this should also be taken into account in the discussion of limits of detection.

11 Test report

The test report shall include at least the following information:

a) reference to this International Standard;

b) identification of the sample;

c) the date and time of sampling, and all necessary sampling data;

d) the date of the analysis;

e) the identity of the analyst;

f) any procedure used that is not specified in this International Standard or regarded as optional;

g) a complete listing of the structure counting data (the following data should be included: grid opening number, structure number, identification category, structure type, length and width of the structure in micrometres, and any comments concerning the structure);

h) a statement of the minimum acceptable identification category and the maximum identification category attempted (refer to tables D.1 and D.2);

i) a statement specifying which identification and structure categories have been used to calculate the concentration values;

j) separate concentration values for chrysotile and amphibole structures, expressed in number of asbestos structures per litre;

k) the 95 % confidence interval limits for the concentration values, expressed in number of asbestos structures per litre;

l) the analytical sensitivity, expressed in number of asbestos structures per litre;

m) the limit of detection, expressed in number of asbestos structures per litre;

n) compositional data for the principal varieties of amphibole, if present;

o) items g) to m) for asbestos fibres and bundles longer than 5 µm;

p) items g) to m) for PCM equivalent asbestos fibres and bundles.

An example of a suitable format for the structure counting data is shown in figures 6 and 7.

Sample analysis information (page 1)

Laboratory name		**Report number**	**Date**
Sample:	456 Queen Street Ashby de la Zouch Exterior sample 1991-09-09		

Air volume:	2 150,0 litres
Area of collection filter:	385,0 mm^2
Level of analysis (chrysotile):	CD or CMQ
Level of analysis (amphibole):	ADQ
Magnification used for fibre counting:	× 20 500
Aspect ratio for fibre definition:	5/1
Mean dimension of grid openings:	95,4 µm
Initials of analyst:	JMW
Number of grid openings examined:	10
Analytical sensitivity:	1,968 structures/l
Number of primary asbestos structures:	13
Number of asbestos structures counted:	26
Number of asbestos structures > 5 µm :	7
Number of asbestos fibres and bundles > 5 µm :	10
Number of PCM equivalent asbestos structures:	3
Number of PCM equivalent asbestos fibres:	5

Figure 6 — Example of format for reporting sample and preparation data

Sample analysis information (pages 2 and following)

Laboratoriy name **Report number** **Date**

Sample: 456 Queen Street
Ashby de la Zouch
Exterior sample 1991-09-09

TEM asbestos structure count — Raw data

Grid	Grid opening	Number of structures		Identification[1]	Structure type	Length	Width	Comments
		primary	total			µm	µm	
A	F4-4	1	1	CD	F	1,7	0,045	
		2	2	CMQ	B	2,6	0,09	
		3	3	ADQ	F	4,0	0,15	Crocidolite
	E3-6	4	4	CD	MC+0	3,5	1,3	
	E5-1	5		CD	MD43	7,5	5,0	
			5	CD	MB	7,7	0,30	
			6	CMQ	MF	5,6	0,045	
			7	CD	MB	5,1	0,30	
			8	CD	MF	1,7	0,045	
B	F4-1	6		CD	CD+0	6,5	3,0	
			9	CD	CB	3,5	0,15	
			10	CD	CF	3,5	0,045	
			11	CMQ	CR+0	2,6	1,9	
	G5-1	7		CD	CD31	6,1	3,2	
			12	CD	CB	5,6	0,3	
			13	CMQ	CF	4,0	0,045	
			14	CMQ	CB	3,2	0,090	
	E4-4	8	15	CD	B	1,5	0,23	
		9	16	AD	F	8,7	0,15	
C	G4-4	10		CMQ	CD42	25	5,6	
			17	CMQ	CB	15	0,15	
			18	CMQ	CF	9,4	0,045	
			19	ADQ	CF	3,6	0,30	Tremolite
			20	CM	CF	4,2	0,045	
	E4-4			No fibres				
	E5-6	11		ADQ	CD+3	9,4	2,5	
			21	ADQ	CF	7,1	0,30	Amosite
			22	ADQ	CF	6,2	0,10	Crocidolite
			23	CM	CB	5,1	0,2	
			24	CM	CR+0	3,3	1,8	
	F4-1	12	25	CMQ	MC10	3,7	2,1	
		13	26	CD	CC+0	7,4	0,5	

1) Identification codes listed in tables D.1 and D.2.

Figure 7 — Example of format for reporting structure counting data

Annex A
(normative)

Determination of operating conditions for plasma asher

A.1 General

During the preparation of TEM specimens from an MEC or cellulose nitrate filter, the spongy structure of the filter is collapsed into a thinner film of polymer by the action of a solvent. Some of the particles on the surface of the original filter become completely buried in the polymer, and the specimen preparation procedure incorporates a plasma etching step to oxidize the surface layer of the polymer. Particles buried by the filter collapsing step are then exposed so that they can become subsequently affixed to the evaporated carbon film without altering their position on the original filter. The amount of etching is critical, and individual ashers vary in performance. Therefore, the plasma asher (7.3.4) shall be calibrated to give a known amount of etching of the surface of the collapsed filter. This is carried out by adjusting the radio-frequency power output and the oxygen flow-rate, and measuring the time taken to completely oxidize an uncollapsed cellulose ester filter with 25 mm diameter of the same type and pore size as those used in the analysis.

A.2 Procedure

Place an unused cellulose ester filter, with 25 mm diameter, of the same type as that being used, in the centre of a glass microscope slide. Position the slide approximately in the centre of the asher chamber. Close the chamber and evacuate to a pressure of approximately 40 Pa, while admitting oxygen to the chamber at a rate of 8 ml/min to 20 ml/min. Adjust the tuning of the system so that the intensity of the plasma in maximized. Measure the time required for complete oxidation of the filter. Determine operating parameters which result in complete oxydation of the filter in a period of approximately 15 min. For etching of collapsed filters, these operating parameters shall be used for a period of 8 min.

NOTE 13 Plasma oxidation at high radio-frequency powers will cause the filter to shrink and curl, followed by sudden violent ignition. At lower powers, the filter will remain in position and will slowly become thinner until it is nearly transparent. It is recommended that a radio-frequency power be used such that violent ignition does not occur. When multiple filters are etched, the rate of etching is reduced, and the system should be calibrated accordingly.

Annex B
(normative)

Calibration procedures

B.1 Calibration of the TEM

B.1.1 Calibration of TEM screen magnification

The electron microscope should be aligned according to the specifications of the manufacturer. Initially, and at regular intervals, calibrate the magnifications used for the analysis using a diffraction grating replica (7.3.11). Adjust the specimen height to the eucentric position before carrying out the calibration. Measure the distance on the fluorescent viewing screen occupied by a convenient number of repeat distances of the grating image, and calculate the magnification. Always repeat the calibration after any instrumental maintenance or change of operating conditions. The magnification of the image on the viewing screen is not the same as that obtained on photographic plates or film. The ratio between these is a constant value for the particular model of TEM.

B.1.2 Calibration of ED camera constant

Calibrate the camera constant of the TEM when used in ED mode. Use a specimen grid supporting a carbon film on which a thin film of gold has been evaporated or sputtered. Form an image of the gold film with the specimen adjusted to the eucentric position and select ED conditions. Adjust the objective lens current to optimize the pattern obtained, and measure the diameters of the innermost two rings either on the fluorescent viewing screen or on a recorded image. Calculate the radius-based camera constant, λL, for both the fluorescent screen and the photographic plate or film, using the following equation:

$$\lambda L = \frac{aD}{2{,}0\sqrt{h^2 + k^2 + l^2}}$$

where

λ is the wavelength, in nanometres, of the incident electrons;

L is the camera length, in millimetres;

a is the unit cell dimension of gold, in nanometres (= 0,407 86 nm);

D is the diameter, in millimetres, of the (hkl) diffraction ring.

Using gold as the calibration material, the radius-based camera constant is given by

$\lambda L = 0{,}117\ 74D$ mm·nm (smallest ring)

$\lambda L = 0{,}101\ 97D$ mm·nm (second ring)

B.2 Calibration of the EDXA system

Energy calibration of the EDXA system for a low energy and high energy peak shall be performed regularly. Calibration of the intensity scale of the EDXA system permits quantitative composition data, at an accuracy of about 10 % of the elemental concentration, to be obtained from EDXA spectra of reference silicate minerals involving the elements Na, Mg, Al, Si, K, Ca, Mn and Fe, and applicable certified reference materials. If quantitative determinations are required for minerals containing other elements, reference standards other than those referred to below will be required. Well-characterized mineral standards permit calibration of any TEM-EDXA combination which meets the instrumental specifications of 7.3.1 and 7.3.2, so that EDXA data from different instruments can be compared. Reference minerals are required for the calibration; the criteria for selection being that they should be silicate minerals with matrices as close as possible to those of the amphiboles or serpentine, and that small individual fragments of the minerals are homogeneous in composition within a few percent.

Determine the compositions of these standards by electron microprobe analysis or chemicals methods. Crush fragments of the same selected mineral standards and prepare filters by dispersal of the crushed material in water and immediate filtration of the suspensions. Prepare TEM specimens from these filters according to the procedures specified in clause 9. These TEM specimens can then be used to calibrate any TEM-EDXA system so that comparable composi-

tional results can be obtained from different instruments.

NOTES

14 The microprobe analysis of the mineral standards are carried out by conventional techniques which can be found in annex J. The mineral is first embedded in a mount of poly(methyl methacrylate) or epoxy resin. The mount is then ground and polished to achieve a flat, polished surface of the mineral fragment. This surface is then analysed, using suitable reference standards, preferably oxide standards of the individual elements wherever these are available. It is necessary to take into account the water concentration in the minerals, which in the case of chrysotile amounts to 13 % by mass. This water content may vary due to losses in the vacuum system.

15 Aqueous suspensions of mineral standards should be filtered immediately after preparation, since alkali and alkali earth metals may be partially leached from minerals containing these elements.

Express the results of the electron microprobe analyses as atomic or mass percentage ratios relative to silicon. X-ray peak ratios of the same elements relative to silicon, obtained from the EDXA system, can then be used to calculate the relationship between peak area ratio and atomic or mass percentage ratio. The technique was described by Cliff and Lorimer (see annex J, reference [8]).

The X-rays generated in a thin specimen by an incident electron beam have a low probability of interacting with the specimen. Thus, mass absorption and fluorescence effects are negligible. In a silicate mineral specimen containing element i, the following equation can be used to perform quantitative analyses in the TEM:

$$\frac{C_i}{C_{Si}} = k_i \times \frac{A_i}{A_{Si}}$$

where

C_i is the concentration or atomic percentage of element i;

C_{Si} is the concentration or atomic percentage of silicon;

A_i is the elemental integrated peak area for element i;

A_{Si} is the elemental integrated peak area for silicon;

k_i is the k-ratio for element i relative to silicon.

For a particular instrumental configuration and a particular particle size, the value of k_i is constant.

To incorporate correction for the particle size effect on peak area ratios (see annex J, references [35] and [36]), extend the Cliff and Lorimer technique by obtaining separate values of the constant k_i for different ranges of fibre diameter. It is recommended that 20 EDXA measurements be made for each range of fibre diameters. Suitable ranges of fibre diameter are:

$< 0{,}25$ µm; 0,25 µm to 0,5 µm; 0,5 µm to 1,0 µm; $> 1{,}0$ µm.

Insert the TEM grid into the transmission electron microscope, obtain an image at the calibrated higher magnification of about × 20 000, and adjust the specimen height to the eucentric point. If the X-ray detector is a side-entry variety, tilt the specimen towards the X-ray detector. Select an isolated fibre or particle less than 0,5 µm in width, and accumulate an EDXA spectrum using an electron probe of suitable diameter. When a well-defined spectrum has been obtained, perform a background subtraction and calculate the background-corrected peak areas for each element listed, using energy windows centred on the peaks. Calculate the ratio of the peak area for each specified element relative to the peak area for silicon. All background-subtracted peak areas used for calibration shall exceed 400 counts.

Repeat this procedure for 20 particles of each mineral standard. Reject analyses of any obviously foreign particles. Calculate the arithmetic mean concentration to peak area ratio, k_i (k-ratio), for each specified element of each mineral standard and for each of the fibre diameter ranges. Periodic routine checks shall be carried out to ensure that there has been no degradation of the detector performance. These k-ratios are used to calculate the elemental concentrations of unknown fibres, using the Cliff and Lorimer relationship.

Annex C
(normative)

Structure counting criteria

C.1 General

In addition to isolated fibres, other assemblages of particles and fibres frequently occur in air samples. Groupings of asbestos fibres and particles, referred to as "asbestos structures", are defined as fibre bundles, clusters and matrices. The numerical result of a TEM examination depends largely on whether the analyst assigns such an assemblage of fibres as a single entity, or as the estimated number of individual fibres which form the assemblage. It is therefore important that a logical system of counting criteria be defined, so that the interpretation of these complex structures is the same for all analysts, and so that the numerical result is meaningful. Imposition of specific structure-counting criteria generally requires that some interpretation, partially based on uncertain information on health effects, be made of each asbestos structure found. It is not the intention of this International Standard to make any interpretations based on health effects, and it is intended that a clear separation shall be made between recording of structure counting data, and later interpretation of those data. The system of coding specified in this International Standard permits a clear morphological description of the structures to be recorded in a concise manner suitable for later interpretation, if necessary, by a range of different criteria, without the necessity for re-examination of the specimens. In particular, the coding system is designed to permit the dimensions of each complex fibrous structure, and also whether these structures contain fibres longer than 5 µm, to be recorded. This approach permits later evaluations of the data to include considerations of particle respirability and comparisons with historical indices of asbestos exposure. Examples of the various types of morphological structure, and the manner in which these shall be recorded, are shown in figure C.1.

C.2 Structure definitions and treatment

Each fibrous structure that is a separate entity shall be designated as a primary structure. Each primary structure shall be designated as a fibre, bundle, cluster or matrix.

C.2.1 Fibre

Any particle with parallel or stepped sides, of minimum length 0,5 µm, and with an aspect ratio of 5/1 or greater, shall be defined as a fibre. For chrysotile asbestos, the single fibril shall be defined as a fibre. A fibre with stepped sides shall be assigned a width equal to the average of the minimum and maximum widths. This average shall be used as the width in determination of the aspect ratio.

C.2.2 Bundle

A grouping composed of apparently attached parallel fibres shall be defined as a bundle, with a width equal to an estimate of the mean bundle width, and a length equal to the maximum length of the structure. The overall aspect ratio of the bundle may have any value, provided that it contains individual constituent fibres with aspect ratios equal to or greater than 5/1. Bundles may exhibit diverging fibres at one or both ends.

C.2.3 Cluster

An aggregate of two or more randomly oriented fibres, with or without bundles, shall be defined as a cluster. Clusters occur as two varieties.

C.2.3.1 disperse cluster (type D): Disperse and open network, in which at least one of the individual fibres or bundles can be separately identified and its dimensions measured;

C.2.3.2 compact cluster (type C): Complex and tightly bound network, in which one or both ends of each individual fibre or bundle is (are) obscured, such that the dimensions of individual fibres and bundles cannot be unambiguously determined.

In practice, clusters can occur in which the characteristics of both types of cluster occur in the same structure. Where this occurs, the structure should be defined as a disperse cluster, and then a logical procedure should be followed by recording structure components according to the counting criteria. The procedure for treatment of clusters is illustrated by examples in figure C.2.

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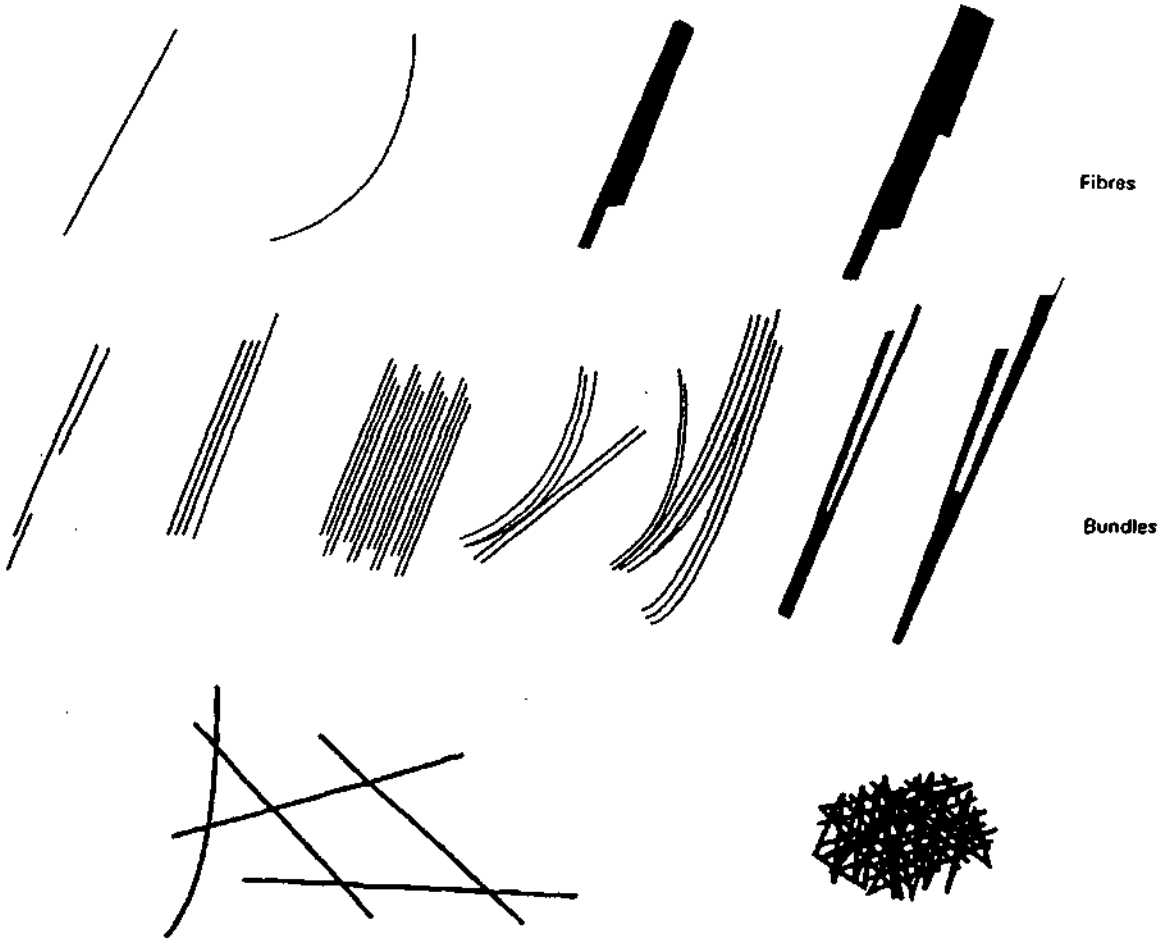


a) Disperse cluster (type D)

b) Compact cluster (type C)

c) Disperse matrix (type D)

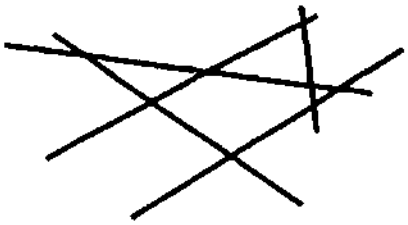
d) Compact matrix (type C)

Figure C.1 — Fundamental morphological structure types

5 µm

Count as 1 compact cluster containing more than 9 fibres (all fibres shorter than 5 µm)

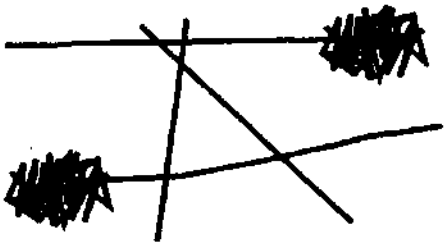
Record as CC+0



5 µm

Count as 1 disperse cluster consisting of 5 fibres, 4 of which are longer than 5 µm

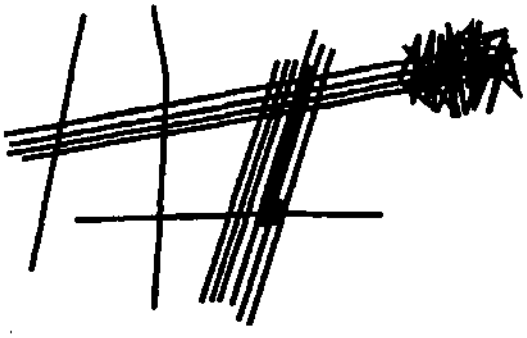
Record as CD54, followed by 5 fibres, each recorded as CF



5 µm

Count as 1 disperse cluster consisting of 4 fibres, 2 of which are longer than 5 µm, and 2 cluster residuals, each containing more than 9 fibres

Record as CD+2, followed by 4 fibres, each recorded as CF, and 2 cluster residual, each recorded as CR+0



5 µm

Count as 1 disperse cluster consisting of 3 fibres, 2 bundles, 1 of which is longer than 5 µm, and 1 cluster residual containing more than 9 fibres

Record as CD+1, followed by 3 fibres, each recorded as CF, 2 bundles, each recorded as CB, and 1 cluster residual recorded as CR+0

Figure C.2 — Examples of recording of complex asbestos clusters

C.2.4 Matrix

One or more fibres, or fibre bundles, may be attached to, or partially concealed by, a single particle or group of overlapping nonfibrous particles. This structure shall be defined as a matrix. The TEM image does not discriminate between particles which are attached to fibres, and those which have by chance overlapped in the TEM image. It is not known, therefore, whether such a structure is actually a complex particle, or whether it has arisen by a simple overlapping of particles and fibres on the filter.

Since a matrix structure may involve more than one fibre, it is important to define in detail how matrices shall be counted. Matrices exhibit different characteristics, and two types can be defined.

C.2.4.1 disperse matrix (type D): Structure consisting of a particle or linked group of particles, with overlapping or attached fibres or bundles in which at least one of the individual fibres or bundles can be separately identified and its dimensions measured.

C.2.4.2 compact matrix (type C): Structure consisting of a particle or linked group of particles, in which fibres or bundles can be seen either within the structure or projecting from it, such that the dimensions of individual fibres and bundles cannot be unambiguously determined.

In practice, matrices can occur in which the characteristics of both types of matrix occur in the same structure. Where this occurs, the structure should be assigned as a disperse matrix, and then a logical procedure should be followed by recording structure components according to the counting criteria. Examples of the procedure which shall be followed are shown in figure C.3.

C.2.5 Asbestos structure larger than 5 µm

Any fibre, bundle, cluster or matrix for which the largest dimension exceeds 5 µm. Asbestos structures larger than 5 µm do not necessarily contain asbestos fibres or bundles longer than 5 µm.

C.2.6 Asbestos fibre or bundle longer than 5 µm

An asbestos fibre of any width, or bundle of such fibres, which has a length exceeding 5 µm.

C.2.7 PCM equivalent structure

Any fibre, bundle, cluster or matrix with an aspect ratio of 3/1 or greater, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm. PCM equivalent structures do not necessarily contain fibres or bundles longer than 5 µm, or PCM equivalent fibres.

C.2.8 PCM equivalent fibre

Any particle with parallel or stepped sides, with an aspect ratio of 3/1 or greater, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm. *For chrysotile, PCM equivalent fibres will always be* bundles.

C.3 Other structure counting criteria

C.3.1 Structures which intersect grid bars

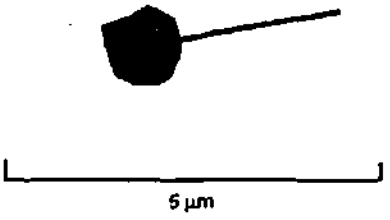
A structure which intersects a grid bar shall only be counted on two sides of the grid opening, as illustrated in figure C.4. Record the dimensions of the structure such that the obscured portions of components are taken to be equivalent to the unobscured portions, *as shown by the broken lines in figure C.4.* For example, the length of a fibre intersecting a grid bar is taken to be twice the unobscured length. Structures intersecting either of the other two sides shall not be included in the count.

C.3.2 Fibres which extend outside the field of view

During scanning of a grid opening, count fibres which extend outside the field of view systematically, so as to avoid double-counting. In general, a rule should be established so that fibres extending outside the field of view in only two quadrants are counted. The procedure is illustrated by figure C.5. Measure the length of each of these fibre by moving the specimen to locate the other end of the fibre, and then return to the original field of view before continuing to scan the specimen. Fibres without terminations within the field of view shall not be counted.

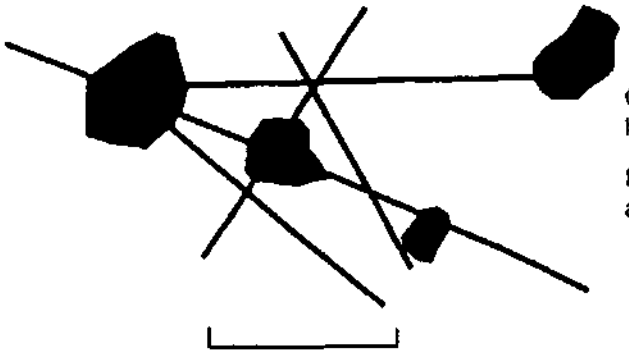
Count as 1 compact matrix, with all fibres shorter than 5 µm

Record as MC+0



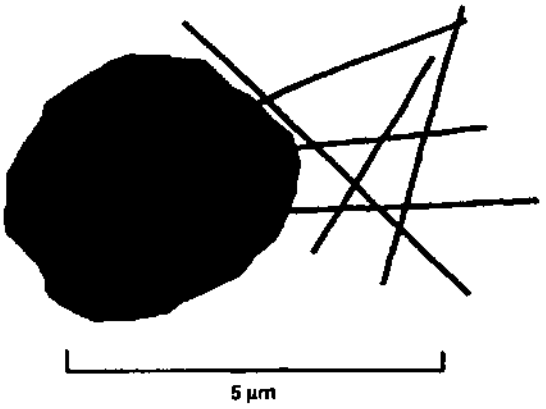
Count as 1 disperse matrix consisting of 1 fibre shorter than 5 µm

Record as MD10, followed by 1 fibre recorded as MF



Count as 1 disperse matrix consisting of 5 fibres, all longer than 5 µm

Record as MD55, followed by 5 fibres, each recorded as MF



Count as 1 disperse matrix, consisting if 3 fibres, 1 of which is longer than 5 µm, and 1 matrix residual containing 3 fibres

Record as MD61, followed by 3 fibres, each recorded as MF, and 1 matrix residual recorded as MR30

Figure C.3 — Examples of recording of complex asbestos matrices

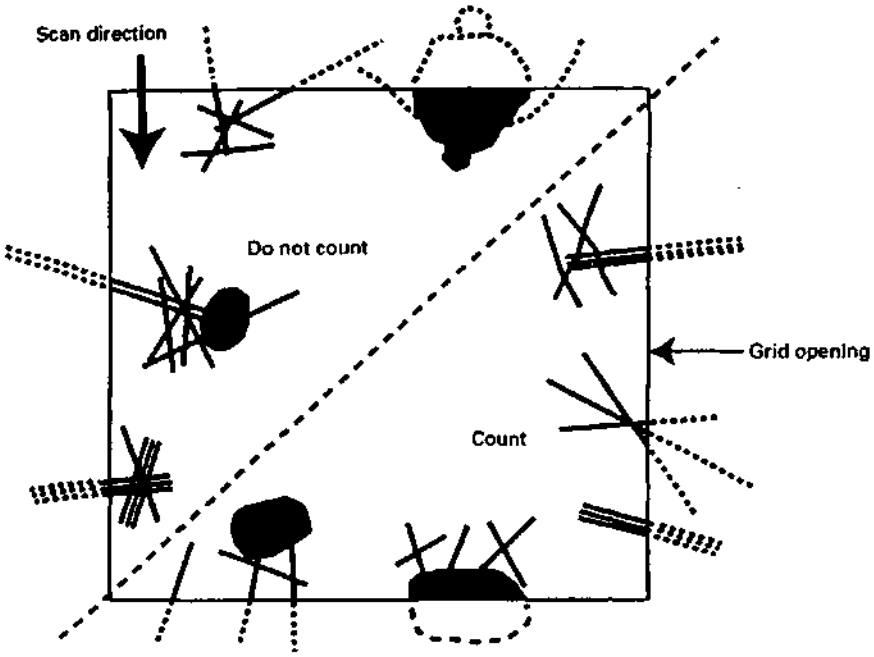


Figure C.4 — Example of counting of structures which intersect grid bars

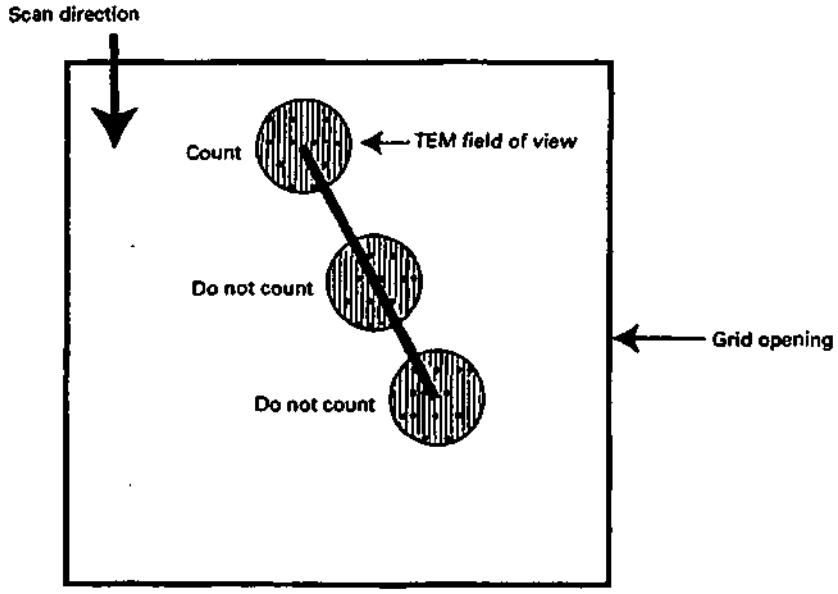


Figure C.5 — Example of counting of fibres which extend outside the field of view

C.4 Procedure for data recording

C.4.1 General

The morphological codes specified are designed to facilitate computer data processing, and to allow recording of a complete representation of the important features of each asbestos structure. The procedure requires that the microscopist classify each primary fibrous structure into one of the four fundamental categories: fibres, bundles, clusters and matrices.

C.4.2 Fibres

On the structure counting form, a fibre as defined in C.2.1 shall be recorded by the designation "F". If the fibre is a separately-counted part of a cluster or matrix, the fibre shall be recorded by the designation "CF", or "MF", depending on whether it is a component of a cluster or matrix.

C.4.3 Bundles

On the structure counting form, a bundle as defined in C.2.2 shall be recorded by the designation "B". If the bundle is a separately-counted part of a cluster or matrix, the bundle shall be recorded by the designation "CB", or "MB", depending on whether it is a component of a cluster or matrix.

C.4.4 Disperse clusters (type D)

On the structure counting form, an isolated cluster of type D as defined in C.2.3 shall be recorded by the designation "CD", followed by a two-digit number. The first digit represents the analyst's estimate of the total number of fibres and bundles comprising the structure. The digit shall be from 1 to 9, or designated as "+" if there are estimated to be more than 9 component fibres or bundles. The second digit shall represent, in the same manner, the total number of fibres and bundles longer than 5 µm contained in the structure. The overall dimensions of the cluster, in two perpendicular directions representing the maximum dimensions, shall be recorded. In order of decreasing length, up to 5 component fibres or bundles shall be separately recorded, using the codes "CF" (cluster fibre) and "CB" (cluster bundle). If, after accounting for prominent component fibres and bundles, a group of clustered fibres remains, this shall be recorded by the designation "CR" (cluster residual). If the remaining clustered fibres are present as more than one localized group, it may be necessary to record more than one cluster residual. Do not record more than 5 cluster residuals for any cluster. A cluster residual shall be measured and assigned a two-digit number, derived in the same manner as specified for the overall cluster. Optionally, if the number of component fibres and bundles in either the original cluster or the cluster residual is outside the range 1 – 9, additional information concerning the number of component fibres and bundles may be noted in the "comments" column.

C.4.5 Compact clusters (type C)

On the structure counting form, an isolated cluster of type C as defined in C.2.3 shall be recorded by the designation "CC", followed by a two-digit number. The two-digit number describing the numbers of component fibres and bundles shall be assigned in the same manner as for clusters of type D. The overall dimensions of the cluster in two perpendicular directions shall be recorded in the same manner as for clusters of type D. By definition, the constitutent fibres and bundles of compact clusters cannot be separately measured; therefore, no separate tabulation of component fibres or bundles can be made.

C.4.6 Disperse matrices (type D)

On the structure counting form, an isolated matrix of type D as defined in C.2.4 shall be recorded by the designation "MD", followed by a two-digit number. The two-digit number shall be assigned in the same manner as for clusters of type D. The overall dimensions of the matrix in two perpendicular directions shall be recorded in the same manner as for clusters of type D. In order of decreasing length, up to 5 component fibres or bundles shall be separately recorded, using the codes "MF" (matrix fibre) and "MB" (matrix bundle). If after accounting for prominent component fibres and bundles, matrix material containing asbestos fibres remains, this shall be recorded by the designation "MR" (matrix residual). If the remaining matrix fibres are present as more than one localized group, it may be necessary to record more than one matrix residual. Do not record more than 5 matrix residuals for any matrix. A matrix residual shall be measured and assigned a two-digit number, derived in the same manner as specified for the overall matrix. Optionally, if the number of component fibres or bundles in either the original matrix or the matrix residual is outside the range 1 – 9, additional information concerning the number of component fibres and bundles may be noted in the "comments" column.

C.4.7 Compact matrices (type C)

On the structure counting form, an isolated matrix of type C as defined in C.2.4 shall be recorded by the

designation "MC", followed by a two-digit number. The two-digit number shall be assigned in the same manner as for clusters of type D. The overall dimensions of the matrix in two perpendicular directions shall be recorded in the same manner as for clusters of type D. By definition, the constitutent fibres and bundles of compact matrices cannot be separately measured; therefore, no separate tabulation of component fibres or bundles can be made.

C.4.8 Procedure for recording of partially obscured fibres and bundles

The proportion of the length of a fibre or bundle that is obscured by other particulates shall be used as the basis for determining whether a fibre or bundle is to be recorded as a separate component or is to be considered as a part of a matrix of type C or part of a matrix residual. If the obscured length could not possibly be more than one-third of the total length, the fibre or bundle shall be considered a prominent feature to be separately recorded. The assigned length for each such partially obscured fibre or bundle shall be equal to the visible length plus the maximum possible contribution from the obscured portion. Fibres or bundles which appear to cross the matrix, and for which both ends can be located approximately, shall be included in the maximum of 5 and recorded according to the counting criteria as separate fibres or bundles. If the obscured length could be more than one third of the total length, the fibre or bundle shall be considered as a part of a compact matrix of type C or part of a matrix residual.

C.5 Special considerations for counting of PCM equivalent structures

Use 3/1 as the minimum aspect ratio for counting of PCM equivalent structures. This aspect ratio definition is required in order to achieve comparability of the results for this size range of structure with historical optical measurements, but use of this aspect ratio definition does not significantly affect the ability to interpret the whole fibre size distribution in terms of a minimum 5/1 aspect ratio. Some applications may require that a count be made of PCM equivalent structures only. The coding system permits discrimination between PCM equivalent structures that contain fibres and bundles longer than 5 µm and those that do not.

NOTE 16 In general, clusters and matrices will yield fewer components as the minimum dimensions specified for countable fibres are increased. Thus, it may be found that a particular structure yields a higher number of component fibres and bundles in a count for all fibre sizes than it does at a reduced magnification when only fibres and bundles longer than 5 µm are being counted. However, the requirement that component fibres and bundles be recorded in decreasing length order ensures that the data are consistent for a particular structure, regardless of the size category of fibres being counted and the magnification in use.

Annex D
(normative)

Fibre identification procedure

D.1 General

The criteria used for identification of asbestos fibres may be selected depending on the intended use of the measurements. In some circumstances, there can be a requirement that fibres shall be unequivocally identified as a specific mineral species. In other circumstances, there can be sufficient knowledge about the sample, so that rigorous identification of each fibre need not be carried out. The time required to perform the analysis, and therefore the cost of analysis, can vary widely depending on the identification criteria considered which are to be sufficiently definitive. The combination of criteria considered definitive for identification of fibres in a particular analysis shall be specified before the analysis is made, and this combination of criteria shall be referred to as the "level" of analysis. Various factors related to instrumental limitations and the character of the sample may prevent satisfaction of all of the specified fibre identification criteria for a particular fibre. Therefore, a record shall be made of the identification criteria which were satisfied for each suspected asbestos fibre included in the analysis. For example, if both ED and EDXA were specified to be attempted for definitive identification of each fibre, fibres with chrysotile morphology which, for some reason, do not give an ED pattern but which do yield an EDXA spectrum corresponding to chrysotile, are categorized in a way which conveys the level of confidence to be placed in the identification.

D.2 ED and EDXA techniques

D.2.1 General

Initially, fibres are classified into two categories on the basis of morphology: those fibres with tubular morphology, and those fibres without tubular morphology. Further analysis of each fibre is conducted using ED and EDXA methods. The following procedures should be used when fibres are examined by ED and EDXA.

The crystal structures of some mineral fibres, such as chrysotile, are easily damaged by the high current densities required for EDXA examination. Therefore, investigation of these sensitive fibres by ED should be completed before attempts are made to obtain EDXA spectra from the fibres. When more stable fibres, such as the amphiboles, are examined, EDXA and ED may be used in either order.

D.2.2 ED techniques

The ED technique can be either qualitative or quantitative. Qualitative ED consists of visual examination, without detailed measurement, of the general characteristics of the ED pattern obtained on the TEM viewing screen from a randomly oriented fibre. ED patterns obtained from fibres with cylindrical symmetry, such as chrysotile, do not change when the fibres are tilted about their axes, and patterns from randomly oriented fibres of these minerals can be interpreted quantitatively. For fibres which do not have cylindrical symmetry, only those ED patterns obtained when the fibre is oriented with a principal crystallographic axis closely parallel with the incident electron beam direction can be interpreted quantitatively. This type of ED pattern shall be referred to as a "zone-axis ED pattern". In order to interpret a zone-axis ED pattern quantitatively, it shall be recorded photographically and its consistency with known mineral structures shall be checked. A computer program may be used to compare measurements of the zone-axis ED pattern with corresponding data calculated from known mineral structures. The zone-axis ED pattern obtained by examination of a fibre in a particular orientation can be insufficiently specific to permit unequivocal identification of the mineral fibre, but is is often possible to tilt the fibre to another angle and to record a different ED pattern corresponding to another zone-axis. The angle between the two zone-axes can also be checked for consistency with the structure of a suspected mineral.

For visual examination of the ED pattern, the camera length of the TEM should be set to a low value of approximately 250 mm and the ED pattern should then be viewed through the binoculars. This procedure minimizes the possible degradation of the fibre by the electron irradiation. However, the pattern is distorted by the tilt angle of the viewing screen. A camera length of at least 2 m should be used when

the ED pattern is recorded, if accurate measurement of the pattern is to be possible. It is necessary that, when obtaining an ED pattern to be evaluated visually or to be recorded, the sample height shall be properly adjusted to the eucentric point and the image shall be focussed in the plane of the selected area aperture. If this is not done, there may be some components of the ED pattern which do not originate from the selected area. In general, it will be necessary to use the smallest available ED aperture.

For accurate measurements of the ED pattern, an internal calibration standard shall be used. A thin coating of gold, or another suitable calibration material, shall be applied to the underside of the TEM specimen. This coating may be applied either by vacuum evaporation or, more conveniently, by sputtering. The polycrystalline gold film yields diffraction rings on every ED pattern and these rings provide the required calibration information.

To form an ED pattern, move the image of the fibre to the centre of the viewing screen, adjust the height of the specimen to the eucentric position, and insert a suitable selected area aperture into the electron beam so that the fibre, or a portion of it, occupies a large proportion of the illuminated area. The size of the aperture and the portion of the fibre shall be such that particles other than the one to be examined are excluded from the selected area. Observe the ED pattern through the binoculars. During the observation, the objective lens current should be adjusted to the point where the most complete ED pattern is obtained. If an incomplete ED pattern is still obtained, move the particle around within the selected area to attempt to optimize the ED pattern, or to eliminate possible interferences from neighbouring particles.

If a zone-axis ED analysis is to be attempted on the fibre, the sample shall be mounted in the appropriate holder. The most convenient holder allows complete rotation of the specimen grid and tilting of the grid about a single axis. Rotate the sample until the fibre image indicates that the fibre is oriented with its length coincident with the tilt axis of the goniometer, and adjust the sample height until the fibre is at the eucentric position. Tilt the fibre until an ED appears which is a symmetrical, two dimensional array of spots. The recognition of zone-axis alignment conditions requires some experience on the part of the operator. During tilting of the fibre to obtain zone-axis conditions, the manner in which the intensities of the spots vary should be observed. If weak reflections occur at some points on a matrix of strong reflections, the possibility of twinning or multiple diffraction exists, and some caution should be exercised in the selection of diffraction spots for measurement and interpretation. A full discussion of electron diffraction and multiple diffraction can be found in the references by J.A. Gard [11] P.B. Hirsch *et al* [14] and H.R. Wenck [42] included in annex J. Not all zone-axis patterns which can be obtained are definitive. Only those which have closely spaced reflections corresponding to low indices in at least one direction should be recorded. Patterns in which all d-spacings are less than about 0,3 nm are not definitive. A useful guideline is that the lowest angle reflections should be within the radius of the first gold diffraction ring (111), and that patterns with smaller distances between reflections are usually the most definitive.

Five spots, closest to the centre spot, along two intersecting lines of the zone-axis pattern shall be selected for measurement, as shown in figure D.1. The distances of these spots from the centre spot and the four angles shown provide the required data for analysis. Since the centre spot is usually very overexposed, it does not provide a well-defined origin for these measurements. The required distances shall therefore be obtained by measuring between pairs of spots symmetrically disposed about the centre spot, preferably separated by several repeat distances. The distances shall be measured with a precision of better than 0,3 mm, and the angles to a precision of better than 2,5°. The diameter of the first or second ring of the calibration pattern (111 and 200) shall also be measured with a precision of better than 0,3 mm.

Using gold as the calibration material, the radius-based camera constant is given by

$$\lambda L = 0{,}117\ 74D \text{ mm·nm (first ring)}$$

$$\lambda L = 0{,}101\ 97D \text{ mm·nm (second ring)}$$

D.2.3 EDXA measurements

Interpretation of the EDXA spectrum may be either qualitative or quantitative. For qualitative interpretation of a spectrum, the X-ray peaks originating from the elements in the fibre are recorded. For quantitative interpretation, the net peak areas, after background subtraction, are obtained for the X-ray peaks originating from the elements in the fibre. This method provides quantitative interpretation for those minerals which contain silicon.

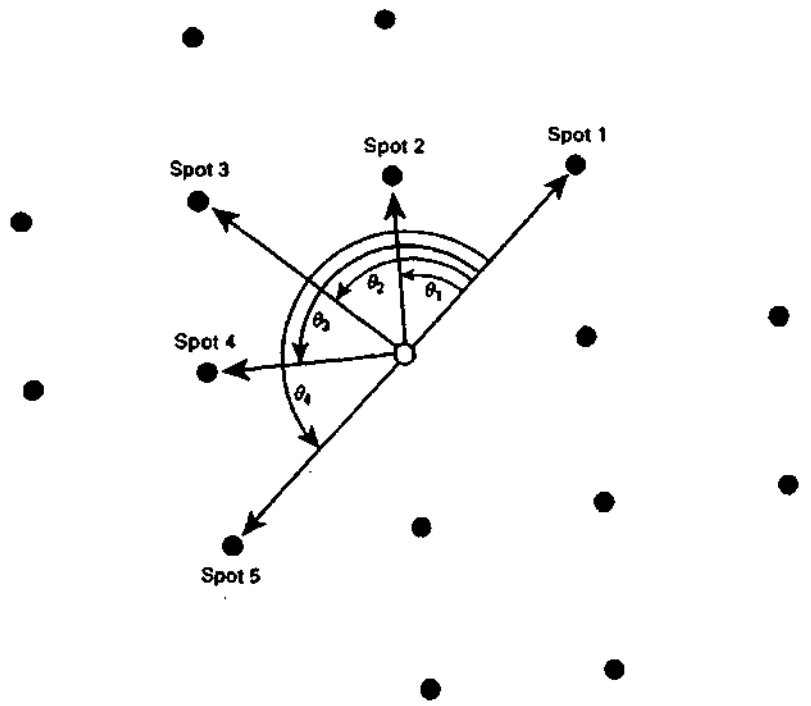


Figure D.1 — Example of measurement of zone-axis SAED patterns

To obtain an EDXA spectrum, move the image of the fibre to the centre of the screen and remove the objective aperture. Select an appropriate electron beam diameter and deflect the beam so that it impinges on the fibre. Depending on the instrumentation, it may be necessary to tilt the specimen towards the X-ray detector and, in some instruments, to use the Scanning Transmission Electron Microscopy (STEM) mode of operation.

The time for acquisition of a suitable spectrum varies with the fibre diameter, and also with instrumental factors. For quantitative interpretation, spectra should have a statistically valid number of counts in each peak. Analyses of small diameter fibres which contain sodium are the most critical, since it is in the low energy range that the X-ray detector is least sensitive. Consequently, it is necessary to acquire a spectrum for a period that is sufficiently long for the sodium to be detected in such fibres. It has been found that satisfactory quantitative an analyses can be obtained if acquisition is continued until the background subtracted silicon $K\alpha$ peak integral exceeds 10 000 counts. The spectrum should then be manipulated to subtract the background and to obtain the net areas of the elemental peaks.

After quantitative EDXA classification of some fibres by computer analysis of the net peak areas, it may be possible to classify further fibres in the same sample on the basis of comparison of spectra at the intrument. Frequently, visual comparisons can be made after somewhat shorter acquisition times.

D.3 Interpretation of fibre analysis data

D.3.1 Chrysotile

The morphological structure of chrysotile is characteristic, and with experience, can be recognized readily. However, a few holder minerals have a similar appearance, and morphological observation by itself is inadequate for most samples. The ED pattern obtained from chrysotile is quite specific for this mineral if the specified characteristics of the pattern correspond to those from reference chrysotile. However, depending on the past history of the fibre, and on a number of other factors, the crystal structure of a particular fibre may be damaged, and it may not yield and ED pattern. In this case, the EDXA spectrum may be the only data available to supplement the morphological observations.

D.3.2 Amphiboles

Since the fibre identification procedure for asbestos fibres other than chrysotile can be involved and time-consuming, computer programmes, such as that developed by B.L. Rhoades (see annex J, reference

[32]), are recommended for interpretation of zone-axis ED patterns. The published literature contains composition and crystallographic data for all of the fibrous minerals likely to be encountered in TEM analysis of air samples, and the compositional and structural data from the unknown fibre should be compared with the published data. Demonstration that the measurements are consistent with the data for a particular test mineral does not uniquely identify the unknown, since the possibility exists that data from other minerals may also be consistent. It is, however, unlikely that a mineral of another structural class could yield data consistent with that from an amphibole fibre identified by quantitative EDXA and two zone-axis ED patterns.

Suspected amphibole fibres should be classified initially on the basis of chemical composition. Either qualitative or quantitative EDXA information may be used as the basis for this classification. From the published data on mineral compositions, a list of minerals which are consistent in composition with that measured for the unknown fibre should be compiled. To proceed further, it is necessary to obtain the first zone-axis ED pattern, according to D.2.2.

It is possible to specify a particular zone-axis pattern for identification of amphibole, since a few patterns are often considered to be characteristic. Unfortunately, for a fibre with random orientation on a TEM grid, no specimen holder and goniometer currently available will permit convenient and rapid location of two preselected zone-axes. The most practical approach has been adopted, which is to accept those low index patterns which are easily obtained, and then to test their consistency with the structures of the minerals already preselected on the basis of the EDXA data. Even the structures of non-amphibole minerals in this preselected list shall be tested against the zone-axis data obtained for the unknown fibre, since non-amphibole minerals in some orientations may yield similar patterns consistent with amphibole structures.

The zone-axis ED interpretation shall include all minerals previously selected from the mineral data file as being chemically compatible with the EDXA data. This procedure will usually shorten the list of minerals for which solutions have been found. A second set of zone-axis data from another pattern obtained on the same fibre can then be processed, either as further confirmation of the identification, or to attempt elimination of an ambiguity. In addition, the angle measured between the orientations of the two zone-axes can be checked for consistency with the structures of the minerals. Caution should be exercised in rationalizing the inter-zone-axis angle, since if the fibre contains $\vec{c}$-axis twinning, the two zone-axis ED patterns may originate from the separate twin crystals. In practice, the full identification procedure will normally be applied to very few fibres, unless precise identification of all fibres is required for a particular reason.

D.4 Fibre classification categories

It is not always possible to proceed to a definitive identification of a fibre; this may be due to instrumental limitations or to the actual nature of the fibre. In many analyses, a definitive identification of each fibre may not actually be necessary if there is other knowledge available about the sample, or if the concentration is below a level of interest. The analytical procedure shall therefore take into account both instrumental limitations and varied analytical requirements. Accordingly, a system for fibre classification is used to permit accurate recording of data. The classifications are shown in tables D.1 and D.2, and are directed towards identification of chrysotile and amphibole respectively. Fibres shall be reported in these categories.

The general principle to be followed in this analytical procedure is first to define the most specific fibre classification which is to be attempted, or the "level" of analysis to be conducted. Then, for each fibre examined, record the classification which is actually achieved. Depending on the intended use of the results, criteria for acceptance of fibres as "identified" can then be established at any time after completion of the analysis.

In an unknown sample, chrysotile will be regarded as confirmed only if a recorded, calibrated ED pattern from one fibre in the CD categories is obtained, or if measurements of the ED pattern are recorded at the instrument. Amphibole will be regarded as confirmed only by obtaining recorded data which indicates exclusively the presence of amphiboles for fibres classified in the AZQ, AZZ or AZZQ categories.

Table D.1 — Classification of fibres with tubular morphology

Category	Description
TM	Tubular Morphology, not sufficiently characteristic for classification as chrysotile
CM	Characteristic Chrysotile Morphology
CD	Chrysotile SAED pattern
CQ	Chrysotile composition by Quantitative EDXA
CMQ	Chrysotile Morphology and composition by Quantitative EDXA
CDQ	Chrysotile SAED pattern and composition by Quantitative EDXA
NAM	Non-Asbestos Mineral

Table D.2 — Classification of fibres without tubular morphology

Category	Description
UF	Unidentified Fibre
AD	Amphibole by random orientation SAED (shows layer pattern of 0,53 nm spacing)
AX	Amphibole by qualitative EDXA. Spectrum has elemental components consistent with amphibole
ADX	Amphibole by random orientation SAED and qualitative EDXA
AQ	Amphibole by Quantitative EDXA
AZ	Amphibole by one Zone-axis SAED pattern
ADQ	Amphibole by random orientation SAED and Quantitative EDXA
AZQ	Amphibole by one Zone-axis SAED pattern and Quantitative EDXA
AZZ	Amphibole by two Zone-axis SAED patterns, with consistent interaxial angle
AZZQ	Amphibole by two Zone-axis SAED patterns, with consistent interaxial angle, and Quantitative EDXA
NAM	Non-Asbestos Mineral

D.4.1 Procedure for classification of fibres with tubular morphology suspected to be chrysotile

Occasionally, fibres are encountered which have tubular morphology similar to that of chrysotile, but which cannot be characterized further either by ED or EDXA. They may be non-crystalline, in which case ED techniques are not useful, or they may be in a position on the grid which does not permit an EDXA spectrum to be obtained. Alternatively, the fibre may be of organic origin, but the morphology and composition may not be sufficiently definitive enough to be disregarded. Accordingly, there is a requirement to record each fibre, and to specify how confidently each fibre can be identified. Classification of fibres will meet with various degrees of success. Figure D.2 shows the classification procedure to be used for fibres which display any tubular morphology. The chart is self explanatory, and every fibre is either rejected as a non-asbestos mineral (NAM), or classified in some way which by some later criterion could still contribute to the chrysotile fibre count.

Morphology is the first consideration, and if this is not similar to that usually seen in chrysotile standard samples, designate the initial classification as TM. Regardless of the doubtful morphology, examine the fibre by ED and EDXA methods according to figure D.2. Where the morphology is more definitive, it may be possible to classify the fibre as having chrysotile morphology (CM).

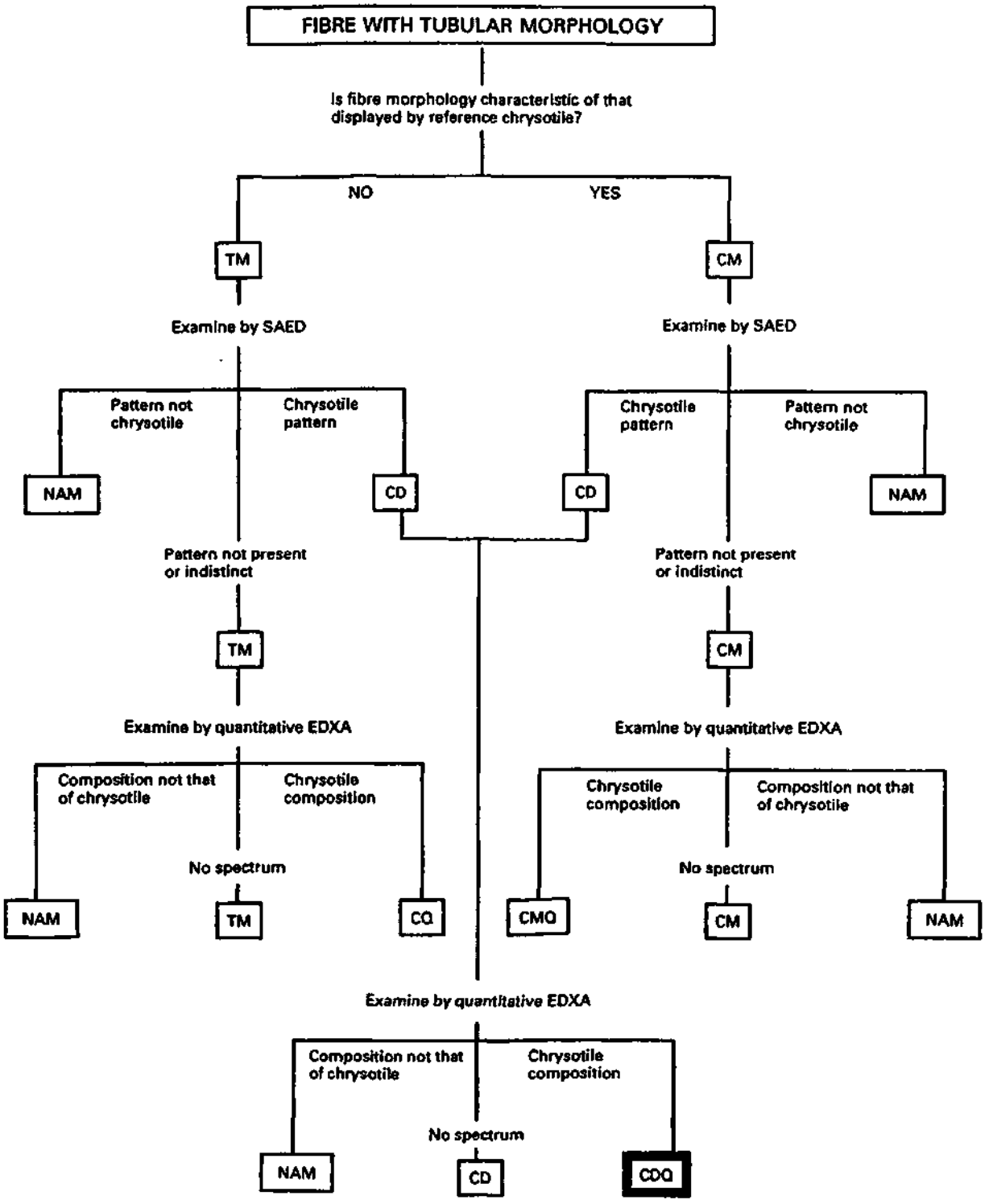


Figure D.2 — Classification chart for fibre with tubular morphology

For classification as CM, the morphological characteristics required are the following:

a) the individual fibrils should have high aspect ratios exceeding 5/1, and be about 30 nm to 40 nm in diameter;

b) the electron scattering power of the fibre at 60 kV to 100 kV accelerating potential should be sufficiently low for the internal structure to be visible;

c) there should be some evidence of an internal structure suggesting a tubular appearance similar to that shown by reference UICC chrysotile, which may degrade in the electron beam.

Examine every fibre having these morphological characteristics by the ED technique, and classify as chrysotile by ED (CD) only those which give diffraction patterns with the precise characteristics shown in figure D.3. The relevant features in this pattern for identification of chrysotile are as follows:

a) the (002) reflections should be examined to determine that they correspond closely to a spacing of 0,73 nm;

b) the layer line repeat distance should correspond to 0,53 nm;

c) there should be "streaking" of the (110) and (130) reflections.

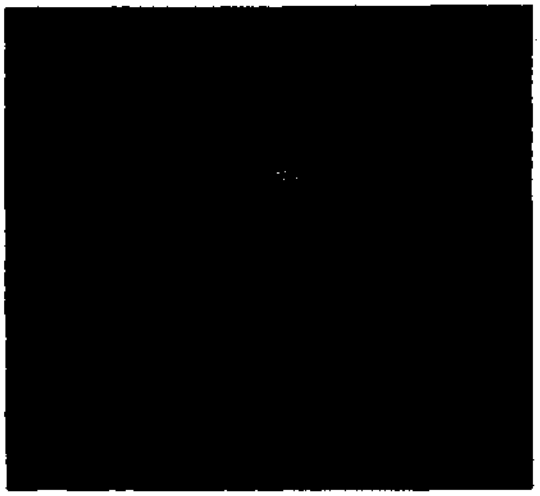


Figure D.3 — Chrysotile SAED pattern

Using the millimetre calibrations on the TEM viewing screen, these observations can readily be made at the instrument. If documentary proof of fibre identification is required, record a TEM micrograph of at least one representative fibre, and record its ED pattern on a separate film or plate. This film or plate shall also carry calibration rings from a known polycrystalline substance such as gold. This calibrated pattern is the only documentary proof that the particular fibre is chrysotile, and not some other tubular or scrolled species such as halloysite, palygorskite, talc or vermiculite. The proportion of fibres which can be successfully identified as chrysotile by ED is variable, and to some extent dependent on both the instrument and the procedures of the operator. The fibres that fail to yield an identifiable ED pattern will remain in the TM or CM categories unless they are examined by EDXA.

In the EDXA analysis of chrysotile there are only two elements which are relevant. For fibre classification, the EDXA analysis shall be quantitative. If the spectrum displays prominent peaks from magnesium and silicon, with their areas in the appropriate ratio, and with only minor peaks from other elements, classify the fibre as chrysotile by quantitative EDXA, in the categories CQ, CMQ, or CDQ, as appropriate.

D.4.2 Procedure for classification of fibres without tubular morphology, suspected to be amphibole

Every particle without tubular morphology and which is not obviously of biological origin, with an aspect ratio of 5/1 or greater, and having parallel or stepped sides, shall be considered as a suspected amphibole fibre. Further examination of the fibre by ED and EDXA techniques will meet with a variable degree of success, depending on the nature of the fibre and on a number of instrumental limitations. It will not be possible to identify every fibre completely, even if time and cost are of no concern. Moreover, confirmation of the presence of amphibole can be achieved only by quantitative interpretation of zone-axis ED patterns, a very time-consuming procedure. Accordingly, for routine samples from unknown sources, this analytical procedure limits the requirement for zone-axis ED work to a minimum of one fibre representative of each compositional class reported. In some samples, it may be necessary to identify more fibres by the zone-axis technique. When analysing samples from well-characterized sources, the cost of identification by zone-axis methods may not be justified.

The 0,53 nm layer spacing of the random orientation ED pattern is not by itself diagnostic for amphibole. However, the presence of $\vec{c}$-axis twinning in many fi-

bres leads to contributions to the layers in the patterns by several individual parallel crystals of different axial orientations. This apparently random positioning of the spots along the layer lines, if also associated with a high fibre aspect ratio, is a characteristic of amphibole asbestos, and thus has some limited diagnostic value. If a pattern of this type is not obtained, the identity of the fibre is still ambiguous, since the absence of a recognizable pattern may be a consequence of an unsuitable orientation relative to the electron beam, or the fibre may be some other mineral species.

Figure D.4 shows the fibre classification chart to be used for suspected amphibole fibres. This chart shows all the classification paths possible in analysis of a suspected amphibole fibre, when examined systematically by ED and EDXA. Two routes are possible, depending on whether an attempt to obtain an EDXA spectrum or a random orientation ED pattern is made first. The normal procedure for analysis of a sample of unknown origin will be to examine the fibre by random orientation ED, qualitative EDXA, quantitative EDXA, and zone-axis ED, in this sequence. The final fibre classification assigned will be defined either by successful analysis at the maximum required level, or by the instrumental limitations. Any instrumental limitations which affect the quality of the results shall be noted. Record the maximum classification achieved for each fibre on the counting sheet in the appropriate column. The various classification categories can then be combined later in any desired way for calculation of the fibre concentration. The complete record of the results obtained when attempting to identify each fibre can also be used to re-assess the data if necessary.

In the unknown sample, zone-axis analysis will be required if the presence of amphibole is to be unequivocally confirmed. For this level of analysis, attempt to raise the classification of every suspected amphibole fibre to the ADQ category by inspection of the random orientation ED pattern and the EDXA spectrum. In addition, examine at least one fibre from each type of suspected amphibole found by zone-axis methods to confirm their identification. In most cases, because information exists about possible sources of asbestos in close proximity to the air sampling location, some degree of ambiguity of identification can be accepted. Lower levels of analysis can therefore be accepted for these situations.

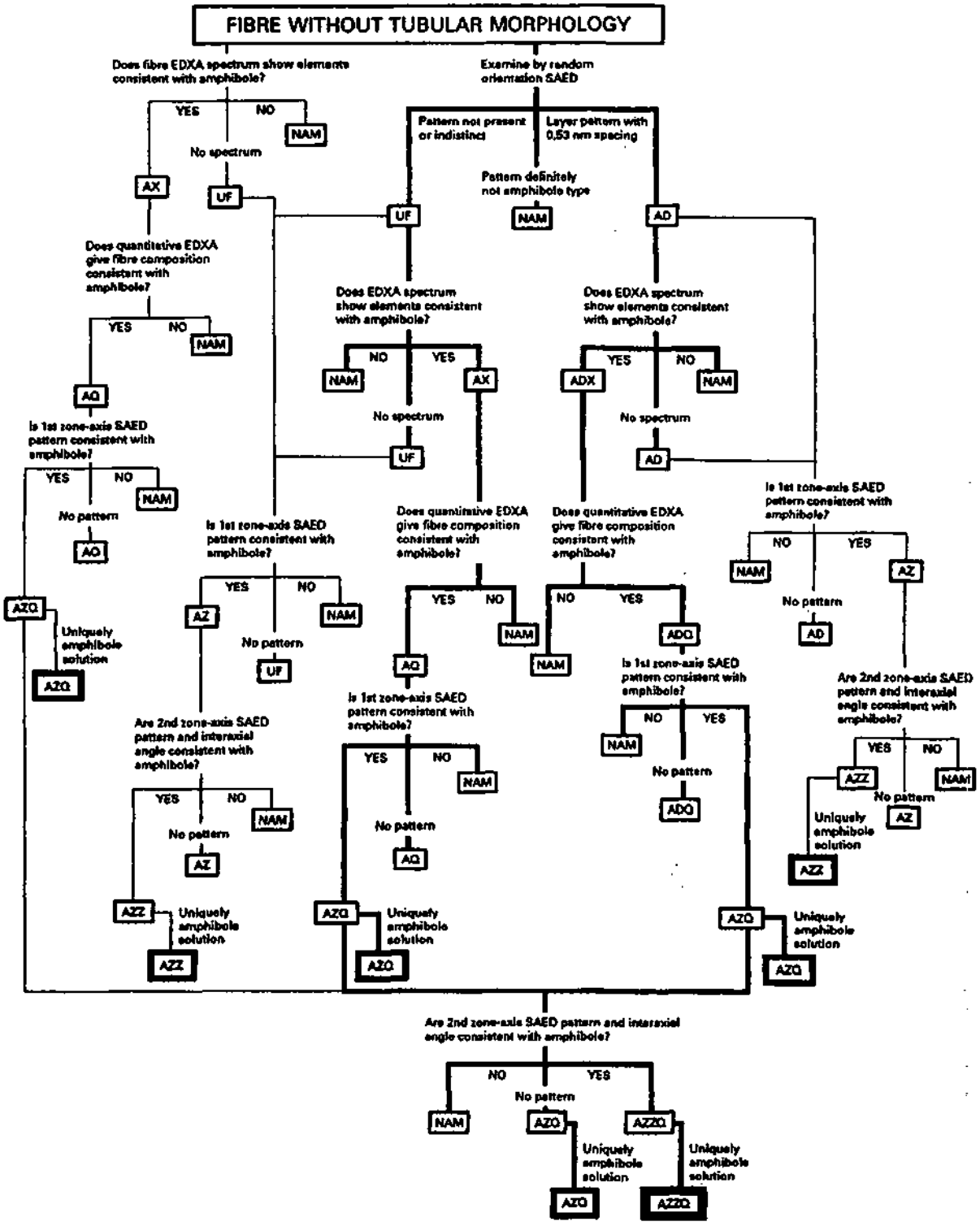


Figure D.4 — Classification chart for fibre without tubular morphology

Annex E
(normative)

Determination of the concentrations of asbestos fibres and bundles longer than 5 µm, and PCM equivalent asbestos fibres

In order to provide increased statistical precision and improved analytical sensitivity for those asbestos fibres and bundles longer than 5 µm, it may be decided to perform additional fibre counting at a lower magnification, taking account only into fibres and bundles within this dimensional range. The result shall be specified as "number of asbestos fibres and bundles longer than 5 µm". For this examination, use a magnification of approximately × 10 000, and continue to assign a morphological code to each structure according to the procedures specified in annex C. Record fibres and bundles only if their lengths exceed 5 µm. Record cluster and matrix components only if their lengths exceed 5 µm.

It may also be decided to provide increased statistical precision and improved analytical sensitivity for fibrous structures longer than 5 µm, with diameters between 0,2 µm and 3,0 µm, which have historically been the basis of risk estimation in the occupational environment (PCM equivalent asbestos fibres). Use a magnification of approximately × 5 000 for this extended fibre count. The result shall be specified as "number of PCM equivalent asbestos fibres". Asbestos structures within this dimensional range do not necessarily incorporate asbestos fibres or bundles longer than 5 µm.

Continue the extended sample examination until 100 asbestos structures have been counted, or until a sufficient area of the specimen has been examined to achieve the desired analytical sensitivity calculated according to table 1. The grid openings examined shall be divided approximately equally between a minimum of two specimen grids.

NOTES

17 The specimen area corresponding to the area of filter examined in the PCM fibre counting methods is 0,785 mm^2, and is equivalent to approximately 100 grid openings of a 200 mesh grid.

18 Some National Standards require that asbestos fibres longer than 2,5 µm, with diameters between 0,2 µm and 3,0 µm be counted. Use a magnification of × 5 000 for counting fibres within these dimensional ranges.

19 The minimum aspect ratio for definition of a fibre in PCM fibre counting methods and in some National Standards is 3/1. Use of a 3/1 aspect ratio is permitted in this International Standard, if this aspect ratio is mentioned in the test report.

The test reports shall include all of the items listed in clause 11.

Annex F
(normative)

Calculation of results

F.1 General

The results should be calculated using the procedures specified below. The results can be conveniently calculated using a computer programme.

F.2 Test for uniformity of distribution of fibrous structures on TEM grids

A chek shall be made using the chi-square test, to determine whether the asbestos structures found on individual grid openings are randomly and uniformly distributed among the grid openings. If the total number found in k grid openings is n, and the areas of the k individual frid openings are designated A_1 to A_k, then the total area of TEM specimen examined is

$$A = \sum_{i=1}^{i=k} A_i$$

The fraction of the total area examined which is represented by the individual grid opening area, p_i, is given by A_i/A. If the structures are randomly and uniformly dispersed over the k grid openings examined, the expected number of structures falling in one grid opening with area A_i is np_i. If the observed number of structures found on that grid opening is n_i, then

$$x^2 = \sum_{i=1}^{i=k} \frac{(n_i - np_i)^2}{np_i}$$

This value shall be compared with significance points of the chi-square distribution, having $(k - 1)$ degrees of freedom. Significance levels lower than 0,1 % may be cause for the sample analysis to be rejected, since this correspond to a very inhomogeneous deposit. If the structure count fails this test, the precision of the result will be uncertain, and if new air samples cannot be collected, additional grid openings may be examined or the sample may be prepared by an indirect method.

F.3 Calculation of the analytical sensitivity

Calculate the required analytical sensitivity S, expressed in number of structures per litre, using the following equation:

$$S = \frac{A_f}{kA_g V}$$

where

A_f is the area, in square millimetres, of sample collection filter;

A_g is the area, in square millimetres, of TEM specimen grid opening;

k is the number of grid openings examined;

V is the volume of air sampled, in litres.

F.4 Calculation of the mean and confidence interval of the structure concentration

In the structure count made according to this International Standard, a number of grid openings have been sampled from a population of grid openings, and it is required to determine the mean grid opening structure count for the population on the basis of this small sample. The interval about the sample mean which, with 95 % confidence, contains the population mean, is also required.

F.4.1 Calculation of the mean structure concentration

Calculate the mean structure concentration C, expressed in number of structures per litre, using the following equation:

$$C = Sn$$

where

S is the analytical sensitivity, expressed in number of structures per litre;

n is the total number of structures found on all grid openings examined.

F.4.2 Calculation of confidence intervals

The distribution of structures on the grid openings should theoretically approximate to a Poisson distribution. Because of fibre aggregation and size-dependent identification effects, the actual structure counting data often does not conform to the Poisson distribution, particularly at high structure counts. An assumption that the structure counting data are distributed according to the Poisson distribution can therefore lead to confidence intervals narrower than are justified by the data. Moreover, if the Poisson distribution is assumed, the variance is related only to the total number of structures counted. Thus, a particular structure count conducted on one grid opening is considered to have the same confidence interval as that for the same number of structures found on many grid openings. However, the area of sample actually counted is very small in relation to the total area of the filter, and for this reason, structures shall be counted on a minimum of four grid openings taken from different areas of the filter in order to ensure that a representative evaluation of the deposit is made.

At high structure counts, where there are adequate numbers of structures per grid opening to allow a sample estimate of the variance to be made, the distribution can be approximated to a Gaussian, with independent values for the mean and variance. Where the sample estimate of variance exceeds that implicit in the Poissonian assumption, use of Gaussian statistics with the variance defined by the actual data is the most conservative approach to calculation of confidence intervals.

At low structure counts, it is not possible to obtain a reliable sample estimate of the variance, and the distribution also becomes asymmetric but not necessarily Poissonian. For 30 structures and below, the distribution becomes asymmetric enough for the fit to a Gaussian to no longer be a reasonable one, and estimates of sample variance are unreliable. Accordingly, for counts below 31 structures, the assumption of a Poisson distribution shall be made for calculation of the confidence intervals.

F.4.3 Example of calculation of Poissonian 95 % confidence intervals

For total structure counts less than 4, the lower 95 % confidence limit corresponds to less than 1 structure. Therefore, it is not meaningful to quote lower confidence interval points for structure counts of less than 4, and the result shall be recorded as "less than" the corresponding one-sided upper 95 % confidence limit of the Poisson distribution, as follows:

0 structure ≙ 2,99 times the analytical sensitivity

1 structure ≙ 4,74 times the analytical sensitivity

2 structures ≙ 6,30 times the analytical sensitivity

3 structures ≙ 7,75 times the analytical sensitivity

For total counts exceeding 4, the 95 % confidence interval shall be calculated using the values shown in table F.1. Table F.1 gives the upper and lower limits of the two-sided Poissonian 95 % confidence interval for structure counts up to 470.

F.4.4 Example of calculation of Gaussian 95 % confidence intervals

Calculate the sample estimate of variance s^2 using the following equation:

$$s^2 = \frac{\sum_{i=1}^{i=k} (n_i - np_i)^2}{(k-1)}$$

where

n_i is the number of structures on the ith grid opening;

n is the total number of structures found in k grid openings;

p_i is the fraction of the total area examined represented by the ith grid opening;

k is the number of grid openings examined.

If the mean value of the structure count is calculated to be n, the upper and lower values of the Gaussian 95 % confidence interval are given respectively by

$$L_u = \frac{n}{k} + \frac{ts}{\sqrt{k}}$$

and

$$L_l = \frac{n}{k} - \frac{ts}{\sqrt{k}}$$

where

L_u is the upper 95 % confidence limit;

L_l is the lower 95 % confidence limit;

n is the total number of structures in all grid openings examined;

t is the value of Student's test (probability 0,975) for $(k - 1)$ degrees of freedom;

s is the standard deviation (square root of sample estimate of variance);

k is the number of grid openings examined.

F.4.5 Summary of procedure for calculation of results

In summary, structure counting data shall be calculated as follows:

No structures detected

The structure concentration shall be reported as less than the concentration equivalent ot the one-sided upper 95 % confidence limit of the Poisson distribution. This is equal to 2,99 times the analytical sensitivity.

From 1 to 3 structures

When 1 to 3 structures are counted, the result shall be reported as less than the corresponding one-sided upper 95 % confidence limit for the Poisson distribution. These are

1 structure ≙ 4,74 times the analytical sensitivity

2 structures ≙ 6,30 times the analytical sensitivity

3 structures ≙ 7,75 times the analytical sensitivity

From 4 to 30 structures

The mean structure concentration and the 95 % confidence intervals shall be reported on the basis of the Poissonian assumption, using the values shown in table F.1.

More than 30 structures

When more 30 structures are counted, both the Gaussian 95 % confidence interval and the Poissonian 95 % confidence interval shall be calculated. The larger of these two intervals shall be used to express the precision of the structure concentration. When the Gaussian 95 % confidence interval is selected for data reporting, the Poissonian 95 % confidence interval shall also be mentioned.

F.5 Calculation of structure length, width, and aspect ratio distributions

The distributions all approximate to logarithmic-normal, and therefore the size range intervals for calculation of the distribution shall be spaced logarithmically. The other characteristics required for the choice of size intervals are that they should allow for a sufficient number of size classes, while still retaining a statistically valid number of structures in each class. Interpretation is also facilitated if each size class repeats at 10 intervals, and if 5 µm is a size class boundary. A ratio from one class to the next of 1,468 satisfies all of these requirements and this value shall be used. The distributions, being approximately logarithmic-normal, when presented graphically, shall be plotted using a logarithmic ordinate scale and a Gaussian abscissa.

F.5.1 Calculation of structure length cumulative number distribution

This distribution allows the fraction of the total number of structures either shorter or longer than a given length to be determined. It is calculated using the following equation:

$$C(P)_k = \frac{\sum_{i=1}^{i=k} n_i}{\sum_{i=1}^{i=P} n_i} \times 100$$

where

$C(P)_k$ is the cumulative number percentage of structures which have lengths less than the upper bound of the kth class;

n_i is the number of structures in the ith length class;

P is the total number of length classes.

F.5.2 Calculation of structure width cumulative number distribution

This distribution allows the fraction of the total number of structures either narrower or wider than a given width to be determined. It is calculated in a similar way to that used in F.5.1, but using the structure widths.

F.5.3 Calculation of structure aspect ratio cumulative number distribution

This distribution allows the fraction of the total number of structures which have aspect ratios either smaller or larger than a given aspect ratio to be determined. It is calculated in a similar way to that used in F.5.1, but using the structure aspect ratios.

Table F.1 — Upper and lower limits of the Poissonian 95 % confidence interval of a count

Structure count	Lower limit	Upper limit	Structure count	Lower limit	Upper limit	Structure count	Lower limit	Upper limit
0	0	3,689[1)]	46	33,678	61,358	92	74,164	112,83
1	0,025	5,572	47	34,534	62,501	93	75,061	113,94
2	0,242	7,225	48	35,392	63,642	94	75,959	115,04
3	0,619	8,767	49	36,251	64,781	95	76,858	116,14
4	1,090	10,242	50	37,112	65,919	96	77,757	117,24
5	1,624	11,669	51	37,973	67,056	97	78,657	118,34
6	2,202	13,060	52	38,837	68,192	98	79,557	119,44
7	2,814	14,423	53	39,701	69,326	99	80,458	120,53
8	3,454	15,764	54	40,567	70,459	100	81,360	121,66
9	4,115	17,085	55	41,433	71,591	110	90,400	132,61
10	4,795	18,391	56	42,301	72,721	120	99,490	143,52
11	5,491	19,683	57	43,171	73,851	130	108,61	154,39
12	6,201	20,962	58	44,041	74,979	140	117,77	165,23
13	6,922	22,231	59	44,912	76,106	150	126,96	176,04
14	7,654	23,490	60	45,785	77,232	160	136,17	186,83
15	8,396	24,741	61	46,658	78,357	170	145,41	197,59
16	9,146	25,983	62	47,533	79,482	180	154,66	208,33
17	9,904	27,219	63	48,409	80,605	190	163,94	219,05
18	10,668	28,448	64	49,286	81,727	200	173,24	229,75
19	11,440	29,671	65	50,164	82,848	210	182,56	240,43
20	12,217	30,889	66	51,042	83,969	220	191,89	251,10
21	13,00	32,101	67	51,922	85,088	230	201,24	261,75
22	13,788	33,309	68	52,803	86,207	240	210,60	272,39
23	14,581	34,512	69	53,685	87,324	250	219,97	283,01
24	15,378	35,711	70	54,567	88,441	260	229,36	293,62
25	16,178	36,905	71	55,451	89,557	270	238,75	304,23
26	16,983	38,097	72	56,335	90,673	280	248,16	314,82
27	17,793	39,284	73	57,220	91,787	290	257,58	325,39
28	18,606	40,468	74	58,106	92,901	300	267,01	335,96
29	19,422	41,649	75	58,993	94,014	310	276,45	346,52
30	20,241	42,827	76	59,880	95,126	320	285,90	357,08
31	21,063	44,002	77	60,768	96,237	330	295,36	367,62
32	21,888	45,175	78	61,657	97,348	340	304,82	378,15
33	22,715	46,345	79	62,547	98,458	350	314,29	388,68
34	23,545	47,512	80	63,437	99,567	360	323,77	399,20
35	24,378	48,677	81	64,328	100,68	370	333,26	409,71
36	25,213	49,840	82	65,219	101,79	380	342,75	420,22
37	26,050	51,000	83	66,111	102,90	390	352,25	430,72
38	26,890	52,158	84	67,003	104,00	400	361,76	441,21
39	27,732	53,315	85	67,897	105,11	410	371,27	451,69
40	28,575	54,469	86	68,790	106,21	420	380,79	462,18
41	29,421	55,622	87	69,684	107,32	430	390,32	472,65
42	30,269	56,772	88	70,579	108,42	440	399,85	483,12
43	31,119	57,921	89	71,474	109,53	450	409,38	493,58
44	31,970	59,068	90	72,370	110,63	460	418,92	504,04
45	32,823	60,214	91	73,267	111,73	470	428,47	514,50

1) The one-sided upper 95 % confidence limit for 0 structures is 2,99.

Annex G
(informative)

Strategies for collection of air samples

G.1 General

An important part of the sampling strategy is a statement of the purpose of the sampling programme. A sufficient number of samples should be collected so that the site is well characterized to the precision and accuracy desired, and also ensure that sample filters appropriately loaded for TEM analysis are obtained from all of the sampling locations.

G.2 Air sample collection in the outdoors environment

Weather conditions restrict the ability to collect satisfactory air samples in the outdoors environment, and whenever possible, sampling should be carried out in low-wind, low-humidity conditions. Detailed records of the weather conditions, windspeed and direction during the sampling period should be made. All available information concerning local topography, and the types and positions of sources should be recorded.

Sequential multipoint sampling is necessary to provide adequate characterization of complex sites and sources. It is recommended that multiple samples are taken upwind and downwind of the site, with a minimum of two samples in the downwind position expected to experience the maximum airborne concentration. The locations of the samplers should be carefully recorded.

G.3 Air sample collection inside buildings

Air samples are often collected inside buildings in which asbestos-containing construction materials are present, in order to determine whether these materials contribute to the asbestos fibre concentration in the building atmosphere. The optimum positions for collection of air samples can only be determined after a complete survey of the building to establish air movement patterns. Multiple samples should be collected in the area where asbestos building materials are present, and control samples should be collected in an adjacent area where no airborne asbestos fibres would be expected. The intakes for air conditioning systems are frequently used as the collection locations for control samples. Whenever possible, static samples should be taken over a period exceeding 4 h during normal activity in the building, at face velocities of between 4 cm/s and 25 cm/s.

Annex H
(informative)

Methods for removal of gypsum fibres

It is common to find fibres of calcium sulfate (gypsum) in airborne particulates collected in buildings and urban environments, and particularly in samples collected where demolition or construction work is in progress. The fibres are readily released when plasters and cement products are disturbed. In some circumstances, particles of calcite or dolomite collected on an air filter can react with atmospheric sulfur dioxide, to form long fibres of gypsum. Gypsum fibres can give rise to high fibre counts by both optical and electron microscopy. The gypsum fibres are often 2 µm to 6 µm long, with aspect ratios greater than 10/1. Sometimes, these fibres appear similar to amphibole asbestos fibres, and in some samples they can be morphologically very similar to chrysotile. In the TEM, the larger fibres have high contrast and at high magnification often exhibit a characteristic mottled appearance which changes under electron beam irradiation. Some gypsum fibres, however, are not easily discriminated from asbestos without examination by EDXA. TEM specimens which contain many such gypsum fibres require an extended examination time in the TEM, because it is necessary to examine each of these fibres by EDXA before it can be rejected.

It is possible to remove gypsum fibres selectively by water extraction. A Jaffe washer (7.3.7), or a condensation washer (7.3.8), should be prepared, but using a water (6.1) as the solvent. The TEM specimens, which have been previously prepared and initially examined in the TEM, should be placed in the washer to allow dissolution of the fibres. If a Jaffe washer is used, the treatment time can be reduced by heating the washer to 90 °C to 100 °C for a few minutes. If a condensation washer is used, the gypsum fibres will be dissolved by treatment for approximately 10 min. The effect of this treatment is to remove the gypsum fibres, leaving carbon replicas (7.3.11) which are readily distinguished from asbestos fibres.

NOTE 20 This procedure should be used only when examination of the untreated TEM specimen grids shows the gypsum fibres to be isolated from any asbestos fibres present. Losses of asbestos fibres may occur if matrices of gypsum and asbestos are exposed to this procedure.

Annex J
(informative)

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Ambient air — Determination of asbestos fibres — Indirect-transfer transmission electron microscopy method

Air ambiant — Dosage des fibres d'amiante — Méthode par microscopie électronique à transmission par transfert indirect

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Contents

Page

1 Scope 1

1.1 Substance determined 1

1.2 Type of sample 1

1.3 Range 1

1.4 Limit of detection 1

2 Terms and definitions 2

3 Abbreviated terms 6

4 Principle 6

5 Apparatus 7

5.1 Air sampling 7

5.2 Specimen preparation laboratory 8

5.3 Equipment for analysis 8

5.4 Consumable supplies 13

6 Reagents 14

7 Air sample collection 14

7.1 Calculation of analytical sensitivity 14

7.2 Sample collection procedure 15

8 Procedure for analysis 15

8.1 General 15

8.2 Cleaning of sample cassettes 16

8.3 Preparation of analytical filters 16

8.4 Preparation of TEM specimens from PC analytical filters 17

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8.5 Preparation of TEM specimens from cellulose ester analytical filters 19
8.6 Criteria for acceptable TEM specimen grids 20
8.7 Procedure for structure counting by TEM 21
8.8 Blank and quality control determinations 24
8.9 Calculation of results 25
9 Performance characteristics 25
9.1 General 25
9.2 Interferences and limitations of fibre identification 25
9.3 Precision and accuracy 26
9.4 Limit of detection 26
10 Test report 27
Annex A (normative) Determination of operating conditions for plasma asher 30
Annex B (normative) Determination and standardization of operating conditions for ultrasonic bath 31
Annex C (normative) Calibration procedures 33
Annex D (normative) Structure-counting criteria 36
Annex E (normative) *Fibre identification procedure* 44
Annex F (normative) Determination of concentrations of asbestos fibres and bundles longer than 5 µm, and of PCM-equivalent asbestos fibres 53
Annex G (normative) Calculation of results 54
Annex H (normative) Test procedure to determine suitability of cellulose ester sample collection filters 60
Annex I (informative) Strategies for collection of air samples 61
Bibliography 62

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 13794 was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 3, *Ambient atmospheres*.

Annexes A to H form a normative part of this International Standard. Annex I is for information only.

Introduction

This International Standard is applicable to the measurement of airborne asbestos in a wide range of ambient air situations, including the interior atmospheres of buildings, and for detailed evaluation of any atmosphere in which asbestos fibres are likely to be present. Because the best available medical evidence indicates that the numerical fibre concentration and the fibre size and type are the relevant parameters for evaluation of the inhalation hazards, a fibre counting and measuring technique is the only logical approach. Most fibres in ambient atmospheres are not asbestos, and therefore there is a requirement for fibres to be identified. Many airborne asbestos fibres in ambient atmospheres have diameters below the resolution limit of the optical microscope. This International Standard is based on transmission electron microscopy, which has adequate resolution to allow detection of small fibres and is currently the only technique capable of unequivocal identification of the majority of individual fibres of asbestos. The fibres found suspended in an ambient atmosphere can often be identified unequivocally, if sufficient measurement effort is expended. However, if each fibre were to be identified in this way, the analysis becomes prohibitively expensive. Because of instrumental deficiencies or because of the nature of the particulate, some fibres cannot be positively identified as asbestos, even though the measurements all indicate that they could be asbestos. Subjective and instrumental factors therefore contribute to this measurement, and consequently a very precise definition of the procedure for identification and enumeration of asbestos fibres is required.

In addition to single fibres and bundles, asbestos is often found in air samples as very complex, aggregated structures which may or may not be also aggregated with other particles. The number of asbestos fibres and bundles incorporated in these complex structures often exceeds the number of isolated fibres and bundles observed, and many of them may be completely obscured in direct-transfer TEM preparations. The method defined in this International Standard incorporates specimen preparation procedures that result in selective concentration of asbestos fibres, and removal of organic and water-soluble materials. These procedures have the effect of dispersing the majority of the complex clusters and aggregates of fibres into their component fibres and bundles so that the asbestos in the air sample can be more accurately quantified. All of the feasible specimen preparation techniques result in some modification of the airborne particulate. Even the collection of particles from a three-dimensional airborne dispersion on to a two-dimensional filter surface can be considered a modification of the particulate, and some of the particles in most samples are modified by the specimen preparation procedures. Although this method results in dispersal of complex clusters and aggregates, it minimizes other effects on the size distribution of fibres and fibre bundles.

This International Standard is necessarily complex, because the instrumental techniques used are complex, and also because a very detailed and logical procedure must be specified to reduce the subjective aspects of the measurement. The method of data recording specified in this International Standard is designed to allow re-evaluation of the fibre counting data as new medical evidence becomes available.

This International Standard describes the method of analysis for a single air filter. However, one of the largest potential errors in characterizing asbestos in ambient atmospheres is associated with the variability between filter samples. For this reason, it is necessary to design a replicate sampling scheme in order to determine the standard's accuracy and precision.

Comparison of results obtained using this indirect-transfer procedure with those from the direct-transfer procedure may not be done *a priori*. A site-specific intercomparison study must be done which takes into account the fibre size and type of asbestos, and also the nature of the source of the airborne asbestos.

Ambient air — Determination of asbestos fibres — Indirect-transfer transmission electron microscopy method

1 Scope

1.1 Substance determined

This International Standard specifies a reference method using transmission electron microscopy (TEM) for determination of the concentration of asbestos structures in ambient atmospheres. The specimen preparation procedure incorporates ashing and dispersion of the collected particulate, so that all asbestos is measured, including the asbestos originally incorporated in particle aggregates or particles of composite materials. The lengths, widths and aspect ratios of the asbestos fibres and bundles are measured, and these, together with the density of the type of asbestos, also allow the total mass concentration of airborne asbestos to be calculated. The method allows determination of the type(s) of asbestos fibre present. The method cannot discriminate between individual fibres of the asbestos and non-asbestos analogues of the same amphibole mineral.

1.2 Type of sample

The method is defined for polycarbonate capillary-pore filters or cellulose ester (either mixed esters of cellulose or cellulose nitrate) filters through which a known volume of air has been drawn. The method is suitable for determination of asbestos in both exterior and building atmospheres.

1.3 Range

The upper limit for the range of concentration that can be measured on the analytical filter is 7 000 structures/mm^2. The lower limit of the range that can be measured on the analytical filter corresponds to detection of 2,99 structures in the area of specimen examined. The air concentrations represented by these values are a function of the volume of air sampled and the degree of dilution or concentration selected during the specimen preparation procedures. The method is particularly applicable for measurements in areas with high suspended-particulate concentrations (exceeding 10 µg/m^3), or where detection and identification of asbestos fibres are likely to be prevented or hindered by other types of particulate in direct-transfer TEM preparations. In theory, there is no lower limit to the dimensions of asbestos fibres which can be detected. In practice, microscopists vary in their ability to detect very small asbestos fibres. Therefore, a minimum length of 0,5 µm has been defined as the shortest fibre to be incorporated in the reported results.

1.4 Limit of detection

The limit of detection theoretically can be lowered indefinitely by filtration of progressively larger volumes of air, concentrating the sample during specimen preparation, and by extending the examination of the specimens in the electron microscope. In practice, the lowest achievable limit of detection for a particular area of TEM specimen examined is controlled by the total suspended particulate concentration remaining after the ashing and aqueous dispersal steps, and this depends on the chemical nature of the suspended particulate. For total suspended particulate concentrations of approximately 10 µg/m^3, corresponding to clean, rural atmospheres, and assuming filtration of 4 000 litres of air, an analytical sensitivity of 0,5 structure/litre can be obtained, equivalent to a limit of detection of 1,8 structures/litre, if an area of 0,195 mm^2 of the TEM specimens is examined. Lower limits of detection can be achieved by increasing the area of the TEM specimen that is examined, or by concentration of the sample during specimen preparation. In order to achieve lower limits of detection for fibres and bundles longer than 5 µm, and for PCM-equivalent fibres, lower magnifications are specified which permit more rapid examination of larger areas of the TEM specimens when the examination is limited to these dimensions of fibre.

2 Terms and definitions

For the purposes of this International Standard, the following terms and definitions apply.

2.1
acicular
shape shown by an extremely slender crystal with cross-sectional dimensions which are small relative to its length, i.e. needle-like

2.2
amphibole
group of rock-forming ferromagnesium silicate minerals, closely related in crystal form and composition, and having the nominal formula:

$$A_{0-1}B_2C_5T_8O_{22}(OH,F,Cl)_2$$

where

A is K, Na

B is Fe^{2+}, Mn, Mg, Ca, Na

C is Al, Cr, Ti, Fe^{3+}, Mg, Fe^{2+}

T is Si, Al, Cr, Fe^{3+}, Ti

NOTE In some varieties of amphibole, these elements can be partially substituted by Li, Pb, or Zn. Amphibole is characterized by a cross-linked double chain of Si-O tetrahedra with a silicon:oxygen ratio of 4:11, by columnar or fibrous prismatic crystals and by good prismatic cleavage in two directions parallel to the crystal faces and intersecting at angles of about 56° and 124°.

2.3
amphibole asbestos
amphibole in an asbestiform habit

2.4
analytical filter
filter through which an aqueous dispersion of ash from the sample collection filter is passed, and from which TEM specimen grids are prepared

2.5
analytical sensitivity
calculated airborne asbestos structure concentration, equivalent to counting of one asbestos structure in the analysis

NOTE 1 It is expressed in structures/litre.

NOTE 2 The method given in this International Standard does not specify an analytical sensitivity.

2.6
asbestiform
specific type of mineral fibrosity in which the fibres and fibrils possess high tensile strength and flexibility

2.7
asbestos
term applied to a group of silicate minerals belonging to the serpentine and amphibole groups which have crystallized in the asbestiform habit, causing them to be easily separated into long, thin, flexible, strong fibres when crushed or processed

NOTE The Chemical Abstracts Service Registry Numbers of the most common asbestos varieties are: chrysotile (12001-29-5), crocidolite (12001-28-4), grunerite asbestos (Amosite) (12172-73-5), anthophyllite asbestos (77536-67-5), tremolite asbestos (77536-68-6) and actinolite asbestos (77536-66-4).

2.8
asbestos structure
term applied to an individual asbestos fibre, or any connected or overlapping grouping of asbestos fibres or bundles, with or without other particles

2.9
ashed filter blank
fibre count made on TEM specimens prepared by the indirect procedure from a blank membrane filter of the type used for collection of air samples

2.10
aspect ratio
ratio of length to width of a particle

2.11
blank
structure count made on TEM specimens prepared from an unused filter in order to determine the background measurement

2.12
camera length
equivalent projection length between the specimen and its electron diffraction pattern, in the absence of lens action

2.13
chrysotile
fibrous mineral of the serpentine group which has the nominal composition

$$Mg_3Si_2O_5(OH)_4$$

NOTE Most natural chrysotile deviates little from this nominal composition. In some varieties of chrysotile, minor substitution of silicon by Al^{3+} may occur. Minor substitution of magnesium by Al^{3+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Mn^{2+} and Co^{2+} may also be present. Chrysotile is the most prevalent type of asbestos.

2.14
cleavage
breaking of a mineral along one of its crystallographic directions

2.15
cleavage fragment
fragment of a crystal that is bounded by cleavage faces

2.16
cluster
structure in which two or more fibres, or fibre bundles, are randomly oriented in a connected grouping

2.17
direct-transfer blank
structure count made on TEM specimens prepared by the direct-transfer procedure from a blank filter of the type used for filtration of aqueous dispersions of ash

2.18
d-spacing
distance between identical adjacent and parallel planes of atoms in a crystal

2.19
electron diffraction
technique in electron microscopy by which the crystal structure of a specimen is examined

2.20
electron scattering power
extent to which a thin layer of substance scatters impinging electrons from their original directions

2.21
empty beaker blank
fibre count made on TEM specimens prepared by the indirect procedure, using an empty beaker as the initial sample

2.22
energy-dispersive X-ray analysis
EDXA
measurement of the energies and intensities of X-rays by use of a solid-state detector and multichannel analyzer system

2.23
eucentric
condition in which the area of interest of an object is placed on a tilting axis, at the intersection of the electron beam with that axis, and is in the plane of focus

2.24
field blank
filter cassette which has been taken to the sampling site, opened and then closed, and the filter subsequently used to determine the background structure count for the measurement

2.25
fibril
single fibre of asbestos which cannot be further separated longitudinally into smaller components without losing its fibrous properties or appearances

2.26
fibre
elongated particle which has parallel or stepped sides

NOTE For the purposes of this International Standard, a fibre is defined to have an aspect ratio equal to or greater than 5:1 and a minimum length of 0,5 µm.

2.27
fibre bundle
structure composed of parallel, smaller-diameter fibres attached along their lengths

NOTE A fibre bundle may exhibit diverging fibres at one or both ends.

2.28
fibrous structure
fibre, or connected grouping of fibres, with or without other particles

2.29
funnel blank
structure count made on TEM specimens prepared by the direct-transfer method from a filter used for filtration of a sample of distilled water

2.30
habit
characteristic crystal growth form, or combination of these forms, of a mineral, including characteristic irregularities

2.31
limit of detection
calculated airborne asbestos structure concentration, equivalent to counting of 2,99 asbestos structures in the analysis

NOTE It is expressed in structures/litre.

2.32
matrix
structure in which one or more fibres, or fibre bundles, touch, are attached to or partially concealed by, a single particle or connected group of nonfibrous particles

2.33
Miller index
set of either three or four integer numbers used to specify the orientation of a crystallographic plane in relation to the crystal axes

2.34
PCM-equivalent fibre
fibre of aspect ratio greater than or equal to 3:1, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm

NOTE For the purposes of this International Standard, PCM is the abbreviated term for phase-contrast optical microscopy.

2.35
PCM-equivalent structure
fibrous structure of aspect ratio greater than or equal to 3:1, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm

NOTE For the purposes of this International Standard, PCM is the abbreviated term for phase-contrast optical microscopy.

2.36
primary structure
fibrous structure that is a separate entity in the TEM image

2.37
replication
procedure in electron microscopy specimen preparation in which a thin copy, or replica, of a surface is made

2.38
selected area electron diffraction
technique in electron microscopy in which the crystal structure of a small area of a sample is examined

2.39
serpentine
group of common rock-forming minerals having the nominal formula

$$Mg_3Si_2O_5(OH)_4$$

2.40
structure
single fibre, fibre bundle, cluster or matrix

2.41
twinning
occurrence of crystals of the same species joined together at a particular mutual orientation, and such that the relative orientations are related by a definite law

2.42
unopened fibre
large diameter asbestos fibre bundle which has not been separated into its constituent fibrils or fibres

2.43
zone-axis
line or crystallographic direction through the centre of a crystal which is parallel to the intersection edges of the crystal faces defining the crystal zone

3 Abbreviated terms

DMF Dimethylformamide

ED Electron diffraction

EDXA Energy dispersive X-ray analysis

FWHM Full width, half maximum

HEPA High efficiency particle absolute

MEC Mixed esters of cellulose

PC Polycarbonate

PCM Phase-contrast optical microscopy

SAED Selected area electron diffraction

SEM Scanning electron microscope

STEM Scanning transmission electron microscope

TEM Transmission electron microscope

UICC Union Internationale Contre le Cancer

4 Principle

A sample of airborne particulate is collected by drawing a measured volume of air through either a capillary-pore polycarbonate (PC) membrane filter of maximum pore size 0,4 µm or a cellulose ester (either mixed esters of cellulose or cellulose nitrate) membrane filter of maximum pore size 0,8 µm by means of a battery-powered or mains-powered pump. A portion of the filter is ashed in an oxygen plasma, and the residual ash is dispersed in distilled water with adjustment of the pH to between 3,0 and 4,0 using acetic acid. Analytical filters are then prepared by filtration of known volumes of this aqueous dispersion through either capillary-pore PC membrane filters of maximum pore size 0,2 µm or cellulose ester membrane filters of maximum pore size 0,22 µm.

TEM specimens are prepared from PC analytical filters by applying a thin film of carbon to the filter surface by vacuum evaporation. Small areas are cut from the carbon-coated filter, supported on TEM specimen grids, and the filter medium is dissolved away by a solvent extraction procedure. This procedure leaves a thin film of carbon which bridges the openings in the TEM specimen grid, and which supports each particle from the original filter in its original position.

Cellulose ester analytical filters are chemically treated to collapse the pore structure of the filter, and the surface of the collapsed filter is then etched in an oxygen plasma to ensure that all particles are exposed. A thin film of carbon is evaporated onto the filter surface and small areas are cut from the filter. These sections are supported on TEM specimen grids and the filter medium is dissolved away by a solvent extraction procedure.

The TEM specimen grids from either preparation method are examined at both low and high magnifications to check that they are suitable for analysis before carrying out a quantitative fibre count on randomly-selected grid openings. If the selected TEM specimen grid has too high a particulate or fibre loading, another specimen grid with a lower filtered aliquot shall be selected for analysis. In the TEM analysis, electron diffraction (ED) is used to examine the crystal structure of a fibre, and its elemental composition is determined by energy-dispersive X-ray analysis (EDXA). For a number of reasons, it is not possible to identify each fibre unequivocally, and fibres are classified according to the techniques which have been used to identify them. A simple code is used to record, for each fibre, the manner in which it was classified. The fibre classification procedure is based on successive inspection of the morphology, the selected area ED pattern, and the qualitative and quantitative EDXAs. Confirmation of the identification of chrysotile is only by quantitative ED, and confirmation of amphibole is only by quantitative EDXA and quantitative zone-axis ED.

In addition to isolated fibres, ambient air samples often contain more complex aggregates of fibres, with or without other particles. Some particles are composites of asbestos fibres with other materials. Individual fibres and these more complex structures are referred to as "asbestos structures". The indirect specimen preparation procedure permits the majority of these complex structures to be dispersed into their constituent fibres and fibre bundles, allowing more precise quantification than is possible using the direct-transfer procedure.

A coding system is used to record the type of fibrous structure, and also to provide the optimum morphological description of each structure. The two codes remove from the microscopist the requirement to interpret the fibre counting data, and allow this evaluation to be made later without the requirement for re-examination of the TEM specimens. Several levels of analysis are specified, the higher levels providing a more rigorous approach to the identification of fibres. The procedure permits a minimum required fibre identification criterion to be defined on the basis of previous knowledge, or lack of it, about the particular sample. Attempts are then made to achieve this minimum criterion for each fibre, and the degree of success is recorded for each fibre. The lengths and widths of all classified structures are recorded.

The number of asbestos structures found on a known area of the microscope sample, together with the volume of air filtered through the sample collection filter, the proportion of the sample collection filter that was ashed, the proportion of the aqueous dispersion that was filtered, and the area of the analytical filter are used to calculate the airborne concentration of asbestos, expressed in asbestos structures/litre of air. The mass concentration of asbestos is calculated using an assumed density for the asbestos variety, and the widths and lengths of the fibres.

5 Apparatus

5.1 Air sampling

5.1.1 Filter cassette, for sample collection.

Field monitors, comprising 25 mm to 50 mm diameter three-piece cassettes with cowls which project less than 2 cm in front of the filter surface, shall be used for sample collection. The cassette shall be loaded with either a capillary pore PC filter of maximum pore size 0,4 µm or a cellulose ester [mixed esters of cellulose (MEC) or cellulose nitrate] filter of maximum pore size 0,8 µm. When the filter is in position, a shrink cellulose band or adhesive tape shall be applied to prevent air leakage. Suitable precautions shall be taken to ensure that the filters are tightly clamped in the assembly so that significant air leakage around the filter cannot occur.

Representative filters from the filter lot shall be analyzed as described in 8.8 for the presence of asbestos structures, and also tested for suitability as described in annex H, before any are used for air sample collection.

NOTE This method permits the use of larger pore size cellulose ester filters for sample collection than the maximum pore size permitted in the direct-transfer method in ISO 10312. The smaller maximum pore size is specified in ISO 10312 in order to ensure that collected particulate and fibres are retained close to the surface of the filter, which is required if the particulate and fibres are to be transferred to the TEM grid with high efficiency by the direct-transfer procedure. In this indirect-transfer method, the depth of penetration of particulate and fibres into the filter medium during sample collection is unimportant, provided that they do not pass through the filter.

5.1.2 Sampling pump, capable of a flowrate sufficient to achieve the desired analytical sensitivity. The face velocity through the filter shall be between 4,0 cm/s and 70 cm/s. The sampling pump used shall provide a nonfluctuating air flow through the filter, and shall maintain the initial volume flowrate to within ± 10 % throughout the sampling period. A constant-flow or critical-orifice controlled pump meets these requirements. Flexible tubing shall be used to connect the filter cassette to the sampling pump. A means for calibration of the flowrate of each pump is also required.

NOTE The sampling efficiency for a particular particle size varies with the face velocity. Depending on the size distribution of the airborne particles, the analytical result may vary with face velocity.

5.1.3 Stand, to hold the filter cassette at the desired height for sampling, isolated from the vibrations of the pump.

5.1.4 Variable area flowmeter, calibrated, with a range suitable for determination of the selected flowrate as required for calibration of the air sampling system.

5.2 Specimen preparation laboratory

Asbestos, particularly chrysotile, is present in varying quantities in many laboratory reagents. Many building materials also contain significant amounts of asbestos or other mineral fibres which may interfere with the analysis if they are inadvertently introduced during preparation of specimens. It is most important to ensure that during preparation, contamination of TEM specimens by any extraneous asbestos fibres is minimized. All specimen preparation steps shall therefore be performed in an environment where contamination of the sample is minimized. The primary requirement of the sample preparation laboratory is that a blank determination shall yield a result which will meet the requirements specified in 8.8. A minimum facility considered suitable for preparation of TEM specimens is a positive pressure, laminar flow hood. However, it has been established that work practices in specimen preparation appear to be more important than the type of clean handling facilities in use. Preparation of samples shall be carried out only after acceptable blank values have been demonstrated.

NOTE It is recommended that activities involving manipulation of bulk asbestos samples not be performed in the same area as TEM specimen preparation, because of the possibilities of contaminating the TEM specimens.

5.3 Equipment for analysis

5.3.1 Transmission electron microscope, operating at an accelerating potential of 80 kV to 120 kV, with a resolution better than 1,0 nm, and a magnification range of approximately 300× to 100 000× shall be used. The ability to obtain a direct screen magnification of about 100 000× is necessary for inspection of fibre morphology; this magnification may be obtained by supplementary optical enlargement of the screen image with a binocular if it cannot be obtained directly. The viewing screen shall be calibrated (such as shown in Figure 1) with concentric circles and a millimetre scale such that the lengths and widths of fibre images down to 1 mm width can be measured in increments of 1 mm.

For Bragg angles less than 0,01 rad the TEM shall be capable of performing ED from an area of 0,6 μm^2 or less, selected from an in-focus image at a screen magnification of 20 000×. This performance requirement defines the minimum separation between particles at which independent ED patterns can be obtained from each particle. If SAED is used, the performance of an individual instrument may normally be calculated using the following relationship:

$$A = 0{,}7854\left(\frac{D}{M} + 2000\,C_s \cdot \theta^3\right)^2$$

where

A is the effective SAED area, expressed in square micrometres (μm^2);

D is the diameter of the SAED aperture, expressed in micrometres;

M is the magnification of the objective lens;

C_s is the spherical aberration coefficient of the objective lens, expressed in millimetres;

θ is the maximum required Bragg angle, expressed in radians.

It is not possible to reduce the effective SAED area indefinitely by the use of progressively smaller SAED apertures, because there is a fundamental limitation imposed by the spherical aberration coefficient of the objective lens.

If zone-axis ED analyses are to be performed, the TEM shall incorporate a goniometer stage which permits the TEM specimen to be either:

a) rotated through 360°, combined with tilting through at least +30° to −30° about an axis in the plane of the specimen; or,

b) tilted through at least +30° to −30° about two perpendicular axes in the plane of the specimen.

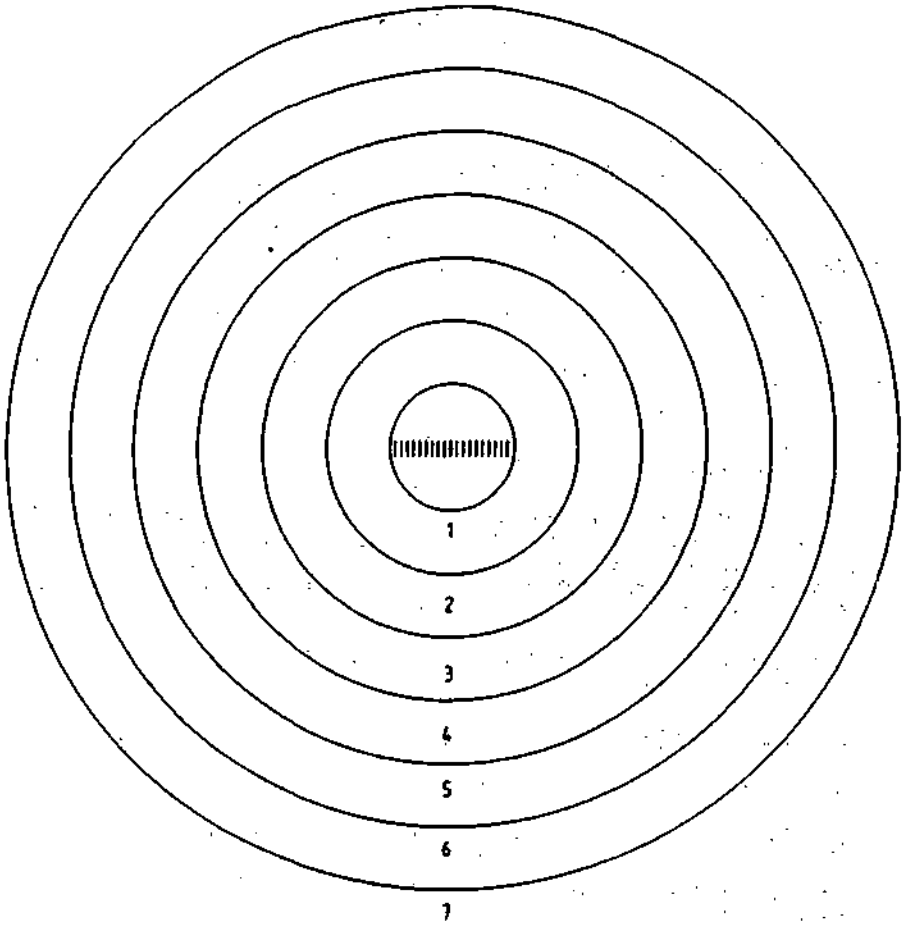


Figure 1 — Example of calibration markings on TEM viewing screen

The analysis is greatly facilitated if the goniometer permits eucentric tilting, although this is not essential. If EDXA and zone-axis ED are required on the same fibre, the goniometer shall be of a type which permits tilting of the specimen and acquisition of EDXA spectra without change of specimen holder.

The TEM shall have an illumination and condenser lens system capable of forming an electron probe smaller than 250 nm in diameter.

NOTE Use of an anticontamination trap around the specimen is recommended if the required instrumental performance is to be obtained.

5.3.2 Energy-dispersive X-ray analyzer, capable of achieving a resolution better than 180 eV (FWHM) on the MnKα peak. Since the performance of individual combinations of TEM and EDXA equipment is dependent on a number of geometrical factors, the required performance of the combination of the TEM and X-ray analyzer is specified in terms of the measured X-ray intensity obtained from a fibre of small diameter, using a known electron-beam diameter. Solid-state X-ray detectors are least sensitive in the low energy region, and so measurement of sodium in crocidolite shall be the performance criterion. The combination of electron microscope and X-ray analyzer shall yield, under routine analytical conditions, a background-subtracted NaKα integrated peak count rate of more than 1 count per second (cps) from a fibre of UICC crocidolite 50 nm in diameter or smaller when irradiated by an electron probe of 250 nm diameter or smaller at an accelerating potential of 80 kV. The peak/background ratio for this performance test shall exceed 1,0.

The EDXA unit shall provide the means for subtraction of the background, identification of element peaks, and calculation of background-subtracted peak areas.

5.3.3 Computer.

Many repetitive numerical calculations are necessary, and these may be performed conveniently by relatively simple computer programs. For analyses of zone-axis ED pattern measurements, a computer with adequate memory is required to accommodate the more complex programs involved.

5.3.4 Plasma asher, with a radio frequency power rating of 50 W or higher, for ashing of sample collection filters and for preparation of TEM specimens from MEC filters. The asher shall be supplied with a controlled oxygen flow, and shall be modified, if necessary, to provide a valve to control the speed of air admission so that rapid air admission does not disturb the particulate.

NOTE It is recommended that filters be fitted to the oxygen supply and the air admission line.

5.3.5 Vacuum coating unit, capable of producing a vacuum better than 0,013 Pa shall be used for vacuum deposition of carbon on the membrane filters. A sample holder is required which will allow a glass microscope slide to be continuously rotated during the coating procedure.

NOTE A mechanism which allows the rotating slide also to be tilted through an angle of approximately 45° during the coating procedure is recommended. A liquid nitrogen cold trap above the diffusion pump may be used to minimize the possibility of contamination of the filter surfaces by oil from the pumping system. The vacuum coating unit may also be used for deposition of the thin film of gold, or other calibration material, when it is required on TEM specimens as an internal calibration of ED patterns.

5.3.6 Sputter coater (optional), with a gold target, for deposition of gold onto TEM specimens as an internal calibration of ED patterns.

Other calibration materials are acceptable.

NOTE Experience has shown that a sputter coater allows better control of the thickness of the calibration material.

5.3.7 Beakers, of borosilicate glass, 50 ml capcity, as containers for plasma ashing of sample collection filters.

5.3.8 Vacuum source, to provide a vacuum of at least 20 kPa for the filtration of aqueous dispersions. A water jet pump is suitable.

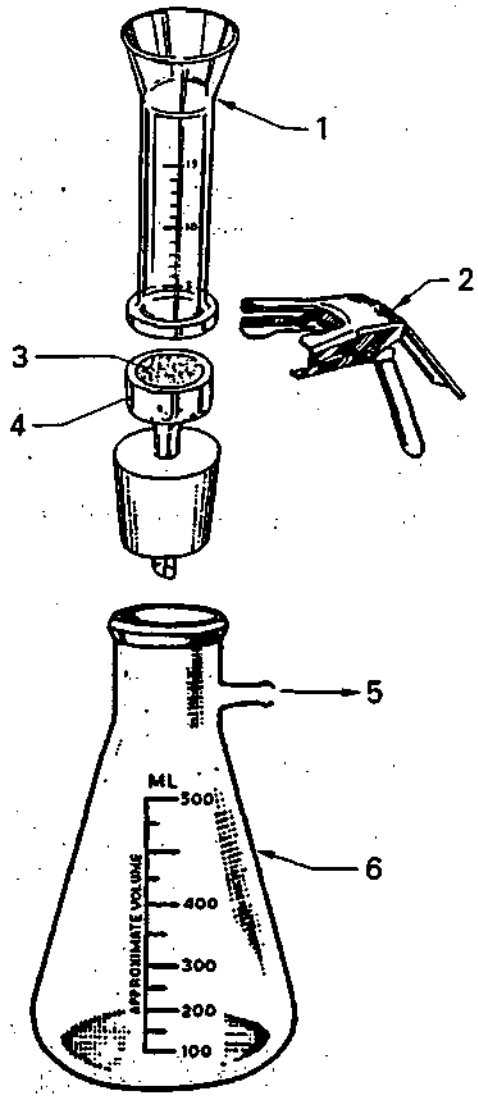
5.3.9 Glass filtration apparatus, for the filtration of aqueous dispersions. This consists of a filtration base with a porous support for the filter, a reservoir with vertical sides, a clamp to hold the reservoir to the filtration base, and a vacuum flask or filtration manifold. A suitable apparatus is shown in Figure 2.

5.3.10 Solvent washer (Jaffe washer), to allow dissolution of the filter polymer while leaving an intact evaporated carbon film supporting the fibres and other particles from the filter surface. One design of a washer which has been found satisfactory for various solvents and filter media is shown in Figure 3.

In general, either chloroform or 1-methyl-2-pyrrolidone is used for dissolving PC filters and dimethylformamide or acetone is used for dissolving cellulose ester filters. The higher evaporation rates of chloroform and acetone require that a reservoir of 10 ml to 50 ml of solvent be used, which may need replenishment during the procedure. Dimethylformamide and 1-methyl-2-pyrrolidone have lower vapour pressures, therefore much smaller volumes of these solvents may be used. It is recommended that all washers be used in a fume hood, and when specimens are not being inserted or removed, during the solvent dissolution the Petri dish lid shall be in place. The washer shall be cleaned before it is used for each batch of specimens.

5.3.11 Condensation washer, for more rapid dissolution of the filter polymer, or if difficulties are experienced in dissolving the filter polymer. The washer shall consist of a flask, condenser and cold-finger assembly, with a heating mantle and means for controlling the temperature. A suitable assembly is shown in Figure 4, for use with either acetone or chloroform as the solvent, depending on the type of filter.

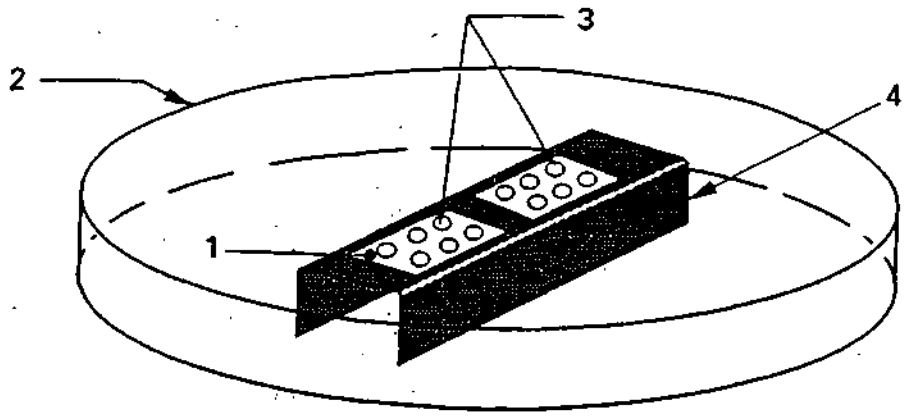
5.3.12 Slide warmer or oven, for heating slides during the preparation of TEM specimens from cellulose ester filters. It shall be capable of maintaining a temperature of 65 °C to 70 °C.



Key

1	Filtration reservoir	4	Filtration base
2	Clamp	5	Vacuum source
3	Porous support	6	Vacuum flask

Figure 2 — Example of filtration apparatus

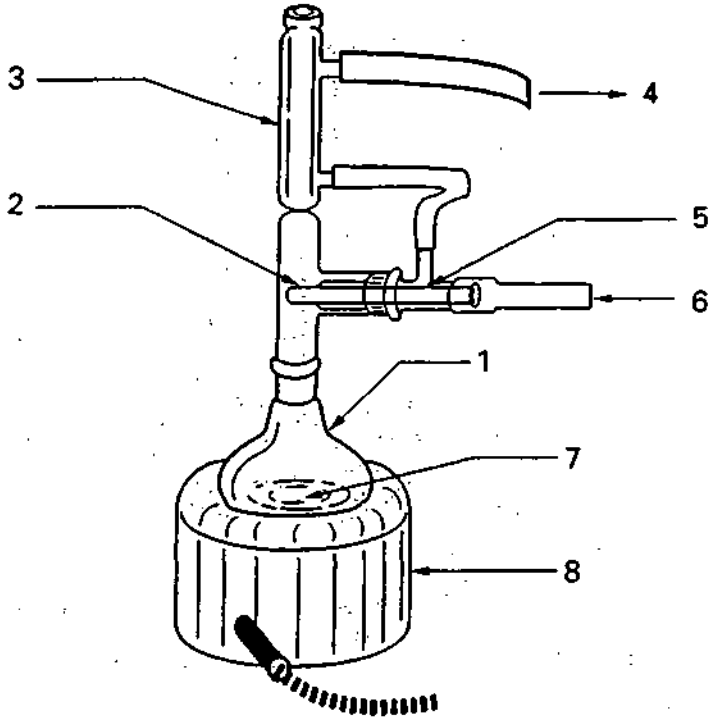


Key

1	Lens tissue	3	Electron microscope specimens
2	Glass Petri dish (100 mm × 15 mm)	4	Stainless steel mesh bridge (50 mesh)

NOTE Solvent is added until the meniscus contacts the underside of the stainless steel mesh.

Figure 3 — Example of design of solvent washer (Jaffe washer)



Key

1	Flask	5	Cold finger
2	Specimens	6	Cold water source
3	Condenser	7	Solvent
4	Water drain	8	Thermostatically controlled heating mantle

Figure 4 — Design of condensation washer

5.3.13 Ultrasonic bath, for aqueous dispersal of ash from sample collection filters, and also for cleaning of apparatus used for TEM specimen preparation. The power of the ultrasonic bath shall be sufficient to allow absorption of energy into a 40 ml volume of water in a 50 ml glass beaker at a rate between 0,05 W/ml to 0,1 W/ml, at a frequency of approximately 50 kHz.

5.3.14 Carbon-grating replica, with about 2 000 parallel lines per millimetre, to calibrate the magnification of the TEM.

5.3.15 Calibration grids for EDXA, prepared from dispersions of calibration minerals, for calibration of the EDXA system. Examples of suitable calibration minerals are riebeckite, chrysotile, halloysite, phlogopite, wollastonite and bustamite. Prepare the mineral used for calibration of the EDXA system for sodium using a gold TEM grid.

5.3.16 Carbon rod sharpener.

NOTE The use of necked carbon rods, or equivalent, allows the carbon to be evaporated on to the filters with a minimum of heating.

5.3.17 Disposable-tip micropipettes, capable of transferring a volume of approximately 50 µl for the pH adjustment, and a volume of approximately 30 µl for the preparation of TEM specimen grids from cellulose ester filters.

5.3.18 Thermometer, with a range from 0 °C to 100 °C, for calibration of the ultrasonic bath.

5.3.19 Stopwatch, for calibration of the ultrasonic bath.

5.4 Consumable supplies

5.4.1 Cellulose ester filters (either MEC or cellulose nitrate) filters of maximum pore size 0,22 µm and diameter appropriate for the liquid filtration apparatus, for filtration of the aqueous suspension of residual ash.

5.4.2 Cellulose ester filters (either MEC or cellulose nitrate) of 5 µm pore size and diameter appropriate for the liquid filtration apparatus, for use with the liquid filtration apparatus.

5.4.3 Polycarbonate filters, of maximum pore size 0,2 µm and diameter appropriate for the liquid filtration apparatus, for filtration of the aqueous suspension of residual ash.

5.4.4 Petri dishes, for storage of analytical filters prepared by filtration of the suspension of residual ash.

5.4.5 Copper electron-microscope grids.

Two hundred mesh TEM grids are recommended. Grids which have grid openings of uniform size such that they meet the requirement of 8.7.2 shall be chosen. To facilitate the relocation of individual grid openings for quality assurance purposes, the use of grids with numerical or alphabetical indexing of individual grid openings is recommended.

5.4.6 Gold electron-microscope grids.

Two-hundred-mesh gold TEM grids are recommended to mount TEM specimens when sodium measurements are required in the fibre identification procedure. Grids which have grid openings of uniform size such that they meet the requirement of 8.7.2 shall be chosen. To facilitate the relocation of individual grid openings for quality assurance purposes, the use of grids with numerical or alphabetical indexing of individual grid openings is recommended.

5.4.7 Aluminium foil, for covering beakers during the plasma ashing and ultrasonic treatment steps. Aluminium foil of 0,015 mm to 0,020 mm thickness is suitable.

5.4.8 Carbon rod electrodes, spectrochemically pure, for use in the vacuum evaporator during carbon coating of filters.

5.4.9 Routine electron microscopy tools and supplies, including fine-point tweezers, scalpel holders and blades, microscope slides, double-sided adhesive tape, lens tissue, gold wire, tungsten filaments and other routine supplies are required.

5.4.10 **Reference asbestos samples**, for preparation of reference TEM specimens of the primary asbestos minerals. The UICC set of minerals is suitable for this purpose.

6 Reagents

WARNING — Use the reagents in accordance with the appropriate health and safety regulations.

6.1 **Fibre-free water**, freshly distilled, or other source of fibre-free, pyrogen-free water shall be used.

6.2 **Chloroform**, analytical grade, distilled in glass [preserved with 1 % (volume fraction) ethanol].

6.3 **1-Methyl-2-pyrrolidone**, analytical grade.

6.4 **1,2-Diaminoethane**, analytical grade.

6.5 **Dimethylformamide**, analytical grade.

6.6 **Glacial acetic acid**, analytical grade.

6.7 **Acetone**, analytical grade.

7 Air sample collection

7.1 Calculation of analytical sensitivity

The desired analytical sensitivity is a parameter that shall be established for the analysis prior to sample collection. The analytical sensitivity is defined as that structure concentration corresponding to the detection of one structure in the analysis. For indirect-transfer methods of TEM specimen preparation, it is a function of the volume of air sampled, the active area of the collection filter, the area of the collection filter that is ashed, the proportion of the aqueous dispersion of ash that is filtered, the area of the analytical filter, and the area of the TEM specimen over which structures are counted. The number of grid openings, k, required to be examined to achieve a particular analytical sensitivity is calculated using the formula:

$$k = \frac{(A_a \cdot V_d)}{(S \cdot A_g \cdot V_f \cdot F_a \cdot V_s)}$$

where

A_a is the active area, expressed in square millimetres, of analytical filter;

V_d is the volume, expressed in millilitres, of water used for dispersal of residual ash;

S is the analytical sensitivity, expressed in structures/litre;

A_g is the mean area of grid openings, expressed in square millimetres;

V_f is the volume, expressed in millilitres, of aqueous dispersion filtered;

F_a is the fraction of sample collection filter ashed;

V_s is the volume, expressed in litres, of air sampled.

Examples of the number of grid openings required to be examined, using various combinations of sampling and analytical parameters, are shown in Table 1.

Table 1 — Examples of minimum number of grid openings required to achieve a particular analytical sensitivity and limit of detection

Analytical sensitivity structures/litre	Limit of detection structures/litre	Minimum number of grid openings						
		Litres of air sampled						
		500	1 000	2 000	3 000	4 000	5 000	10 000
0,1	0,3	1 297	649	325	217	163	130	65
0,2	0,6	649	325	163	109	82	65	33
0,3	0,9	433	217	109	73	55	44	22
0,4	1,2	325	163	82	55	41	33	17
0,5	1,5	260	130	65	44	33	26	13
0,7	2,1	186	93	47	31	24	19	10
1,0	3,0	130	65	33	22	17	13	7
2,0	6,0	65	33	17	11	9	7	4
3,0	9,0	44	22	11	8	6	5	4
4,0	12	33	17	9	6	5	4	4
5,0	15	26	13	7	5	4	4	4
7,0	21	19	10	5	4	4	4	4
10	30	13	7	4	4	4	4	4

NOTE In Table 1, it is assumed that one half of the sample collection filter is ashed, the volume of water used to disperse the ash is 40 ml, the volume of aqueous dispersion filtered is 34 ml, the active area of the analytical filter is 199 mm^2, and the TEM grid openings are square with a linear dimension of 65 µm. The limit of detection is defined as the upper 95 % confidence limit of the Poisson distribution for a count of zero structures. In the absence of background, this is equal to 2,99 times the analytical sensitivity. Non-zero backgrounds observed during analysis of blank filters will degrade the limit of detection.

7.2 Sample collection procedure

Air samples shall be collected using cassettes as described in 5.1.1. During sampling, the filter cassette shall be supported on a stand which is isolated from the vibrations of the pump. The cassette shall be held facing vertically downwards at a height of approximately 1,5 m to 2,0 m above ground/floor level, and connected to the pump with a flexible tube. Measure the sampling flowrate at the front end of the cassette, both at the beginning and end of the sampling period, using a calibrated variable-area flowmeter temporarily attached to the inlet of the cassette. The mean value of these two measurements shall be used to calculate the total air volume sampled.

The variable area flowmeter shall be cleaned before use, to avoid transfer of fibre contamination from the flowmeter to the sample being collected.

Basic strategies for monitoring environmental sources of airborne asbestos are described in annex I. After sampling, a cap shall be placed over the open end of the cassette, and the cassette packed with the filter face-upwards for return to the laboratory. Field blank filters shall also be included, as described in 8.8, and processed through the remaining analytical procedures along with the samples.

8 Procedure for analysis

8.1 General

The procedures for ashing of sample collection filters, dispersion of the residual ash, and filtration of the aqueous dispersion are identical for the two types of sample collection filter. The techniques used to prepare TEM specimens from the analytical filters are different for PC and cellulose ester filters. The preparation method to be used shall be

that described in either 8.4 or 8.5, depending on the type of membrane filter used for filtration of the aqueous dispersions. Cleaning of the sample cassettes before they are opened, preparation of the carbon evaporator, criteria for acceptable specimen grids, and the requirement for blank determinations are identical for the two preparation techniques. TEM examination, structure counting, fibre identification and reporting of results are independent of the type of filter or preparation technique used.

The ability to meet the blank sample criteria is dependent on the cleanliness of equipment and supplies. Consider all supplies, such as microscope slides and glassware, as potential sources of asbestos contamination. It is necessary to wash all glassware before it is used. Wash any tools or glassware which come into contact with the air sampling filters or TEM specimen preparations both before use, and between handling of individual samples. Where possible, use disposable supplies.

NOTE In order to ensure that all particulate contamination and fibres are detached from glassware, it is recommended that all surfaces be washed by rubbing vigorously with clean paper towel soaked in detergent, and rinsed several times under a continuous flow of water. The glassware should then be rinsed at least twice using fibre-free water.

8.2 Cleaning of sample cassettes

Asbestos fibres can adhere to the exterior surfaces of air-sampling cassettes, and these fibres can be inadvertently transferred to the sample during handling. To prevent this possibility of contamination, and after ensuring that the cassette is tightly sealed, wipe the exterior surfaces of each sampling cassette with wet paper towel before it is taken into the clean facility or laminar-flow hood.

8.3 Preparation of analytical filters

8.3.1 Selection of filter area for ashing

Depending on the analytical sensitivity to be achieved, ash either a quarter or a half of the sample collection filter. Using freshly cleaned tweezers, remove the sample collection filter from the sampling cassette, and place it onto a second cleaned glass microscope slide which is used as a cutting surface. Using a freshly-cleaned curved scalpel blade, cut the required area of the filter by rocking the blade from the point, pressing it into contact with the filter. Repeat the process as necessary.

8.3.2 Ashing of sample collection filters

Place the selected filter portion into a 50 ml borosilicate glass beaker, ensuring that the side of the filter with the collected particulate faces downwards. Cover the beaker with a 6 cm × 6 cm square piece of aluminium foil of thickness 0,015 mm to 0,02 mm, and bend the foil over the edges of the beaker so that it forms a tight seal. Perforate the foil in 10 to 20 positions with a needle to allow for gas exchange during plasma ashing. Depending on the size of the plasma asher chamber, several sample filters may be ashed simultaneously. At least one empty beaker, prepared in the same way, shall be placed in the asher chamber along with each batch of samples ashed. Operate the plasma asher using the minimum power at which a glow discharge is observed, until the filters appear to be completely ashed. Increase the plasma asher power to the maximum, and operate under these conditions for a minimum period of 3 h. After the ashing treatment is completed, admit air to the chamber and remove the beakers.

NOTE Losses of particulate and fibres from the beaker will occur if the plasma asher is operated at excessive radio-frequency power. During ashing of MEC or cellulose nitrate filters, a critical point is reached during the oxidation at which a sudden, violent ignition occurs if the radio-frequency power is excessive. This results in loss of fibres from the beaker, contamination of the interior surfaces of the chamber, and possible cross-contamination of the samples. The same considerations apply to the ashing of PC filters. The ashing procedure specified avoids these problems. It is recommended that the ashing of a blank filter be observed closely during the final stages of oxidation, in order to ensure that the radio-frequency power setting is such that a sudden ignition does not occur.

8.3.3 Aqueous dispersal of residual ash from sample collection filters

Remove the aluminium foil from the top of the beaker. Using a disposable-tip pipette, add 50 µl of glacial acetic acid, and then add 40 ml of freshly distilled water. Cover the beaker with a new 6 cm × 6 cm square piece of aluminium foil, and bend the foil over the edges of the beaker so that it forms a tight seal. Place the beaker into the ultrasonic bath and operate the bath at the calibrated power (see annex B) for a period of 5 min.

8.3.4 Assembly of system for filtration of aqueous dispersions

Filtration of the aqueous dispersions is a very critical procedure because it is important to obtain uniform deposits of particulate on the analytical filters. The following procedure shall be used.

a) Set up the filtration system and connect to a vacuum source;

b) add freshly distilled water to the filtration unit base component until there is a raised meniscus;

c) place a 5 µm pore size cellulose ester filter onto the water meniscus. The filter will centralize. Apply the vacuum very briefly in order to bring the filter into contact with the base component;

d) add freshly distilled water to the top of the cellulose ester filter, and place the analytical filter (either a 0,2 µm maximum pore size capillary-pore PC filter or a 0,22 µm maximum pore size cellulose ester filter) onto the water surface. Apply the vacuum very briefly again in order to bring both filters into contact with the base component;

e) install the filtration reservoir and clamp the assembly together.

8.3.5 Filtration of aqueous dispersions

Before filtering the aqueous dispersions, prepare a funnel blank by filtration of 40 ml of freshly distilled water.

The volume of the aqueous dispersion to be filtered depends on either the particulate concentration or the asbestos fiber concentration. The volume of aqueous dispersion required to produce an analytical filter with a suitable particulate or fiber loading for analysis often cannot be predicted, and it is usually necessary to prepare several analytical filters corresponding to filtration of different aliquots. The aqueous dispersions are not stable, and extended exposure of chrysotile to acidic environments causes leaching of magnesium to occur from the fibres; it is therefore necessary to prepare all analytical filters immediately. Uniform deposits of particulate on the analytical filters cannot be assured if liquid volumes smaller than 5 ml are filtered using filtration systems of 199 mm^2 active area; accordingly, where it is required to filter volumes smaller than 5 ml, the aliquot shall be diluted with freshly distilled water to a volume exceeding 5 ml.

Pour the aliquot of the dispersion into the filtration reservoir, and apply the vacuum. If the volume of the aliquot is larger than the capacity of the filtration reservoir, do not allow the level of liquid in the reservoir to fall below 5 cm depth before the remaining volume is added. Failure to observe this precaution may result in disturbance of the filtered particulate and non-uniform deposition.

With the vacuum still applied, unclamp the filtration assembly and remove the filtration reservoir. Using clean tweezers, remove the analytical filter and transfer it to a Petri dish. Allow the filter to dry before placing the cover on the Petri dish.

For the beaker blank, prepare only one analytical filter by filtration of the entire 40 ml dispersion.

NOTE It is recommended that analytical filters corresponding to volumes of 1 ml, 5 ml and 34 ml be prepared, unless there is reason to suspect that even the lowest volume will yield an analytical filter of unacceptably high loading. If the particulate or fiber concentration is thought to be such that it is required to filter an aliquot of volume lower than 1 ml, use a dilution procedure in which 1 ml of the original dispersion is transferred to a clean beaker and diluted with freshly distilled water to a total volume of 100 ml. After stirring to ensure complete mixing, aliquots of 10 ml and 30 ml from this diluted dispersion can then be filtered, corresponding to volumes of 0,1 ml and 0,3 ml of the original dispersion. From the original dispersion, volumes of 1 ml, 5 ml and 33 ml are also filtered, giving five analytical filters with a concentration range of a factor of 330. The requirement for washing of the filtration apparatus is minimized if the aliquots are filtered in order of increasing concentration. If aqueous dispersions are known to have high concentrations of asbestos fibres, the level of cross-contamination from one sample to the next can be determined by interposing additional funnel blanks. Wash the filtration apparatus between filtrations of different samples.

8.4 Preparation of TEM specimens from PC analytical filters

8.4.1 Selection of filter area for carbon-coating

Use a cleaned microscope slide to support representative portions of polycarbonate (PC) filter during the carbon evaporation. Double-sided adhesive tape is used to hold the filter portions to the glass slide. Take care not to

stretch the PC filters during handling. Using freshly cleaned tweezers, remove the PC filter from the Petri dish, and place it on a second cleaned glass microscope slide which is used as a cutting surface. Using a freshly-cleaned curved scalpel blade, cut the filter by rocking the blade from the point, pressing it into contact with the filter. Repeat the process as necessary. Several such portions may be mounted on the same microscope slide. The scalpel blade and tweezers shall be washed and dried between the handling of each filter. Identify the filter portions by writing on the glass slide.

8.4.2 Carbon-coating of filter portions

Place the glass slide holding the filter portions on the rotating-tilting device, approximately 10 cm to 12 cm from the evaporation source, and evacuate the evaporator chamber to a vacuum better than 0,013 Pa. The evaporation of carbon shall be performed in very short bursts, separated by a few seconds to allow the electrodes to cool. If evaporation of carbon is too rapid, the strips of PC filter will begin to curl, and crosslinking of the surface will occur. This crosslinking produces a layer of polymer which is relatively insoluble in chloroform, and it will not be possible to prepare satisfactory TEM specimens. The thickness of carbon required is dependent on the size of particles on the filter; approximately 30 nm to 50 nm has been found to be satisfactory. If the carbon film is too thin, large particles will break out of the film during the later stages of preparation, and there will be few complete and undamaged grid openings on the specimen. Too thick a carbon film will lead to a TEM image which is lacking in contrast, and the ability to obtain ED patterns will be compromised. The carbon film thickness should be the minimum possible, while retaining most of the grid openings of the TEM specimen intact.

8.4.3 Use of the Jaffe washer

Place several pieces of lens tissue, as shown in Figure 3, on the stainless steel bridge, and fill the washer with chloroform or 1-methyl-2-pyrrolidone to a level where the meniscus contacts the underside of the mesh, resulting in saturation of the lens tissue.

Using a curved scalpel blade, cut three 3 mm square pieces of carbon-coated PC filter from the carbon-coated filter portion. Select three squares to represent the centre and the outer periphery of the active surface of the filter. Place each square of filter, carbon side up, on a TEM specimen grid, and place the grid and filter onto the saturated lens tissue in the Jaffe washer. Place the three specimen grids from one sample on the same piece of lens tissue. Any number of separate pieces of lens tissue may be placed in the same Jaffe washer. Cover the Jaffe washer with the lid, and allow the washer to stand for at least 8 h.

NOTE It has been found that some PC filters will not completely dissolve in the Jaffe washer, even after exposure to chloroform for as long as 3 days. This problem is more severe if the surface of the filter was overheated during the carbon evaporation. It has been found that the problem of residual undissolved filter polymer can be overcome in several ways:

a) a mixture of 20 % 1,2-diaminoethane (ethylenediamine) and 80 % 1-methyl-2-pyrrolidone, used in a Jaffe washer, completely dissolves PC filters in 15 min, even if the surface of the filter has been overheated. To use this solvent, place the grids directly on the stainless steel mesh of the Jaffe washer; do not use the lens paper. After a period of 15 min, transfer the stainless steel bridge into another Petri dish, and add distilled water until the meniscus contacts the underside of the mesh. After approximately 15 min, remove the mesh and allow the grids to dry;

b) used in a Jaffe washer, 1-methyl-2-pyrrolidone has been found to be a more effective solvent than chloroform for PC filters. This solvent is more effective if the lens paper not used and grids are placed directly on the stainless steel mesh of the Jaffe washer. A dissolution period of 2 h to 6 h has been found to be satisfactory. After dissolution is complete, remove the stainless steel mesh from the Jaffe washer and allow the grids to dry. 1-Methyl-2-pyrrolidone evaporates very slowly. If it is required to dry the grids more rapidly, transfer the stainless steel bridge into another Petri dish, and add distilled water until the meniscus contacts the underside of the mesh. After approximately 15 min, remove the mesh and allow the grids to dry;

c) condensation washing of the grids, using chloroform as the solvent, after the initial Jaffe washer treatment, can often remove much of the residual filter medium in a period of approximately 30 min. To carry out this procedure, transfer the piece of lens tissue supporting the specimen grids to the cold finger of the condensation washer, which has achieved stable operating conditions. Operate the washer for approximately 30 min after inserting the grids.

8.4.4 Rapid preparation of TEM specimens from PC filters

TEM specimens can be prepared rapidly from PC filters, if desired, using the filter dissolution procedure described in item a) of the note in 8.4.3. Alternatively, the specimens may be washed for approximately 1 h in a Jaffe washer, followed by washing for 30 min in a condensation washer using chloroform as the solvent.

8.5 Preparation of TEM specimens from cellulose ester analytical filters

8.5.1 Selection of area of filter for preparation

Using clean tweezers, remove the filter from the Petri dish, and place it on a cleaned microscope slide. Using a clean, curved scalpel blade, cut out a portion of the filter.

8.5.2 Preparation of solution for collapsing cellulose ester filters

Mix 35 ml of dimethylformamide, 15 ml of glacial acetic acid and 50 ml of freshly distilled water. Store this mixture in a clean bottle. The mixture is stable and suitable for use for up to 3 months after preparation.

8.5.3 Filter-collapsing procedure

Using a micropipette with a disposable tip, place 15 µl/cm^2 to 25 µl/cm^2 of the collapsing solution on a cleaned microscope slide and, using the end of the pipette tip, spread the liquid over the area to be occupied by the filter portion. Place the filter portion, active surface upwards, on top of the solution, lowering the edge of the filter at an angle of about 20° so that air bubbles are not created. Remove any solution not absorbed by the filter by allowing a paper tissue to contact the liquid at the edge of the filter. More than one filter portion may be placed on one slide. Place the slide either on a thermostatically controlled slide warmer at a temperature of 65 °C to 70 °C, or in an oven at this temperature, for 10 min. The filter collapses slowly to about 15 % of its original thickness. The procedure leaves a thin, transparent polymer film, with particles and fibres embedded in the upper surface.

8.5.4 Plasma etching of the filter surface

The conditions required in the particular plasma asher shall be established using the procedure defined in annex A. Place the microscope slide holding the collapsed filter portions in the plasma asher, and etch for the time and under the conditions determined. Take care to ensure that the correct conditions are used. After etching, admit air slowly to the chamber and remove the microscope slide.

The air admission valve of the plasma asher shall be adjusted such that the time taken for the chamber to reach atmospheric pressure exceeds 2 min. Rapid air admission may disturb particulate on the surface of the etched filter.

8.5.5 Carbon-coating

Carbon-coat the microscope slide holding the collapsed filter portions as described in 8.4.2.

8.5.6 Use of the Jaffe washer

Place several pieces of lens tissue on the stainless steel bridge, and fill the washer with dimethylformamide or acetone to a level where the meniscus contacts the underside of the mesh, resulting in saturation of the lens tissue.

Place the specimens in the Jaffe washer as described in 8.4.3. Specimens are normally cleared after approximately 4 h.

8.5.7 Rapid preparation of TEM specimens from cellulose ester filters

An alternative washing procedure may be used to prepare TEM specimens from cellulose ester filters more rapidly than can be achieved by the Jaffe washing procedure. After the specimens have been washed in a Jaffe washer for approximately 1 h, transfer the piece of lens tissue supporting the specimens to the cold finger of a condensation washer operating with acetone as the solvent. Operate the condensation washer for approximately 30 min. This treatment removes all remaining filter polymer.

Dimethylformamide shall not be used in a condensation washer.

8.6 Criteria for acceptable TEM specimen grids

Valid data cannot be obtained unless the TEM specimens meet specified quality criteria. Incomplete dispersion of the residual ash from the sample collection filter, or non-uniform deposition of particulate due to the use of incorrect filtration procedures are of major concern. Examine in the TEM all three of the TEM grids corresponding to the largest filtered aliquot at a magnification sufficiently low (300× to 1 000×) so that complete grid openings can be inspected. If large areas of undispersed ash are observed on widely separated grid openings, the sample collection filter was either incompletely ashed, or the filter medium may be unsuitable for this preparation procedure. Reject all of the filters and grids. If it is suspected that the filters are unsuitable for this analytical procedure, use the procedure specified in annex H to confirm that the filter medium is suitable for analysis by this method, and reprepare the sample from another sector of the sample collection filter.

If the particulate deposit on the TEM grids corresponding to filtration of the largest filtered aliquot appears to be uniform, it is then necessary to select the set of TEM grids with an appropriate loading for fibre counting. Reject the grids if:

a) the carbon replica covers less than approximately 75 % of the area of the TEM specimen grids;

b) the TEM specimen has not been cleared of filter medium by the filter dissolution step;

   If the TEM specimen exhibits areas of undissolved filter medium, and if at least two of the three specimen grids are not cleared, either additional solvent washing shall be carried out, or new specimens shall be prepared from the analytical filter.

c) the sample is overloaded with particulate;

   If the specimen grid exhibits more than approximately 10 % obscuration on the majority of the grid openings, the specimen shall be designated as over-loaded. This filter cannot be analyzed satisfactorily because the grid is too heavily loaded with debris to allow separate examination of individual particles by ED and EDXA, and obscuration of fibres by other particulate may lead to under-estimation of the structure count. Specimens prepared from a filter corresponding to filtration of a smaller aliquot shall be selected.

d) the particulate deposits on the specimen are not uniformly distributed from one grid opening to the next;

   If the particulate deposits on the specimen are obviously not uniform from one grid opening to the next, the specimen shall be designated as non-uniform. This condition is caused by incorrect installation of the filters for filtration of the aqueous dispersions, or inadequate mixing during dilution of a small volume aliquot. Satisfactory analysis of this filter may not be possible unless a large number of grid openings is examined.

e) the TEM grid is too heavily loaded with fibrous structures to make an accurate count;

   Accurate counts cannot be made if the grid has more than approximately 7 000 structures/mm^2. Select specimens prepared from a filter corresponding to filtration of a smaller aliquot.

f) more than approximately 25 % of the grid openings have broken carbon film over the whole grid opening;

   Since the breakage of carbon film is usually more frequent in areas of heavy deposit, counting of the intact openings can lead to an underestimate of the structure count. Prepare specimens from another sector of the analytical filter, or select grids corresponding to filtration of a smaller aliquot.

   If the specimens are rejected because unacceptable numbers of grid openings exhibit broken carbon replica, an additional carbon coating may be applied to the carbon-coated filter, and new specimen grids prepared. The larger particles can often be supported by using a thicker carbon film. If this action does not produce acceptable specimen grids, this filter cannot be analyzed and grids prepared from an analytical filter with a lower particulate loading shall be selected.

8.7 Procedure for structure counting by TEM

8.7.1 General

The examination consists of a count of asbestos structures which are present on a specified number of grid openings. Fibrous structures are classified into groups on the basis of morphological observations, ED patterns and EDXA spectra. The total number of asbestos structures to be counted depends on the statistical precision desired. In the absence of asbestos structures, the area of the TEM specimen grids to be examined depends on the analytical sensitivity required. The precision of the structure count depends not only on the total number of structures counted, but also on their uniformity from one grid opening to the next. Additional structure counting will be necessary if greater precision is required.

In order that the estimate of the structure density on the sampling filter is not based on the structure deposits found within the small area represented by one specimen grid, examine grid openings on two of the three specimen grids prepared. Then combine the results for the calculation of the structure density. Make structure counts at a magnification of approximately 20 000×, and terminate at the end of the examination of the grid opening on which the 100th asbestos structure is observed, except continue the count until a minimum of four grid openings have been examined. Otherwise, continue the structure count to that number of grid openings at which the specified analytical sensitivity has been achieved.

NOTE The normal range for the number of grid openings which should be examined is from 4 to 20. If insufficient air has been sampled through the filter, the calculation in 8.7.4 can indicate that an impractically large number of grid openings should be examined. When this situation occurs, a larger value of analytical sensitivity may have to be accepted.

8.7.2 Measurement of mean grid-opening area

Measure the mean grid-opening area for the type of TEM specimen grid in use. The standard deviation of the mean of 10 openings selected from 10 grids should be less than 5 %. Optionally, or if the 5 % standard deviation criterion cannot be demonstrated, measure at a calibrated magnification the dimensions of each grid opening examined in the TEM.

8.7.3 TEM alignment and calibration procedures

Before structure counting is performed, align the TEM according to instrumental specifications. Calibrate the TEM and EDXA system according to the procedures in annex C.

8.7.4 Determination of stopping point

Before structure counting is begun, calculate the area of specimen to be examined in order to achieve the selected analytical sensitivity. Calculate the maximum number of grid openings to be examined, using the formula:

$$k = \frac{(A_a \cdot V_d)}{(S \cdot A_g \cdot V_f \cdot F_a \cdot V_s)}$$

where

k is the number of grid openings to be examined, rounded to the next highest integer;

A_a is the area, expressed in square millimetres (mm^2), of sample filter;

V_d is the volume, expressed in millilitres (ml), of water used for dispersal of residual ash;

S is the required analytical sensitivity, expressed in structures/litre;

A_g is the area, expressed in square millimetres (mm^2), of TEM specimen grid opening;

V_f is the volume, expressed in millilitres (ml), of aqueous dispersion filtered;

F_a is the fraction of sample collection filter ashed;

V_s is the volume, expressed in litres (l), of air sampled.

8.7.5 General procedure for structure counting and size analysis

Use at least two specimen grids prepared from the filter in the structure count. Select at random several grid openings from each grid, and combine the data in the calculation of the results.

Use a form similar to that shown in Figure 5 to record the structure-counting data. Insert the first specimen grid into the TEM.

In order to facilitate quality assurance measurements which require re-examination of the same grid opening by different microscopists, insert the grid into the specimen holder in a standard orientation with the grid bars parallel and perpendicular to the axis of the specimen holder. This will provide scan directions parallel to the edges of the grid opening. Ensure that all microscopists begin scanning at the same starting point on the grid opening, and that they use similar scan patterns. This procedure permits rapid relocation of fibrous structures for further examination if necessary.

Select a typical grid opening and set the screen magnification to the calibrated value (approximately 20 000×). Adjust the sample height until the features in the centre of the TEM viewing screen are at the eucentric point. Set the goniometer tilt angle to zero. In column 1 of the structure-counting form, record the number or letter used to identify the grid. In column 2, record the identification of the particular grid opening. Position the specimen so that the grid opening is positioned with one corner visible on the screen. Move the image by adjustment of only one translation control, carefully examining the sample for fibrous structures, until the opposite side of the grid opening is encountered. Move the image by a predetermined distance less than one screen diameter, using the other translation control, and scan the image in the reverse direction. Continue the procedure in this manner until the entire grid opening has been inspected in a pattern similar to that shown in Figure 6.

When a fibrous structure is detected, assign a sequential number to the primary structure in column 3, perform the identification procedures required as detailed in annex E, and enter the appropriate compositional classification on the structure counting form in column 5. Assign a morphological classification to the structure according to the procedures in annex D, and record this in column 6. Measure on the TEM viewing screen the length and width of the image of the primary structure, in millimetres, and record these measurements in columns 7 and 8. For a disperse cluster or matrix, assign a compositional classification and a morphological classification to each structure component, measure the length and width, and enter the data in columns 4 to 8. Use column 4 of the structure-counting form to tabulate the sequential number of total structures, taking into account structure components. If non-asbestos fibres are observed, note their presence and type, if known.

After a fibrous structure has been examined and measured, relocate the original field of view accurately before continuing scanning of the specimen. Failure to do this may cause fibres to be overlooked or counted twice. Continue the examination until the completion of the grid opening on which the 100th fibrous structure has been recorded, or until the number of grid openings required to achieve the specified analytical sensitivity, calculated according to 8.7.4, have been examined, whichever occurs first. The data shall be drawn approximately equally from a minimum of two grids. Regardless of the value calculated according to 8.7.4, fibrous structures on a minimum of four grid openings shall be counted.

8.7.6 Measurement of concentration of asbestos fibres and bundles longer than 5 mm

Give consideration to improving the statistical validity for measurement of asbestos fibres and bundles longer than 5 µm by additional examination of the TEM specimens at a lower magnification, taking account only of these long fibres and bundles. Perform this extended examination for fibres and bundles longer than 5 µm in accordance with the procedures described in Annex F. Use a magnification of approximately 10 000× for counting of all asbestos fibres and bundles longer than 5 µm, or approximately 5 000× if only fibres and bundles within the diameter range 0,2 µm to 3,0 µm are to be counted. Continue the count until completion of the grid opening on which 100 fibres and bundles have been recorded, or until sufficient area of the specimen has been examined to achieve the desired analytical sensitivity. Only those structures which are identified as, or are suspected to be, either chrysotile or one of the amphibole minera… will be reported in either the original or the extended TEM examination. This restriction is intended to ensure that the best statistical validity is obtained for the materials of interest.

TEM ASBESTOS STRUCTURE COUNT

Page of

Report Number: _____

Sample Number: _____

Filename: _____

Sample description: _____

_____

_____

Preparation date: _____ By: _____

Analysis date: _____ By: _____

Computer entry date: _____ By: _____

Air volume: _____ l

Sample filter area: _____ mm^2

Fraction ashed: _____

Dispersal volume: _____ ml

Volume filtered: _____ ml

Analytical filter area: _____ mm^2

Magnification: _____ ×

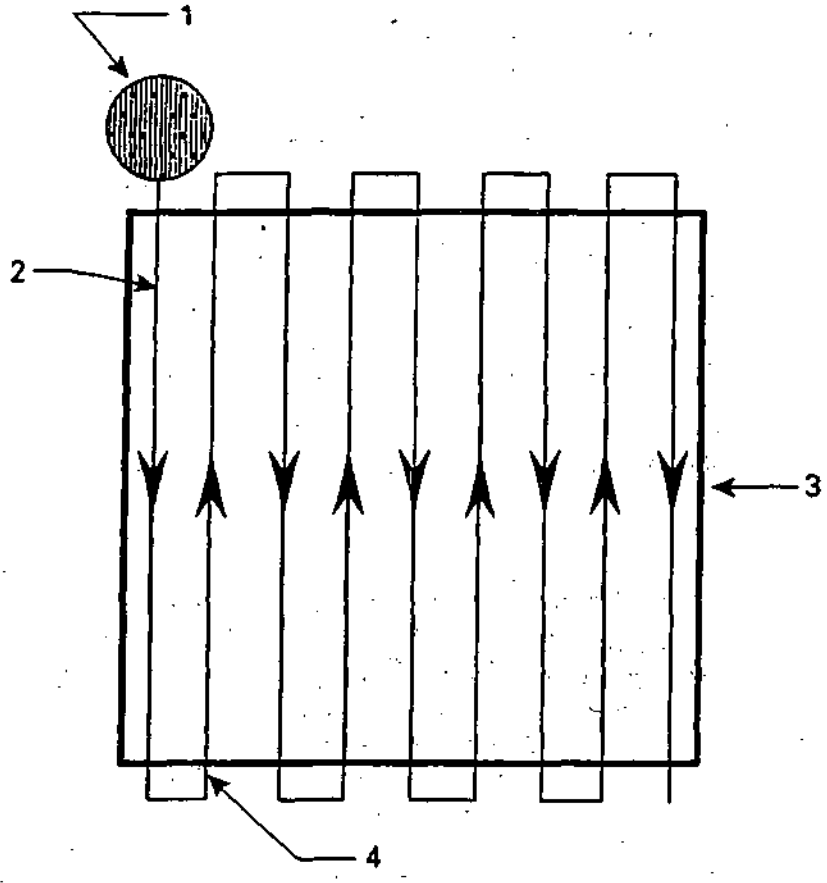
Grid-opening dimensions: _____ µm

Level of analysis (C): _____

(A): _____

Grid	Grid opening	Structure		Class	Structure	Length mm	Width mm	Comments
		Primary	Total					

Figure 5 — Example of structure-counting form



Key

1	TEM field of view	3	Grid opening
2	First pass	4	Second pass

Figure 6 — Example of scanning procedure for TEM specimen examination

8.7.7 Estimation of mass concentration of asbestos fibres and bundles

If the primary objective of the analysis is to estimate the mass concentration, adopt a structure-counting strategy that allows large structures that contribute most to the mass concentration to be counted with greater statistical reliability. Follow the procedures described in annex D.

8.8 Blank and quality control determinations

Before air samples are collected, analyse a minimum of two unused filters from each lot of 100 filters to determine the mean background asbestos structure count of the analytical procedure. If the mean background count for all types of asbestos structure is found to be more than 10 structures/mm^2, or if the mean background fibre count for asbestos fibres longer than 5 µm is more than 1,0 fibre/mm^2, determine the origin of the contamination and correct before air samples are collected.

NOTE The background contamination originating from the sample collection filters is determined by ashing one filter, a group of three filters, and a group of 10 filters. The background contamination originating from a single filter is calculated from any observed incremental increase between these measurements. If a constant background value is obtained for these three measurements, the contamination originates at some other point in the analytical procedure.

Include at least one open field blank and one closed field blank with the samples from each survey. To prepare an open field blank, transport an unused filter cassette to the sampling site and remove the front cap. Locate the open cassette in a similar position and orientation as the samples for the whole period of sample collection, but do not pump air through the filter. After sample collection is completed, replace the front cap and submit the cassette for analysis as the open field blank. To prepare the closed field blank, transport an unused filter cassette to the sampling site and keep it closed.

To ensure that contamination by extraneous asbestos fibres during specimen preparation is insignificant compared with the results reported on samples, establish a continuous programme of blank measurements. Process at least one funnel blank and one empty beaker blank along with each batch of samples. In addition, include at least one unused filter with every group of samples prepared on one microscope slide.

Initially, and also at intervals afterwards, ensure that samples of known asbestos concentrations can be analysed satisfactorily. Since there is a subjective component in the structure-counting procedure, re-counts of some specimens shall be made by different microscopists, in order to minimize the subjective effects. Such re-counts provide a means of maintaining comparability between counts made by different microscopists. Characterize the variability between and within microscopists and between laboratories. These quality assurance measurements shall constitute approximately 10 % of the analyses. Repeat results should not differ at the 5 % significance level.

8.9 Calculation of results

Calculate the results using the procedures detailed in annex G. Prior to TEM examination of the specimens, the level of analysis was specified. Before the results are calculated, specify the compositional and morphological classifications to be included in the result. Conduct the chi-squared uniformity test using the number of primary asbestos structures found on each grid opening, prior to application of the cluster- and matrix-counting criteria. Calculate the concentration result using the numbers of asbestos structures reported after application of the cluster- and matrix-counting criteria.

9 Performance characteristics

9.1 General

It is important to use this analytical method in conjunction with a continuous quality control programme. The quality control programme should include use of standard samples, blank samples, and both inter- and intra-laboratory analyses.

9.2 Interferences and limitations of fibre identification

Unequivocal identification of every chrysotile fibre is not possible, due to both instrumental limitations and the nature of some of the fibres. The requirement for a calibrated ED pattern eliminates the possibility of an incorrect identification of the fibre selected. However, there is a possibility of misidentification of fibres for which both the morphologies and the ED patterns are reported on the basis of visual inspection only. 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 ED pattern.

As in the case of chrysotile fibres, complete identification of every amphibole fibre is not possible due to instrumental limitations and the nature of some of the fibres. Moreover, complete identification of every amphibole fibre is not practical due to the limitations of both time and cost. Particles of a number of other minerals having compositions similar to those of some amphiboles could be erroneously classified as amphibole when the classification criteria do not include zone-axis ED techniques. However, the requirement for quantitative EDXA measurements on all fibres as support for the random orientation ED technique makes misidentification very unlikely, particularly when other similar fibres in the same sample have been identified as amphibole by zone-axis methods. The possibility of misidentification is further reduced with increasing aspect ratio, since it is rare for the minerals with which amphibole may be confused to display an asbestiform habit.

9.3 Precision and accuracy

9.3.1 Precision

The analytical precision that can be obtained is dependent upon the number of structures counted, and also on the uniformity of the particulate deposit on the analytical filter. Assuming that the structures are randomly deposited on the analytical filter, if 100 structures are counted and the loading is at least 3,5 structures/grid opening, computer modelling of the counting procedure shows that a coefficient of variation of about 10 % can be expected. As the number of structures counted decreases, the precision will also decrease approximately as $\sqrt{N}$, where N is the number of structures counted. In practice, particulate deposits obtained by filtration of aqueous dispersions of particles are rarely ideally distributed, and it is found that the precision is correspondingly reduced. Degradation of precision is a consequence of several factors, such as:

a) non-uniformity of the filtered particulate deposit;

b) distortion of the fibre distribution by application of the structure-counting criteria;

c) variation between microscopists in their interpretation of the fibrous structures; and,

d) variation between microscopists in their ability to detect and identify fibres.

The 95 % confidence interval about the mean for a single structure-concentration measurement using this analytical method should be approximately ± 25 % when 100 structures are counted over 10 grid openings.

9.3.2 Accuracy

There is no independent method available to determine the accuracy.

NOTE It has been demonstrated that, after carbon-coating of PC membrane filters, particulate material is transferred to the TEM specimens without measurable losses. However, if the filters are heavily loaded with particulate, some material may be lost before they are carbon-coated. Good comparability between the direct-transfer capillary-pore PC procedure and the direct-transfer cellulose ester filter procedure has been demonstrated for laboratory-generated aerosols of chrysotile asbestos collected at a face velocity of 8 cm/s. Using laboratory-generated and field-collected aerosols of chrysotile asbestos, it has been shown that, compared with the direct-transfer procedure, this indirect specimen preparation procedure results in comparable numbers of fibres longer than 5 µm, and less than a factor of two increase in the numbers of fibres longer than 2,5 µm. This indirect specimen preparation procedure may produce results different from those given by the direct-transfer procedure for aerosols of materials in which asbestos is blended with other components.

9.3.3 Inter- and intra-laboratory analyses

Inter- and intra-laboratory analyses are required in order to monitor systematic errors that may develop among microscopists when using this method. These analyses should be designed to test both the overall method and the performance of individual microscopists. Repeat preparation of TEM grids from different sectors of a filter, followed by examination of the grids by a different microscopist, is a test for reproducibility of the whole method. However, non-uniformity of the particulate deposit on the filter may lead to differences which are not related to the performance of the microscopists. Verified fibre-counting (counting of asbestos structures on the same grid opening of a TEM grid by two or more operators, followed by resolution of any discrepancies) may be used both as a training aid and to determine the performance of different microscopists. The use of indexed TEM grids as defined in 5.4.5 and 5.4.6 is recommended in order to facilitate relocation of specific grid openings.

9.4 Limit of detection

The limit of detection of the method can be varied by choice of the volume of air sampled, the proportion of the sample collection filter which is ashed, the proportion of the aqueous dispersion of ash which is filtered, the area of the analytical filter, and the area of the specimen examined in the TEM. It is also a function of the background of asbestos structures on unused filters. A limit of detection shall be quoted for each sample analysis.

NOTE In practice, the lowest limit of detection is frequently determined by the total suspended particulate concentration, since each particle on the analytical filter must be separated from adjacent ones by a sufficient distance that the particle can be identified without interference. Particulate loadings greater than about 25 µg/cm² on the analytical filters usually preclude preparation of TEM specimens. If the analysis is to be performed with an acceptable expenditure of time, the area of the specimen examined in the TEM for structures of all sizes is limited in most cases to between 10 and 20 grid openings. In

typical ambient or building atmospheres, it has been found that an analytical sensitivity of 1 structure/litre can be achieved. In some circumstances, where the atmosphere is exceptionally clean, this can be reduced to 0,1 structure/litre or lower. For fibres and bundles longer than 5 µm, the reduced magnifications specified permit larger areas of the TEM specimens to be examined in with an acceptable expenditure of time, resulting in proportionately lower limits of detection. If no structures are found in the analysis, the upper 95 % confidence limit can be quoted as the upper bound of the concentration, corresponding to 2,99 times the analytical sensitivity if a Poisson distribution of structures on the filter is assumed. This 95 % confidence limit for zero structures counted is taken as the detection limit. Since there is sometimes contamination of unused sample filters by asbestos structures, this should also be taken into account in the discussion of limits of detection.

10 Test report

The test report shall include at least items a) to m) as follows:

a) reference to this International Standard;

b) identification of the sample;

c) date and time of sampling, and all necessary sampling data;

d) date of the analysis;

e) identity of the analyst;

f) all necessary specimen preparation data;

g) any procedure used not specified in this International Standard or regarded as an optional procedure;

h) statement of the minimum acceptable identification category and the maximum identification category attempted (refer to Tables D.1 and D.2);

i) statement specifying which identification and structure categories have been used to calculate the concentration values;

j) the analytical sensitivity, expressed in asbestos structures/litre;

k) the limit of detection, expressed in asbestos structures/litre;

l) separate concentration values for chrysotile and amphibole structures, expressed in asbestos structures/litre;

m) the 95 % confidence interval limits for the concentration values, expressed in asbestos structures/litre.

The following items n) and o) shall be recorded, but their inclusion in the test report is optional:

n) a complete listing of the structure-counting data. The following data should be included: grid opening number, structure number, identification category, structure type, length and width of the structure expressed in micrometres, and any comments concerning the structure;

o) compositional data for the principal varieties of amphibole, if present.

Inclusion of the following items p), q) and r) in the test report is optional:

p) items h) to m) for all asbestos fibres and bundles longer than 5 µm;

q) items h) to m) for PCM-equivalent asbestos fibres;

r) estimated mass concentrations of chrysotile and amphibole.

An example of a suitable format for the structure-counting data is shown in Figures 7 and 8.

SAMPLE ANALYSIS INFORMATION

Laboratory name **Report number** **Date**

SAMPLE: 456 Queen Street
Ashby de la Zouch
Exterior sample 1991-09-09

Air volume:	5 750,0 l
Area of collection filter:	385,0 mm²
Volume flowrate:	10,0 l/min
Fraction of filter ashed:	0,50
Volume of water used for dispersal of ash:	40,0 ml
Volume of dispersion filtered:	34,0 ml
Active area of analytical filter:	199,0 mm²
Level of analysis (chrysotile):	CD or CMQ
Level of analysis (amphibole):	ADQ
Magnification used for structure counting:	20 500×
Aspect ratio for fibre definition:	5:1
Mean dimension of grid openings:	95,4 µm
Initials of analyst:	JMW
Number of grid openings examined:	10
Analytical sensitivity:	*0,895 structures/l*
Number of primary asbestos structures:	13
Number of asbestos structures counted:	26
Number of asbestos structures > 5 µm:	7
Number of asbestos fibres and bundles > 5 µm:	10
Number of PCM-equivalent asbestos structures:	2
Number of PCM-equivalent asbestos fibres:	5

Figure 7 — Example of format for reporting of sample and preparation data

SAMPLE ANALYSIS INFORMATION (Page 2 and higher)

Laboratory name Report number Date

SAMPLE: 456 Queen Street
Ashby de la Zouch
Exterior sample 1991-09-09

TEM ASBESTOS STRUCTURE COUNT — RAW DATA

Grid	Grid opening	Structures Primary	Structures Total	Identification[a]	Structure type	Length µm	Width µm	Comments
A	F4-4	1	1	CD	F	1,7	0,045	
		2	2	CMQ	B	3,6	0,09	
		3	3	ADQ	F	4,0	0,15	Crocidolite
	E3-6	4	4	CD	MC+0	3,5	1,3	
	E5-1	5		CD	MD 43	7,5	5,0	
			5	CD	MB	7,7	0,30	
			6	CMQ	MF	5,6	0,045	
			7	CD	MB	5,1	0,30	
			8	CD	MF	1,7	0,045	
B	F4-1	6		CD	CD+0	6,5	3,0	
			9	CD	CB	3,5	0,15	
			10	CD	CF	3,5	0,045	
			11	CMQ	CR+0	2,6	1,9	
	G5-1	7		CD	CD31	6,1	3,2	
			12	CD	CB	5,6	0,30	
			13	CMQ	CF	4,0	0,045	
			14	CMQ	CB	3,2	0,090	
	E4-4	8	15	CD	B	1,5	0,23	
		9	16	AD	F	8,7	0,15	
C	G4-4	10		CMQ	CD42	25,	5,6	
			17	CMQ	CB	15,	0,15	
			18	CMQ	CF	9;4	0,045	
			19	ADQ	CF	3,6	0,30	Tremolite
			20	CM	CF	4,2	0,045	
	E4-4			No Fibres				
	E5-6	11		ADQ	CD+3	9,4	2,5	
			21	ADQ	CF	7,1	0,30	Amosite
			22	ADQ	CF	6,2	0,10	Crocidolite
			23	CM	CB	5,1	0,20	
			24	CM	CR+0	3,3	1,8	
	F4-1	12	25	CMQ	MC10	3,7	2,1	
		13	26	CD	CC+0	7,4	0,5	

[a] Identification codes listed in Tables E.1 and E.2

Figure 8 — Example of format for reporting of structure-counting data

Annex A
(normative)

Determination of operating conditions for plasma asher

A.1 General

The plasma asher is used for complete ashing of sample collection filters, and also for an etching procedure during the preparation of TEM specimens from cellulose ester analytical filters.

For complete ashing of either PC or cellulose ester sample collection filters, operate the plasma asher at the minimum power at which a glow discharge can be maintained, until the filter appears to be completely ashed. In order to ensure that the filter has been completely ashed, then operate the plasma asher at full power for longer than 3 h.

During preparation of TEM specimens from a cellulose ester analytical filter, the spongy structure of the filter is collapsed into a thinner film of polymer by action of a solvent. Some of the particles on the surface of the original filter become completely buried in the polymer, and the specimen preparation procedure incorporates a plasma etching step to oxidize the surface layer of the polymer. Particles buried by the filter-collapsing step are then exposed, so that they can become subsequently affixed in the evaporated carbon film without altering their position on the original filter. The amount of etching is critical, and individual ashers vary in performance. Therefore, calibrate the plasma asher to give a known amount of etching of the collapsed-filter surface. This is carried out by adjusting the radio-frequency power output and the oxygen flowrate, and measuring the time taken to completely oxidize an uncollapsed 25 mm diameter cellulose ester filter of the same type and pore size as the analytical filters.

A.2 Procedure

Place an unused, 25 mm diameter MEC or cellulose nitrate filter of the type being used in the centre of a glass microscope slide. Position the slide approximately in the centre of the asher chamber. Close the chamber and evacuate to a pressure of approximately 40 Pa, while admitting oxygen to the chamber at a rate of 8 cm^3/min to 20 cm^3/min. Adjust the tuning of the system so that the intensity of the plasma is maximized. Measure the time required for complete oxidation of the filter. Determine operating parameters which result in complete oxidation of the filter in a period of approximately 15 min. For etching of collapsed filters, use these operating parameters for a period of 8 min.

NOTE Plasma oxidation at high radio-frequency powers will cause uncollapsed cellulose ester filters to shrink and curl, often followed by sudden violent ignition. At lower powers, the filter will remain in position and will become slowly thinner until it becomes nearly transparent. When multiple filters are etched, the rate of etching is reduced, and the system should be calibrated accordingly.

Annex B
(normative)

Determination and standardization of operating conditions for ultrasonic bath

B.1 General

The ultrasonic bath is used for dispersal of the residual ash from sample collection filters. Since prolonged ultrasonic treatment of asbestos dispersions can affect the size distribution of asbestos structures and thereby change the structure concentration, it is important to standardize the rate of ultrasonic energy absorption by the aqueous dispersion and the time for which the sample is exposed to ultrasonic treatment.

Depending on the design of the ultrasonic bath, the rate of energy deposition into the contents of the sample beaker will vary with the position of the sample in the bath and the thickness of the glass. Accordingly, calibrate in the positions used, and always place the sample beakers in these positions. During operation, the temperature of the water in the ultrasonic bath will rise, and will reach an equilibrium temperature. Since energy transfer to the sample beaker increases with the temperature of the water in the bath, operate the bath at its equilibrium temperature.

Ultrasonic energy absorbed by the sample reappears as heat, and the rate of absorption of ultrasonic energy can be measured calorimetrically. Use the measurement procedure specified in B.2.

B.2 Procedure

Ensure that the level of water in the ultrasonic bath is adjusted to that which will be used. Operate the bath until it reaches its equilibrium temperature, accelerating this process if necessary by initially using hot water in the bath. Place 40 ml of water at approximately 20 °C into a 50 ml beaker, and measure its temperature. With the bath switched off, place the beaker into the position to be calibrated. After a period of 60 s to 90 s, remove the beaker and measure the temperature of the contents. Discard the contents of the beaker. Using another 40 ml of water at approximately 20 °C, measure the initial temperature of the contents and, with the ultrasonic bath operating, the final temperature when the beaker has spent the same time in the bath as for the first measurement. Calculate the rate of energy absorption by the sample using the formula:

$$R = 4{,}185 \times \frac{(\theta_2 - \theta_1)}{t}$$

where

R is the rate of energy absorption, expressed in watts per millilitre (W/ml);

θ_2 is the temperature rise with ultrasonic bath operating, expressed in degrees Celsius (°C);

θ_1 is the temperature rise with ultrasonic bath not operating, expressed in degrees Celsius (°C);

t is the time, expressed in seconds (s).

If the rate of energy absorption is higher than the specified range, use a variable transformer as the power supply for the ultrasonic bath, and adjust the operating conditions so that the rate of energy absorption is in the range 0,05 W/ml to 0,1 W/ml, as defined by this measurement procedure.

Always operate the ultrasonic bath at its equilibrium temperature, because the energy transfer to the beaker increases with temperature. If a thermometer is used for temperature measurement when calibrating the bath, do

not immerse the thermometer in the beaker of water during the exposure to the bath, because the presence of the thermometer increases the absorption of energy during ultrasonic treatment. Alternatively, a low thermal capacity thermocouple may be used for temperature measurement.

Annex C
(normative)

Calibration procedures

C.1 Calibration of the TEM

C.1.1 Calibration of TEM screen magnification

The electron microscope should be aligned according to the specifications of the manufacturer. Initially, and at regular intervals, calibrate the magnifications used for the analysis using a diffraction grating replica. Adjust the specimen height to the eucentric position before carrying out the calibration. On the fluorescent viewing screen, measure the distance occupied by a convenient number of repeat distances of the grating image, and calculate the magnification. Always repeat the calibration after any instrumental maintenance or change in operating conditions. The magnification of the image on the viewing screen is not the same as that obtained on photographic plates or film. The ratio between these is a constant value for the particular model of TEM.

C.1.2 Calibration of ED camera constant

Calibrate the camera constant of the TEM when used in ED mode. Use a specimen grid supporting a carbon film on which a thin film of gold has been evaporated or sputtered. Form an image of the gold film with the specimen adjusted to the eucentric position and select ED conditions. Adjust the objective-lens current to optimize the pattern obtained, and measure either on the fluorescent viewing screen or on a recorded image the diameters of the innermost two rings. Calculate the radius-based camera constant, $\lambda \cdot L$, for both the fluorescent screen and the photographic plate or film, from the relationship:

$$\lambda \cdot L = \frac{a \cdot D}{2,0\sqrt{h^2 + k^2 + l^2}}$$

where

λ is the wavelength of the incident electrons, expressed in nanometres (nm);

L is the camera length, expressed in millimetres (mm);

a is the unit cell dimension of gold, expressed in nanometres (0,40786 nm);

D is the diameter of the (hkl) diffraction ring, expressed in millimetres (mm).

Using gold as the calibration material, the radius-based camera constant is given by:

$\lambda \cdot L = 0,11774D$ mm·nm (smallest ring);

$\lambda \cdot L = 0,10197D$ mm·nm (second ring).

C.1.3 Calibration of the EDXA system

Perform energy calibration of the EDXA system for a low-energy and a high-energy peak regularly. Calibration of the intensity scale of the EDXA system permits quantitative composition data, at an accuracy of about 10 % of the elemental concentration, to be obtained from EDXA spectra of reference silicate minerals containing the elements Na, Mg, Al, Si, K, Ca, Mn and Fe, and relevant certified reference materials. If quantitative determinations are required for minerals containing other elements, reference standards other than those referred to below will be required. Well-characterized mineral standards permit calibration of any TEM-EDXA combination which meets the

instrumental specifications of 5.3.1 and 5.3.2, so that EDXA data from different instruments can be compared. Reference minerals are required for the calibration; the criteria for selection being that they should be silicate minerals with matrices as close as possible to those of the amphiboles or serpentine, and that individual small fragments of the minerals are homogeneous in composition within a few percent.

Determine the compositions of these standards by electron microprobe analysis or chemical methods. Crush fragments of the same selected mineral standards and prepare filters by dispersal of the crushed material in water and immediate filtration of the suspensions. Prepare TEM specimens from these filters by the procedures described in clause 8. These TEM specimens can then be used to calibrate any TEM-EDXA system so that comparable compositional results can be obtained from different instruments.

NOTE The microprobe analyses of the mineral standards are made by conventional techniques which can be found in the Bibliography. In summary, the mineral is first embedded in a mount of polymethyl methacrylate or epoxy resin. The mount is then ground and polished to achieve a flat, polished surface of the mineral fragment. This surface is then analyzed, using suitable reference standards, preferably oxide standards of the individual elements wherever these are available. It is necessary to take account of the water concentration in the minerals, which in the case of chrysotile amounts to 13 % by mass. This water content may vary due to losses in the vacuum system.

Aqueous suspensions of mineral standards should be filtered immediately after preparation, since alkali and alkaline earth metals may be partially leached from minerals containing these elements.

Express the results of the electron microprobe analyses as atomic or mass percentage ratios relative to silicon. X-ray peak ratios of the same elements relative to silicon, obtained from the EDXA system, can then be used to calculate the relationship between peak area ratio and atomic or mass percentage ratio. This technique is described by Cliff and Lorimer (see Bibliography).

The X-rays generated in a thin specimen by an incident electron beam have a low probability of interacting with the specimen. Thus mass absorption and fluorescence effects are negligible. In a silicate mineral specimen containing element i, the following relationship can be used to perform quantitative analyses in the TEM.

$$\frac{C_i}{C_{Si}} = k_i \cdot \frac{A_i}{A_{Si}}$$

where

C_i is the concentration or atomic proportion of element i;

C_{Si} is the concentration or atomic proportion of silicon;

A_i is the elemental integrated peak area for element i;

A_{Si} is the elemental integrated peak area for silicon;

k_i is the k-ratio for element i relative to silicon (a constant).

NOTE For a particular instrumental configuration and a particular particle size, the value of k_i is constant.

To incorporate correction for the particle-size effect on peak area ratios (see Small *et al.* in Bibliography), extend the Cliff and Lorimer technique by obtaining separate values of the constant k_i for different ranges of fibre diameter. It is recommended that 20 EDXA measurements be made for each range of fibre diameter. Suitable ranges of fibre diameter are < 0,25 µm; 0,25 µm to 0,5 µm; 0,5 µm to 1,0 µm and > 1,0 µm.

Insert the TEM grid into the TEM, obtain an image at the calibrated higher magnification of about 20 000×, and adjust the specimen height to the eucentric point. If the X-ray detector is a side-entry variety, tilt the specimen towards the X-ray detector. Select an isolated fibre or particle less than 0,5 µm in width, and accumulate an EDXA spectrum using an electron probe of suitable diameter. When a well-defined spectrum has been obtained, perform a background subtraction and calculate the background-corrected peak areas for each element listed, using energy windows centred on the peaks. Calculate the ratio of the peak area for each specified element relative to the peak area for silicon. All background-subtracted peak areas used for calibration shall exceed 400 counts.

Repeat this procedure for 20 particles of each mineral standard. Reject analyses of any obviously foreign particles. Calculate the arithmetic mean concentration to peak area ratio, k_i (k-ratio), for each specified element of each mineral standard and for each of the fibre diameter ranges. Periodic routine checks shall be carried out to ensure that there has been no degradation of the detector performance. These k-ratios are used to calculate the elemental concentrations of unknown fibres, using the Cliff and Lorimer relationship.

Annex D
(normative)

Structure-counting criteria

D.1 Introduction

In addition to isolated fibres, assemblages of particles and fibres frequently occur in air samples. Groupings of asbestos fibres and particles, referred to as "asbestos structures", are defined as fibre bundles, clusters and matrices. The numerical result of a TEM examination depends strongly on whether the analyst assigns such an assemblage of fibres as a single entity, or as the estimated number of individual fibres which form the assemblage. It is therefore important that a logical system of counting criteria be defined, so that the interpretation of these complex structures is the same for all analysts, and so that the numerical result is meaningful. Imposition of specific structure-counting criteria generally requires that some interpretation, partially based on uncertain health effects information, be made of each asbestos structure found. It is not the intention of this International Standard to make any interpretations based on health effects, and it is intended that a clear separation be made between recording of structure-counting data, and later interpretation of those data. The system of coding specified in this International Standard permits a clear morphological description of the structures to be recorded in a concise manner suitable for later interpretation, if necessary, by a range of different criteria, without the necessity for re-examination of the specimens. In particular, the coding system is designed to permit recording of the dimensions of each complex fibrous structure, and also whether these structures contain fibres longer than 5 µm. This approach permits later evaluations of the data to include considerations of particle respirability and comparisons with historical indices of asbestos exposure. Examples of the various types of morphological structure, and the manner in which these shall be recorded, are shown in Figure D.1.

D.2 Structure definitions and treatment

Each fibrous structure that is a separate entity shall be designated as a primary structure. Each primary structure shall be designated as a fibre, bundle, cluster or matrix. These structures are discussed in D.2.1 to D.2.8.

D.2.1 Fibre

Any particle with parallel or stepped sides, of minimum length 0,5 µm, and with an aspect ratio of 5:1 or greater, shall be defined as a fibre. For chrysotile asbestos, the single fibril shall be defined as a fibre. A fibre with stepped sides shall be assigned a width equal to the average of the minimum and maximum widths. This average shall be used as the width in determination of the aspect ratio.

D.2.2 Bundle

A grouping composed of apparently attached parallel fibres shall be defined as a bundle, with a width equal to an estimate of the mean bundle width, and a length equal to the maximum length of the structure. The overall aspect ratio of the bundle may be any value, provided that it contains individual constituent fibres having aspect ratios equal to or greater than 5:1. Bundles may exhibit diverging fibres at one or both ends.

D.2.3 Cluster

An aggregate of two or more randomly oriented fibres, with or without bundles, shall be defined as a cluster. Clusters occur as two varieties:

a) disperse cluster (type D): a disperse and open network, in which at least one of the individual fibres or bundles can be separately identified and its dimensions measured;

b) compact cluster (type C): a complex and tightly bound network, in which one or both ends of each individual fibre or bundle are obscured, such that the dimensions of individual fibres and bundles cannot be unambiguously determined.

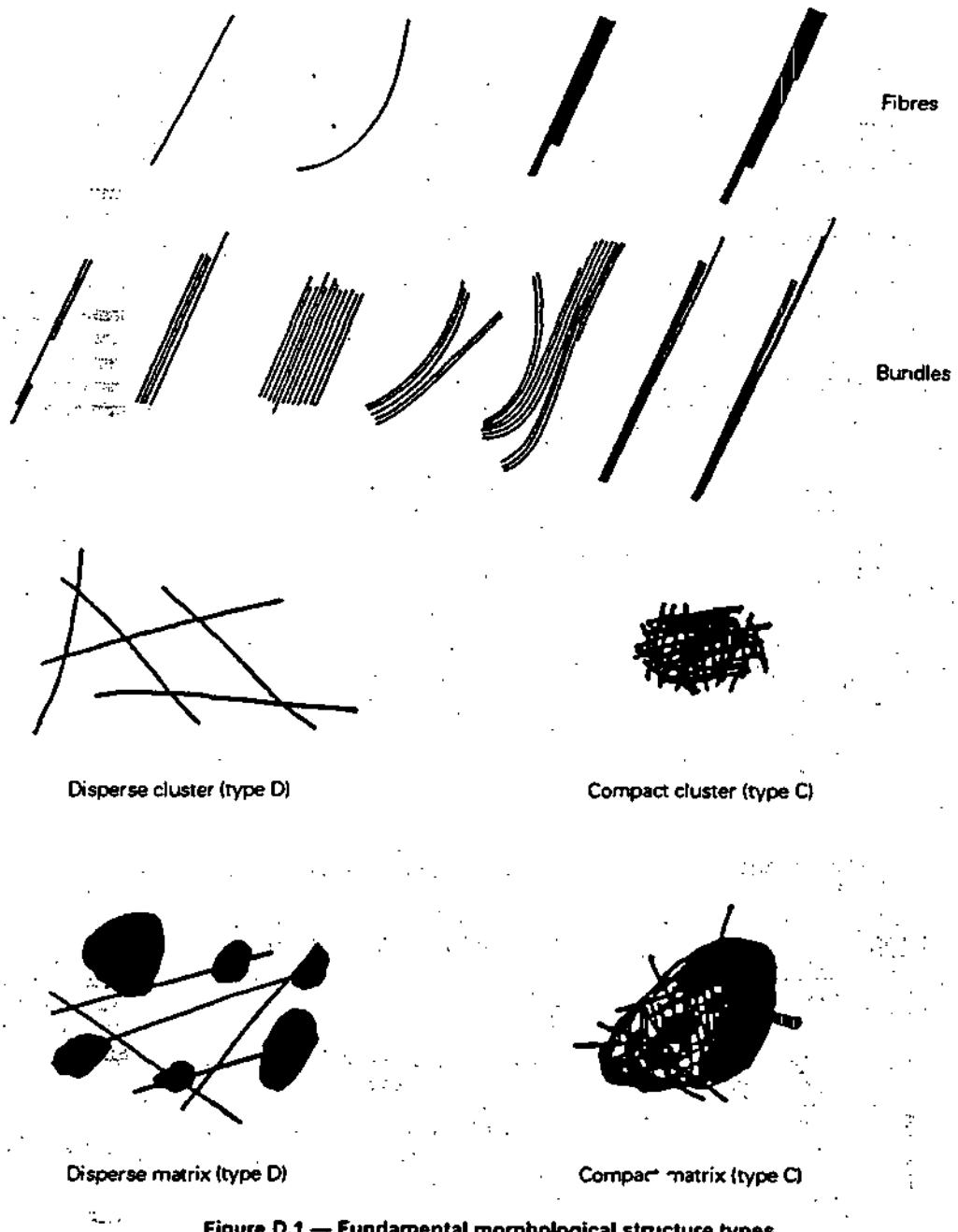


Figure D.1 — Fundamental morphological structure types

In practice, clusters can occur in which the characteristics of both types of cluster occur in the same structure. Where this occurs, the structure should be assigned as a disperse cluster, and then a logical procedure should be followed by recording structure components according to the counting criteria. The procedure for treatment of clusters is illustrated by examples in Figure D.2.

D.2.4 Matrix

One or more fibres, or fibre bundles, may be attached to, or partially concealed by, a single particle or group of overlapping non-fibrous particles. This structure shall be defined as a matrix. The TEM image does not discriminate between particles which are attached to fibres and those which have by chance overlapped in the TEM image. It is not known, therefore, whether such a structure is actually a complex particle, or whether it has arisen by a simple overlapping of particles and fibres on the filter.

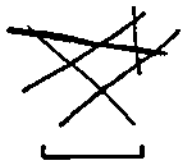
Since a matrix structure may involve more than one fibre, it is important to define in detail how matrices shall be counted. Matrices exhibit different characteristics, and two types can be defined:

a) disperse matrix (type D): a structure consisting of a particle or linked group of particles, with overlapping or attached fibres or bundles in which at least one of the individual fibres or bundles can be separately identified and its dimensions measured;

b) compact matrix (type C): a structure consisting of a particle or linked group of particles, in which fibres or bundles can be seen either within the structure or projecting from it, such that the dimensions of individual fibres and bundles cannot be unambiguously determined.

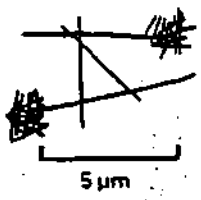
Count as 1 compact cluster
(all fibres shorter than 5 µm)

Record as CC+0



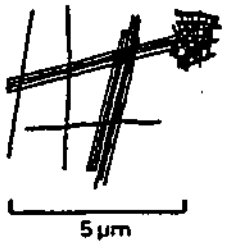
Count as a disperse cluster consisting of 5 fibres,
4 of which are longer than 5 µm

Record as CD54, followed by 5 fibres each recorded as CF



Count as a disperse cluster consisting of 4 fibres,
2 of which are longer than 5 µm, and 2 cluster residuals

Record as CD+2, followed by 4 fibres each recorded as
CF, and 2 cluster residuals each recorded as CR+0



Count as a disperse cluster consisting of 3 fibres,
2 bundles, 1 of which is longer than 5 µm, and 1 cluster residual

Record as CD+1, followed by 3 fibres each recorded as CF,
2 bundles each recorded as CB, and one cluster residual
recorded as CR+0

Figure D.2 — Examples of recording of complex asbestos clusters

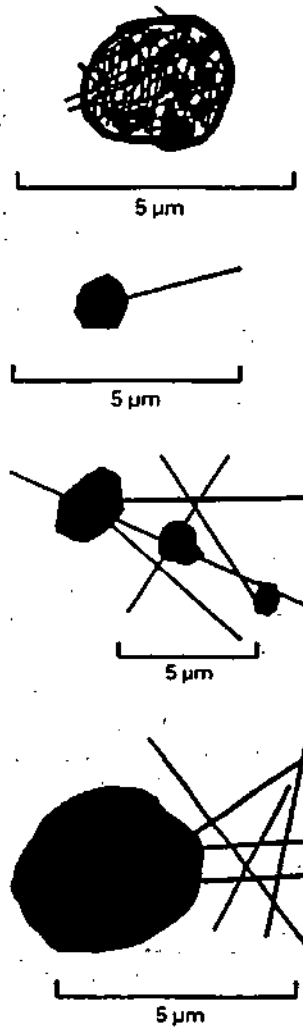
In practice, matrices can occur in which the characteristics of both types of matrix occur in the same structure. Where this occurs, the structure should be assigned as a disperse matrix, and then a logical procedure should be followed by recording structure components according to the counting criteria. Examples of the procedure which shall be followed are shown in Figure D.3.

D.2.5 Asbestos structure larger than 5 mm

Any fibre, bundle, cluster or matrix for which the largest dimension exceeds 5 µm. Asbestos structures larger than 5 µm do not necessarily contain asbestos fibres or bundles longer than 5 µm.

D.2.6 Asbestos fibre or bundle longer than 5 mm

An asbestos fibre of any width, or bundle of such fibres, which has a length exceeding 5 µm.



Count as 1 compact matrix, with all fibres shorter than 5 µm

Record as MC+0

Count as 1 disperse matrix containing 1 fibre shorter than 5 µm

Record as MD10, followed by 1 fibre recorded as MF

Count as 1 disperse matrix containing 5 fibres, all longer than 5 µm

Record as MD55, followed by 5 fibres each recorded as MF

Count as 1 disperse matrix, containing 3 fibres, 1 of which is longer than 5 µm, and 1 matrix residual

Record as MD61, followed by 3 fibres each recorded as MF, and 1 matrix residual recorded as MR30

Figure D.3 — Examples of recording of complex asbestos matrices

D.2.7 PCM-equivalent structure

Any fibre, bundle, cluster or matrix with an aspect ratio of 3:1 or greater, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm. PCM-equivalent structures do not necessarily contain fibres or bundles longer than 5 µm, or PCM-equivalent fibres.

D.2.8 PCM-equivalent fibre

Any particle with parallel or stepped sides, with an aspect ratio of 3:1 or greater, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm. For chrysotile, PCM-equivalent fibres will always be bundles.

D.3 Other structure-counting criteria

D.3.1 Structures which intersect grid bars

Count a structure which intersects a grid bar for two sides of the grid opening only, as illustrated in Figure D.4. Record the dimensions of the structure such that the obscured portions of components are taken to be equivalent to the unobscured portions, as shown by the broken lines in Figure D.4. For example, the length of a fibre intersecting a grid bar is taken to be twice the unobscured length. Structures intersecting either of the other two sides shall not be included in the count.

D.3.2 Fibres which extend outside the field of view

During scanning of a grid opening, count fibres which extend outside the field of view systematically, so as to avoid double-counting. In general, a rule should be established so that fibres extending outside the field of view in only two quadrants are counted. The procedure is illustrated by Figure D.5. Measure the length of each such fibre by moving the specimen to locate the other end of the fibre, and then return to the original field of view before continuing to scan the specimen. Fibres without terminations within the field of view shall not be counted.

D.4 Procedure for data recording

D.4.1 Introduction

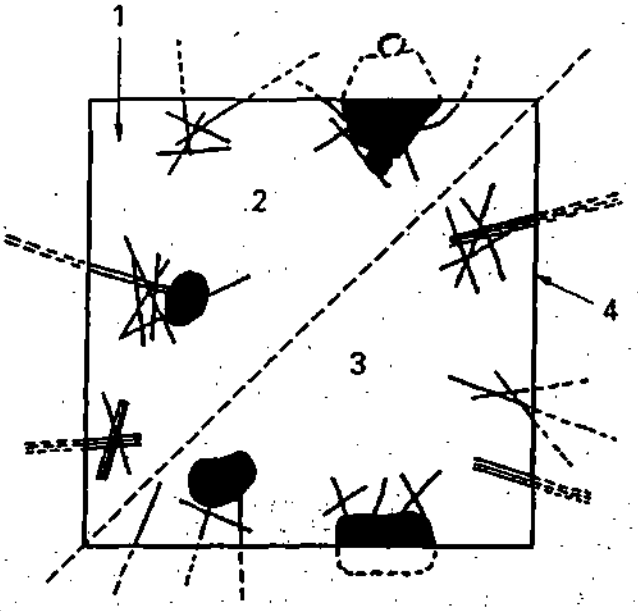
The morphological codes specified are designed to facilitate computer data-processing, and to allow recording of a complete representation of the important features of each asbestos structure. The procedure requires that the microscopist classify each primary fibrous structure into one of the four fundamental categories: fibres, bundles, clusters and matrices.

D.4.2 Fibres

On the structure-counting form, a fibre as defined in D.2.1 shall be recorded by the designation "F". If the fibre is a separately-counted part of a cluster or matrix, the fibre shall be recorded by the designation "CF", or "MF", depending on whether it is a component of a cluster or matrix.

D.4.3 Bundles

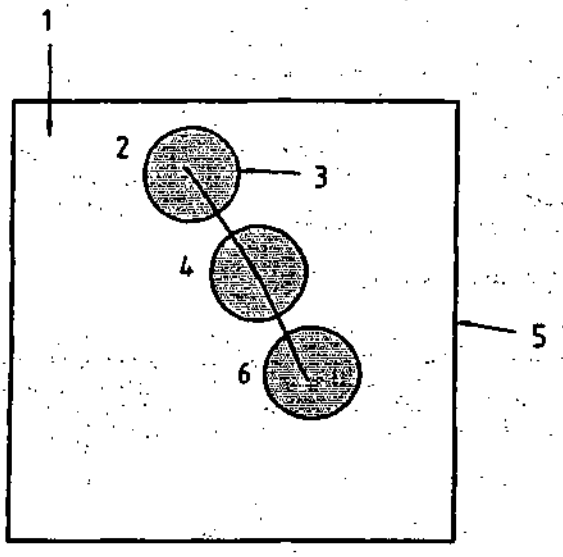
On the structure-counting form, a bundle as defined in D.2.2 shall be recorded by the designation "B". If the bundle is a separately-counted part of a cluster or matrix, the bundle shall be recorded by the designation "CB", or "MB", depending on whether it is a component of a cluster or matrix.



Key

1	Scan direction	3	Count
2	Do not count	4	Grid opening

Figure D.4 — Example of counting of structures which intersect grid bars



Key

1	Scan direction	4	Do not count
2	Count	5	Grid opening
3	TEM field of view	6	Do not count

Figure D.5 — Example of counting of fibres which extend outside the field of view

D.4.4 Disperse clusters (type D)

On the structure-counting form, an isolated cluster of type D as defined in D.2.3 shall be recorded by the designation "CD", followed by a two-digit number. The first digit represents the analyst's estimate of the total number of fibres and bundles comprising the structure. The digit shall be from 1 to 9, or designated as "+" if there are estimated to be more than 9 component fibres or bundles. The second digit shall represent, in the same manner, the total number of fibres and bundles longer than 5 µm contained in the structure. The overall dimensions of the cluster in two perpendicular directions representing the maximum dimensions shall be recorded. In order of decreasing length, up to five component fibres or bundles shall be separately recorded, using the codes "CF" (cluster fibre) and "CB" (cluster bundle). If, after accounting for prominent component fibres and bundles, a group of clustered fibres remains, this shall be recorded as "CR" (cluster residual). If the remaining clustered fibres are present as more than one localized group, it may be necessary to record more than one cluster residual. Do not record more than five cluster residuals for any cluster. A cluster residual shall be measured and assigned a two-digit number, derived in the same manner as specified for the overall cluster. Optionally, if the number of component fibres and bundles in either the original cluster or the cluster residual is outside of the range of 1 to 9, additional information concerning the number of component fibres and bundles may be noted in the "comments" column.

D.4.5 Compact clusters (type C)

On the structure-counting form, an isolated cluster of type C as defined in D.2.3 shall be recorded by the designation "CC", followed by a two-digit number. The two-digit number describing the numbers of component fibres and bundles shall be assigned in the same manner as for clusters type D. The overall dimensions of the cluster in two perpendicular directions shall be recorded in the same manner as for clusters type D. By definition, the constitutent fibres and bundles of compact clusters cannot be separately measured, therefore no separate tabulation of component fibres or bundles can be made.

D.4.6 Disperse matrices (type D)

On the structure counting form, an isolated matrix of type D as defined in D.2.4 shall be recorded by the designation "MD", followed by a two-digit number. The two digit number shall be assigned in the same manner as for clusters type D. The overall dimensions of the matrix in two perpendicular directions shall be recorded in the same manner as for clusters type D. In order of decreasing length, up to five component fibres or bundles shall be separately recorded, using the codes "MF" (matrix fibre) and "MB" (matrix bundle). If, after accounting for prominent component fibres and bundles, matrix material containing asbestos fibres remains, this shall be recorded as "MR" (matrix residual). If the remaining matrix fibres are present as more than one localized group, it may be necessary to record more than one matrix residual. Do not record more than five matrix residuals for any matrix. A matrix residual shall be measured and assigned a two-digit number, derived in the same manner as specified for the overall matrix. Optionally, if the number of component fibres and bundles in either the original matrix or the matrix residual is outside of the range of 1 to 9, additional information concerning the number of component fibres and bundles may be noted in the "comments" column.

D.4.7 Compact matrices (type C)

On the structure counting form, an isolated matrix of type C as defined in D.2.4 shall be recorded by the designation "MC", followed by a two-digit number. The two-digit number shall be assigned in the same manner as for clusters type D. The overall dimensions of the matrix in two perpendicular directions shall be recorded in the same manner as for clusters type D. By definition, the constituent fibres and bundles of compact matrices cannot be separately measured, therefore no separate tabulation of component fibres or bundles can be made.

D.4.8 Procedure for recording partially obscured fibres and bundles

The proportion of the length of a fibre or bundle that is obscured by other particulates shall be used as the basis for determining whether a fibre or bundle is to be recorded as a separate component or is to be considered a part of a matrix type C or a part of a matrix residual. If the obscured length could not possibly be more than one-third of the total length, the fibre or bundle shall be considered a prominent feature to be separately recorded. The assigned length for each such partially-obscured fibre or bundle shall be equal to the visible length plus the maximum possible contribution from the obscured portion. Fibres or bundles which appear to cross the matrix, and for which both ends can be located approximately, shall be included in the maximum count of five and recorded according to the counting criteria as separate fibres or bundles. If the obscured length could be more than one-third of the total length, the fibre or bundle shall be considered as a part of a compact matrix type C or part of a matrix residual.

D.5 Special considerations for counting PCM-equivalent structures

Use 3:1 as the minimum aspect ratio for counting PCM-equivalent structures. This aspect ratio definition is required in order to achieve comparability of the results for this size range of structure with historical optical measurements, but use of this aspect ratio definition does not significantly affect the ability to interpret the whole fibre size distribution in terms of a minimum 5:1 aspect ratio. Some applications may require that a count be made of PCM-equivalent structures only. The coding system permits discrimination between PCM-equivalent structures that contain fibres and bundles longer than 5 µm and those that do not.

NOTE In general, clusters and matrices will yield fewer components as the minimum dimensions specified for countable fibres are increased. Thus it may be found that a particular structure yields a higher number of component fibres in a count for all fibre sizes than it does at a reduced magnification when only fibres and bundles longer than 5 µm are being counted. However, the requirement that component fibres and bundles be recorded in order of decreasing length ensures that the data are consistent for a particular structure, regardless of the size category of fibres being counted and the magnification in use.

D.6 Special considerations for estimation of the mass concentration of asbestos fibres and bundles

If the primary objective of the analysis is to estimate the mass concentration, it is necessary to adopt a structure-counting strategy that allows large structures that contribute most to the mass concentration to be counted with greater statistical reliability. The number of structures that must be counted in order to achieve a reliable estimate of the mass concentration depends primarily on the range of diameter distribution. The mass concentration measurement is most sensitive to fibres and bundles of large diameters, which are generally statistically infrequent relative to the smaller fibres and bundles. If the diameter distribution is narrow, such as that found in a dispersion of chrysotile fibrils, the mass concentration can be measured with approximately the same precision as that of the numerical concentration. If the diameter distribution is broad, mass concentration estimates derived from TEM examinations to determine numerical concentrations are statistically unreliable. The strategy specified below is designed to give greater statistical significance to the large structures, which contribute most to the mass concentration.

Initially, establish the largest width of asbestos fibre or bundle that can be detected on the grid by a cursory survey, at a reduced magnification, of approximately 50 grid openings. Calculate the volume of this structure. Adjust the magnification of the TEM to a value such that a width of 1 mm on the fluorescent screen corresponds to approximately 10 % of the width of the previously-selected large structure. Carry out a routine TEM examination at this magnification, terminating the examination at the end of the grid opening on which the integrated volume of all structures recorded is at least 10 times the volume of the orginally-selected structure. For asbestos structures which intercept the grid bars, measure only the unobscured parts of the fibres and bundles within the grid opening, for the purposes of calculation of the mass concentration of asbestos. Disregard the procedure indicated in D.3.1 and Figure D.4.

Annex E
(normative)

Fibre identification procedure

E.1 Introduction

The criteria used for identification of asbestos fibres may be selected as a function of the intended use of the measurements. In some circumstances, there can be a requirement that fibres shall be unequivocally identified as a specific mineral species. In other circumstances there can be sufficient knowledge about the sample that rigorous identification of each fibre need not be carried out. The time required to perform the analysis, and therefore the cost of analysis, can vary widely depending on the identification criteria which are considered to be sufficiently definitive. The combination of criteria considered definitive for identification of fibres in a particular analysis shall be specified before the analysis is made, and this combination of criteria shall be referred to as the "Level" of analysis. Various factors related to instrumental limitations and the character of the sample may prevent satisfaction of all of the specified fibre identification criteria for a particular fibre. Therefore, a record shall be made of the identification criteria which were satisfied for each suspected asbestos fibre included in the analysis. For example, if both ED and EDXA were specified to be attempted for definitive identification of each fibre, fibres with chrysotile morphology which, for some reason, do not give an ED pattern but which do yield an EDXA spectrum corresponding to chrysotile, are categorized in a way which conveys the level of confidence to be placed in the identification.

E.2 ED and EDXA techniques

E.2.1 General

Initially, classify fibres into two categories on the basis of morphology: those fibres with tubular morphology, and those fibres without tubular morphology. Conduct further analysis of each fibre using ED and EDXA methods. The following procedures should be used when fibres are examined by ED and EDXA.

The crystal structures of some mineral fibres, such as chrysotile, are easily damaged by the high current densities required for EDXA examination. Therefore, investigation of these sensitive fibres by ED shall be completed before attempts are made to obtain EDXA spectra from the fibres. When more stable fibres, such as the amphiboles, are examined, EDXA and ED may be used in either order.

E.2.2 ED techniques

The ED technique can be either qualitative or quantitative. Qualitative ED consists of visual examination, without detailed measurement, of the general characteristics of the ED pattern obtained on the TEM viewing screen from a randomly oriented fibre. ED patterns obtained from fibres with cylindrical symmetry, such as chrysotile, do not change when the fibres are tilted about their axes, and patterns from randomly oriented fibres of these minerals can be interpreted quantitatively. For fibres which do not have cylindrical symmetry, only those ED patterns obtained when the fibre is oriented with a principal crystallographic axis closely parallel with the incident electron-beam direction can be interpreted quantitatively. This type of ED pattern shall be referred to as a zone-axis ED pattern. In order to interpret a zone-axis ED pattern quantitatively, it shall be recorded photographically and its consistency with known mineral structures shall be checked. A computer program may be used to compare measurements of the zone-axis ED pattern with corresponding data calculated from known mineral structures. The zone-axis ED pattern obtained by examination of a fibre in a particular orientation can be insufficiently specific to permit unequivocal identification of the mineral fibre, but it is often possible to tilt the fibre to another angle and to record a different ED pattern corresponding to another zone-axis. The angle between the two zone-axes can also be checked for consistency with the structure of a suspected mineral.

For visual examination of the ED pattern, the camera length of the TEM should be set to a low value of approximately 250 mm and the ED pattern then should be viewed through the binoculars. This procedure minimizes

the possible degradation of the fibre by the electron irradiation. However, the pattern is distorted by the tilt angle of the viewing screen. A camera length of at least 2 m should be used when the ED pattern is recorded, if accurate measurement of the pattern is to be possible. It is necessary that, when obtaining an ED pattern to be evaluated visually or to be recorded, the sample height shall be properly adjusted to the eucentric point and the image shall be focussed in the plane of the selected area aperture. If this is not done there may be some components of the ED pattern which do not originate from the selected area. In general, it is necessary to use the smallest available ED aperture.

For accurate measurements of the ED pattern, an internal calibration standard shall be used. Apply a thin coating of gold, or other suitable calibration material, to the underside of the TEM specimen. This coating may be applied either by vacuum evaporation or, more conveniently, by sputtering. The polycrystalline gold film yields diffraction rings on every ED pattern and these rings provide the required calibration information.

To form an ED pattern, move the image of the fibre to the centre of the viewing screen; adjust the height of the specimen to the eucentric position, and insert a suitable selected area aperture into the electron beam so that the fibre, or a portion of it, occupies a large proportion of the illuminated area. The size of the aperture and the portion of the fibre shall be such that particles other than the one to be examined are excluded from the selected area. Observe the ED pattern through the binoculars. During the observation, the objective lens current should be adjusted to the point where the most complete ED pattern is obtained. If an incomplete ED pattern is still obtained, move the particle around within the selected area to attempt to optimize the ED pattern, or to eliminate possible interferences from neighbouring particles.

If a zone-axis ED analysis is to be attempted on the fibre, the sample shall be mounted in the appropriate holder. The most convenient holder allows complete rotation of the specimen grid and tilting of the grid about a single axis. Rotate the sample until the fibre image indicates that the fibre is oriented with its length coincident with the tilt axis of the goniometer, and adjust the sample height until the fibre is at the eucentric position. Tilt the fibre until an ED appears which is a symmetrical, two dimensional array of spots. The recognition of zone-axis alignment conditions requires some experience on the part of the operator. During tilting of the fibre to obtain zone-axis conditions, the manner in which the intensities of the spots vary should be observed. If weak reflections occur at some points on a matrix of strong reflections, the possibility of twinning or multiple diffraction exists, and some caution should be exercised in the selection of diffraction spots for measurement and interpretation. A full discussion of electron diffraction and multiple diffraction can be found in the references by Gard, Hirsch *et al.*, and Wenk in the Bibliography. Not all zone-axis patterns which can be obtained are definitive. Only those which have closely-spaced reflections corresponding to low indices in at least one direction should be recorded. Patterns in which all d-spacings are less than about 0,3 nm are not definitive. A useful guideline is that the lowest angle reflections should be within the radius of the first gold diffraction ring (111), and that patterns with smaller distances between reflections are usually the most definitive.

Five spots, closest to the centre spot, along two intersecting lines of the zone-axis pattern shall be selected for measurement, as illustrated in Figure E.1. The distances of these spots from the centre spot and the four angles shown provide the required data for analysis. Since the centre spot is usually very over-exposed, it does not provide a well-defined origin for these measurements. The required distances shall therefore be obtained by measuring between pairs of spots symmetrically disposed about the centre spot, preferably separated by several repeat distances. The distances must be measured with a precision of better than 0,3 mm, and the angles to a precision of better than 2,5°. The diameter of the first or second ring of the calibration pattern (111 and 200) must also be measured with a precision of better than 0,3 mm.

Using gold as the calibration material, the radius-based camera constant is given by:

$\lambda \cdot L = 0{,}11774D$ mm·nm (first ring)

$\lambda \cdot L = 0{,}10197D$ mm·nm (second ring)

E.2.3 EDXA measurements

Interpretation of the EDXA spectrum may be either qualitative or quantitative. For qualitative interpretation of a spectrum, the X-ray peaks originating from the elements in the fibre are recorded. For quantitative interpretation, the net peak areas, after background subtraction, are obtained for the X-ray peaks originating from the elements in the fibre. This method provides quantitative interpretation for those minerals which contain silicon.

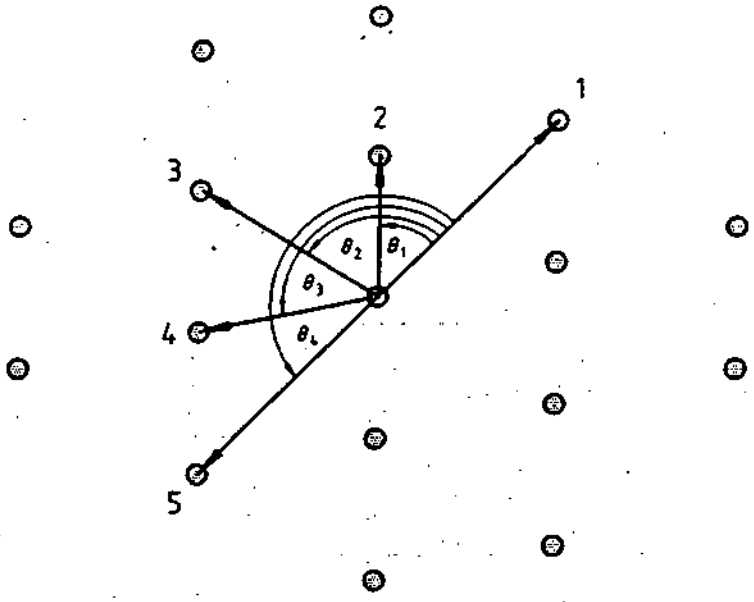


Figure E.1 — Example of measurement of five spots in zone-axis SAED patterns

To obtain an EDXA spectrum, move the image of the fibre to the centre of the screen and remove the objective aperture. Select an appropriate electron beam diameter and deflect the beam so that it impinges on the fibre. Depending on the instrumentation, it may be necessary to tilt the specimen towards the X-ray detector and, in some instruments, to use a scanning transmission electron microscopy (STEM) mode of operation.

The time for acquisition of a suitable spectrum varies with the fibre diameter, and also with instrumental factors. For quantitative interpretation, spectra should have a statistically valid number of counts in each peak. Analyses of small-diameter fibres which contain sodium are the most critical, since it is in the low energy range that the X-ray detector is least sensitive. Accordingly, it is necessary to acquire a spectrum over a sufficiently long period that the presence of sodium can be detected in such fibres. It has been found that satisfactory quantitative analyses can be obtained if acquisition is continued until the background-subtracted silicon Kα peak integral exceeds 10 000 counts. The spectrum should then be manipulated to subtract the background and to obtain the net areas of the elemental peaks.

After quantitative EDXA classification of some fibres by computer analysis of the net peak areas, it may be possible to classify further fibres in the same sample on the basis of comparison of spectra at the instrument. Frequently, visual comparisons can be made after somewhat shorter acquisition times.

E.3 Interpretation of fibre analysis data

E.3.1 Chrysotile

The morphological structure of chrysotile is characteristic, and with experience, can be recognized readily. However, a few other minerals have similar appearance, and morphological observation by itself is inadequate for most samples. The ED pattern obtained from chrysotile is quite specific for this mineral if the specified characteristics of the pattern correspond to those from reference chrysotile. However, depending on the past history of the fibre, and on a number of other factors, the crystal structure of a particular fibre may be damaged, and it may not yield an ED pattern. In this case, the EDXA spectrum may be the only data available to supplement the morphological observations.

E.3.2 Amphiboles

Since the fibre identification procedure for asbestos fibres other than chrysotile can be involved and time-consuming, computer programmes such as that developed by Rhoades (see Bibliography) are recommended for interpretation of zone-axis ED patterns. The published literature contains composition and crystallographic data for all of the fibrous minerals likely to be encountered in TEM analysis of air samples, and the compositional and structural data from the unknown fibre should be compared with the published data. Demonstration that the measurements are consistent with the data for a particular test mineral does not uniquely identify the unknown, since the possibility exists that data from other minerals may also be consistent. It is, however, unlikely that a mineral of another structural class could yield data consistent with that from an amphibole fibre identified by quantitative EDXA and two zone-axis ED patterns.

Suspected amphibole fibres should be classified initially on the basis of chemical composition. Either qualitative or quantitative EDXA information may be used as the basis for this classification. From the published data on mineral compositions, a list of minerals which are consistent in composition with that measured for the unknown fibre should be compiled. To proceed further, it is necessary to obtain the first zone-axis ED pattern, according to the instructions in E.2.2.

It is possible to specify a particular zone-axis pattern for identification of amphibole, since a few patterns are often considered to be characteristic. Unfortunately, for a fibre with random orientation on a TEM grid, no specimen holder and goniometer currently available will permit convenient and rapid location of two preselected zone-axes. The most practical approach has been adopted, which is to accept those low index patterns which are easily obtained, and then to test their consistency with the structures of the minerals already preselected on the basis of the EDXA data. Test the structures of non-amphibole minerals in this preselected list against the zone-axis data obtained for the unknown fibre, since non-amphibole minerals in some orientations may yield similar patterns consistent with amphibole structures.

The zone-axis ED interpretation shall include all minerals previously selected from the mineral data file as being chemically compatible with the EDXA data. This procedure usually shortens the list of minerals for which solutions have been found. A second set of zone-axis data from another pattern obtained on the same fibre can then be processed, either as further confirmation of the identification, or to attempt elimination of an ambiguity. In addition, the angle measured between the orientations of the two zone-axes can be checked for consistency with the structures of the minerals. Caution should be exercised in rationalizing the inter-zone axis angle, since if the fibre contains *c*-axis twinning the two zone-axis ED patterns may originate from the separate twin crystals. In practice, the full identification procedure will normally be applied to very few fibres, unless for a particular reason precise identification of all fibres is required.

E.4 Fibre classification categories

It is not always possible to proceed to a definitive identification of a fibre; this may be due to instrumental limitations or to the actual nature of the fibre. In many analyses a definitive identification of each fibre may not actually be necessary if there is other knowledge available about the sample, or if the concentration is below a level of interest. The analytical procedure must therefore take account of both instrumental limitations and varied analytical requirements. Accordingly, a system for fibre classification is used to permit accurate recording of data. The classifications are shown in Tables E.1 and E.2, and are directed towards identification of chrysotile and amphibole respectively. Fibres shall be reported in these categories.

The general principle to be followed in this analytical procedure is first to define the most specific fibre classification which is to be attempted, or the "level" of analysis to be conducted. Then, for each fibre examined, record the classification which is actually achieved. Depending on the intended use of the results, criteria for acceptance of fibres as "identified" can then be established at any time after completion of the analysis.

In an unknown sample, chrysotile shall be regarded as confirmed only if a recorded, calibrated ED pattern from one fibre in the CD categories is obtained, or if measurements of the ED pattern are recorded at the instrument. Amphibole shall be regarded as confirmed only by obtaining recorded data which yields exclusively amphibole solutions for fibres classified in the AZQ, AZZ or AZZQ categories.

Table E.1 — Classification of fibres with tubular morphology

TM	-	Tubular Morphology, not sufficiently characteristic for classification as chrysotile
CM	-	Characteristic Chrysotile Morphology
CD	-	Chrysotile SAED pattern
CQ	-	Chrysotile composition by Quantitative EDXA
CMQ	-	Chrysotile Morphology and composition by Quantitative EDXA
CDQ	-	Chrysotile SAED pattern and composition by Quantitative EDXA
NAM	-	Non-Asbestos Mineral

Table E.2 — Classification of fibres without tubular morphology

UF	-	Unidentified Fibre
AD	-	Amphibole by random-orientation SAED (shows layer pattern of 0,53 nm spacing)
AX	-	Amphibole by qualitative EDXA. Spectrum has elemental components consistent with amphibole
ADX	-	Amphibole by random-orientation SAED and qualitative EDXA
AQ	-	Amphibole by Quantitative EDXA
AZ	-	Amphibole by one Zone Axis SAED pattern
ADQ	-	Amphibole by random orientation SAED and Quantitative EDXA
AZQ	-	Amphibole by one Zone Axis SAED pattern and Quantitative EDXA
AZZ	-	Amphibole by two Zone Axis SAED patterns with consistent interaxial angle
AZZQ	-	Amphibole by two Zone Axis SAED patterns, with consistent interaxial angle, and Quantitative EDXA
NAM	-	Non-Asbestos Mineral

E.4.1 Procedure for classification of fibres with tubular morphology, suspected to be chrysotile

Occasionally, fibres are encountered which have tubular morphology similar to that of chrysotile, but which cannot be characterized further either by ED or EDXA. They can be non-crystalline, in which case ED techniques are not useful, or they can be in a position on the grid which does not permit an EDXA spectrum to be obtained. Alternatively, the fibre can be of organic origin, but the morphology and composition may not be sufficiently definitive that it can be disregarded. Accordingly, it is necessary to record each fibre, and to specify how confidently each fibre can be identified. Classification of fibres will meet with various degrees of success. Figure E.2 shows the classification procedure which shall be used for fibres which display any tubular morphology. The chart is self-explanatory, and every fibre is either rejected as a non-asbestos mineral (NAM), or classified in some way which by some later criterion could still contribute to the chrysotile fibre count.

Morphology is the first consideration, and if this is not similar to that usually seen in chrysotile standard samples, designate the initial classification as TM. Regardless of the doubtful morphology, examine the fibre by ED and EDXA methods according to Figure E.2. Where the morphology is more definitive, it may be possible to classify the fibre as having chrysotile morphology (CM).

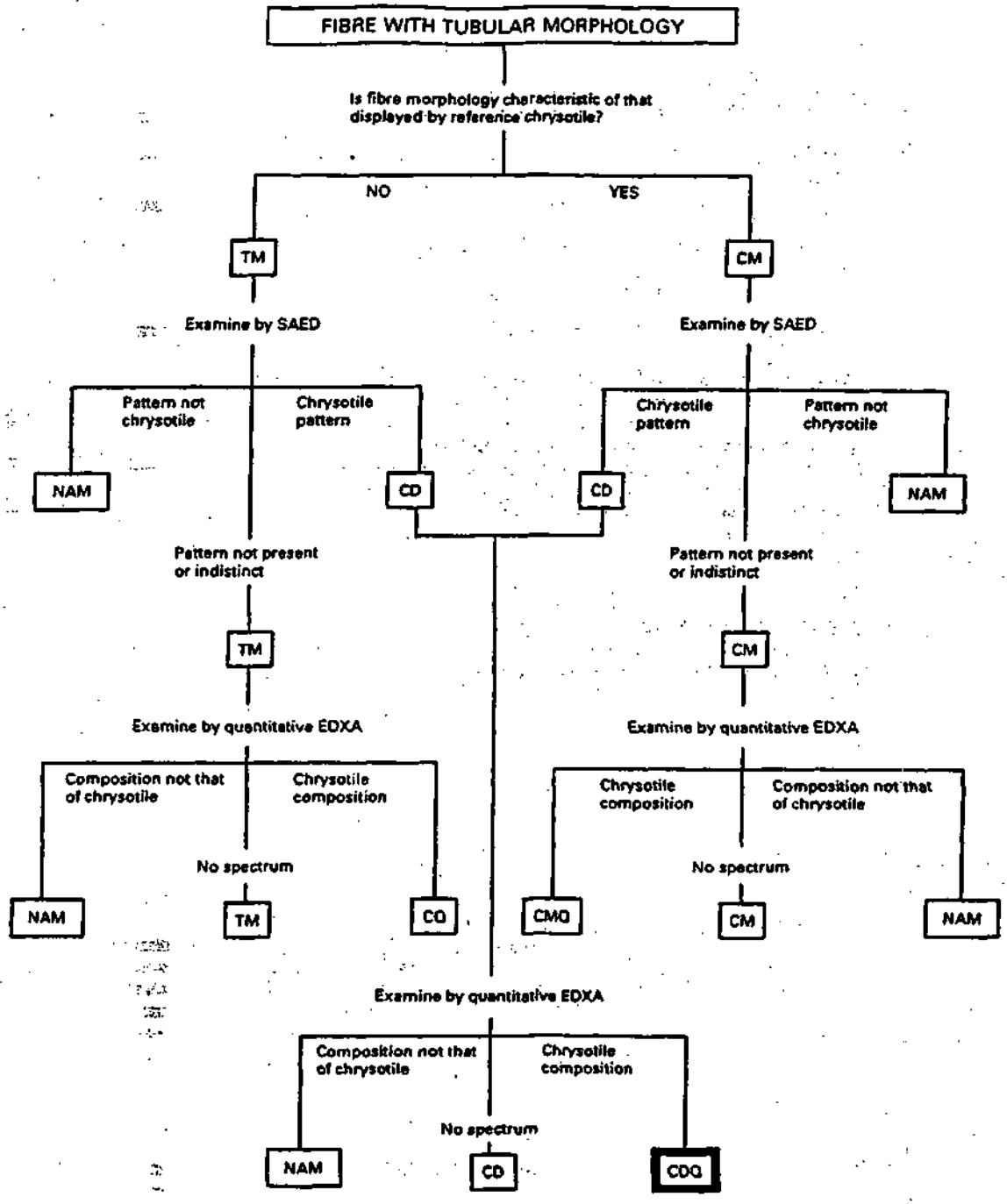


Figure E.2 — Classification chart for fibre with tubular morphology

For classification as CM, the morphological characteristics required are:

a) the individual fibrils have high aspect ratios, exceeding 5:1, and are about 30 nm to 40 nm in diameter;

b) the electron-scattering power of the fibre at 60 kV to 100 kV accelerating potential is sufficiently low for internal structure to be visible; and,

c) there is some evidence of internal structure suggesting a tubular appearance similar to that shown by reference UICC chrysotile, which may degrade in the electron beam.

Examine every fibre having these morphological characteristics by the ED technique, and classify as chrysotile by ED (CD) only those which give diffraction patterns with the precise characteristics shown in Figure E.3. The relevant features in this pattern for identification of chrysotile are as follows:

a) the (002) reflections shall be examined to determine that they correspond closely to a spacing of 0,73 nm;

b) the layer line repeat distance corresponds to 0,53 nm; and,

c) there is "streaking" of the (110) and (130) reflections.

Using the millimetre calibrations on the TEM viewing screen, these observations can readily be made at the instrument. If documentary proof of fibre identification is required, record a TEM micrograph of at least one representative fibre, and record its ED pattern on a separate film or plate. This film or plate shall also carry calibration rings from a known polycrystalline substance, such as gold. This calibrated pattern is the only documentary proof that the particular fibre is chrysotile, and not some other tubular or scrolled species such as halloysite, palygorskite, talc or vermiculite. The proportion of fibres which can be successfully identified as chrysotile by ED is variable, and to some extent dependent on both the instrument and the procedures of the operator. The fibres that fail to yield an identifiable ED pattern will remain in the TM or CM categories unless they are examined by EDXA.

In the EDXA analysis of chrysotile there are only two elements which are relevant. For fibre classification, the EDXA analysis shall be quantitative. If the spectrum displays prominent peaks from magnesium and silicon, with their areas in the appropriate ratio, and with only minor peaks from other elements, classify the fibre as chrysotile by quantitative EDXA, in the categories CQ, CMQ, or CDQ, as appropriate.

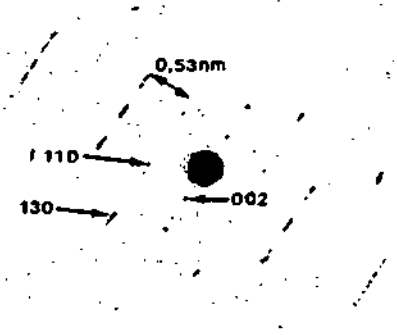


Figure E.3 — Chrysotile SAED pattern

E.4.2 Procedure for classification of fibres without tubular morphology, suspected to be amphibole

Every particle without tubular morphology and which is not obviously of biological origin, with an aspect ratio of 5:1 or greater, and having parallel or stepped sides, shall be considered as a suspected amphibole fibre. Further examination of the fibre by ED and EDXA techniques will meet with a variable degree of success, depending on the nature of the fibre and on a number of instrumental limitations. It is not possible to identify every fibre completely, even if time and cost are of no concern. Moreover, confirmation of the presence of amphibole can be achieved only by quantitative interpretation of zone-axis ED patterns, a very time-consuming procedure. Accordingly, for routine samples from unknown sources, this analytical procedure limits the requirement for zone-axis ED work to a minimum of one fibre representative of each compositional class reported. In some samples, it may be necessary to identify more fibres by the zone-axis technique. When analyzing samples from well-characterized sources, the cost of identification by zone-axis methods may not be justified.

The 0,53 nm layer spacing of the random-orientation ED pattern is not by itself diagnostic for amphibole. However, the presence of *c*-axis twinning in many fibres leads to contributions to the layers in the patterns by several individual parallel crystals of different axial orientations. This apparently random positioning of the spots along the layer lines, if also associated with a high fibre aspect ratio, is a characteristic of amphibole asbestos, and thus has some limited diagnostic value. If a pattern of this type is not obtained, the identity of the fibre is still ambiguous, since the absence of a recognizable pattern can be a consequence of an unsuitable orientation relative to the electron beam, or the fibre can be some other mineral species.

Figure E.4 shows the fibre classification chart which shall be used for suspected amphibole fibres. This chart shows all the classification paths possible in analysis of a suspected amphibole fibre, when examined systematically by ED and EDXA. Two routes are possible, depending on whether an attempt to obtain an EDXA spectrum or a random-orientation ED pattern is made first. The normal procedure for analysis of a sample of unknown origin is to examine the fibre by random-orientation ED, qualitative EDXA, quantitative EDXA, and zone-axis ED, in this sequence. The final fibre classification assigned is defined either by successful analysis at the maximum required level, or by the instrumental limitations. Any instrumental limitations which affect the quality of the results shall be noted. Record the maximum classification achieved for each fibre on the counting sheet in the appropriate column. The various classification categories can then be combined later in any desired way for calculation of the fibre concentration. The complete record of the results obtained when attempting to identify each fibre can also be used to re-assess the data if necessary.

In the unknown sample, zone-axis analysis will be required if the presence of amphibole is to be unequivocally confirmed. For this level of analysis, attempt to raise the classification of every suspected amphibole fibre to the ADQ category by inspection of the random orientation ED pattern and the EDXA spectrum. In addition, examine at least one fibre from each type of suspected amphibole found by zone-axis methods to confirm their identification. In most cases, because information exists about possible sources of asbestos in close proximity to the air sampling location, some degree of ambiguity of identification can be accepted. Lower levels of analysis can therefore be accepted for these situations.

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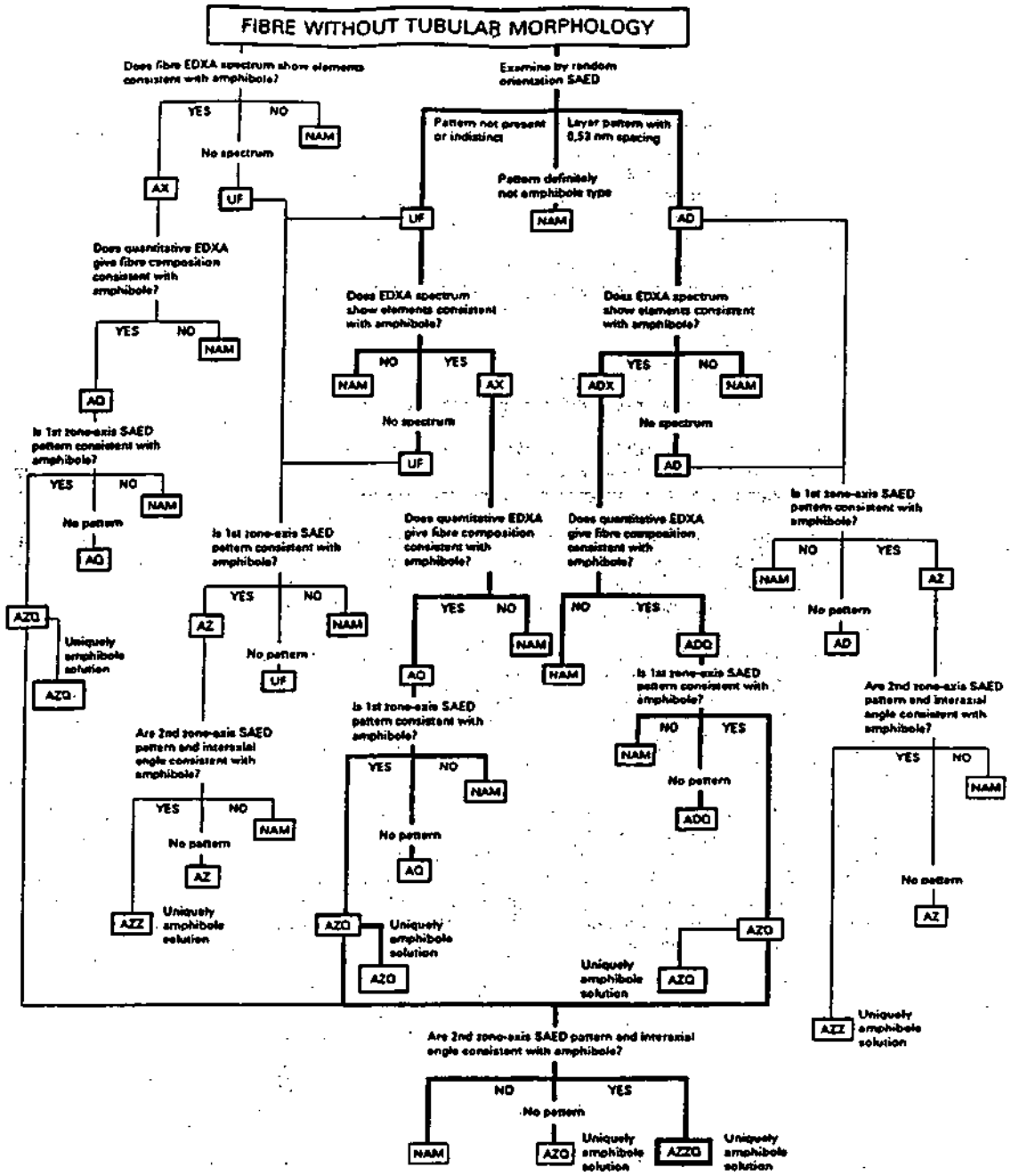

Figure E.4 — Classification chart for fibre without tubular morphology

Annex F
(normative)

Determination of concentrations of asbestos fibres and bundles longer than 5 µm, and of PCM-equivalent asbestos fibres

In order to provide increased statistical precision and improved analytical sensitivity for those asbestos fibres and bundles longer than 5 µm, it may be decided to perform additional fibre counting at a lower magnification, taking account only of fibres and bundles within this dimensional range. The result shall be specified as "asbestos fibres and bundles longer than 5 µm". For this examination, use a magnification of approximately 10 000×, and continue to assign a morphological code to each structure according to the procedures in annex D. Record fibres and bundles only if their lengths exceed 5 µm. Record cluster and matrix components only if their lengths exceed 5 µm.

It may also be decided to provide increased statistical precision and improved analytical sensitivity for fibrous structures longer than 5 µm that have diameters between 0,2 µm and 3,0 µm, which have historically been the basis of risk estimation in the occupational environment (PCM-equivalent asbestos fibres). Use a magnification of approximately 5 000× for this extended fibre count. The result shall be specified as "PCM-equivalent asbestos fibres". Asbestos structures within this dimensional range do not necessarily incorporate asbestos fibres or bundles longer than 5 µm.

Continue the extended sample examination until 100 asbestos structures have been counted, or until a sufficient area of the specimen has been examined to achieve the desired analytical sensitivity calculated according to Table 1. The grid openings examined shall be divided approximately equally between a minimum of two specimen grids.

NOTE The specimen area corresponding to the area of filter examined in the PCM fibre-counting methods is 0,785 mm², and is equivalent to approximately 100 grid openings of a 200 mesh grid.

Some national standards require that asbestos fibres longer than 2,5 µm, with diameters between 0,2 µm and 3,0 µm be counted. Use a magnification of 5 000× for counting fibres within these dimensional ranges.

The minimum aspect ratio for definition of a fibre in PCM fibre-counting methods and in some national standards is 3:1. Use of a 3:1 aspect ratio is permitted in this International Standard, if this aspect ratio is specified in the test report.

The test reports shall include all of the items specified in clause 10.

Annex G
(normative)

Calculation of results

G.1 Introduction

Calculate the results using the procedures described below. The results can be conveniently calculated using a computer program.

G.2 Test for uniformity of distribution of fibrous structures on TEM grids

A check shall be made using the chi-squared test, to determine whether the asbestos structures found on individual grid openings are randomly and uniformly distributed among the grid openings. If the total number found in k grid openings is n, and the areas of the k individual grid openings are designated A_1 to A_k, then the total area of TEM specimen examined is:

$$A = \sum_{i=1}^{i=k} A_i$$

The fraction of the total area examined which is represented by the individual grid opening area, p_i, is given by A_i/A. If the structures are randomly and uniformly dispersed over the k grid openings examined, the expected number of structures falling in one grid opening with area A_i is $n \cdot p_i$. If the observed number of structures found on that grid opening is n_i, then:

$$\chi^2 = \sum_{i=1}^{i=k} \frac{n_i - n \cdot p_i^2}{n \cdot p_i}$$

This value shall be compared with significance points of the χ^2 distribution, having $(k - 1)$ degrees of freedom. Significance levels lower than 0,1 % may be cause for the sample analysis to be rejected, since this corresponds to a very heterogeneous deposit. If the structure count fails this test, the precision of the result will be uncertain. The precision of the result may be improved by examination of additional grid openings. Inhomogeneous deposits of particulate occur either as a result of incomplete dispersal of the residues after ashing, or during filtration of the aqueous dispersions. Accordingly, the optimum approach is to prepare new TEM specimens from the original sample collection filter.

G.3 Calculation of the analytical sensitivity

Calculate the analytical sensitivity S, in structures/litre, using the formula:

$$S = \frac{(A_a \cdot V_d)}{(k \cdot A_g \cdot V_f \cdot F_a \cdot V_s)}$$

where

S is the required analytical sensitivity, expressed in structures/litre;

A_a is the active area, expressed in square millimetres (mm^2), of analytical filter;

V_d is the volume of water, expressed in millilitres (ml), used for dispersal of residual ash;

k is the number of grid openings examined;

A_g is the area, expressed in square millimetres (mm^2), of TEM specimen grid opening;

V_f is the volume, expressed in millilitres (ml), of aqueous dispersion filtered;

F_a is the fraction of sample collection filter ashed;

V_s is the volume, expressed in litres (l), of air sampled.

G.4 Calculation of the mean and confidence interval of the structure concentration

G.4.1 General

In the structure count made according to this International Standard, a number of grid openings have been sampled from a population of grid openings, and the mean grid opening structure count for the population shall be determined on the basis of this small sample. The interval about the sample mean, which, with 95 % confidence, contains the population mean, is also required.

G.4.2 Calculation of the mean structure concentration

Calculate the mean structure concentration, C, in structures/litre:

$$C = S \cdot n$$

where

S is the analytical sensitivity, expressed in structures/litre;

n is the total number of structures found on all grid openings examined.

G.4.3 Calculation of confidence intervals

The distribution of structures on the grid openings should theoretically approximate to a Poisson distribution. Because of fibre aggregation and size-dependent identification effects, the actual structure counting data often does not conform to the Poisson distribution, particularly at high structure counts. An assumption that the structure-counting data are distributed according to the Poisson distribution can therefore lead to confidence intervals narrower than are justified by the data. Moreover, if the Poisson distribution is assumed, the variance is related only to the total number of structures counted. Thus a particular structure count conducted on one grid opening is considered to have the same confidence interval as that for the same number of structures found on many grid openings. However, the area of sample actually counted is very small in relation to the total area of the filter, and for this reason structures shall be counted on a minimum of four grid openings taken from different areas of the filter in order to ensure that a representative evaluation of the deposit is made.

At high structure counts, where there are adequate numbers of structures per grid opening to allow a sample estimate of the variance to be made, the distribution can be approximated to a Gaussian, with independent values for the mean and variance. Where the sample estimate of variance exceeds that implicit in the Poissonian assumption, use of Gaussian statistics with the variance defined by the actual data is the most conservative approach to calculation of confidence intervals.

At low structure counts, it is not possible to obtain a reliable sample estimate of the variance, and the distribution also becomes asymmetric but not necessarily Poissonian. For 30 structures and below, the distribution becomes sufficiently asymmetric that the fit to a Gaussian is no longer a reasonable one, and estimates of sample variance are unreliable. Accordingly, for counts below 31 structures, the assumption of a Poisson distribution shall be made for calculation of the confidence intervals.

G.4.4 Example of calculation of Poissonian confidence intervals

For total structure counts less than four, the lower 95 % confidence limit corresponds to less than one structure. Therefore, it is not meaningful to quote lower confidence interval points for structure counts of less than four, and the result shall be specified as "less than" the corresponding one-sided upper 95 % confidence limit of the Poisson distribution. These are as follows:

0 structures: 2,99 times the analytical sensitivity

one structure: 4,74 times the analytical sensitivity

two structures: 6,30 times the analytical sensitivity

three structures: 7,75 times the analytical sensitivity

For total counts exceeding four, the 95 % confidence interval shall be calculated using the values shown in Table G.1. Table G.1 gives the upper and lower limits of the two-sided Poissonian 95 % confidence interval for structure counts up to 470.

G.4.5 Example of calculation of Gaussian 95 % confidence intervals

Calculate the sample estimate of variance s^2 as follows:

$$s^2 = \sum_{i=1}^{i=k} \frac{(n_i - n \cdot p_i)^2}{(k-1)}$$

where

n_i is the number of structures on the i'th grid opening;

n is the total number of structures found in k grid openings;

P_i is the fraction of the total area examined represented by the i'th grid opening;

k is the number of grid openings.

If the mean value of the structure count is calculated to be n, the upper and lower values of the Gaussian 95 % confidence interval are given by:

where

$$n_L = \frac{n}{k} - \frac{t \cdot s}{\sqrt{k}}$$

$$n_U = \frac{n}{k} + \frac{t \cdot s}{\sqrt{k}}$$

n_U is the upper 95 % confidence limit;

n_L is the lower 95 % confidence limit;

n is the total number of structures in all grid openings examined;

t is the value of Student's "t" (probability 0,975) for (k-1) degrees of freedom;

s is the standard deviation (square root of sample estimate of variance);

k is the number of grid openings.

G.5.3 Calculation of structure-width cumulative number distribution

This distribution allows the fraction of the total number of structures either narrower or wider than a given width to be determined. It is calculated in a manner similar to that used in G.5.2, but using the structure widths.

G.5.4 Calculation of structure aspect-ratio cumulative number distribution

This distribution allows the fraction of the total number of structures which have aspect ratios either smaller or larger than a given aspect ratio to be determined. It is calculated in a manner similar to that used in G.5.2, but using the structure aspect ratios.

G.6 Calculation of an estimate of mass concentration of asbestos fibres and bundles

An estimate of the mass concentration of asbestos fibres and bundles can be calculated from the results of a TEM examination conducted according to the procedure specified in D.6. It is calculated using the relationship:

$$M = 0{,}25 \cdot \pi \cdot S \cdot D \cdot 10^{-3} \cdot \sum_{j=1}^{j=n} w_j^2 \cdot L_j$$

where

M is the estimate of mass concentration of asbestos, expressed in nanograms per cubic metre (ng/m^3);

S is the analytical sensitivity, expressed in structures/litre;

w_j is the width of the j'th structure, expressed in micrometres (µm);

L_j is the length of the j'th structure, expressed in micrometres (µm);

D is the density of the variety of asbestos, expressed in kilograms per cubic metre (kg/m^3);

n is the total number of asbestos fibres and bundles recorded in k grid openings

In the above relationship, assume the densities of the asbestos varieties to be as follows: chrysotile 2 550 kg/m^3, crocidolite 3 370 kg/m^3, amosite 3 430 kg/m^3, anthophyllite 3 000 kg/m^3, tremolite 3 000 kg/m^3, actinolite 3 100 kg/m^3. Densities of the asbestos varieties vary with composition, but the values specified are sufficiently accurate for the purposes of this calculation.

NOTE The above calculation does not include contributions from compact clusters, compact matrices, cluster residuals or matrix residuals, because the total volume of the constituent asbestos fibres in these structures cannot be estimated reliably.

Table G.1 — Upper and lower limits of the Poissonian 95 % confidence interval of a count

Structure count	Lower	Upper	Structure count	Lower	Upper	Structure count	Lower	Upper
0	0	*3,689	46	33,678	61,358	92	74,164	112,83
1	0,025	5,572	47	34,534	62,501	93	75,061	113,94
2	0,242	7,225	48	35,392	63,642	94	75,959	115,04
3	0,619	8,767	49	36,251	64,781	95	76,858	116,14
4	1,090	10,242	50	37,112	65,919	96	77,757	117,24
5	1,624	11,669	51	37,973	67,056	97	78,657	118,34
6	2,202	13,060	52	38,837	68,192	98	79,557	119,44
7	2,814	14,423	53	39,701	69,326	99	80,458	120,53
8	3,454	15,764	54	40,567	70,459	100	81,360	121,66
9	4,115	17,085	55	41,433	71,591	110	90,400	132,61
10	4,795	18,391	56	42,301	72,721	120	99,490	143,52
11	5,491	19,683	57	43,171	73,851	130	108,61	154,39
12	6,201	20,962	58	44,041	74,979	140	117,77	165,23
13	6,922	22,231	59	44,912	76,106	150	126,96	176,04
14	7,654	23,490	60	45,785	77,232	160	136,17	186,83
15	8,396	24,741	61	46,658	78,357	170	145,41	197,59
16	9,146	25,983	62	47,533	79,482	180	154,66	208,33
17	9,904	27,219	63	48,409	80,605	190	163,94	219,05
18	10,668	28,448	64	49,286	81,727	200	173,24	229,75
19	11,440	29,671	65	50,164	82,848	210	182,56	240,43
20	12,217	30,889	66	51,042	83,969	220	191,89	251,10
21	13,000	32,101	67	51,922	85,088	230	201,24	261,75
22	13,788	33,309	68	52,803	86,207	240	210,60	272,39
23	14,581	34,512	69	53,685	87,324	250	219,97	283,01
24	15,378	35,711	70	54,567	88,441	260	229,36	293,62
25	16,178	36,905	71	55,451	89,557	270	238,75	304,23
26	16,983	38,097	72	56,335	90,673	280	248,16	314,82
27	17,793	39,284	73	57,220	91,787	290	257,58	325,39
28	18,606	40,468	74	58,106	92,901	300	267,01	335,96
29	19,422	41,649	75	58,993	94,014	310	276,45	346,52
30	20,241	42,827	76	59,880	95,126	320	285,90	357,08
31	21,063	44,002	77	60,768	96,237	330	295,36	367,62
32	21,888	45,175	78	61,657	97,348	340	304,82	378,15
33	22,715	46,345	79	62,547	98,458	350	314,29	388,68
34	23,545	47,512	80	63,437	99,567	360	323,77	399,20
35	24,378	48,677	81	64,328	100,68	370	333,26	409,71
36	25,213	49,840	82	65,219	101,79	380	342,75	420,22
37	26,050	51,000	83	66,111	102,90	390	352,25	430,72
38	26,890	52,158	84	67,003	104,00	400	361,76	441,21
39	27,732	53,315	85	67,897	105,11	410	371,27	451,69
40	28,575	54,469	86	68,790	106,21	420	380,79	462,18
41	29,421	55,622	87	69,684	107,32	430	390,32	472,65
42	30,269	56,772	88	70,579	108,42	440	399,85	483,12
43	31,119	57,921	89	71,474	109,53	450	409,38	493,58
44	31,970	59,068	90	72,370	110,63	460	418,92	504,04
45	32,823	60,214	91	73,267	111,73	470	428,47	514,50

*The one-sided upper 95 % confidence limit for zero structures is 2,99.

Annex H
(normative)

Test procedure to determine suitability of cellulose ester sample collection filters

H.1 Introduction

The residue remaining after ashing of unused cellulose ester filters primarily consists of aggregates of small particles of silica. This residue does not interfere with the analysis, other than imposing a limit on the degree of concentration that can be achieved. The residue from some varieties of cellulose ester filter, in addition to these small particles, also contains fragments of a silica film which originates from the surface of the original filter. The majority of the particles originally on the surface of the filter are bonded together by this silica film, which shrinks to a small area during the plasma ashing procedure. This fragment of silica film usually does not disperse completely in water. The resulting TEM specimens exhibit a much reduced particulate deposition on most of the grid openings, which will lead to a serious negative bias in the result. A few large fragments of silica film to which most of the particles are attached may be observed on a few of the grid openings. Membrane filters which exhibit this type of behaviour are unsuitable for use with this analytical method. Before air samples are collected, filters from the actual lot number to be used shall be tested in order to ensure that they do not exhibit this defect.

H.2 Test procedure

The optimum method for testing the suitability of the cellulose ester filters is to compare specimen grids prepared by the indirect-transfer procedure with grids prepared from the same filter using the direct-transfer procedure. The filter used for this test shall have a uniform deposit of single asbestos fibres, collected by air deposition, on its surface. The performance of a filter is satisfactory if it yields a numerical recovery close to 100 % for the indirect-transfer preparation, and also if no evidence of the effect described in H.1 is observed on the grids prepared by the indirect-transfer procedure.

Alternatively, blank filters can be tested by the following procedure. Evaporate a film 5 nm to 10 nm thick of carbon onto approximately 5 cm^2 of the uncollapsed cellulose ester filter to be tested. Prepare a Jaffe washer using a 3 cm × 3 cm piece of type 304 stainless steel woven cloth with approximately 80 mesh repeats per centimetre. Add dimethylformamide to the washer until the meniscus just contacts the underside of the mesh. Place the carbon-coated filter portion on the mesh, with the carbon side facing upwards. After a period of approximately 2 h, using a pipette, remove the dimethylformamide and re-fill the Jaffe washer with new dimethylformamide. After a further period of 2 h, remove the mesh supporting the carbon replica from the Jaffe washer and place it into a clean 50 ml borosilicate glass beaker. Allow the solvent on the mesh to evaporate. After the mesh is dry, cover the beaker with aluminium foil and perforate the foil as specified in clause 10. Ash the carbon replica in the same way as specified for ashing of filters in clause 10. The particulate residues from the carbon replica remain loosely attached to the stainless steel mesh after this operation. Leaving the stainless steel mesh in the beaker throughout, prepare TEM specimen grids by the procedure specified in clause 10 for analysis of blank filters. Examine the TEM specimens for the presence of tabular particles, from which the EDXA spectrum exhibits only a Si Kα peak. If the concentration of such tabular particles is more than 100 particles/mm^2, the filters are unsatisfactory for use with this analytical method.

Annex I
(informative)

Strategies for collection of air samples

I.1 Introduction

An important part of the sampling strategy is a statement of the purpose of the sampling programme. A sufficient number of samples should be collected so that the site is well characterized to the precision and accuracy desired, and also ensure that sample filters appropriately-loaded for TEM analysis are obtained from all of the sampling locations.

I.2 Air sample collection in the outdoors environment

Weather conditions restrict the ability to collect satisfactory air samples in the outdoors environment, and whenever possible, sampling should be carried out in low-wind, low-humidity conditions. Detailed records of the weather conditions, wind speed and direction during the sampling period should be made. All available information concerning local topography, and the types and positions of sources, should be recorded.

Sequential multipoint sampling is necessary to provide adequate characterization of complex sites and sources. It is recommended that multiple samples be taken upwind and downwind of the site, with a minimum of two samples in the downwind position expected to experience the maximum airborne concentration. The locations of the samplers should be carefully recorded.

I.3 Air sample collection inside buildings

Air samples are often collected inside buildings in which asbestos-containing construction materials are present, in order to determine whether these materials contribute to the asbestos concentration in the building atmosphere. The optimum positions for collection of air samples can be determined only after a complete survey of the building to establish air movement patterns. Multiple samples should be collected in the area where asbestos building materials are present, and control samples should be collected in an adjacent area where no airborne asbestos fibres would be expected. The intakes for air-conditioning systems are frequently used as the control sample collection locations. Whenever possible, static samples should be taken over a period exceeding 4 h during normal activity in the building, at face velocities of between 4 cm/s and 70 cm/s.

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Tabbed Page: 5

ASBESTOS and OTHER FIBERS by PCM 7400

Various MW: Various CAS: Various RTECS: Various

METHOD: 7400, Issue 2 **EVALUATION: FULL** **Issue 1: Rev. 3 on 15 May 1989**
Issue 2: 15 August 1994

OSHA : 0.1 asbestos fiber (> 5 µm long)/cc; 1 f/cc/30 min excursion; carcinogen
MSHA: 2 asbestos fibers/cc
NIOSH: 0.1 f/cc (fibers > 5 µm long)/400 L; carcinogen
ACGIH: 0.2 crocidolite; 0.5 amosite; 2 chrysotile and other asbestos, fibers/cc; carcinogen

PROPERTIES: solid, fibrous, crystalline, anisotropic

SYNONYMS [CAS #]: actinolite [77536-66-4] or ferroactinolite [15669-07-5]; amosite [12172-73-5]; anthophyllite [77536-67-5]; chrysotile [12001-29-5]; serpentine [18786-24-8]; crocidolite [12001-28-4]; tremolite [77536-68-6]; amphibole asbestos [1332-21-4]; refractory ceramic fibers [142844-00-6]; fibrous glass.

SAMPLING

SAMPLER: FILTER (0.45- to 1.2-µm cellulose ester membrane, 25-mm; conductive cowl on cassette)

FLOW RATE*: 0.5 to 16 L/min

VOL-MIN*: 400 L @ 0.1 fiber/cc
-MAX*: (step 4, sampling)
*Adjust to give 100 to 1300 fiber/mm^2

SHIPMENT: routine (pack to reduce shock)

SAMPLE STABILITY: stable

BLANKS: 2 to 10 field blanks per set

ACCURACY

RANGE STUDIED: 80 to 100 fibers counted

BIAS: See EVALUATION OF METHOD

OVERALL PRECISION ($\hat{S}_{rt}$): 0.115 to 0.13 [1]

ACCURACY: See EVALUATION OF METHOD

MEASUREMENT

TECHNIQUE: LIGHT MICROSCOPY, PHASE CONTRAST

ANALYTE: fibers (manual count)

SAMPLE PREPARATION: acetone - collapse/triacetin - immersion

COUNTING RULES: described in previous version of this method as "A" rules [1,3]

EQUIPMENT:
1. positive phase-contrast microscope
2. Walton-Beckett graticule (100-µm field of view) Type G-22
3. phase-shift test slide (HSE/NPL)

CALIBRATION: HSE/NPL test slide

RANGE: 100 to 1300 fibers/mm^2 filter area

ESTIMATED LOD: 7 fibers/mm^2 filter area

PRECISION ($\hat{S}_r$): 0.10 to 0.12 [1]; see EVALUATION OF METHOD

APPLICABILITY: The quantitative working range is 0.04 to 0.5 fiber/cc for a 1000-L air sample. The LOD depends on sample volume and quantity of interfering dust, and is <0.01 fiber/cc for atmospheres free of interferences. The method gives an index of airborne fibers. It is primarily used for estimating asbestos concentrations, though PCM does not differentiate between asbestos and other fibers. Use this method in conjunction with electron microscopy (e.g., Method 7402) for assistance in identification of fibers. Fibers < ca. 0.25 µm diameter will not be detected by this method [4]. This method may be used for other materials such as fibrous glass by using alternate counting rules (see Appendix C).

INTERFERENCES: If the method is used to detect a specific type of fiber, any other airborne fiber may interfere since all particles meeting the counting criteria are counted. Chain-like particles may appear fibrous. High levels of non-fibrous dust particles may obscure fibers in the field of view and increase the detection limit.

OTHER METHODS: This revision replaces Method 7400, Revision #3 (date 5/15/89).

REAGENTS:

1. Acetone,* reagent grade.
2. Triacetin (glycerol triacetate), reagent grade.

* See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Sampler: field monitor, 25-mm, three-piece cassette with ca. 50-mm electrically conductive extension cowl and cellulose ester filter, 0.45- to 1.2-µm pore size, and backup pad.
   NOTE 1: Analyze representative filters for fiber background before use to check for clarity and background. Discard the filter lot if mean is ≥5 fibers per 100 graticule fields. These are defined as laboratory blanks. Manufacturer-provided quality assurance checks on filter blanks are normally adequate as long as field blanks are analyzed as described below.
   NOTE 2: The electrically conductive extension cowl reduces electrostatic effects. Ground the cowl when possible during sampling.
   NOTE 3: Use 0.8-µm pore size filters for personal sampling. The 0.45-µm filters are recommended for sampling when performing TEM analysis on the same samples. However, their higher pressure drop precludes their use with personal sampling pumps.
   NOTE 4: Other cassettes have been proposed that exhibit improved uniformity of fiber deposit on the filter surface, e.g., bellmouthed sampler (Envirometrics, Charleston, SC). These may be used if shown to give measured concentrations equivalent to sampler indicated above for the application.
2. Personal sampling pump, battery or line-powered vacuum, of sufficient capacity to meet flow-rate requirements (see step 4 for flow rate), with flexible connecting tubing.
3. Wire, multi-stranded, 22-gauge; 1", hose clamp to attach wire to cassette.
4. Tape, shrink- or adhesive-.
5. Slides, glass, frosted-end, pre-cleaned, 25 x 75-mm.
6. Cover slips, 22- x 22-mm, No. 1-1/2, unless otherwise specified by microscope manufacturer.
7. Lacquer or nail polish.
8. Knife, #10 surgical steel, curved blade.
9. Tweezers.

EQUIPMENT:

10. Acetone flash vaporization system for clearing filters on glass slides (see ref. [5] for specifications or see manufacturer's instructions for equivalent devices).
11. Micropipets or syringes, 5-µL and 100- to 500-µL.
12. Microscope, positive phase (dark) contrast, with green or blue filter, adjustable field iris, 8 to 10X eyepiece, and 40 to 45X phase objective (total magnification ca. 400X); numerical aperture = 0.65 to 0.75.
13. Graticule, Walton-Beckett type with 100-µm diameter circular field (area = 0.00785 mm2) at the specimen plane (Type G-22). Available from Optometrics USA, P.O. Box 699, Ayer, MA 01432 [phone (508)-772-1700], and McCrone Accessories and Components, 850 Pasquinelli Drive, Westmont, IL 60559 [phone (312) 887-7100].
    NOTE: The graticule is custom-made for each microscope. (see APPENDIX A for the custom-ordering procedure).
14. HSE/NPL phase contrast test slide, Mark II. Available from Optometrics USA (address above).
15. Telescope, ocular phase-ring centering.
16. Stage micrometer (0.01-mm divisions).

SPECIAL PRECAUTIONS: Acetone is extremely flammable. Take precautions not to ignite it. Heating of acetone in volumes greater than 1 mL must be done in a ventilated laboratory fume hood using a flameless, spark- free heat source.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. To reduce contamination and to hold the cassette tightly together, seal the crease between the cassette base and the cowl with a shrink band or light colored adhesive tape. For personal sampling, fasten the (uncapped) open-face cassette to the worker's lapel. The open face should be oriented downward.
    NOTE: The cowl should be electrically grounded during area sampling, especially under conditions of low relative humidity. Use a hose clamp to secure one end of the wire (Equipment, Item 3) to the monitor's cowl. Connect the other end to an earth ground (i.e., cold water pipe).
3. Submit at least two field blanks (or 10% of the total samples, whichever is greater) for each set of samples. Handle field blanks in a manner representative of actual handling of associated samples in the set. Open field blank cassettes at the same time as other cassettes just prior to sampling. Store top covers and cassettes in a clean area (e.g., a closed bag or box) with the top covers from the sampling cassettes during the sampling period.
4. Sample at 0.5 L/min or greater [6]. Adjust sampling flow rate, Q (L/min), and time, t (min), to produce a fiber density, E, of 100 to 1300 fibers/mm^2 ($3.85 \cdot 10^4$ to $5 \cdot 10^5$ fibers per 25-mm filter with effective collection area A_c= 385 mm^2) for optimum accuracy. These variables are related to the action level (one-half the current standard), L (fibers/cc), of the fibrous aerosol being sampled by:

$$t = \frac{A_c \cdot E}{Q \cdot L \cdot 10^3}, \text{ min.}$$

NOTE 1: The purpose of adjusting sampling times is to obtain optimum fiber loading on the filter. The collection efficiency does not appear to be a function of flow rate in the range of 0.5 to 16 L/min for asbestos fibers [7]. Relatively large diameter fibers (>3 µm) may exhibit significant aspiration loss and inlet deposition. A sampling rate of 1 to 4 L/min for 8 h is appropriate in atmospheres containing ca. 0.1 fiber/cc in the absence of significant amounts of non-asbestos dust. Dusty atmospheres require smaller sample volumes (≤400 L) to obtain countable samples. In such cases take short, consecutive samples and average the results over the total collection time. For documenting episodic exposures, use high flow rates (7 to 16 L/min) over shorter sampling times. In relatively clean atmospheres, where targeted fiber concentrations are much less than 0.1 fiber/cc, use larger sample volumes (3000 to 10000 L) to achieve quantifiable loadings. Take care, however, not to overload the filter with background dust. If ≥ 50% of the filter surface is covered with particles, the filter may be too overloaded to count and will bias the measured fiber concentration.

NOTE 2: OSHA regulations specify a minimum sampling volume of 48 L for an excursion measurement, and a maximum sampling rate of 2.5 L/min [3].

5. At the end of sampling, replace top cover and end plugs.
6. Ship samples with conductive cowl attached in a rigid container with packing material to prevent jostling or damage.
   NOTE: Do not use untreated polystyrene foam in shipping container because electrostatic forces may cause fiber loss from sample filter.

SAMPLE PREPARATION:

NOTE 1: The object is to produce samples with a smooth (non-grainy) background in a medium with refractive index ≤1.46. This method collapses the filter for easier focusing and produces permanent (1 - 10 years) mounts which are useful for quality control and interlaboratory comparison. The aluminum "hot block" or similar flash vaporization techniques may be used outside the laboratory [2]. Other mounting techniques meeting the above criteria may also be used (e.g., the laboratory fume hood procedure for generating acetone vapor as described in Method 7400 - revision of 5/15/85, or the non-permanent field mounting technique used in P&CAM 239 [3,7,8,9]). Unless the effective filtration area is known, determine the area and record the information referenced against the sample ID number [1,9,10,11].

NOTE 2: Excessive water in the acetone may slow the clearing of the filter, causing material to be washed off the surface of the filter. Also, filters that have been exposed to high humidities prior to clearing may have a grainy background.

7. Ensure that the glass slides and cover slips are free of dust and fibers.
8. Adjust the rheostat to heat the "hot block" to ca. 70 °C [2].
   NOTE: If the "hot block" is not used in a fume hood, it must rest on a ceramic plate and be isolated from any surface susceptible to heat damage.
9. Mount a wedge cut from the sample filter on a clean glass slide.
   a. Cut wedges of ca. 25% of the filter area with a curved-blade surgical steel knife using a rocking motion to prevent tearing. Place wedge, dust side up, on slide.
      NOTE: Static electricity will usually keep the wedge on the slide.

b. Insert slide with wedge into the receiving slot at base of "hot block". Immediately place tip of a micropipet containing ca. 250 µL acetone (use the minimum volume needed to consistently clear the filter sections) into the inlet port of the PTFE cap on top of the "hot block" and inject the acetone into the vaporization chamber with a slow, steady pressure on the plunger button while holding pipet firmly in place. After waiting 3 to 5 sec for the filter to clear, remove pipet and slide from their ports.
   CAUTION: Although the volume of acetone used is small, use safety precautions. Work in a well-ventilated area (e.g., laboratory fume hood). Take care not to ignite the acetone. Continuous use of this device in an unventilated space may produce explosive acetone vapor concentrations.
c. Using the 5-µL micropipet, immediately place 3.0 to 3.5 µL triacetin on the wedge. Gently lower a clean cover slip onto the wedge at a slight angle to reduce bubble formation. Avoid excess pressure and movement of the cover glass.
   NOTE: If too many bubbles form or the amount of triacetin is insufficient, the cover slip may become detached within a few hours. If excessive triacetin remains at the edge of the filter under the cover slip, fiber migration may occur.
d. Mark the outline of the filter segment with a glass marking pen to aid in microscopic evaluation.
e. Glue the edges of the cover slip to the slide using lacquer or nail polish [12]. Counting may proceed immediately after clearing and mounting are completed.
   NOTE: If clearing is slow, warm the slide on a hotplate (surface temperature 50 °C) for up to 15 min to hasten clearing. Heat carefully to prevent gas bubble formation.

CALIBRATION AND QUALITY CONTROL:

10. Microscope adjustments. Follow the manufacturers instructions. At least once daily use the telescope ocular (or Bertrand lens, for some microscopes) supplied by the manufacturer to ensure that the phase rings (annular diaphragm and phase-shifting elements) are concentric. With each microscope, keep a logbook in which to record the dates of microscope cleanings and major servicing.
    a. Each time a sample is examined, do the following:
       (1) Adjust the light source for even illumination across the field of view at the condenser iris. Use Kohler illumination, if available. With some microscopes, the illumination may have to be set up with bright field optics rather than phase contract optics.
       (2) Focus on the particulate material to be examined.
       (3) Make sure that the field iris is in focus, centered on the sample, and open only enough to fully illuminate the field of view.
    b. Check the phase-shift detection limit of the microscope periodically for each analyst/microscope combination:
       (1) Center the HSE/NPL phase-contrast test slide under the phase objective.
       (2) Bring the blocks of grooved lines into focus in the graticule area.
          NOTE: The slide contains seven blocks of grooves (ca. 20 grooves per block) in descending order of visibility. For asbestos counting the microscope optics must completely resolve the grooved lines in block 3 although they may appear somewhat faint, and the grooved lines in blocks 6 and 7 must be invisible when centered in the graticule area. Blocks 4 and 5 must be at least partially visible but may vary slightly in visibility between microscopes. A microscope which fails to meet these requirements has resolution either too low or too high for fiber counting.
       (3) If image quality deteriorates, clean the microscope optics. If the problem persists, consult the microscope manufacturer.
11. Document the laboratory's precision for each counter for replicate fiber counts.
    a. Maintain as part of the laboratory quality assurance program a set of reference slides to be used on a daily basis [13]. These slides should consist of filter preparations including a range of loadings and background dust levels from a variety of sources including both field and reference samples (e.g., PAT, AAR, commercial samples). The Quality Assurance Officer

should maintain custody of the reference slides and should supply each counter with a minimum of one reference slide per workday. Change the labels on the reference slides periodically so that the counter does not become familiar with the samples.

b. From blind repeat counts on reference slides, estimate the laboratory intra- and intercounter precision. Obtain separate values of relative standard deviation (S_r) for each sample matrix analyzed in each of the following ranges: 5 to 20 fibers in 100 graticule fields, >20 to 50 fibers in 100 graticule fields, and >50 to 100 fibers in 100 graticule fields. Maintain control charts for each of these data files.

NOTE: Certain sample matrices (e.g., asbestos cement) have been shown to give poor precision [9]

12. Prepare and count field blanks along with the field samples. Report counts on each field blank.

NOTE 1: The identity of blank filters should be unknown to the counter until all counts have been completed.

NOTE 2: If a field blank yields greater than 7 fibers per 100 graticule fields, report possible contamination of the samples.

13. Perform blind recounts by the same counter on 10% of filters counted (slides relabeled by a person other than the counter). Use the following test to determine whether a pair of counts by the same counter on the same filter should be rejected because of possible bias: Discard the sample if the absolute value of the difference between the square roots of the two counts (in fiber/mm^2) exceeds 2.77 (X)S_r', where X = average of the square roots of the two fiber counts (in fiber/mm^2) and $S_r' = \frac{S_r}{2}$, where S_r is the intracounter relative standard deviation for the appropriate count range (in fibers) determined in step 11. For more complete discussions see reference [13].

NOTE 1: Since fiber counting is the measurement of randomly placed fibers which may be described by a Poisson distribution, a square root transformation of the fiber count data will result in approximately normally distributed data [13].

NOTE 2: If a pair of counts is rejected by this test, recount the remaining samples in the set and test the new counts against the first counts. Discard all rejected paired counts. It is not necessary to use this statistic on blank counts.

14. The analyst is a critical part of this analytical procedure. Care must be taken to provide a non-stressful and comfortable environment for fiber counting. An ergonomically designed chair should be used, with the microscope eyepiece situated at a comfortable height for viewing. External lighting should be set at a level similar to the illumination level in the microscope to reduce eye fatigue. In addition, counters should take 10-to-20 minute breaks from the microscope every one or two hours to limit fatigue [14]. During these breaks, both eye and upper back/neck exercises should be performed to relieve strain.
15. All laboratories engaged in asbestos counting should participate in a proficiency testing program such as the AIHA-NIOSH Proficiency Analytical Testing (PAT) Program for asbestos and routinely exchange field samples with other laboratories to compare performance of counters.

MEASUREMENT:

16. Center the slide on the stage of the calibrated microscope under the objective lens. Focus the microscope on the plane of the filter.
17. Adjust the microscope (Step 10).

NOTE: Calibration with the HSE/NPL test slide determines the minimum detectable fiber diameter (ca. 0.25 µm) [4].

18. Counting rules: (same as P&CAM 239 rules [1,10,11]: see examples in APPENDIX B).

a. Count any fiber longer than 5 µm which lies entirely within the graticule area.
   (1) Count only fibers longer than 5 µm. Measure length of curved fibers along the curve.
   (2) Count only fibers with a length-to-width ratio equal to or greater than 3:1.

b. For fibers which cross the boundary of the graticule field:
   (1) Count as 1/2 fiber any fiber with only one end lying within the graticule area, provided that the fiber meets the criteria of rule a above.

(2) Do not count any fiber which crosses the graticule boundary more than once.
(3) Reject and do not count all other fibers.

c. Count bundles of fibers as one fiber unless individual fibers can be identified by observing both ends of a fiber.
d. Count enough graticule fields to yield 100 fibers. Count a minimum of 20 fields. Stop at 100 graticule fields regardless of count.

19. Start counting from the tip of the filter wedge and progress along a radial line to the outer edge. Shift up or down on the filter, and continue in the reverse direction. Select graticule fields randomly by looking away from the eyepiece briefly while advancing the mechanical stage. Ensure that, as a minimum, each analysis covers one radial line from the filter center to the outer edge of the filter. When an agglomerate or bubble covers ca. 1/6 or more of the graticule field, reject the graticule field and select another. Do not report rejected graticule fields in the total number counted.

NOTE 1: When counting a graticule field, continuously scan a range of focal planes by moving the fine focus knob to detect very fine fibers which have become embedded in the filter. The small-diameter fibers will be very faint but are an important contribution to the total count. A minimum counting time of 15 seconds per field is appropriate for accurate counting.

NOTE 2: This method does not allow for differentiation of fibers based on morphology. Although some experienced counters are capable of selectively counting only fibers which appear to be asbestiform, there is presently no accepted method for ensuring uniformity of judgment between laboratories. It is, therefore, incumbent upon all laboratories using this method to report <u>total</u> fiber counts. If serious contamination from non-asbestos fibers occurs in samples, other techniques such as transmission electron microscopy must be used to identify the asbestos fiber fraction present in the sample (see NIOSH Method 7402). In some cases (i.e., for fibers with diameters >1 µm), polarized light microscopy (as in NIOSH Method 7403) may be used to identify and eliminate interfering non-crystalline fibers [15].

NOTE 3: Do not count at edges where filter was cut. Move in at least 1 mm from the edge.

NOTE 4: Under certain conditions, electrostatic charge may affect the sampling of fibers. These electrostatic effects are most likely to occur when the relative humidity is low (below 20%), and when sampling is performed near the source of aerosol. The result is that deposition of fibers on the filter is reduced, especially near the edge of the filter. If such a pattern is noted during fiber counting, choose fields as close to the center of the filter as possible [5].

NOTE 5: Counts are to be recorded on a data sheet that provides, as a minimum, spaces on which to record the counts for each field, filter identification number, analyst's name, date, total fibers counted, total fields counted, average count, fiber density, and commentary. Average count is calculated by dividing the total fiber count by the number of fields observed. Fiber density (fibers/mm^2) is defined as the average count (fibers/field) divided by the field (graticule) area (mm^2/field).

CALCULATIONS AND REPORTING OF RESULTS

20. Calculate and report fiber density on the filter, E (fibers/mm^2), by dividing the average fiber count per graticule field, F/n_f, minus the mean field blank count per graticule field, B/n_b, by the graticule field area, A_f (approx. 0.00785 mm^2):

$$E = \frac{\left(\frac{F}{n_f} - \frac{B}{n_b}\right)}{A_f}, \text{ fibers/mm}^2.$$

NOTE: Fiber counts above 1300 fibers/mm^2 and fiber counts from samples with >50% of filter area covered with particulate should be reported as "uncountable" or "probably biased." Other fiber counts outside the 100-1300 fiber/mm^2 range should be reported as having "greater than optimal variability" and as being "probably biased."

21. Calculate and report the concentration, C (fibers/cc), of fibers in the air volume sampled, V (L), using the effective collection area of the filter, A_c (approx. 385 mm^2 for a 25-mm filter):

$$C = \frac{(E)(A_c)}{V \cdot 10^3}.$$

NOTE: Periodically check and adjust the value of A_c, if necessary.

22. Report intralaboratory and interlaboratory relative standard deviations (from Step 11) with each set of results.

NOTE: Precision depends on the total number of fibers counted [1,16]. Relative standard deviation is documented in references [1,15-17] for fiber counts up to 100 fibers in 100 graticule fields. Comparability of interlaboratory results is discussed below. As a first approximation, use 213% above and 49% below the count as the upper and lower confidence limits for fiber counts greater than 20 (Fig. 1).

EVALUATION OF METHOD:

A. This method is a revision of P&CAM 239 [10]. A summary of the revisions is as follows:

1. Sampling:
   The change from a 37-mm to a 25-mm filter improves sensitivity for similar air volumes. The change in flow rates allows for 2-m^3 full-shift samples to be taken, providing that the filter is not overloaded with non-fibrous particulates. The collection efficiency of the sampler is not a function of flow rate in the range 0.5 to 16 L/min [10].
2. Sample Preparation Technique:
   The acetone vapor-triacetin preparation technique is a faster, more permanent mounting technique than the dimethyl phthalate/diethyl oxalate method of P&CAM 239 [2,4,10]. The aluminum "hot block" technique minimizes the amount of acetone needed to prepare each sample.
3. Measurement:
   a. The Walton-Beckett graticule standardizes the area observed [14,18,19].
   b. The HSE/NPL test slide standardizes microscope optics for sensitivity to fiber diameter [4,14].
   c. Because of past inaccuracies associated with low fiber counts, the minimum recommended loading has been increased to 100 fibers/mm^2 filter area (a total of 78.5 fibers counted in 100 fields, each with field area = .00785 mm^2.) Lower levels generally result in an overestimate of the fiber count when compared to results in the recommended analytical range [20]. The recommended loadings should yield intracounter S_r in the range of 0.10 to 0.17 [21,22,23].

B. Interlaboratory comparability:
   An international collaborative study involved 16 laboratories using prepared slides from the asbestos cement, milling, mining, textile, and friction material industries [9]. The relative standard deviations (S_r) varied with sample type and laboratory. The ranges were:

	Intralaboratory S_r	Interlaboratory S_r	Overall S_r
AIA (NIOSH A Rules)*	0.12 to 0.40	0.27 to 0.85	0.46
Modified CRS (NIOSH B Rules)**	0.11 to 0.29	0.20 to 0.35	0.25

* Under AIA rules, only fibers having a diameter less than 3 µm are counted and fibers attached to particles larger than 3 µm are not counted. NIOSH A Rules are otherwise similar to the AIA rules.
** See Appendix C.

A NIOSH study conducted using field samples of asbestos gave intralaboratory S_r in the range 0.17 to 0.25 and an interlaboratory S_r of 0.45 [21]. This agrees well with other recent studies [9,14,16].

At this time, there is no independent means for assessing the overall accuracy of this method. One measure of reliability is to estimate how well the count for a single sample agrees with the mean count from a large number of laboratories. The following discussion indicates how this estimation can be carried out based on measurements of the interlaboratory variability, as well as showing how the results of this method relate to the theoretically attainable counting precision and to measured intra- and interlaboratory S_r. (NOTE: The following discussion does not include bias estimates and should not be taken to indicated that lightly loaded samples are as accurate as properly loaded ones).

Theoretically, the process of counting randomly (Poisson) distributed fibers on a filter surface will give an S_r that depends on the number, N, of fibers counted:

$$S_r = 1/(N)^{1/2} \qquad (1)$$

Thus S_r is 0.1 for 100 fibers and 0.32 for 10 fibers counted. The actual S_r found in a number of studies is greater than these theoretical numbers [17,19,20,21].

An additional component of variability comes primarily from subjective interlaboratory differences. In a study of ten counters in a continuing sample exchange program, Ogden [15] found this subjective component of intralaboratory S_r to be approximately 0.2 and estimated the overall S_r by the term:

$$\frac{[N + (0.2 \cdot N)^2]^{1/2}}{N} \qquad (2)$$

Ogden found that the 90% confidence interval of the individual intralaboratory counts in relation to the means were +2 S_r and - 1.5 S_r. In this program, one sample out of ten was a quality control sample. For laboratories not engaged in an intensive quality assurance program, the subjective component of variability can be higher.

In a study of field sample results in 46 laboratories, the Asbestos Information Association also found that the variability had both a constant component and one that depended on the fiber count [14]. These results gave a subjective interlaboratory component of S_r (on the same basis as Ogden's) for field samples of ca. 0.45. A similar value was obtained for 12 laboratories analyzing a set of 24 field samples [21]. This value falls slightly above the range of S_r (0.25 to 0.42 for 1984-85) found for 80 reference laboratories in the NIOSH PAT program for laboratory-generated samples [17].

A number of factors influence S_r for a given laboratory, such as that laboratory's actual counting performance and the type of samples being analyzed. In the absence of other information, such as from an interlaboratory quality assurance program using field samples, the value for the subjective component of variability is chosen as 0.45. It is hoped that the laboratories will carry out the recommended interlaboratory quality assurance programs to improve their performance and thus reduce the S_r.

The above relative standard deviations apply when the population mean has been determined. It is more useful, however, for laboratories to estimate the 90% confidence interval on the mean count from a single sample fiber count (Figure 1). These curves assume similar shapes of the count distribution for interlaboratory and intralaboratory results [16].

For example, if a sample yields a count of 24 fibers, Figure 1 indicates that the mean interlaboratory count will fall within the range of 227% above and 52% below that value 90% of the time. We can apply these percentages directly to the air concentrations as well. If, for instance, this sample (24 fibers counted) represented a 500-L volume, then the measured concentration is 0.02 fibers/mL (assuming 100 fields counted, 25-mm filter, 0.00785 mm^2 counting field area). If this same sample were counted by a group of laboratories, there is a 90% probability that the mean would fall between 0.01 and 0.08 fiber/mL. These limits should be reported in any comparison of results between laboratories.

Note that the S_r of 0.45 used to derive Figure 1 is used as an estimate for a random group of laboratories. If several laboratories belonging to a quality assurance group can show that their interlaboratory S_r is smaller, then it is more correct to use that smaller S_r. However, the estimated S_r of 0.45 is to be used in the absence of such information. Note also that it has been found that S_r can be higher for certain types of samples, such as asbestos cement [9].

Quite often the estimated airborne concentration from an asbestos analysis is used to compare to a regulatory standard. For instance, if one is trying to show compliance with an 0.5 fiber/mL standard using a single sample on which 100 fibers have been counted, then Figure 1 indicates that the 0.5 fiber/mL standard must be 213% higher than the measured air concentration. This indicates that if one measures a fiber concentration of 0.16 fiber/mL (100 fibers counted), then the mean fiber count by a group of laboratories (of which the compliance laboratory might be one) has a 95% chance of being less than 0.5 fibers/mL; i.e., $0.16 + 2.13 \times 0.16 = 0.5$.

It can be seen from Figure 1 that the Poisson component of the variability is not very important unless the number of fibers counted is small. Therefore, a further approximation is to simply use +213% and −49% as the upper and lower confidence values of the mean for a 100-fiber count.

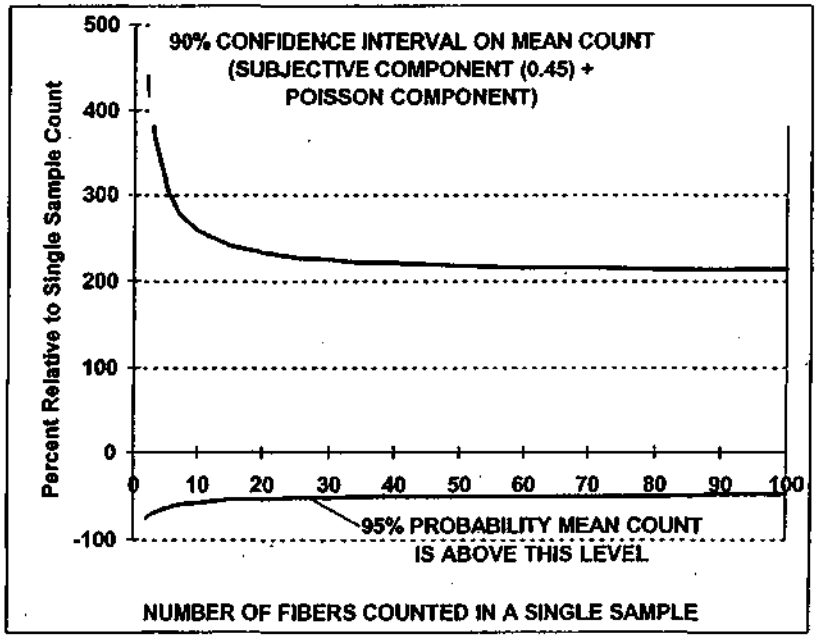

Figure 1. Interlaboratory Precision of Fiber Counts

The curves in Figures 1 are defined by the following equations:

$$UCL = \frac{2X + 2.25 + [(2.25 + 2X)^2 - 4(1 - 2.25 S_r^2) X^2]^{1/2}}{2(1 - 2.25 S_r^2)} \quad (3)$$

$$LCL = \frac{2X + 4 - [(4 + 2X)^2 - 4(1 - 4 S_r^2) X^2]^{1/2}}{2(1 - 4 S_r^2)} \quad (4)$$

where S_r = subjective interlaboratory relative standard deviation, which is close to the total interlaboratory S_r when approximately 100 fibers are counted.
X = total fibers counted on sample
LCL = lower 95% confidence limit.
UCL = upper 95% confidence limit.
Note that the range between these two limits represents 90% of the total range.

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METHOD WRITTEN BY:

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APPENDIX A: CALIBRATION OF THE WALTON-BECKETT GRATICULE:

Before ordering the Walton-Beckett graticule, the following calibration must be done to obtain a counting area (D) 100 µm in diameter at the image plane. The diameter, d_c (mm), of the circular counting area and the disc diameter must be specified when ordering the graticule.

1. Insert any available graticule into the eyepiece and focus so that the graticule lines are sharp and clear.
2. Set the appropriate interpupillary distance and, if applicable, reset the binocular head adjustment so that the magnification remains constant.
3. Install the 40 to 45X phase objective.
4. Place a stage micrometer on the microscope object stage and focus the microscope on the graduated lines.
5. Measure the magnified grid length of the graticule, L_o (µm), using the stage micrometer.
6. Remove the graticule from the microscope and measure its actual grid length, L_a (mm). This can best be accomplished by using a stage fitted with verniers.
7. Calculate the circle diameter, d_c (mm), for the Walton-Beckett graticule:

$$d_c = \frac{L_a}{L_o} \times D. \qquad (5)$$

Example: If L_o = 112 µm, L_a = 4.5 mm and D = 100 µm, then d_c = 4.02 mm.

8. Check the field diameter, D (acceptable range 100 µm ± 2 µm) with a stage micrometer upon receipt of the graticule from the manufacturer. Determine field area (acceptable range 0.00754 mm^2 to 0.00817 mm^2).

APPENDIX B: COMPARISON OF COUNTING RULES:

Figure 2 shows a Walton-Beckett graticule as seen through the microscope. The rules will be discussed as they apply to the labeled objects in the figure.

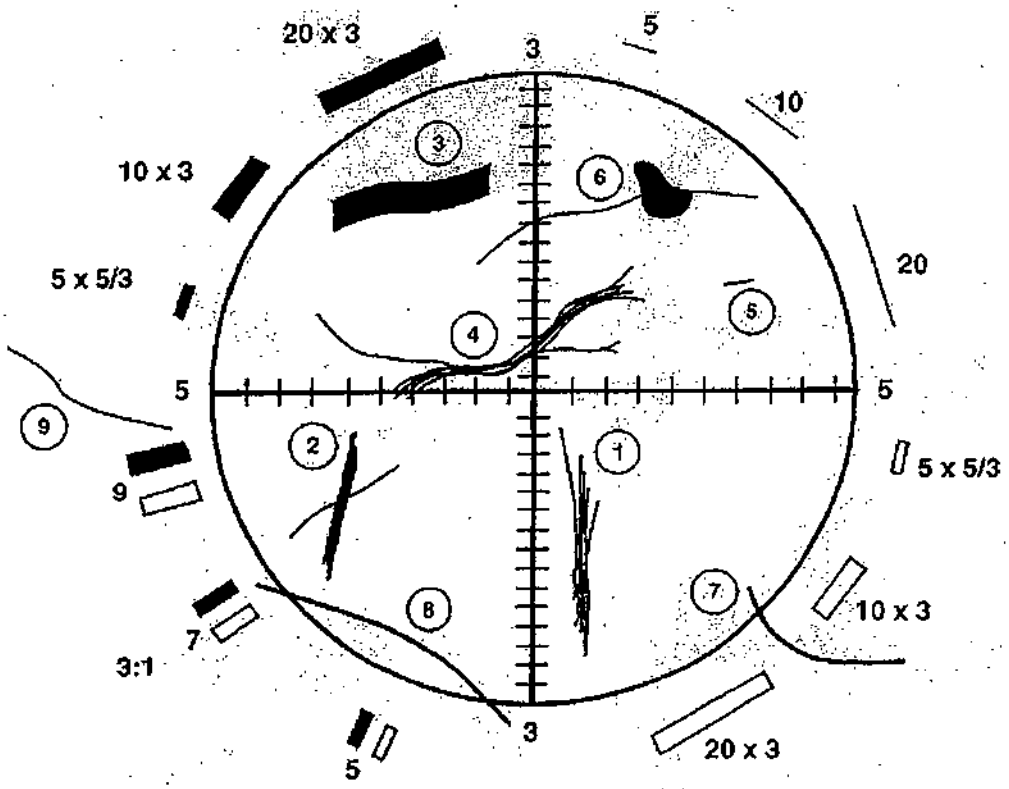

Figure 2. Walton-Beckett graticule with fibers.

These rules are sometimes referred to as the "A" rules.

FIBER COUNT

Object	Count	DISCUSSION
1	1 fiber	Optically observable asbestos fibers are actually bundles of fine fibrils. If the fibrils seem to be from the same bundle the object is counted as a single fiber. Note, however, that all objects meeting length and aspect ratio criteria are counted whether or not they appear to be asbestos.
2	2 fiber	If fibers meeting the length and aspect ratio criteria (length >5 µm and length-to-width ratio >3 to 1) overlap, but do not seem to be part of the same bundle, they are counted as separate fibers.
3	1 fiber	Although the object has a relatively large diameter (>3 µm), it is counted as fiber under the rules. There is no upper limit on the fiber diameter in the counting rules. Note that fiber width is measured at the widest compact section of the object.
4	1 fiber	Although long fine fibrils may extend from the body of a fiber, these fibrils are considered part of the fiber if they seem to have originally been part of the bundle.
5	Do not count	If the object is ≤5 µm long, it is not counted.
6	1 fiber	A fiber partially obscured by a particle is counted as one fiber. If the fiber ends emanating from a particle do not seem to be from the same fiber and each end meets the length and aspect ratio criteria, they are counted as separate fibers.
7	1/2 fiber	A fiber which crosses into the graticule area one time is counted as 1/2 fiber.
8	Do not count	Ignore fibers that cross the graticulate boundary more than once. count
9	Do not count	Ignore fibers that lie outside the graticule boundary.

APPENDIX C. ALTERNATE COUNTING RULES FOR NON-ASBESTOS FIBERS

Other counting rules may be more appropriate for measurement of specific non-asbestos fiber types, such as fibrous glass. These include the "B" rules given below (from NIOSH Method 7400, Revision #2, dated 8/15/87), the World Health Organization reference method for man-made mineral fiber [24], and the NIOSH fibrous glass criteria document method [25]. The upper diameter limit in these methods prevents measurements of non-thoracic fibers. It is important to note that the aspect ratio limits included in these methods vary. NIOSH recommends the use of the 3:1 aspect ratio in counting fibers.

It is emphasized that hybridization of different sets of counting rules is not permitted. Report specifically which set of counting rules are used with the analytical results.

"B" COUNTING RULES:

1. Count only ends of fibers. Each fiber must be longer than 5 µm and less than 3 µm diameter.
2. Count only ends of fibers with a length-to-width ratio equal to or greater than 5:1.
3. Count each fiber end which falls within the graticule area as one end, provided that the fiber meets rules 1 and 2 above. Add split ends to the count as appropriate if the split fiber segment also meets the criteria of rules 1 and 2 above.
4. Count visibly free ends which meet rules 1 and 2 above when the fiber appears to be attached to another particle, regardless of the size of the other particle. Count the end of a fiber obscured by another particle if the particle covering the fiber end is less than 3 µm in diameter.
5. Count free ends of fibers emanating from large clumps and bundles up to a maximum of 10 ends (5 fibers), provided that each segment meets rules 1 and 2 above.
6. Count enough graticule fields to yield 200 ends. Count a minimum of 20 graticule fields. Stop at 100 graticule fields, regardless of count.
7. Divide total end count by 2 to yield fiber count.

APPENDIX D. EQUIVALENT LIMITS OF DETECTION AND QUANTITATION

	fiber density on filter*		fiber concentration in air, f/cc	
	fibers per 100 fields	fibers/mm^2	400-L air sample	1000-L air sample
	200	255	0.25	0.10
	100	127	0.125	0.05
LOQ	80	102	0.10	0.04
	50	64	0.0625	0.025
	25	32	0.03	0.0125
	20	25	0.025	0.010
	10	12.7	0.0125	0.005
	8	10.2	0.010	0.004
LOD	5.5	7	0.00675	0.0027

* Assumes 385 mm^2 effective filter collection area, and field area = 0.00785 mm^2, for relatively "clean" (little particulate aside from fibers) filters.

Tabbed Page: 6

ASBESTOS (bulk) by PLM 9002

various MW: various CAS: 1332-21-4 RTECS: C16475000

METHOD: 9002, Issue 2 **EVALUATION: PARTIAL** **Issue 1: 15 May 1989**
Issue 2: 15 August 1994

EPA Standard (Bulk): 1% **PROPERTIES:** solid, fibrous, crystalline, anisotropic

SYNONYMS [CAS #]: actinolite [77536-66-4], or ferroactinolite [15669-07-5]; amosite [12172-73-5]; anthophyllite [77536-67-5]; chrysotile [12001-29-5]; serpentine [18786-24-8]; crocidolite [12001-28-4]; tremolite [77536-68-6]; amphibole.

SAMPLING		MEASUREMENT	
BULK SAMPLE:	1 to 10 grams	**TECHNIQUE:**	MICROSCOPY, STEREO AND POLARIZED LIGHT, WITH DISPERSION STAINING
SHIPMENT:	seal securely to prevent escape of asbestos	**ANALYTE:**	actinolite asbestos, amosite, anthophyllite asbestos, chrysotile, crocidolite, tremolite asbestos
SAMPLE STABILITY:	stable	**EQUIPMENT:**	microscope, polarized light; 100-400X dispersion staining objective, stereo microscope: 10-45X
BLANKS:	none required	**RANGE:**	1% to 100% asbestos
		ESTIMATED LOD:	<1% asbestos [1]
ACCURACY		**PRECISION:**	not determined
RANGE STUDIED:	<1% to 100% asbestos		
BIAS:	not determined		
PRECISION:	not determined		
ACCURACY:	not determined		

APPLICABILITY: this method is useful for the qualitative identification of asbestos and the semi-quantitative determination of asbestos content of bulk samples. The method measures percent asbestos as perceived by the analyst in comparison to standard area projections, photos, and drawings, or trained experience. The method is not applicable to samples containing large amounts of fine fibers below the resolution of the light microscope

INTERFERENCES: Other fibers with optical properties similar to the asbestos minerals may give positive interferences. Optical properties of asbestos may be obscured by coating on the fibers. Fibers finer than the resolving power of the microscope (ca. 0.3 µm) will not be detected. Heat and acid treatment may alter the index of refraction of asbestos and change its color.

OTHER METHODS: This method (originally designated as method 7403) is designed for use with NIOSH Methods 7400 (phase contrast microscopy) and 7402 (electron microscopy/EDS). The method is similar to the EPA bulk asbestos method [1].

REAGENTS:

1. Refractive index (RI) liquids for Dispersion Staining: high-dispersion (HD) series, 1.550, 1.605, 1.620.
2. Refractive index liquids: 1.670, 1.680, and 1.700.
3. Asbestos reference samples such as SRM #1866, available from the National Institute of Standards and Technology.*
4. Distilled Water (optional).
5. Concentrated HCl: ACS reagent grade.

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Sample containers: screw-top plastic vials of 10- to 50-mL capacity.
2. Microscope, polarized light, with polarizer, analyzer, port for retardation plate, 360 graduated rotating stage, substage condenser with iris, lamp, lamp iris, and:
   a. Objective lenses: 10X, 20X, and 40X or near equivalent.
   b. Ocular lense: 10X minimum.
   c. Eyepiece reticle: crosshair.
   d. Dispersion staining objective lens or equivalent.
   e. Compensator plate: ca. 550 nm± 20 nm, retardation: "first order red" compensator.
3. Microscope slides: 75 mm x 25 mm.
4. Cover slips.
5. Ventilated hood or negative-pressure glove box.
6. Mortar and pestle: agate or porcelain.
7. Stereomicroscope, ca. 10 to 45X.
8. Light source: incandescent or fluorescent.
9. Tweezers, dissecting needles, spatulas, probes, and scalpels.
10. Glassine paper or clean glass plate.
11. Low-speed hand drill with coarse burr bit (optional).

SPECIAL PRECAUTIONS: Asbestos, a human carcinogen, should be handled only in an exhaust hood (equipped with a HEPA filter) [2]. Precautions should be taken when collecting unknown samples, which may be asbestos, to preclude exposure to the person collecting the sample and minimize the disruption to the parent material [3]. Disposal of asbestos-containing materials should follow EPA Guidelines [4].

SAMPLING:

1. Place 1 to 10 g of the material to be analyzed in a sample container.
   NOTE: For large samples (i.e., whole ceiling tiles) that are fairly homogenous, a representative small portion should be submitted for analysis. Sample size should be adjusted to ensure that it is representative of the parent material.
2. Make sure that sample containers are taped so they will not open in transit.
3. Ship the samples in a rigid container with sufficient packing material to prevent damage or sample loss.

SAMPLE PREPARATION:

4. Visually examine samples in the container and with a low-magnification stereomicroscope in a hood. (If necessary, a sample may be carefully removed from the container and placed on glassine transfer paper or clean glass plate for examination). Break off a portion of the sample and examine the edges for emergent fibers. Note the homogeneity of the sample. Some hard tiles can be broken, and the edges examined for emergent fibers. If fibers are found, make an estimate of the amount and type of fibers present, confirm fiber type (step 14) and quantify (step 15).
5. In a hood, open sample container and with tweezers remove small, representative portions of the sample.
   1. If there are obvious separable layers, sample and analyze each layer separately.

b. If the sample appears to be slightly inhomogeneous, mix it in the sample container with tweezers or a spatula before taking the portion of analysis. Alternatively, take small representative portions of each type of material and place on a glass slide.
c. On hard tiles that may have thin, inseparable layers, use a scalpel to cut through all the layers for a representative sample. Then cut it into smaller pieces after placing RI liquid on it before trying to reduce the thickness. Alternatively, use a low-speed hand drill equipped with a burr bit to remove material from hard tiles. Avoid excessive heating of the sample which may alter the optical properties of the material.
   NOTE: This type of sample often requires ashing or other specialized preparation, and may require transmission electron microscopy for detection of the short asbestos fibers which are characteristic of floor tiles.
d. If the sample has large, hard particles, grind it in a mortar. Do not grind so fine that fiber characteristics are destroyed.
e. If necessary, treat a portion of the sample in a hood with an appropriate solvent to remove binders, tars, and other interfering materials which may be present in the sample. Make corrections for the non-asbestos material removed by this process.
   NOTE: Other methods of sample preparation such as acid washing and sodium metaphosphate treatment and ashing may be necessary, especially to detect low concentrations of asbestos. If needed, use as described in Reference [1].

6. After placing a few drops of RI liquid on the slide, put a small portion of sample in the liquid. Tease apart with a needle or smash small clumps with the flat end of a spatula or probe, producing a uniform thickness or particles so that better estimates of projected area percentages can be made. Mix the fibers and particles on the slide so that they are as homogeneous as possible.
   NOTE: An even dispersion of sample should cover the entire area under the cover slip. some practice will be necessary to judge the right amount of material to place on the slide. Too little sample may not give sufficient information and too much sample cannot be easily analyzed.

CALIBRATION AND QUALITY CONTROL:

7. Check for contamination each day of operation. Wipe microscope slides and cover slips with lens paper before using. Check refractive index liquids. Record results in a separate logbook.
8. Verify the refractive indices of the refractive index liquids used once per week of operation. Record these checks in a separate logbook.
9. Follow the manufacturer's instructions for illumination, condenser alignment and other microscope adjustments. Perform these adjustments prior to each sample set.
10. Determine percent of each identified asbestos species by comparison to standard projections (Figure 1) [1]. If no fibers are detected in a homogeneous sample, examine at least two additional preparations before concluding that no asbestos is present.
11. If it appears that the preparation technique might not be able to produce a homogeneous or representative sample on the slide, prepare a duplicate slide and average the results. Occasionally, when the duplicate results vary greatly, it will be necessary to prepare additional replicate slides and average all the replicate results. Prepare duplicate slides of at least 10% of the samples analyzed. Average the results for reporting.
12. Analyze about 5% blind samples of known asbestos content.
13. Laboratories performing this analytical method should participate in the National Voluntary Laboratory Accreditation Program [5] or a similar interlaboratory quality control program. Each analyst should have complete formal training in polarized light microscopy and its application to crystalline materials. In lieu of formal training, laboratory training in asbestos bulk analysis under the direction of a trained asbestos bulk analyst may be substituted. Owing to the subjective nature of the method, frequent practice is essential in order to remain proficient in estimating projected area percentages.

QUALITATIVE ASSESSMENT:

14. Scan the slide to identify any asbestos minerals using the optical properties of morphology,

refractive indices, color, pleochroism, birefringence, extinction characteristics, sign of elongation, and dispersion staining characteristics.

NOTE: Identification of asbestos using polarized light microscopy is unlike most other analytical methods. The quality of the results is dependent on the skill and judgment of the analyst. This method does not lend itself easily to a step-wise approach. Various procedures devised by different analysts may yield equivalent results. The following step-wise procedure repeatedly utilizes the sample preparation procedure previously outlined.

a. Prepare a slide using 1.550 HD RI liquid. Adjust the polarizing filter such that the polars are partially crossed, with ca. 15 offset. Scan the preparation, examining the morphology for the presence of fibers. If no fibers are found, scan the additional preparations. If no fibers are found in any of the preparations, report that the sample does not contain asbestos, and stop the analysis at this point.

b. If fibers are found, adjust the polarizing filter such that the polars are fully crossed. If all of the fibers are isotropic (disappear at all angles of rotation) then those fibers are not asbestos. Fibrous glass and mineral wool, which are common components of suspect samples, are isotropic. If only isotropic fibers are found in the additional preparations, report no asbestos fibers detected, and stop the analysis.

c. If anisotropic fibers are found, rotate the stage to determine the angle of extinction. Except for tremolite-actinolite asbestos which has oblique extinction at 10-20 , the other forms of asbestos exhibit parallel extinction (Table 1). Tremolite may show both parallel and oblique extinction.

d. Insert the first order red compensator plate in the microscope and determine the sign of elongation. All forms of asbestos have a positive sign of elongation except for crocidolite. If the sign of elongation observed is negative, go to step "g."

NOTE: To determine the direction of the sign of elongation on a particular microscope configuration, examine a known chrysotile sample and note the direction (NE-SW or NW-SE) of the blue coloration. Chrysotile has a positive sign of elongation.

e. Remove the first-order red compensator and uncross the polarizer. Examine under plane polarized light for blue and gold-brown Becke colors at the fiber-oil interface (i.e., index of refraction match). Becke colors are not always evident. Examine fiber morphology for twisted, wavy bundles of fibers which are characteristic of chrysotile. Twisted, ribbon-like morphology with cellular internal features may indicate cellulose fibers. It may be necessary to cross the polars partially in order to see the fibers if the index of refraction is an exact match at 1.550. If the fibers appear to have higher index of refraction, go to step "h," otherwise continue.

f. Identification of chrysotile. Insert the dispersion staining objective. Observation of dispersion staining colors of blue and blue-magenta confirms chrysotile. Cellulose, which is a common interfering fiber at the 1.550 index of refraction, will not exhibit these dispersion staining colors. If chrysotile is found, go to step 15 for quantitative estimation.

g. Identification of crocidolite. Prepare a slide in 1.700 RI liquid. Examine under plane-polarized light (uncrossed polars); check for morphology of crocidolite. Fibers will be straight, with rigid appearance, and may appear blue or purple-blue. Crocidolite is pleochroic, i.e., it will appear to change its color (blue or gray) as it is rotated through plane polarized light. Insert the dispersion staining objective. The central stop dispersion staining color are red magenta and blue magenta, however, these colors are sometimes difficult to impossible to see because of the opacity of the dark blue fibers. If observations above indicate crocidolite, go to step 15 for quantitative estimation.

h. Identification of amosite. Prepare a slide in 1.680 RI liquid. Observed the fiber morphology for amosite characteristics: straight fibers and fiber bundles with broom-like or splayed ends. If the morphology matches amosite, examine the fibers using the dispersion staining objective. Blue and pale blue colors indicate the cummingtonite form of amosite, and gold and blue colors indicate the grunerite form of amosite. If amosite is confirmed by this test, go to step 15 for quantitative estimation, otherwise continue.

i. Identification of anthophyllite-tremolite-actinolite. Prepare a slide in 1.605 HD RI liquid. Examine morphology for comparison to anthophyllite-tremolite-actinolite asbestos. The refractive indices for these forms of asbestos vary naturally within the species. Anthophyllite can be distinguished from actinolite and tremolite by its nearly parallel extinction. Actinolite has a light to dark green color under plane-polarized light and exhibits some pleochroism. For all

three, fibers will be straight, single fibers possibly with some larger composite fibers. Cleavage fragments may also be present. Examine using the central stop dispersion staining objective. Anthophyllite will exhibit central stop colors of blue and gold/gold-magenta; tremolite will exhibit pale blue and yellow; and actinolite will exhibit magenta and golden-yellow colors.

NOTE: In this refractive index range, wollastonite is a common interfering mineral with similar morphology including the presence of cleavage fragments. It has both positive and negative sign of elongation, parallel extinction, and central stop dispersion staining colors of pale yellow and pale yellow to magenta. If further confirmation of wollastonite versus anthophyllite is needed, go to step "j". If any of the above forms of asbestos were confirmed above, go to step 15 for quantitative estimation. If none of the tests above confirmed asbestos fibers, examine the additional preparations and if the same result occurs, report the absence of asbestos in this sample.

j. Wash a small portion of the sample in a drop of concentrated hydrochloric acid on a slide. Place the slide, with cover slip in place, on a warm hot plate until dry. By capillary action, place 1.620 RI liquid under the cover clip and examine the slide. Wollastonite fibers will have a "cross-hatched" appearance across the length of the fibers and will not show central stop dispersion colors. Anthophyllite and tremolite will still show their original dispersion colors.

NOTE: There are alternative analysis procedures to the step-wise approach outlined above which will yield equivalent results. Some of these alternatives are:

i. Perform the initial scan for the presence of asbestos using crossed polars as well as the first-order red compensator. This allows for simultaneous viewing of birefringent and amorphous materials as well as determine their sign of elongation. Some fibers which are covered with mortar may best be observed using this configuration.

ii. Some analysts prefer to mount their first preparation in a RI liquid different than any asbestos materials and conduct their initial examination under plane-polarized light.

iii. If alternative RI liquids are used from those specified, dispersion staining colors observed will also change. Refer to an appropriate reference for the specific colors associated with asbestos in the RI liquids actually used.

QUANTITATIVE ASSESSMENT:

15. Estimate the content of the asbestos type present in the sample using the 1.550 RI preparation. Express the estimate as an area percent of all material present, taking into account the loading and distribution of all sample material on the slide. Use Figure 1 as an aid in arriving at your estimate. If additional unidentified fibers are present in the sample, continue with the qualitative measurement (step 14).

NOTE: Point-counting techniques to determine percentages of the asbestos minerals are not generally recommended. The point-counting method only produces accurate quantitative data when the material on the slide is homogeneous and has a uniform thickness, which is difficult to obtain [6]. The point-counting technique is, recommended by the EPA to determine the amount of asbestos in bulk [1]; however, in the more recent Asbestos Hazard Emergency Response Act (AHERA) regulations, asbestos quantification may be performed by a point-counting or equivalent estimation method [7].

16. Make a quantitative estimate of the asbestos content of the sample from the appropriate combination of the estimates from both the gross and microscopic examinations. If asbestos fibers are identified, report the material as "asbestos-containing". Asbestos content should be reported as a range of percent content. The range reported should be indicative of the analyst's precision in estimating asbestos content. For greater quantities use Figure 1 in arriving at your estimate.

EVALUATION OF METHOD:

The method is compiled from standard techniques used in mineralogy [8-13], and from standard laboratory procedures for bulk asbestos analysis which have been utilized for several years. These

techniques have been successfully applied to the analysis of EPA Bulk Sample Analysis Quality Assurance Program samples since 1982 [1,5]. However, no formal evaluation of this method, as written, has been performed.

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METHOD WRITTEN BY:

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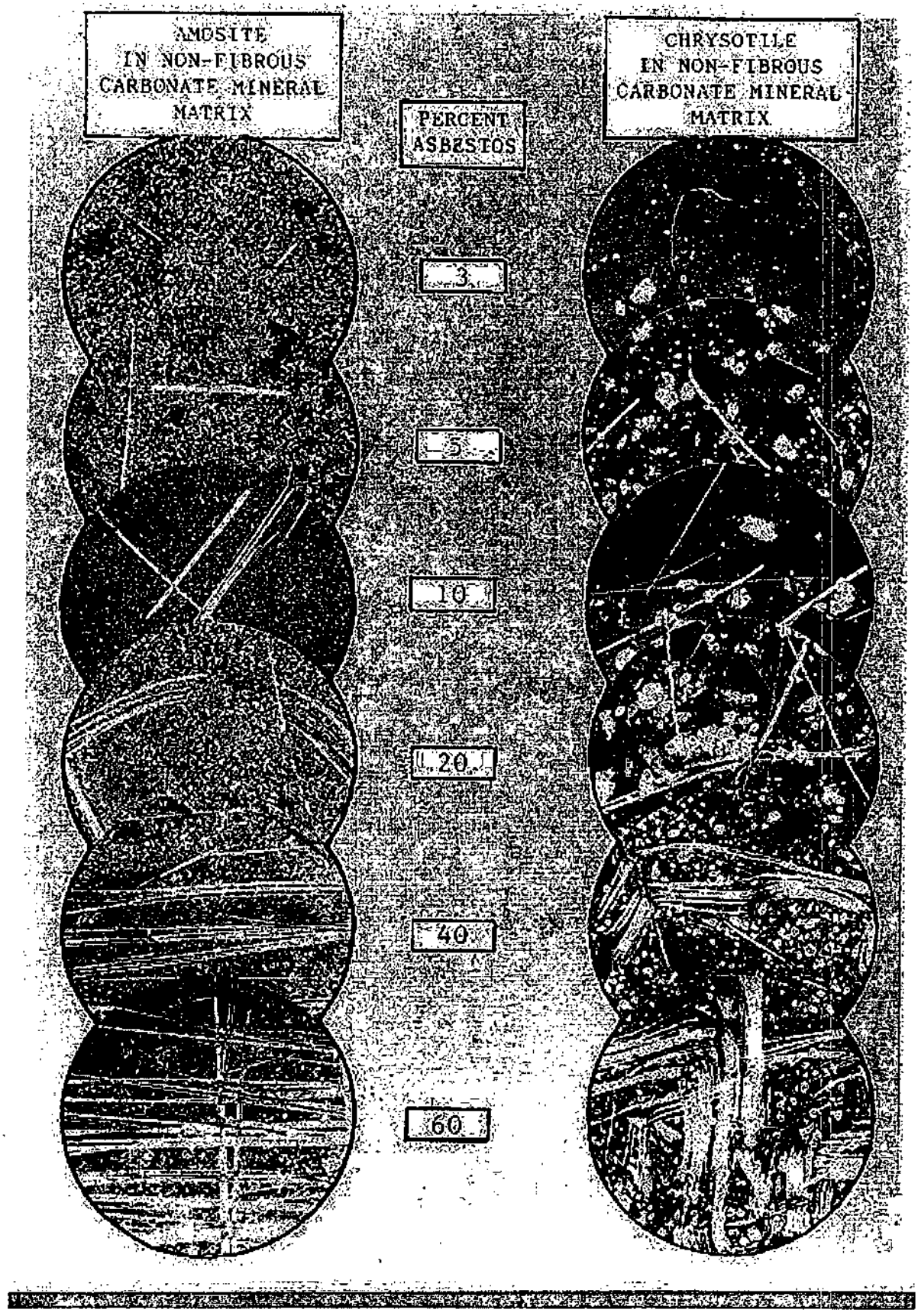

Figure 1. Percent estimate comparator

Table 1. Optical Properties of Asbestos Fibers

		Refractive Index (Approximate Values)		
Mineral	Morphology and Color	⊥ to Elongation	to Elongation	Birefringence
Chrysotile	Wavy fibers with kinks. Splayed ends on larger bundles. Colorless to light brown upon being heated. Nonpleochroic. Aspect ratio typically >10:1.	1.54	1.55	0.002 - 0.014
Cummingtonite-Grunerite (Amosite)	Straight fibers and fiber bundles. Bundle ends appear broom-like or splayed. Colorless to brown upon heating. May be weakly pleochroic. Aspect ratio typically >10:1.	1.67	1.70	0.02 - 0.03
Crocidolite (Riebeckite)	Straight fibers and fiber bundles. Longer fibers show curvature. Splayed ends on bundles. Characteristic blue color. Pleochroic. Aspect ratio typically >10:1.	1.71	1.70	0.014 - 0.016 Interference colors may be masked by blue color.
Anthophyllite	Straight fibers and fiber bundles. Cleavage fragments may be present. Colorless to light brown. Nonpleochroic to weakly pleochroic. Aspect ratio generally <10:1.	1.61	1.63	0.019 - 0.024
Tremolite-Actinolite	Straight and curved fibers. Cleavage fragments common. Large fiber bundles show splayed ends. Tremolite is colorless. Actinolite is green and weakly to moderately pleochroic. Aspect ratio generally <10:1.	1.60 - 1.62 (tremolite) 1.62 - 1.67 (actinolite)	1.62 - 1.64 (tremolite) 1.64 - 1.68 (actinolite)	0.02 - 0.03

Table 1. Optical Properties of Asbestos Fibers (Continued)

			Central Stop Dispersion Staining Colors		
Mineral	Extinction	Sign of Elongation	RI Liquid	⊥ to Vibration	∥ to Vibration
Chrysotile	Parallel to fiber length	+ (length slow)	1.550 HD	Blue	Blue-magenta
Cummingtonite-Grunerite (Amosite) Cummingtonite Grunerite	Parallel to fiber length	+ (length slow)	1.670 Fibers subjected to high temperatures will not dispersion-stain. 1.680 1.680	Red magenta to blue pale blue blue	Yellow blue gold
Crocidolite (Riebeckite)	Parallel to fiber length	- (length fast)	1.700 1.680	Red magenta yellow	Blue-magenta pale yellow
Anthophyllite	Parallel to fiber length	+ (length slow)	1.605 HD 1.620 HD	Blue Blue-green	Gold to gold-magenta Golden-yellow
Tremolite-Actinolite	Oblique - 10 to 20 for fragments. Some composite fibers show extinction.	+ (length slow)	1.605 HD	Pale blue (tremolite) Yellow (actinolite)	Yellow (tremolite) Pale yellow (actinolite)

HD = high-dispersion RI liquid series.

Tabbed Page: 7

Development of a method for the determination of low contents of asbestos fibres in bulk material

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Asbestos is a category 1 carcinogen under the EU classification, but in the absence of a method to quantify asbestos in a matrix at the 0.1% level, there has been a delay in implementing relevant directives to asbestos. An analytical scheme for identification and quantification of asbestos using polarised light microscopy (PLM) and phase contrast optical microscopy (PCM) has now been developed. When used on artificial mixtures by an experienced laboratory, it achieved the required target performance, at 0.1% asbestos concentration by mass in a bulk sample, to obtain a result, which with 90% probability, is correct within a factor of two. The method of identification by PLM and quantification by PCM has been assessed by interlaboratory comparisons. The method begins with an initial identification using PLM, and depending on asbestos type and matrix a combination of preparation procedures are used to produce the analytical filter. A gentle comminution method was used which reduces the risk of overmilling. The asbestos mass percentage on the filter is quantified using PCM in combination with a PLM attachment for identification of possible non-asbestos fibres. The final method is supported by efficient methods for fibre identification for size determination and calculation of total fibre volume. A statistical analysis of mass concentration estimates was made and the effect of preferred orientation of fibres on the analytical filter was quantified.

Keywords: Asbestos mass determination; optical microscopy; bulk material; comminution

Asbestos is a category 1 carcinogen under the EU classification system (EU Directive 548/67/EEC and EU Directive 88/379/EEC) based on epidemiological and animal toxicological evidence. However, in the absence of a method to quantify asbestos at the 0.1% level in a matrix, there has been a delay in implementing the directives to asbestos. An analytical scheme for identification and quantification of asbestos was thus developed. The following target performance was specified. The method should, at 0.1% asbestos concentration by mass in a bulk sample of grain size less than 2 mm obtain a result, which with 90% probability, is correct within a factor of two.

Asbestos is a technical term used for the fibrous forms of several naturally occurring silicate minerals, which have been exploited for their useful properties. Like most other minerals, the chemical composition of asbestos varies within a certain range. In practice, six commercial varieties of asbestos have been regulated by the EU: One serpentine mineral, chrysotile (CAS No. 12001-29-5); and five amphibole minerals, amosite (grunerite asbestos; CAS No. 12172-73-5); crocidolite (riebeckite asbestos; CAS No. 12001-28-4); tremolite asbestos (CAS No. 77536-68-6); anthophyllite asbestos (CAS No. 77536-67-5); and actinolite asbestos (CAS No. 77536-66-4).

The International Standards Organisation defines asbestos as follows:[1]

'Asbestos: a collective term applied to specific serpentine and amphibole minerals which have been crystallised in the asbestiform habit, causing them to separate into long, thin, strong fibres when crushed or processed. The most common forms are . . .' the EU regulated varieties.

Asbestos occurs in a large number of metamorphic rocks either as a constituent of the rock or as late formed fillings of veins and cavities. When present in quantities of greater than 1%, the deposit may be commercially exploited to extract the fibres for use as insulation or for further processing into a wide range of commercial products. If present in the rock at less than 1%, the asbestos is essentially a contaminant of the mineral or stone product that is being mined or quarried. In practice asbestos can be present in quarried rock (*e.g.*, dolomitic marble and granite), minerals (*e.g.*, talc and vermiculite), sediment materials (*e.g.*, clays) and commercial products (*e.g.*, building materials, fillers). No one single analytical method is suitable or cost-effective for all these greatly different bulk materials. The approach thus was to develop an analytical scheme built on several building blocks each of which were based on the simplest possible existing methods, modified or developed as needed.

Shape is a major determinant of toxicity and thus hazard. This requires use of microscopical methods. It was decided to base the method on polarised light microscopy (PLM) and phase contrast optical microscopy (PCM) as this would ensure that a sufficient number of testing facilities would be available, and would keep costs low. Also the fibre mass is usually concentrated in the thicker (visible in PCM) fibres, and further support for choosing optical microscopy also comes from the fact that the PCM method is used for routine quantification of risk, once fibres have become airborne (EC Directive 83/477).

The importance of 'shape' presents a unique and very difficult challenge to the sample preparation steps and to quantification. External conditions of growth have a large effect on the morphology of the crystal and the same mineral may occur in different habits (morphological types). The morphology of amphibole asbestos is complex and is based on the preferential growth of long thin fibrils along the *c*-axis of the amphibole crystal, and which are usually held together to form relatively loose bundles or clusters of fibres. The metamorphic condition within any particular area may give rise to a

continuous range of morphological forms only some of which may be asbestos. The temperature, pressure, and concentration of mineral forming medium may vary from place to place within the same rock. Therefore, serpentine may occur as chrysotile, antigorite or lizardite. The amphiboles may form acicular crystals (Fig. 1), fibres (Fig. 2) or prismatic crystals which by cleaving may generate elongated cleavage fragments (Fig. 3). These fragments are part of a crystal, while asbestos fibres are aggregates of numerous fibrils. Ultimately, it may not be possible either to define the exact amount of asbestos by mass or by number in a material, as there is no strict dividing line between the formation of acicular crystals and asbestos.

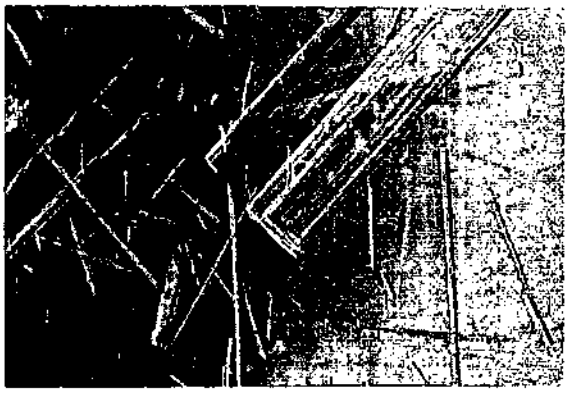

Fig. 1 Photomicrograph under oblique illumination of lamellar and acicular crystals of tremolite defined as 'acicular tremolite'. Ala di Stura, Italy.

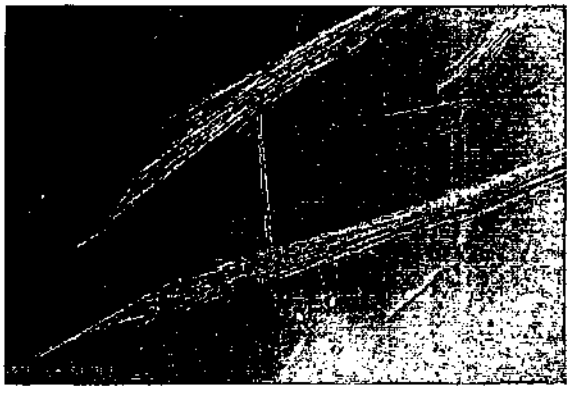

Fig. 2 Photomicrograph under oblique illumination of single fibres and bundles of fibres of tremolite defined as 'asbestiform tremolite'. Jamestown, California.

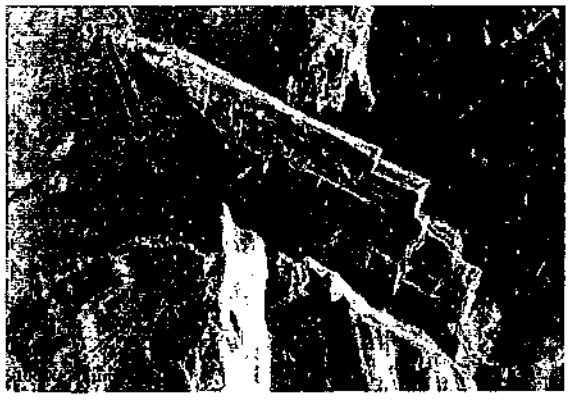

Fig. 3 Photomicrograph under oblique illumination of cleavage fragment from prismatic tremolite. Shinness, Lairg.

The method is needed for classifying hazard, *i.e.*, the potential of a material to release fibres. A standard challenge was developed to maximise the amount of fibres originally present in clumps or bound in the matrix then be released in the sample and thus be detected as fibres, while minimising the risk of destroying fibres by overmilling. It was not the purpose of the challenge to relate directly to exposure risk from airborne fibres, which is handled under EC Directive 83/477/EEC. However, for such fibres the shape criteria is: width < 3 μm, length to width ratio (aspect ratio, AR) $> 3:1$, length > 5 μm . Since asbestos fibres in a bulk sample can only disintegrate into fibres with smaller width or into shorter fibres during use, the implications for the present method is that all fibre widths, but only fibres longer than 5 μm, will need to be evaluated in the bulk material. The aspect ratio AR < 3 is also used as an exclusion criteria in the method.

Further problems related to shape arise from the use of PCM for quantification. The analyte is observed as discrete entities and their volume has to be estimated from the projected image. Thus *efficient counting and sizing methods and a mechanism* for extrapolation from the two to the three dimensional shape had to be developed.

Experimental

Overview

The method begins with an initial identification using PLM, and depending on asbestos type and matrix a combination of preparation procedures are used to produce the analytical filter. The asbestos mass percentage on this filter is quantified using PCM in combination with a PLM attachment for identification of possible non-asbestos fibres. Although measurement of extinction angle, birefringence, pleochroism, texture, and elongation would have allowed classification of fibres, it was decided to use only a standard PCM with polariser and analyser and to rely on observations that a non-specialist microscopist could reliably and reproducibly record. The analytical scheme allows additional analysis by scanning (SEM) or transmission electron microscopy (TEM) for difficult samples. The flow diagram, Fig. 4, illustrates the sample preparation scheme. The exact choice of individual steps must be made by an experienced person, who can write the detailed procedure to be applied in the laboratory for each specific type of sample. The individual steps are described below.

For development of this method a basic stock of 12 artificial materials were produced.[2] They comprised: (1) New York talc undiluted, 20–40% anthophyllite; (2) New York talc, (1) dilution 1 : 10; (3) New York talc, (2) dilution 1 : 10; (4) acid washed dolomite, contains tremolite asbestos; (5) 1% amosite in soil, spiked; (6) fibrous wollastonite, no asbestos; (7) vermiculite with tremolite asbestos, spiked, 0.05%; (8) amosite present as asbestos cement fragments in demolition waste, spiked, 0.25%; (9) chrysotile in olivine sand, spiked, 0.01%; (10) crocidolite in sepiolite, spiked, 0.1%; (11) amosite in talc, spiked, 0.15%; and (12) dolomite.

Initial identification of fibres using polarised light microscopy (PLM).

The method begins with a preliminary evaluation by stereo microscopy. Based upon the observation of physical appearance, the fibres are mounted in the most suitable refractive index liquid. PLM is used to identify fibres using morphology, pleochroism, birefringence, extinction, and sign of elongation. The refractive index is assessed by observing relief, Becke line,

or dispersion staining colours. The method is described in detail in Burdett.[3]

Optical properties alone may not be sufficient to distinguish between tremolite and actinolite from some sources (because these minerals are members of a 'solid solution series' for which there is continuously varying composition giving a continuous range of refractive index) or between tremolite and anthophyllite (because they have similar birefringence and ranges of refractive index). Should such distinctions be necessary, additional methods (such as electron microscopy) may be used.

Fibre counting and sizing using PCM–PLM

Four types of microscopical methods are commonly used for quantification of asbestos in bulk samples:

(1) Comparison with reference slides, see for example method 9002 in NIOSH.[4] This method is semi-quantitative at best, and is not suitable for concentrations below 1% m/m.

(2) Determination of fibre number per unit weight of material. The result will depend critically on the preparation step prior to microscopy.

(3) Projected area percentage determined by point counting.[4] This method is inherently biased.[5] The result will also depend on the preparation step prior to microscopy.

(4) Volume determination based on measured fibre length and projected width.

Because of the limitations of the methods 1–3, quantification was based on volume determination.

When counting fibres using PCM, rules are needed to decide if a fibre not completely contained in the field of view has to be included and with which statistical weight. The following rules and weights for a fibre overlapping the field of view were

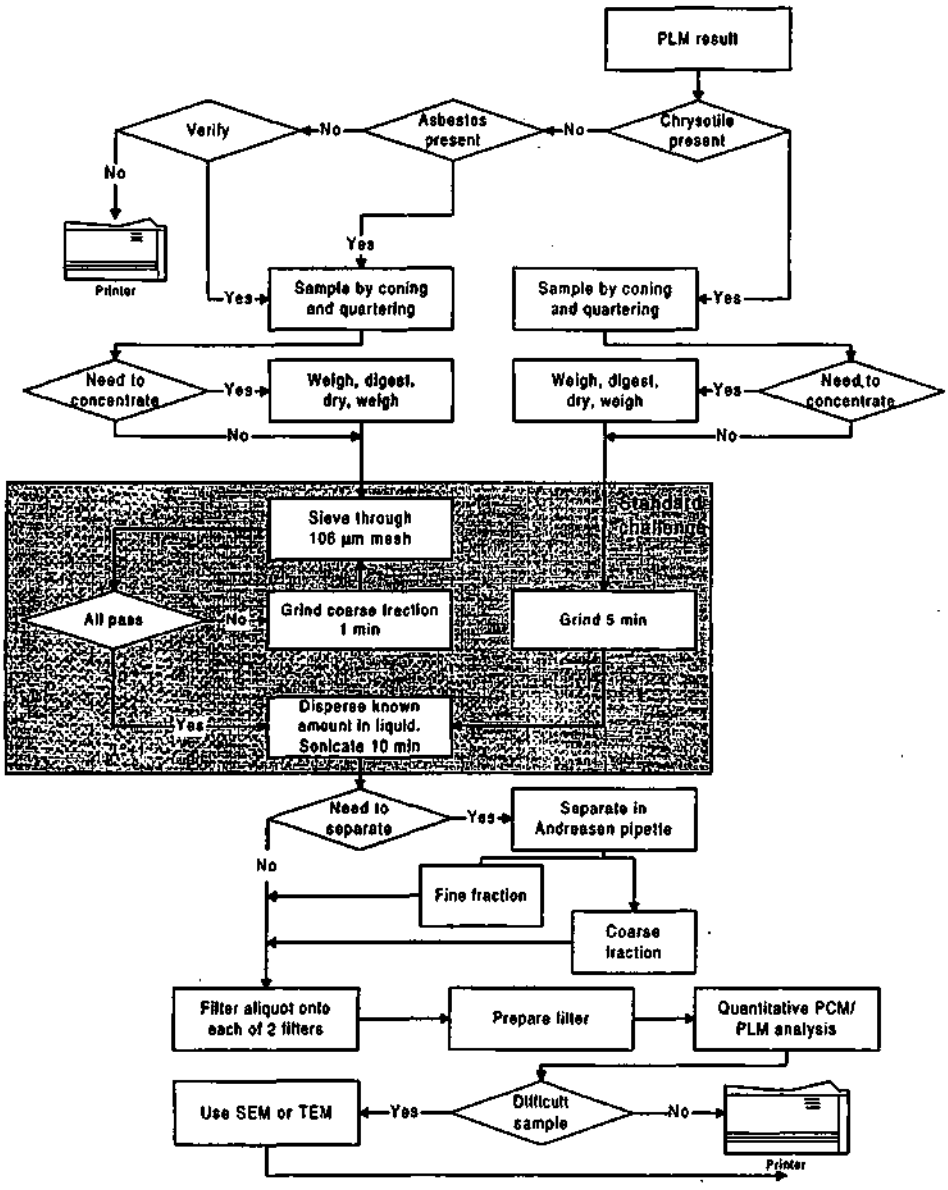

Fig. 4 Preparation of sample for PLM–PCM quantiative analysis.

originally considered: by number of endpoints within field, divided by two, termed $R(\frac{1}{2})$ rule; by 0 if lower endpoint in field, by 1 if upper endpoint in field, $R(1)$ rule; ratio of fibre length within field to total length, R(field) rule. This is equivalent to the rule used in practice; only the length of fibre within the field is measured, and a weight 1 is assigned.

The properties of these counting rules were studied using Monte Carlo simulations.[2,6] It was found that the R(field) counting rule gave the smallest variance, and volume determination thus is based on this rule. However, since the fibre size and distribution is needed for discriminating according to aspect ratio, the total length is also observed, and each fibre weighted according to counting rule $R(\frac{1}{2})$.

To improve accuracy and precision of sizing the original Walton–Beckett graticule was modified.[7] The graticule can be obtained from Graticules Ltd., Morley Road, Tonbridge, Kent, UK TN9 1RN (Reference number 9/950/427). It has a central circular counting area, designed to have a diameter in the object plane of 100 μm, and divided into quadrants by two lines scaled in units of 3 μm and 5 μm, respectively. Unlike the original Walton–Beckett design the shapes around the outside of the circle have been chosen to assist in the estimation of fibre lengths and diameters as well as those depicting typical countable fibres. Fibre length is measured to the nearest 5 μm for all fibres longer than 5 μm (no maximum fibre length). Fibre width is measured to the nearest 0.5 μm, and placed in one of the categories < 0.5, 0.5, 1.0, 1.5 μm, *etc.* Fibres falling into the thinnest category, < 0.5 μm are given the width 0.2 μm. Given that the fibre mass is associated with thick fibres, it was thought that this approximation would not significantly bias the mass percentage estimates.

Sample pre-treatment and concentration

The analytical scheme assumes that samples are received with grain size less than 2 mm. Choice of initial amount of and sample size reduction by coning and quartering should follow BS 5309.[8]

Sample pre-treatment and concentration is essential as it sets free fibres from any binding matrix material, improves the detection limit, and reduces effects of sample inhomogeneities. Ashing and digestion can be used. Ashing of dry, organic material is performed at 450 °C. Acid digestion of carbonates is performed using 1 M HCl at room temperature, which will leave chrysotile unharmed. In some cases, special digestion methods must be used, such as solvent extraction of *e.g.*, vinyl floor tiles and acid–alkali digestion for extracting amphiboles from a vermiculite matrix.[9]

Comminution

Comminution is a key step and represents the major part of the standard challenge. A range of milling methods were initially considered. The final selection for the study were the following three methods:

(1) The McCrone Micronizing Mill. It uses a wet technique, is relatively small, and is compact. The comminution process is standardised.

(2) A steel percussion mortar, which is used by mineralogists for comminuting minerals without damaging crystal structure.

(3) Mortar and pestle. The main reason for its inclusion was that this technique is widely used for preparation of powders.

Milling of acid washed dolomite and of talc in a McCrone mill showed that with increasing milling time the response for tremolite as measured by X-ray diffractometry, fibre length, and more important diameter, continued to decrease, whereas the total number of fibres per unit weight of material increased (Table 1). After 45 min milling of the acid-washed dolomite no fibres were visible in PLM. The risk of overmilling has also been demonstrated by others.[10,11]

Grinding for equal time periods of acid washed dolomite, olivine sand and demolition waste lead to different degrees of comminution. Zero milling time underestimated fibre mass, and produced fewer fibres for those samples that contained fibres bound in a matrix (Table 2). Thus milling is needed but the McCrone mill could not be used in a one-pass procedure ensuring that all particles would pass a 106 μm sieve, without risk of overmilling.

The percussion mortar was constructed from a commercial steel mortar with pestle and cylinder. A 'pile driver' ensured a reproducible stroke on the pestle. Calcite, tremolite and quartz were comminuted, and the resulting size distribution determined by sieving. An almost linear relationship was found between the potential energy of the pile driver and the median of the diameter distribution.

The key problem in using manual grinding by mortar and pestle is to standardise the pressure and movement of the pestle. This problem was resolved by using a large mortar with a heavy pestle and by moving the pestle in a circular movement at 100 rev min^{-1}, without pressing the pestle. The degree of grinding can be changed by changing the grinding time. Calcite was ground to below 100 μm in 0.25 min, tremolite in 1 min, and quartz in 10 min. This mortar was finally chosen because the test showed that most types of asbestos are more easily comminuted by ceramic mortar than by percussion mortar. An exception is chrysotile which can neither be easily comminuted by ceramic mortar nor by percussion mortar. Furthermore a mortar is readily available and inexpensive. It is easy to clean,

Table 1 Size distribution parameters, diffractometric responses, and fibre number after different time-controlled comminution

Sample	Grinding time/min	Length/μm*	Diameter/μm*	Counts s^{-1} for 400 μg†	Number $\times 10^5$/mg	RSD (%)
Tremolite in acid-washed dolomite						
	0	22 (2.2)	1.6 (2.6)	18.7 (9.2)	3.82	16
	5	14.2 (2.4)	1.2 (2.4)	46.6 (6.9)	15.6	8
	15	11 (2.2)	1.05 (2.2)	39 (3.4)	14.5	8
	30	9.3 (2.0)	0.75 (2.2)	31.7 (7.0)	32.5	6
	45	8.0 (1.9)	0.66 (2.0)	24.3 (1.7)	49.4	5
Tremolite in talc						
	0	11 (2.1)	4.3 (2.1)	144 (12.5)	10.6	8.1
	5	9.2 (1.8)	3.7 (1.8)	130 (11.8)	14.1	7.6
	15	8.4 (1.8)	2.8 (1.7)	96.2 (4.8)	26.3	6.6
	30	6.4 (1.8)	2.3 (1.8)	69.9 (2.4)	37.0	5.0
	45	6.0 (1.7)	2.2 (1.7)	4.3 (5.8)	34.6	4.5

* Geometric mean (geometric standard deviation in parentheses). † X-ray diffractometric response in counts s^{-1}, arithmetic mean (standard deviation in parentheses).

or more specific: cleaning by grinding a portion of quartz sand followed by washing in water eliminates the risk of cross contamination with fibres.

In order to prevent over-comminution the grinding is carried out by repeated grinding for 1 min and sieving (106 μm sieve) to remove the generated fine fraction from the material in the mortar.

It is not possible to sieve chrysotile, as the wire screen will trap the fibres. Comminution of chrysotile to below 100 μm remains a challenge and no suitable method of reducing chrysotile to below 100 μm could be found. However, because the concept of a standard challenge is essential it was decided also to grind chrysotile, disregarding that this may be difficult to a degree depending on the chrysotile concentration and the matrix. The grinding time was set at 5 min, being an expert judgement.

Fibre dispersion

The final residue is dispersed in water for preparation of the analytical filter. Dispersion is assisted by ultrasonic treatment and use of a dispersing agent. The effect of using acetic acid as a dispersion agent on the fibre size, fibre mass and number concentration (measured by PCM) was determined on acid washed dolomite containing tremolite asbestos. It was found that dispersion in water adjusted to pH 3.0–4.0 with acetic acid did not change the size distribution. This was consistent with the results that within experimental uncertainties, no change in concentration by mass or by number could be detected. However, since use of acetic acid facilitates dispersion of chrysotile, and since its use is recommended by ISO,[12] use of water with adjustment of the pH between 3–4 using acetic acid was adopted. Sonication for 30 min and 1 h gave a statistically significant lower mass pertentage (two-sample t-test, $p = 0.02$) and higher fibre number concentration ($p < 0.001$) of tremolite asbestos. On the other hand, sonication reduced by a factor of 2 the RSD both for mass and for number concentrations. Use of a high energy ultrasonic probe will actually break down the fibres, but an ultrasonic bath used for a short time will only disperse fibres.[12] A sonication time of 10 min was finally chosen. After sonication, the suspension is filtered onto a 0.8 μm membrane filter and cleared using the acetone–triacetin method.[13]

Determination of fibre volume

When using microscopy only two dimensions; the fibre length (L_i) and the projected fibre width (B_i) is determined for each fibre, i. The volume W_i of the single fibres has to be estimated as

$$W_i = L_i (B_i)^2$$

Obviously the true cross section area will usually not be square and fibres may exhibit preferred orientation on the filter. Thus some kind of correction factor is needed. Suppose that the true volume W^{true} is known. Then the shape correction factor, k, is defined as

$$\sum_i W_i^{true} = k \cdot \sum_i L_i B_i^2$$

The correction factor applies to the total volume of a fibre population, not to the volume of individual fibres. The exact value of k was left open until the entire method had been developed and recovery studies were made. The overall best fit of PCM quantification of samples with known asbestos concentration was obtained for $k = 0.48$.

Determination of asbestos mass percentage

Filter samples are prepared in replicate from each dispersion. For both filters 50 fibres are counted or 100 fields per filter, whichever comes first. At least 20 fields are counted. Each fibre is observed under crossed polarisers. The stage is rotated 180° and it is observed if any part of the fibre is illuminated (undulating or partial extinction). If this occurs, the microscope stage is rotated through 360°. An asbestos fibre will extinguish at four positions each 90° apart. If extinction occurs to within ±5° of the vibration orientation of the polariser or analyser, the fibre is classified as asbestos. All other fibres are classified as non-asbestos. These rules do not apply for thin fibres, which should always be classified as asbestos. The lower limit of width is approximately 1 μm, but may be larger, depending on the birefringence. The experience of the microscopist is essential. Pure samples of the regulated fibre types should be analysed at regular intervals to maintain familiarity with the performance of the classification procedures.

Chrysotile may be distinguished from amosite, crocidolite, tremolite asbestos, anthophyllite asbestos, and actinolite asbestos by its yarn-like morphology. Amphibole asbestos fibres are straight but long fibres can bend in curves with a large radius of curvature.

For each individual fibre, i, W_i (μm³) is calculated as

$$W_i = L_{f,i} B_i^2$$

where $L_{f,i}$ is length of fibre within counting field and B_i is width of fibre i, both measured in μm. The total fibre volume in the investigated number of fields is calculated as:

$$\hat{W}_A = k_A \sum_{\text{all A}} W_i$$

$$\hat{W}_C = k_C \sum_{\text{all C}} W_i$$

A refers to all individual fibres classified as amphibole and C to all individual fibres classified as chrysotile. The volume is corrected for influence of cross section shape by the factor k. where $k_A = 0.48$ and $k_C = \pi/4$.

It was found that W_i were not log-normally distributed. Thus maximum-likelihood estimators or minimum variance estimators[14] assuming log normality should not be used.

Rules for determining effective volume of fibre bundles and clusters were not specifically developed. Fibre densities were compiled from the literature.[15–17] The fibre density may be lower than the non-fibrous mineral, which is given in the tables

Table 2 Results from residual maximum likelihood (REML) analysis of milling time. Mass percentage estimates were calculated using a cross-sectional correction factor of $k = 0.48$. The predicted mean is that calculated from the model

Sample no.	Description	Milling time/min	Predicted mean (mass %)	Confidence interval (95%)
5	1.0% Amosite in sand	0	0.94	0.66–1.34
		0.5	1.92	1.07–3.43
		1	1.09	0.78–1.54
8	0.25% Amosite in demolition waste	0	0.055	0.033–0.094
		0.5	0.25	0.12–0.50
		1	0.15	0.077–0.31
		5	0.18	0.11–0.30
		20	0.25	0.12–0.50
9	0.01% Chrysotile in olivine sand	0	0.0061	0.0005–0.0691
		5	0.0015	0.0001–0.0157
10	0.1% Crocidolite in sepiolite	0	0.15	0.12–0.18
		1	0.10	0.078–0.14
		5	0.12	0.085–0.16

of densities. Thus for chrysotile a density of 2.5 g cm^{-3} is adopted. A common density of 3.0 g cm^{-3} is applied for all amphiboles, as the PCM–PLM method does not always distinguish between the different amphibole asbestos.

The mass (g) of fibres on the filter is calculated as:

$$M_{s,A} = \frac{10^{-12}\hat{W}_A\rho_A A}{NG}$$

$$M_{s,C} = \frac{10^{-12}\hat{W}_C\rho_C A}{NG}$$

where ρ_A = 3.0 (g cm^{-3}); ρ_C = 2.5 (g cm^{-3}); N = number of fields analysed; G = graticule area (mm^2); and A = dust covered area of filter (mm^2).

The asbestos mass percentage is calculated as:

$$P = \frac{100F}{M_A}\left[M_{s,A} + M_{s,C}\right]$$

$$M_A = \frac{M_d Q_p}{Q_{tot}}$$

where M_d = mass of powder (g) dispersed in total volume Q_{tot} (cm^3); Q_p = volume of liquid (cm^3) from dispersion filtered onto filter surface A; M_A mass of sample on filter (g); and F = mass fraction remaining after pre-concentration.

Discrimination by aspect ratio

In regulatory methods, an aspect ratio, AR > 3:1 has been widely used to discriminate between a particle and a fibre. However, crushing non-asbestos forms of anthophyllite, tremolite and hornblende produces fragments with AR > 3:1. In order to differentiate between asbestos and non-asbestos fibres it has been argued that a higher minimum aspect ratio (*i.e.* 10:1 to 20:1) should be used.[18–20] Aspect ratio distributions of particles from six different samples including amosite in soil and demolition waste, tremolite in vermiculite, crocidolite in sepiolite, chrysotile in olivine sand and New York talc have been measured.[2] It was found that an aspect ratio of about 8:1 is the best compromise to exclude most cleavage fragments without excluding more than about 10% of the asbestos fibres. Unfortunately, the crude sizing bins used for the PCM sizing do not allow this precision and the 5 μm length bins and 0.5 μm width bins make a 10:1 aspect ratio the pragmatic choice. Also the 10:1 aspect ratio is shown on the graticule.

Aspect ratio discrimination is used as follows. If a non-asbestos fibre population is indicated (the majority of fibres having AR < 10) an estimate of the non-asbestos component can be made by removing all fibres with AR < 10. This allows an estimate of the remaining possible asbestos fibres to be made. It is important that this additional discriminant analysis is reported as an observation and the initial result without applying the discrimination must be reported.

Results

PLM identification

The PLM method was tested on 57 samples taken from the stock of 12 materials. Very good agreement between the PLM result and the true asbestos component for all 57 samples analysed was obtained. Only for undiluted and diluted New York talc, and digested dolomite, were there any differences. However, for all of these samples the differences were for samples containing tremolite or anthophyllite. For all other samples the type and proportions of asbestos found by PLM were consistent with the known components of the original mixtures. For a sub-sample of 22 samples, analysis was repeated. All of the original results were reproduced.

Ashing

On 'difficult-to ash' samples containing bitumen, paint and epoxy the relative standard deviation (RSD) of the ash content was 1% or less (at 50% ash). For bitumen, with an ash content of 2.1%, RSD was 5%. On samples of dolomite containing tremolite, the RSD of the residue was 8%.

Comminution

The mortar and pestle method resulted in a reproducible comminution as can be seen from Table 3.

Chrysotile containing samples will still contain large particles, as the repeated grinding/sieving procedure cannot be used. For such samples, separation of the ground material into a fine and coarse fraction using, *e.g.*, a modified Andreasen pipette and analysis of both fractions may be the only solution.[2] The coarse fraction should be inspected by PLM at a ×100 total magnification. If asbestos is present these fibres should be sized and included in the final calculation of asbestos mass percentage.

Statistical analysis of asbestos number and mass percentage estimates

During the development work a considerable number of samples were analysed by two counters at one laboratory using quantitative PCM and the standard Walton–Beckett graticule. A statistical analysis of the results was performed to determine accuracy and precision of the estimated mass percentages. A residual maximum likelihood (REML) analysis was used which included effects for counter, milling time, accuracy and precision, sub-samples and microscope slides.

Accuracy

Mass percentage estimates were calculated for samples with known asbestos content using maximum-likelihood estimation of fibre volumes. The ratio of observed to nominal asbestos content was calculated and the shape correction factor was adjusted to maximise the number of observations giving ratios in the target range 0.5 to 2. This gave a value k = 0.48, and Fig. 5 illustrates the final result. The number of evaluations falling within the target range were 87, 100, 80, 92 and 83% for samples 5, 7, 8, 10 and 11, respectively. An overall total of 88% of individual results were within the target performance range. The analytical results were obtained using the normal Walton–Beckett graticule and the value may need adjustment when using the modified graticule. Also it will have to be validated against a broader range of amphibole asbestos samples. For chrysotile a circular cross-section is assumed (k = π/4). The value of the correction factor k is in agreement with the results of Pooley and Clark[21] from which it was deduced[22] that the

Table 3 Reproducibility of comminution by ceramic mortar and pestle. Material, grunerite, grinding time, 5 min; no. of fibres sized, 108.

	Length, L/μm		Width, B/μm	
Run	Geometric mean	Geometric standard deviation	Geometric mean	Geometric standard deviation
1	14	2.1	2.6	2.0
2	15	2.1	2.6	2.3
3	15	2.0	1.8	2.6

following correction factors would apply: k = 0.71 for crocidolite, k = 0.44 for amosite, and k = 0.39 for anthophyllite. Results using direct observation of fibre cross sections for estimating k will be published elsewhere.

Precision

Due to the discrete nature of the analyte, there will be an inevitable variability in the fibre volume estimate. This variability is controlled by the total number of fibres sized and by the variance of the individual fibre volumes.[23] The actual precision of the analytical protocol was considered in terms of the variability of the mass percentage estimates obtained from samples for which the nominal asbestos content was known and for which sufficient evaluations had been performed (including repeat suspensions, repeat counts and where appropriate, different milling times). The samples included were numbers 5 (1.0% amosite), 8 (0.25% amosite), 9 (0.01% chrysotile) and 10 (0.1% crocidolite). The REML analysis produced predicted confidence intervals for mass percentages from samples 5, 8, 9 and 10, which are summarised in Table 2. The confidence intervals (CI) are ± 2 mean standard errors and represent approximately a 95% confidence interval.

Table 4 summarises the means and standard deviations for counters 1 and 2 for samples 5, 7, 8, 9, 10 and 11 calculated as the ratio of observed to true measurements. Notice that k minimised the number of ratios outside the target range not the sum of square distances of the mean from one.

There was no systematic effect of counter evident from mean ratios. However, counter 1 almost always produced a wider range of individual results than counter 2.

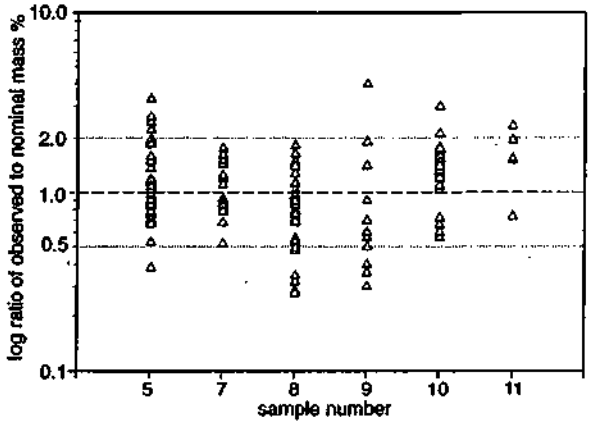

Fig. 5 Logarithmic ratio of observed to nominal mass percentage asbestos for samples 5, 7, 8, 9, 10 and 11. Also plotted are lines representing ratios of 1.0, 0.5 and 2.0. (The target value and lower and upper acceptable values, respectively).

Table 4 Mean and standard deviations (s) of mass percentages calculated as ratios of observed to nominal mass percentage estimates (k = 0.48)

Sample number	Counter 1		Counter 2	
	Mean	s	Mean	s
5	1.33	0.80	1.23	0.43
7	0.87	0.22	1.24	0.34
8*	0.72	0.37	1.01	0.40
9	1.67	1.93	0.60	0.61
10	1.14	0.42	1.21	0.37
11	1.39	0.60	1.93	0.40

* Zero milling time not included.

The statistical analysis showed that accuracy and precision of asbestos mass percentage estimates were generally good. Accuracy although ultimately dependent on the most appropriate correction factor, k, was close to the method's target performance criteria. The method was best applied to material containing amphibole asbestos. The method was generally unreliable for materials containing chrysotile.

PLM intercomparison

Two round robins, each involving four samples have been completed in which a number of EU laboratories and a selected group of UK laboratories accredited by the United Kingdom Accreditation Service (UKAS) for both the sampling and analysis of bulk asbestos were invited. The laboratories were asked to report whether asbestos was present in the sample and the type of asbestos found. In addition, the laboratories were asked to provide the visually estimated volume percentage of asbestos. No method for this was circulated, but it was seen as an opportunity to measure the bias that would be present if laboratories attempt to estimate asbestos content.

The laboratories were assessed by a scoring system based on analytical errors. Details will be published elsewhere. In summary, none of the laboratories made errors which were analytically unacceptable and which would have serious consequences if committed in reality. Some errors were made which were analytically unacceptable but that might not have significant consequences; such as the failure to detect a proportion of one asbestos component in the presence of an already detected amphibole asbestos, the false positive identification of a very small amount of asbestos in the absence of any other asbestos, or the failure to detect such a component. More errors were made which were analytically almost acceptable and would have no significant consequences; such as the failure to detect a very small proportion of any asbestos in the presence of an already detected amphibole asbestos, a false positive identification of one or more asbestos in the presence of an already detected amphibole asbestos, or the identification of tremolite as actinolite or anthophyllite, *etc.* This error rate represents what specially selected laboratories can achieve and thus what the method is likely to achieve. The result stresses the importance of training and experience of the microscopist and an adequate quality assurance programme. Details will be published elsewhere.

PCM–PLM intercomparison

An intercomparison in two parts of the PCM–PLM quantification method has been conducted, and details will be given elsewhere. The participants received a prepared slide and a simple bulk sample in the first round and five bulk samples in the second. Results were received from 12 in the first and from 11 in the second round. The results are shown in Fig. 6. One laboratory in particular (number 6) was known to be considerably more experienced than most and had already undertaken a considerable amount of research in developing a similar method for asbestos quantification. One sample contained only chrysotile and most laboratories reported the presence of a varying proportion of amphibole fibres incorrectly applying a correction factor, k, for each amphibole fibre reported. The resulting mass percentage estimates for this sample are therefore generally underestimated by an unknown amount. The findings for this first interlaboratory exchange indicate that with care, experience and perhaps considerable practice, it is possible to achieve analytical results approaching the target performance range.

Discussion

Use of optical microscopy limits the lower limit of visibility of fibres. Optical properties such as isotropic–non-isotropic and parallel–non-parallel extinction can only be observed if fibres have a minimum width *B*. For observed fibre width below about 1 μm no such observations can be made. Thus to minimise risk of false negatives (stating compliance with target concentration when the true concentration exceed the limit), such fibres have to be classified as asbestos. This increases the risk of false positives. However, it is always possible to use electron microscopy for further analysis of samples, *e.g.*, if the result was > 0.1% m/m and there is reason to believe that a considerable part of the fibre mass is not asbestos. A risk of false negatives cannot be entirely eliminated, *e.g.*, for samples containing extremely thin fibres in large quantities. A person experienced in mineralogy may assess mineral constituents which combined with knowledge of sample origin may give clues about this risk. The method was generally unreliable for materials containing chrysotile.

Microscope samples will always contain particles from coarser materials comminuted during the production process and/or during the standard challenge. This may contaminate the sample with elongated cleavage fragments, which according to the present definition in EU regulation are not asbestos. A considerable part of our research, and of the effort to be spent by laboratories during analysis, stems from the need to separate those fragments from the legally defined asbestos. It is interesting to notice the recent decision to classify the bio-durable man-made ceramic fibres as a category 2 carcinogen.[24] This could support the following hypothesis: provided cleavage fragments if inhaled have the same bio-durability as their asbestiform counterparts of the same size and shape, a difference in health effect is unlikely. If this hypothesis is true, it implies that the analyst at present has to spend large efforts on differentiating between asbestos fibres and fragments from the same parent rock, with no benefit to health protection.

In the interlaboratory comparison one of the most experienced laboratories came very close to the target performance. This is promising if compared with the experience from the Asbestos Fibre Regular Informal Counting Arrangement (AFRICA) scheme.[25] This scheme has shown that: (1) There is a learning curve for microscopists, and it should be expected that full time training over two weeks and supervision during one month's work with the method is needed to obtain reliable results. (2) Even for the much easier task of only counting fibre number on prepared microscope slides, the AFRICA scheme cannot after years of operation obtain a target performance as the one specified in the present project. Thus the original target performance may have to be relaxed.

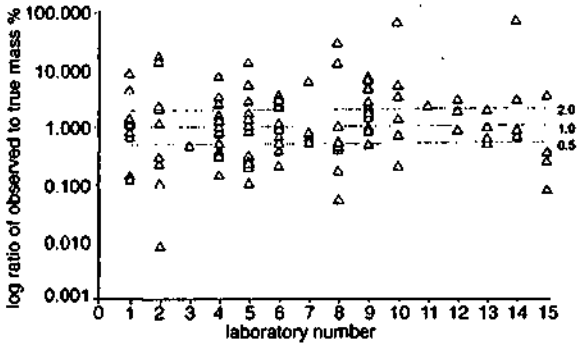

Fig. 6 Logarithmic ratio of observed to nominal mass percentage asbestos for round 1 and 2. Also plotted are lines representing ratios of 1.0, 0.5 and 2.0. (The target value and lower and upper acceptable values, respectively).

This work was conducted under European Community Contract No MAT1-CT93-0003.

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Tabbed Page: 8

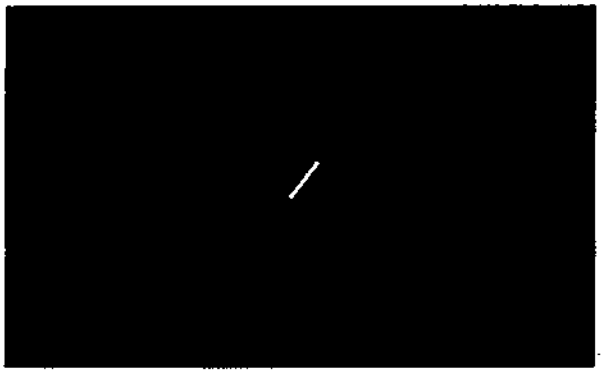
DRAFT 28 August 2002

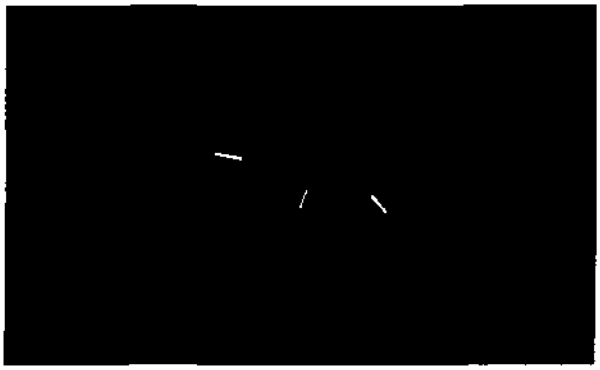
Reference Charts for Visual Estimate of Area Percentage in SEM Analysis

(For Monitor Screen with Aspect Ratio 1.64)

Shu-Chun Su, Ph.D.
S & L Consulting International
2002

- **These charts were specifically created to aid the visual estimation of fibrous structures in SEM images for the specified screen aspect ratio.**
- **The aspect ratios of the fibrous objects used in these charts range from 12:1 to 20:1, centering around 16:1, to mimic Libby amphiboles.**
- **For each percentage, three charts were created with different numbers of fibrous objects to approximate real-world situations.**
- **The area percentages of fibrous objects in all charts have been analyzed using Image-Pro Plus 4.5 and confirmed to be accurate within ± 0.005% of the specified values.**
- **For each percentage, one set of positives and one set of negatives are provided so that an analyst can choose the one that suits his or her vision.**
- **As shown by the charts of 0.5%, 0.8%, 1.0% and 1.5%, it is not easy to visually ascertain whether the total percentage of asbestos structures in an FOV (field of view) is above or below 1.0%.**
- **As shown by the charts of 20% and 30%, mental grouping structures together without the aid of appropriate reference charts tends to overestimate the actual concentration.**
- **Comments and suggestions to improve these charts are welcome. Please contact the author at 302-995-3498 or *scsu@delanet.com*.**





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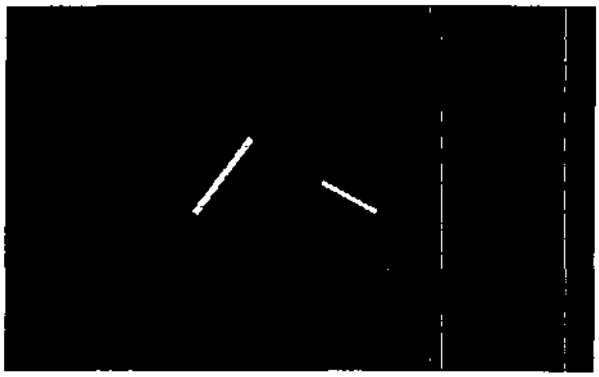

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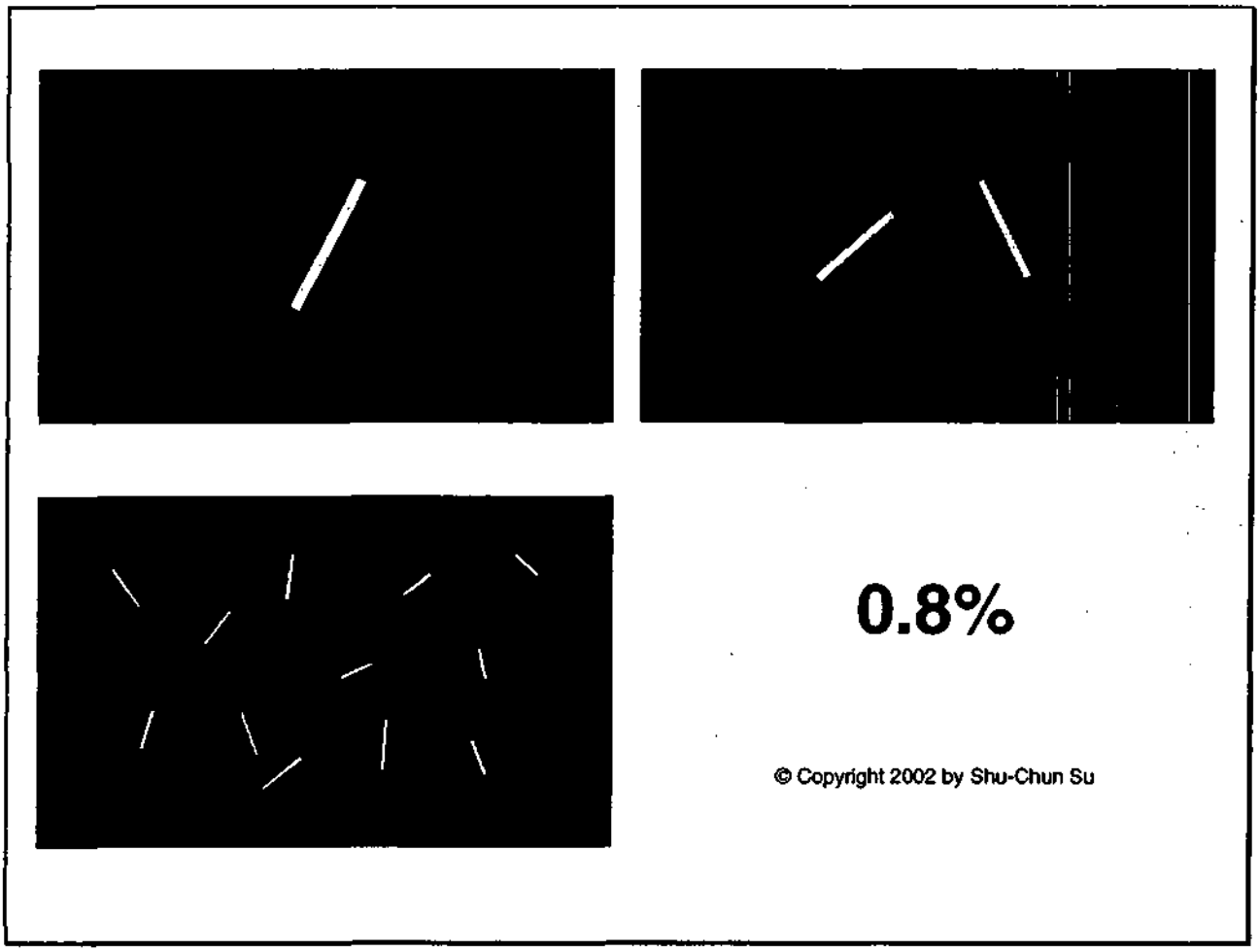
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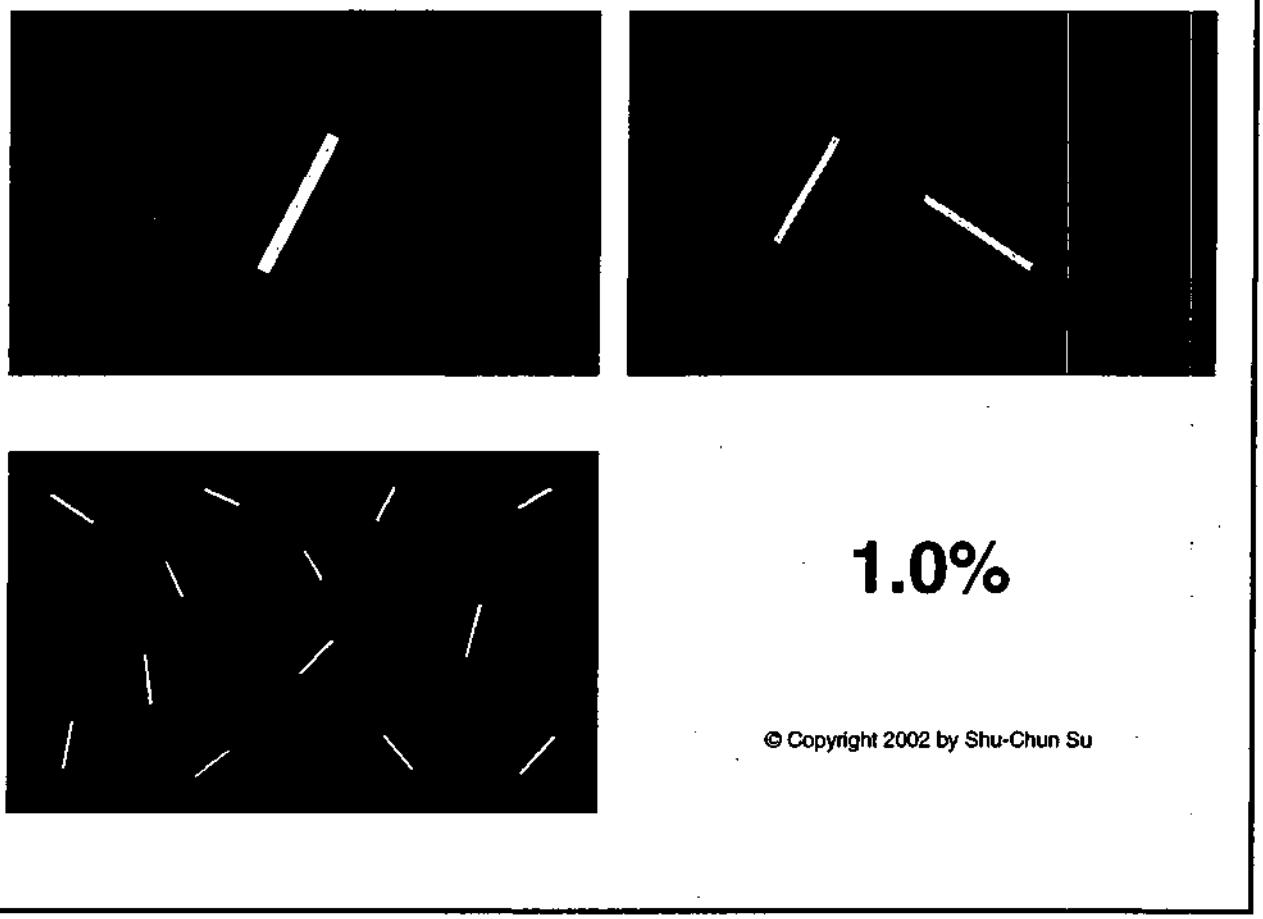
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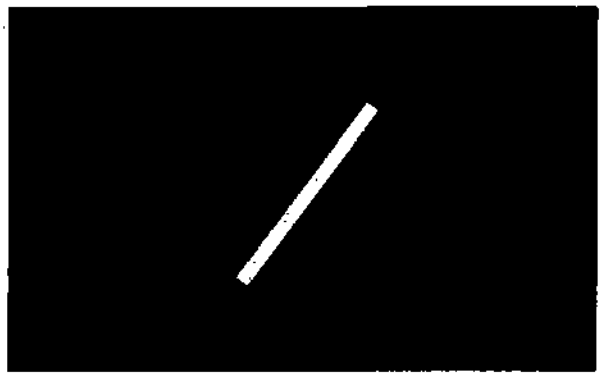

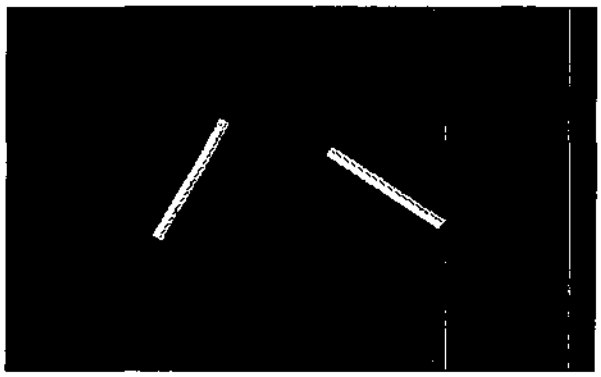

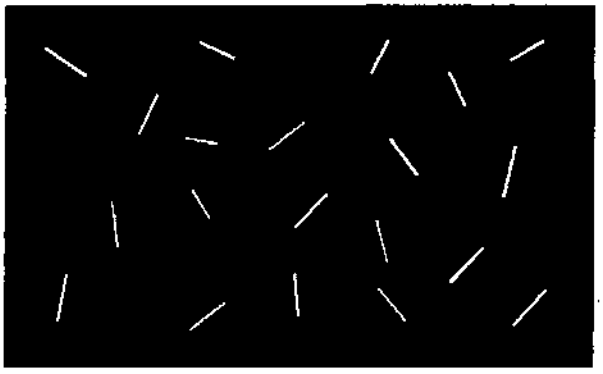

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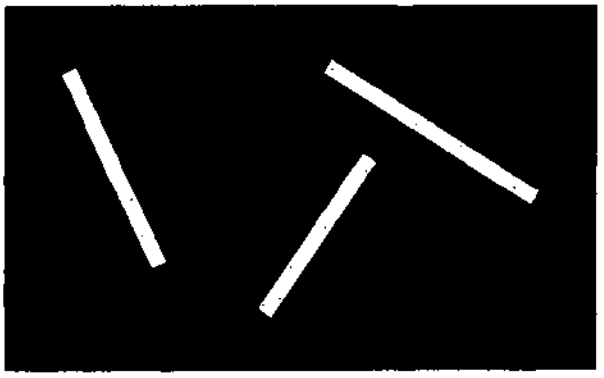

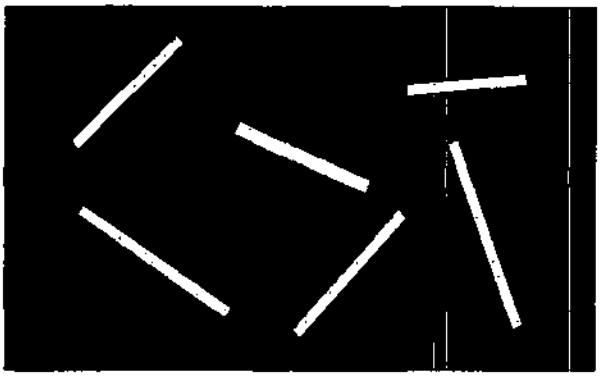

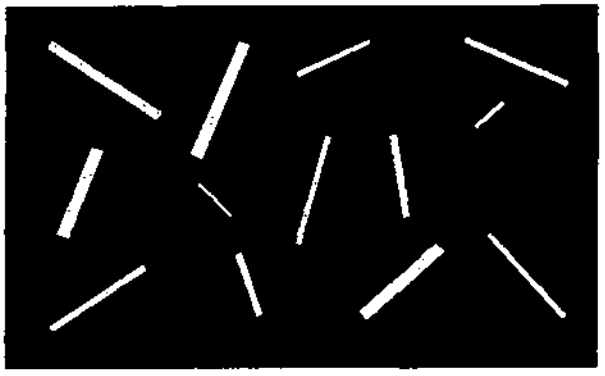

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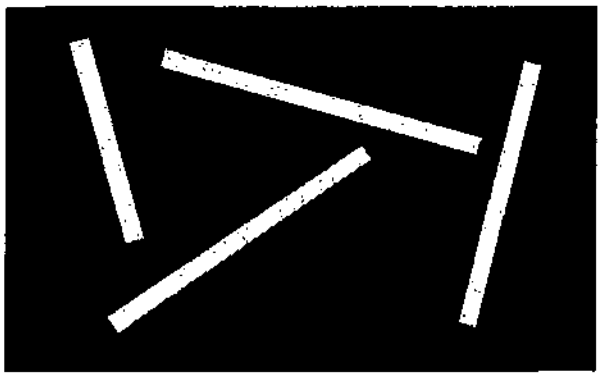

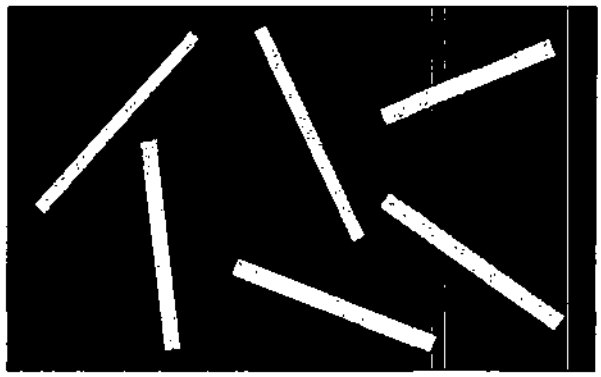

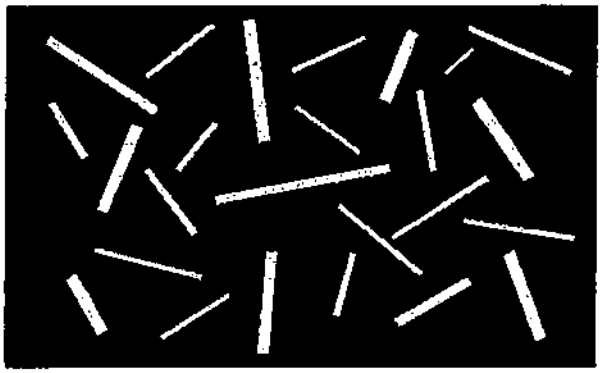

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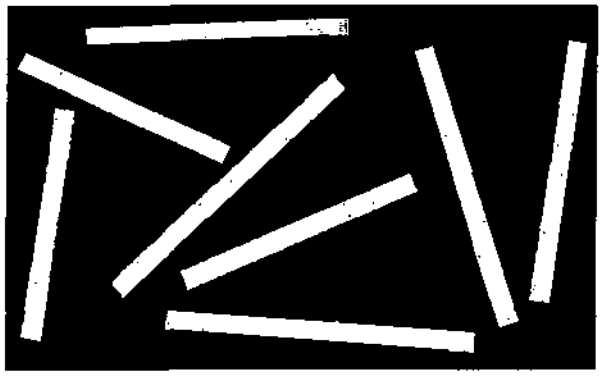

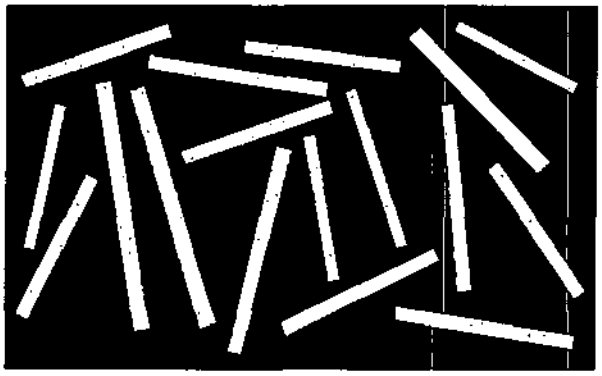

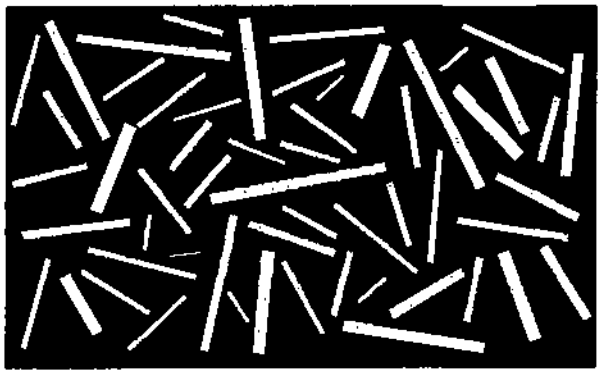

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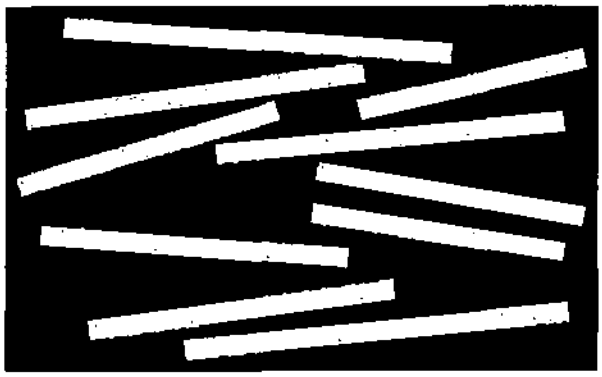

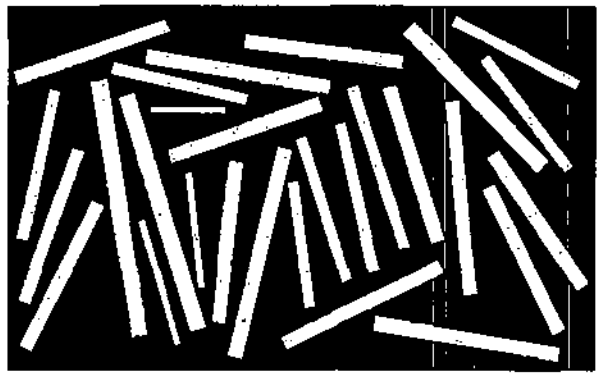

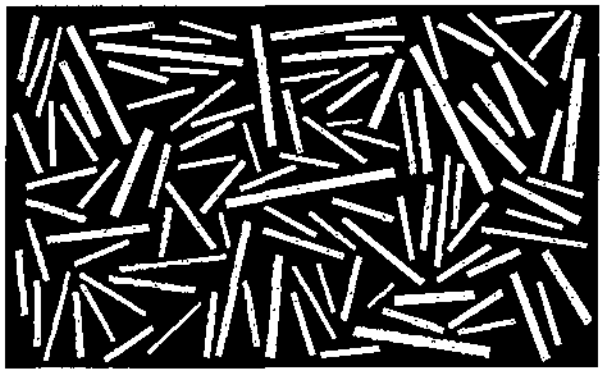

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Tabbed Page: 9

Section 9

Analysis of Asbestos Fibers in Soil by Polarized Light Microscopy (SRC-Libby-03) is under revision (December 2005).

Date: March 3, 2003 SOP No. SRC-LIBBY-03 (Revision 0)

Title: **ANALYSIS OF ASBESTOS FIBERS IN SOIL BY POLARIZED LIGHT MICROSCOPY**

Author: William Brattin

SYNOPSIS: A semi-quantitative method for identifying and quantifying asbestos fibers in soil using polarized light microscopy (PLM) is provided. This method is based on NIOSH Method 9002, EPA Method 600/R-93/116, and CARB Method 435, with project-specific modifications intended specifically for use at the Libby Superfund Site.

APPROVALS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
USEPA Region 8	Joey Cuddade	3/3/03
Syracuse Research Corp.	W J Brattin	3/3/03

Revision	Date	Principal Changes
0	03/03/03	--

1.0 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide a standard approach for semi-quantitative analysis of asbestos in samples of soil or other soil-like materials using polarized light microscopy (PLM). This SOP is specifically intended for application at the Libby Superfund site.

2.0 SCOPE AND APPLICATION

This method is intended mainly for analysis of asbestos in soil or other similar soil-like media. This method is appropriate for the analysis of all types of asbestos fibers, including both chrysotile and amphiboles, including those that are characteristic of the Libby site.

3.0 RESPONSIBILITIES

It is the responsibility of the laboratory supervisor to ensure that all analyses and quality assurance procedures are performed in accord with this SOP, and to identify and take appropriate corrective action to address any deviations that may occur during sample preparation or analysis. The laboratory supervisor should also communicate with project managers at EPA or their oversight contractors any situations where a change from the SOP may be useful, and must receive approval from EPA for any deviation or modification from the SOP before proceeding with sample preparation and analysis.

4.0 METHOD DESCRIPTION

The soil sample to be evaluated for asbestos content by PLM is examined under stereomicroscopy and under PLM (3-5 slides per sample) to estimate the amount of asbestos present. Quantification of the amount of asbestos present may be done either using a visual estimation approach or by a point counting approach, as specified in the Chain of Custody request. In either case, the concentration of Libby amphibole asbestos in the sample is estimated in terms of mass fraction (i.e., percent asbestos by weight) based on the use of project-specific reference materials.

5.0 DETAILED METHOD

5.1 Basic Methods

All qualitative and quantitative analyses are to be performed in general accordance with the methods and techniques specified in NIOSH 9002, EPA 600/R-93/116, and CARB Method 435. Project-specific modification, clarifications, and requirements are provided below.

5.2 Visual Estimation Approach

5.2.1 Classification of Asbestos Mineral Type

Based on fiber attributes (morphology, refractive index, color, birefringence, etc.), asbestos in the sample is classified into one of three categories:

Code	Description	Notes
LA	Libby Amphibole	Refractive index values for LA span the standard values for tremolite/actinolite (EPA 1993), but may include values for other similar amphiboles (e.g., winchite, richterite) characteristic of the mine at Libby. Based on analysis of 4 different samples from the mine (Wylie and Verkouteren 2000; USGS, unpublished data; Verkouteren, personal communication), observed refractive indices of Libby amphiboles range from about 1.629-1.640γ and 1.614-1.623α, with a birefringence of about 0.017. The full range of refractive indices of samples from the mine may be somewhat greater.
OA	Other amphibole	Includes amphibole forms (e.g., amosite, crocidolite, anthophyllite) that are not thought to occur in significant amount at the mine in Libby
C	Chrysotile	

5.2.2 Estimation of LA Mass Percent

The visual area estimation is a semi-quantitative approach that requires the microscopist to estimate the area fraction of the total material present in a field of view that consists of asbestos material. Because this estimation may be difficult, especially at low concentration values, and

because the desired output is an estimate of mass fraction (rather than area fraction), all visual estimates of Libby amphibole content will be performed using a set of site-specific reference materials as a frame of reference. These reference material will contain either 0.2 % or 1.0% Libby amphibole by weight, and have been prepared for analysis using the same approach as for field samples. Because the ability to visualize asbestos under stereomicroscopy and PLM may depend in part on the attributes of the soil matrix, reference materials have been prepared using two different site-specific soils, referred to as "brown" and "tan". These two soil types are generally similar in their principal mineral components, but may differ in the relative amounts of trace mineral components and in their organic content.

The microscopist will examine the field soil and determine which type of reference material (tan or brown) most closely resembles the field sample. (If the field sample is substantially different in appearance than either type of reference material, this should be noted in the sample results). Then, using the two reference concentrations (0.2% and 1.0%) of that soil type as a visual guide, the microscopist will evaluate the field sample and report the results as follows:

PLM Laboratory Report			Description
Qual	Conc (wt.%)	Bin	
ND		A	Asbestos was not observed in the field sample
Tr		B1	Asbestos was observed in the field sample at a level that appeared to be lower than the 0.2% reference material
<	1	B2	Asbestos was observed in the field sample at a level that appeared to exceed the 0.2% reference material but was less than the 1% reference material.
	1, 2, 3, etc	C	Asbestos was observed in the field sample at a level that appeared to equal or exceed the 1% standard. In this case, the mass percent is estimated quantitatively.

"ND" (not detected) in the Qualifier column is used for all samples in which asbestos is not observed under stereomicroscopy and is also not detected in five (5) different PLM slides prepared using representative sub-samples of the test material. These samples are assigned to **Bin A**.

"Tr" (trace) in the Qualifier column is used for all samples in which asbestos is observed either under stereomicroscopy or in at least one out of 3-5 PLM slides prepared from representative sub-samples of the test material, and in which the amount of asbestos present appears to be less than the 0.2 % reference material (tan and/or brown, whichever is most similar in appearance to the test material). These samples are assigned to **Bin B1**.

"<" (less than) in the Qualifier column and 1 in the Concentration column is used for all samples in which asbestos is observed either under stereomicroscopy or in PLM slides prepared from representative sub-samples of the test material, and in which the amount of asbestos present appears to be similar to or greater than the 0.2 % reference material but less than the 1% reference material (tan and/or brown, whichever is most similar in appearance to the test material). These samples are assigned to **Bin B2**.

A numeric value (1, 2, 3, etc) in the Concentration column without an entry in the Qualifier column is used for all samples in which asbestos is observed either under stereomicroscopy or in PLM slides prepared from representative sub-samples of the test material, and in which the amount of asbestos present appears to be similar to or greater than the 1 % reference material (tan and/or brown, whichever is most similar in appearance to the test material). These samples are assigned to **Bin C**.

Note that because these reference materials are based on Libby amphibole, they are not appropriate for estimating the mass percent of other types of asbestos (chrysotile, other types of asbestos). Therefore, if any asbestos types besides Libby amphibole are observed, the reported values for those samples should be in units of area percent.

5.3 Point Counting Approach

5.3.1 Counting Procedure

Any analysis in which evaluation by point counting is requested will be performed in general accordance with the descriptions provided in EPA/600/R-93/116 and CARB Method 435. The total number of particles to be counted (generally 400 or 1000) will be specified in the Chain of Custody request.

Take eight sub-samples of the soil sample and mount each separately with the appropriate refractive index liquid. The preparations should not be heavily loaded. Each sample should be uniformly dispersed to avoid overlapping particles and allow 25-50% empty area within the fields of view.

An ocular reticule (point array) or cross-hair is used to visually superimpose points on the microscope field of view. Count 1/8 of the total points required on each of the 8 slides (e.g., 50 non-empty points per slide for a 400 point count and 125 non-empty points per slide for a 1000 point count). For each non-empty point counted, assign the particle that is present at the point into one of four bins:

- Not asbestos
- Libby asbestos (LA)
- Other asbestos (OA)
- Chrysotile asbestos (C)

In order for a particle to be counted as asbestos, the aspect ratio must be ≥ 3:1.

After the required total number of non-empty points have been counted, record the total number of points in the LA, OA and C bins on the point counting data sheet.

5.3.2 Estimation of Mass Percent

Like visual estimation, the output of the point counting approach is an estimate of area fraction, not mass fraction. For this site, point-count estimates of area fraction for Libby amphibole particles will be converted into estimates of mass fraction using a standard curve approach.

The standard curve will be prepared using a series of site-specific reference materials containing 0%, 0.2%, 0.5%, 1%, or 2% Libby amphibole. The area fraction of each reference material will be estimated by the point counting approach in quadruplicate. The standard curve will be prepared by plotting the mean area fraction determined by point counting versus the mass percent in the reference material. The mass fraction of a field sample will be determined by measuring the area fraction of the field sample and locating the mass fraction that corresponds to that area fraction on the standard curve.

Because the standard curve is based on Libby amphibole, it is not appropriate to utilize this standard curve for other types of asbestos. Therefore, if any asbestos types besides Libby amphibole are observed, the reported values for those samples should be in units of area percent.

6.0 APPARATUS AND MATERIALS

Polarized light microscope, with lens and filters
Stereomicroscope (approximately 10-45x)
Petri dish for stereomicroscopic sample examination
Spatula and forceps
Glass slides and cover slips
Refractive Index (RI) oils
Reference Materials
- Tan soil, 0.2% LA by mass
- Tan soil, 1.0% LA by mass
- Brown Soil, 0.2% LA by mass
- Brown soil, 1.0% LA by mass

Laboratory log book
Data recording sheet (Attachment 1)

7.0 QUALITY ASSURANCE/QUALITY CONTROL

7.1 Precision and Accuracy

PLM by visual estimation and point counting are both semi-quantitative methods. For the purposes of this project, the accuracy and precision of the method are evaluated by measuring the frequency with which samples are assigned to the correct "bins". Data on precision and accuracy of bin assignment will be collected in the future and used to establish performance criteria for this project.

7.2 Method Proficiency

At present, sufficient data are not available to establish a quantitative procedure for method proficiency demonstration. As results become available, a procedure will be established and applied, based on the analysis of a set of blind Performance Evaluation materials and assessing

the frequency of correct bin assignments. If the assignments reported by a laboratory are within acceptance criteria bounds (see Section 7.1), then that laboratory will be deemed proficient. If not, remedial actions must be taken to address the errors before work may begin by that laboratory.

8.0 RECORDS

8.1 PLM Data Forms

Analysts will record analytical results using the electronic data sheets developed for the Libby project, as presented in Attachment 1. Note that there are two different electronic forms; one is for use in visual area estimation, and the other is for use in point counting. Once completed and checked, these spreadsheets are submitted to EPA for upload into the database. The laboratory should retain all original records for use in resolving any questions until otherwise instructed by EPA.

8.2 Instrument Maintenance Logbook

An individual instrument maintenance logbook should be kept for each piece of equipment in use at the laboratory. All maintenance activities must be recorded in the appropriate logbook.

8.3 Data Storage and Archival

Electronic Data. Each day of data acquisition, all electronic files will be saved onto two separate media. For example, the data may be saved onto a computer hard drive, but must also be backed up onto a type of portable media such as CD-ROM, floppy disc, or tape. Portable media will be maintained in a single location with limited access.

Hardcopy Data. All data sheets and micrographs must be stored in a secured location with limited access (e.g., locking file cabinet) when not in use.

Copies (hardcopy and electronic) of the raw analytical data will be submitted to USEPA for archival.

9.0 REFERENCES

CARB 435. California Environmental Protection Agency Air Resources Board, Method 435, Determination of Asbestos Content in Serpentine Aggregate. June 6, 1991.

EPA. 1993. Method for the Determination of Asbestos in Bulk Building Materials. United States Environmental Protection Agency, Office of Research and Development. EPA/600/R-93/116. July 1993.

EPA. 2003. Technical Memo 8. Procedure for Combining Mass Fraction Estimates for Coarse and Fine Fractions of Soil. Prepared by US EPA Region 8 with technical assistance from Syracuse Research Corporation.

NIOSH. 1994. Asbestos (Bulk) by PLM. NIOSH Manual of Analytical Methods, Fourth Edition. National Institute of Occupational Safety and Health. August 15, 1994.

Wylie AG and Verkouteren JR. 2000. Amphibole Asbestos from Libby, Montana: Aspects of Nomenclature. American Mineralogist 85:1540-1542.

ATTACHMENT 1

PLM DATA RECORDING SHEETS

PLM (VE and PC) Data Sheet and EDD v4.xls

PLM VISUAL ESTIMATION DATA RECORDING SHEET

Laboratory Name _____

Job Number _____

Date Received _____

SOP Name/Revision _____

EPA Index ID	Index Suffix ID	QA Type (NOT QA or LD)	Lab Sample ID	Date Analyzed	Analyst Name	Sample Appearance	Ref Material (S or T)	Libby Amphibole		Other Amphibole			Chrysotile		Deviation?	Comments (list below)
								Qual (ND, Tr, <)	Mass Fract (%)	Qual (ND, Tr, <)	Area Fract (%)	QA Type (AMOS, ANTH, CROC, UNK)	Qual (ND, Tr, <)	Area Fract (%)		

Comments (Use back if needed)

Tabbed Page: 10

LIBBY STANDARD OPERATING PROCEDURE

SRC-LIBBY-04 (REVISION 0): MEASUREMENT OF DUST LOADING

Date: June 27, 2003 SOP No. SRC-LIBBY-04 (Rev. 0)

Title: MEASUREMENT OF DUST LOADING

Author: William Brattin Syracuse Research Corporation

SYNOPSIS: A standardized method for measuring dust loading is described. This method is based on NIOSH Method 0500, with site-specific modifications intended for use at the Libby Superfund site.

Received by QA Unit:

APPROVALS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
EPA Region 8	[signature]	6/30/03
Syracuse Research Corp.	W S Brattin	6/27/03

Rev. No.	Date	Reason for Revision
0	6/27/03	--

1.0 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to provide a standardized method for collection and weighing dust from a known area in order to estimate dust loading (mg/cm^2). This procedure will be used by employees of USEPA Region 8 and by contractors/subcontractors supporting USEPA Region 8 projects and tasks for the Remedial Investigation work performed at the Libby, Montana site.

2.0 RESPONSIBILITIES

The Field Sampling Team Leader is responsible for ensuring that dust samples are collected in accord with SOP ASTM D5755-95, as amended in this SOP. The Laboratory Director is responsible for ensuring that dust samples provided to the laboratory for evaluation by this SOP are handled and evaluated in accord with the requirements of this SOP. It is also the responsibility of the Laboratory Director to communicate the need for any deviations from the SOP with the appropriate USEPA Region 8 Remedial Project Manager or Regional Chemist.

3. 0 EQUIPMENT

- Microvac apparatus and filter cassette as described in ASTM D5755-95
- Template of known area to mark off area to be vacuumed
- Environmental chamber that controls temperature and humidity
- Filtration device and filters

4.0 METHOD SUMMARY

Dust is vacuumed from within the boundary from a 100-cm^2 template in accord with ASTM D5755-95. In some cases, a composite sample from more than one template area may be collected. The dust is transferred from the cassette onto a pre-weighed filter, and the dust loading is calculated as the mass of dust divided by the area vacuumed.

5.0 SAMPLE COLLECTION

Dust samples will be collected in accord with current standard operating procedures at the Libby site, which is based on ASTM Method D 5755-95. The following project-specific modifications apply:

1. The number of templates vacuumed into the cassette should be sufficient that the total mass of dust in the cassette can be measured without undue difficulty. This is expected to vary from home to home. The default approach is to collect dust from a total of 5 templates (500 cm^2). These templates should be collected from locations that are representative of areas where residents may be exposed, including bare floors, carpets,

upholstery, counter tops, etc. Samples should not be collected from poorly-accessible areas (e.g., under the refrigerator). Because of the possibility that flow through the vacuum may decrease if the filter becomes overloaded, the flow rate must be checked at the beginning and end of each template collected. If flow rate through the vacuum decreases more than 10%, no further templates should be collected.

2. Submit the dust cassettes under chain-of-custody to the analytical laboratory.

6.0 DUST WEIGHING

Dust loading is determined by analytical transfer of the dust sample from the cassette to a filter of known mass. Determine the mass of the dust collected onto a cassette as described below.

6.1 Weight Filter

Pre-equilibrate a filter in an environmental chamber as described in NIOSH 0500. The relative humidity should be 50% ± 5%, and the temperature should be 20°C ± 1°C.

Record the mass of the filter to the nearest 0.1 mg.

6.2 Transfer Dust to Pre-Weighed Filter

Place the pre-weighed filter into a filtration device, and use de-ionized water to rinse the contents of the dust microvac cassette into the filtration device. Remove excess water by vacuum, and dry the filter under a heat lamp.

6.3 Weight Filter Plus Dust

Placed the dried filter back into the environmental chamber. After equilibration, re-weigh the filter plus dust to the nearest 0.1 mg, and calculate the mass of dust by difference.

7.0 CALCULATION OF DUST LOADING

Calculate dust loading (DL) as follows:

$$DL\ (mg/cm^2) = \text{Mass of dust (mg)} / [\text{Area of template } (cm^2) \cdot \text{number of templates collected}]$$

8.0 DOCUMENTATION

The laboratory must maintain a log to document that the analytical balance used to weigh dust samples is accurate. Calibration of the analytical balance should occur once per day when dust weights are being measured.

The laboratory must maintain a log to document that conditions within the environmental chamber remains at the desired temperature and humidity settings. Calibration of the environmental chamber must occur once per day when the environmental chamber is in use.

All weight measurements will be maintained in the laboratory log book and transmitted to EPA using the electronic data reporting sheet provided in Attachment 1.

9.0 QUALITY ASSURANCE

There are no quality assurance samples to be run as part of this project.

10.0 DECONTAMINATION

All non-disposable equipment used during sample collection and preparation must be decontaminated prior to use. If dust soil particles are visible on any of the equipment, repeat the decontamination procedure until the equipment is clean.

11.0 REFERENCES

American Society for Testing and Materials. 1995. Standard Test method for Microvacuum sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Concentrations. ASTM Method D 5755-95.

NIOSH. 1994. Method 0500 (Issue 2). Particulates Not Otherwise Regulated, Total. NIOSH Manual of Analytical Methods, Fourth Edition. 8/15/94.

ATTACHMENT 1

ELECTRONIC DATA REPORTING SHEET

See Excel file "Dust Loading Data Sheet.xls"

(Check with Volpe or SRC to determine the most recent version number)

Tabbed Page: 11

Date: May 20, 2003 SOP No. SRC-LIBBY-01 (Revision 1)

Title: QUALITATIVE ESTIMATION OF ASBESTOS IN COARSE SOIL BY VISUAL EXAMINATION USING STEREOMICROSCOPY AND POLARIZED LIGHT MICROSCOPY

Author Sally M. L. Gibson Syracuse Research Corporation

SYNOPSIS: A standardized method is described for the examination of the coarse fraction (>1/4") of soil samples using stereomicroscopy and polarized light microscopy (PLM) to identify, segregate, and estimate the mass percent of asbestos in the sample matrix.

Received by QA Unit:

APPROVALS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
EPA Region 8	[signature]	5/20/03
Syracuse Research Corp.	[signature]	5/20/03

Revision	Date	Reason for Revision
0	11/12/02	--
1	05/20/03	Provided clarification on dealing with very small particles.

1.0 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to provide a standardized screening method for the visual examination of the coarse fraction of previously sieved soil samples for evidence of asbestos mineral content using stereomicroscopy with confirmation of asbestos content by polarized light microscopy (PLM). This SOP incorporates salient components of EPA Test Method 600/R-93/116 *Method for Determination of Asbestos in Bulk Building Materials* and National Institute of Occupational Safety and Health (NIOSH) Method 9002 *Asbestos (bulk) by PLM,* Issue 2.

This procedure will be used by employees of contractors/subcontractors supporting USEPA Region 8 projects and tasks for the Libby, Montana, site. Deviations from the procedure outlined in this document must be approved by the USEPA Region 8 Remedial Project Manager or Regional Chemist prior to initiation of sample analysis.

2.0 PREREQUISITE TRAINING

Visual examination will be performed according to this SOP by a laboratory accredited by the National Voluntary Laboratory Accreditation Program (NVLAP) and by analysts proficient either by education or experience in asbestos mineral identification by stereomicroscopy and PLM. Analyst familiarity with the procedural applications prescribed in EPA Test Method 600/R-93/116 and NIOSH Method 9002 is required.

Training as described in the Sampling and Analysis Plan, Remedial Investigation, Contaminant Screening Study, Libby Asbestos Site, Operable Unit 4, (CSS SQAPP [CDM 2002]) will be provided to laboratory personnel or laboratories with less than one year of project-specific experience by "mentors" from either Reservoir Environmental Services, Inc. or EMSL.

3.0 RESPONSIBILITIES

The CDM Laboratory Coordinator (LC) is responsible for overseeing the activities of the CDM Soil Preparation Laboratory and subcontracted laboratories performing sample analysis for the Libby, Montana, project. The LC is also responsible for checking all work performed and verifying that the work satisfies the specific tasks outlined by this SOP and the CSS SQAPP. It

is the responsibility of the LC to communicate with the project personnel and subcontracted laboratory regarding specific analysis objectives and anticipated situations that require any deviation from the CSS SQAPP SOPs. In addition, it is the responsibility of the LC to communicate the need for any deviations from this SOP with the CDM Project Manager, USEPA Region 8 personnel (Remedial Project Manager or Regional Chemist.)

Subcontracted laboratory analysts performing the visual examination are responsible for adhering to the applicable tasks outlined in this SOP and substantiating components of the reference procedures (EPA 1993; NIOSH 1994) with the modifications contained herein.

4.0 EQUIPMENT

- Analytical balance - accurate to 0.01 g, range of 0.01 g to 1000 g (for weighing total sample)
- Analytical balance - accurate to 1 mg (for weighing asbestos)
- Traceable standards - major asbestos types
- Microscope - binocular stereomicroscope, 5-60X approximate magnification
- Microscope - polarized light, binocular or monocular with a cross hair reticle (or functional equivalent) and magnification of at least 8X
  - 10X, 20X, and 40X objectives
  - 360 degree rotatable stage
  - substage condenser with iris diaphragm
  - polarizer and analyzer which can be placed at 90 degrees to one another and calibrated relative to the cross-line reticle in the ocular
  - port for wave plates and compensators
  - wave retardation plate (Red I Compensator) with ~550 nanometer retardation and known slow and fast vibration directions
- Light Sources - incandescent or fluorescent

- Tweezers, dissecting needles, scalpels, probes, razor knives, etc. - standard sample manipulation instruments/tools
- Microscope slides and cover slips
- Refractive index liquids
- Pre-tared glassine paper, glass plates, weigh boats, petri dishes, watchglasses, etc. - laboratory sample containers
- HEPA-filtered or Class 1 biohazard hood negative pressure
- Three-ring binder book- binders will contain Microscopic Examination Logbook Sheets (Attachment 1)

5.0 METHOD

Soils from the Libby, Montana site will be dried, sieved, and prepared according to the most recent revision of SOP ISSI-LIBBY-01, Soil Sample Preparation. The coarse fraction of the soil sample is defined as that portion of the sample which does not pass through a 1/4" sieve. The coarse fraction will be weighed, placed in a zip-top plastic bag, and labeled as described in Camp, Dresser, and McKee (CDM) SOP 1-3 (with project-specific modifications). The samples will be packaged and shipped by the soil preparation laboratory as described in CDM SOP 2-1 (with project-specific modifications) and transferred to the laboratory via chain-of-custody procedures described in CDM SOP 1-2 (with project-specific modifications).

The following sections describe the stereomicroscopic and PLM examination. Materials tentatively characterized as asbestos by stereomicroscopy will be isolated and subjected to confirmation by PLM. The mass % of Libby amphibole asbestos, other amphibole asbestos, and chrysotile asbestos in the coarse soil fraction will be calculated from the mass of each asbestos type positively identified by PLM and the original sample weight. Figure 1 provides an overview of the process.

5.1 Stereomicroscopic Examination

The laboratory will receive the coarse fraction soil samples from the CDM Soil Preparation Laboratory. The entire sample will be weighed and placed in an appropriate container. The weight of each coarse sample will be recorded, along with the sample identification, on the Microscope Examination Logbook Sheet. The sample will be subject to stereomicroscopic examination and particle segregation as depicted Figure 1. The stereomicroscopic examination to identify and segregate asbestos includes:

- using multiple fields of view over the entire sample
- probing the sample by turning pieces over and breaking clumps where possible
- manipulating the sample using appropriate instruments/tools
- observing homogeneity, texture, friability, color and extent of any observed asbestos in the sample(s)

NOTE: Although the coarse fraction is prepared by sieving with a 1/4" screen, particles smaller than 1/4" may be present in the fraction due to adherence between coarse and fine particles. This may even include some very fine asbestos fibers. Because of the technical difficulty, the analyst should not attempt to physically segregate and weigh particles smaller than about 2-3 mm (1/10 inch). A particle this size is expected to have a mass of about 10-20 mg, which is less than 0.1% of a sample whose total mass is 25 grams. If no particles larger than 2-3 mm are present, this should be noted in the data sheet for each category of asbestos using the following code system:

- ND = No asbestos observed
- Tr = Trace levels of asbestos observed but not quantified

The weight fraction for any asbestos type marked "ND" or "Tr" in a given sample is not calculated and is left blank.

As the sample is examined, the analyst will continue segregation of the sample until the entire coarse soil fraction has been characterized as either "non-asbestos" or "tentatively identified asbestos." The tentatively identified asbestos particles will be examined by PLM, as described below. The stereomicroscopist will initial and date the Microscopy Examination Logbook Sheet.

5.2 PLM

The coarse material tentatively identified as asbestos by stereomicroscopic examination will be subject to confirmation using PLM, as described in SOP SRC-LIBBY-03 (Revision 0) ("Analysis of Asbestos Fibers in Soil by Polarized Light Microscopy"). The PLM examination will be used to confirm that the particles tentatively classified as asbestos are actually asbestos, and will be assign each particles to one of three categories:

LA = Libby amphibole
OA = Other amphibole
C = Chrysotile

If OA is observed, the type of OA observed should be noted in the data sheet using the following code system:

- AMOS = Amosite
- ANTH = Anthophyllite
- CROC = Crocidolite
- UNK = Unknown

The total weight of each type of positively identified asbestos (LA, OA, C) will be determined and recorded on the Microscopic Examination Logbook Sheet, along with the analyst's initials and the date of the examination.

6.0 QUALITY ASSURANCE

Laboratories performing the examination must be accredited by NVLAP. "Calibration" should be verifiable for each microscopist in terms of project-specific training and the successful analysis of materials of known asbestos content (NVLAP test samples, in-house standards) similar to those anticipated to be observed in Libby, Montana soils. Additionally, references such as photographs of the asbestos minerals illustrating distinguishing properties should be available benchside during characterization.

Quality control samples as described in ISSI-LIBBY-01 (i.e., preparation duplicates) will not submitted for the coarse materials samples. The entire coarse fraction will be subject to examination.

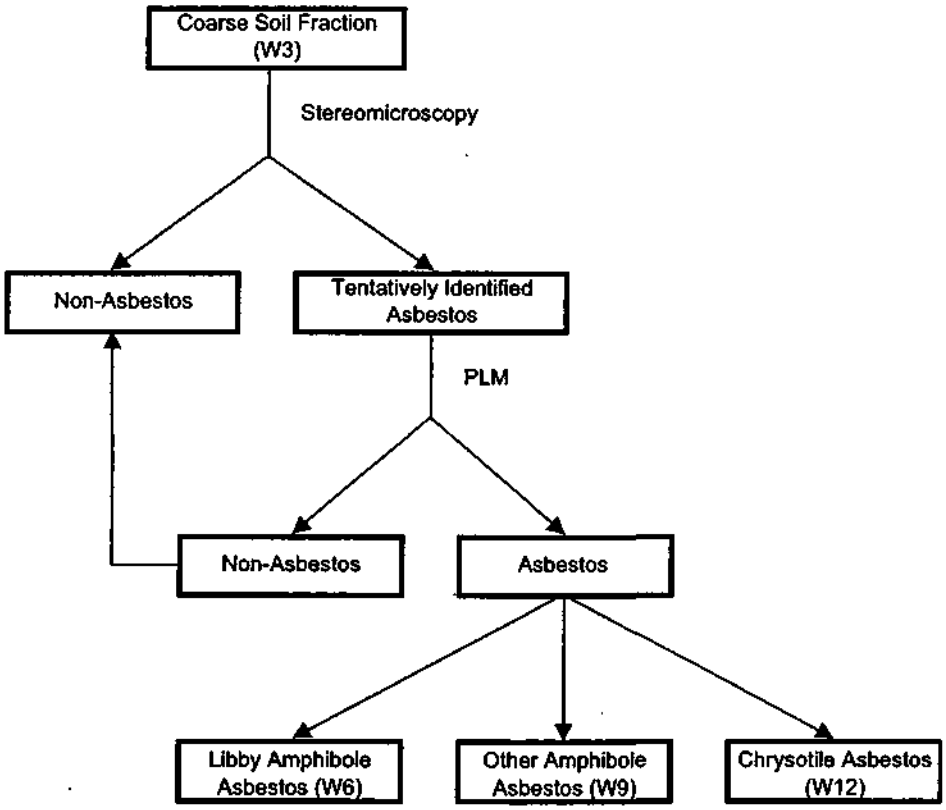
7.0 REFERENCES

CDM 2002. *Sampling and Analysis Plan, Remedial Investigation, Contaminant Screening Study, Libby Asbestos Site, Operable Unit 4.* 3282-116-PP-SAMP-14187. Camp, Dresser and McKee Denver, Colorado. April.

NIOSH 1994. National Institute of Occupational Safety and Health (NIOSH) Method 9002 *Asbestos (bulk) by PLM*, Issue 2.

USEPA 1993. *Method for Determination of Asbestos in Bulk Building Materials*. 600/R-93/116.

Figure 1. Overview of Sample Examination Process



W3 = Original coarse soil fraction mass (g)
W6 = If present in measurable quantities, mass (mg) of Libby amphibole
W9 = If present in measurable quantities, mass (mg) of other amphibole
W12 = If present in measurable quantities, mass (mg) of chrysotile

Codes used in the Illustration (e.g., W3) correspond to Data Log Sheet

ATTACHMENT 1

MICROSCOPIC EXAMINATION LOGBOOK SHEET

See attached electronic file "SRC-LIBBY-01 Data sheet and EDD v6a.xls"

Example hard copy of data entry sheet shown on next page (for illustration purposes only).

Data Log Sheet v6 for SOP SRC-LIBBY-01
Stereomicroscopic and Gravimetric Analysis of Coarse Soil

Lab Name:

SOP Version:

Lab Job No.

Page _____ of _____

Calculated automatically in the "Electronic Data Entry" form. Do not enter data here.

EPA Index ID	Index Suffix	Lab Job-Sample No.	Status (1 = Assigned, 2 = Missing, 3 = Complete, 4 = Cancelled)	QA Type (Not QA)	Total Sample Weights (g): Tare Weight (g) Empty Container	Mass of Sample + Container (g)	Mass of Sample (g)	Analysis Details: Analyst Initials	Analysis Date	Mass of Asbestos Particles (mg): Using Amphibole (LA) Adhesive: LA Qual* (ND, Tr)	Tare Weight - Container (mg)	Mass of LA + Container (mg)	Mass (mg) LA	Other Amphibole (OA) Adhesive: OA Qual* (ND, Tr)	OA Type** (AMOS, ANTH, CROC, UNK)	Tare Weight - Container (mg)	Mass of OA + Container (mg)	Mass (mg) OA	Chrysotile (C) Asbestos: C Qual* (ND, Tr)	Tare Weight - Container (mg)	Mass of C + Container (mg)	Mass (mg) C	% LA	% OA	% C	Comments (see Notes below)

Notes:

*Qualifier codes: ND = No asbestos observed.
Tr = Trace levels observed but not quantified.

**QA Type codes: AMOS = Amosite
ANTH = Anthophyllite
CROC = Crocidolite
UNK = Unknown

Comment Codes (user-defined):

Tabbed Page: 12

LIBBY SUPERFUND SITE STANDARD OPERATING PROCEDURE
APPROVED FOR USE AT THE LIBBY SUPERFUND SITE ONLY

Date: October 28, 2005 SOP No. SRC-LIBBY-02 (Rev. 2)

Title: QUANTIFICATION OF ASBESTOS IN SOIL BY SEM/EDS

Author: William Brattin[a] Syracuse Research Corporation

[a] This SOP is adapted from methods developed by the US Geological Survey (USGS 2002) and by EMSL Analytical Inc. (EMSL 2000), along with input from other Libby analytical team members.

SYNOPSIS: A method for estimating the asbestos content (mass percent) of soil using Scanning Electron Microscopy (SEM) and Energy-Dispersive X-Ray Spectroscopy (EDS) is described. The method is intended mainly for analysis of amphibole asbestos similar to that derived from the mine in Libby, and is intended mainly for soil samples that contain less than 1% asbestos by mass.

Received by QA Unit:

APPROVALS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
EPA Region 8:	Mary Goddard	10-28-05
Syracuse Research Corp.:	W J Brattin	10/28/05

Revision Number	Revision Date	Changes
0	4/28/2003	--
1	6/26/03	Add clarifications on purpose and limitations
2	10/28/05	Add details on counting and stopping rules

1.0 PURPOSE

The purpose of this document is to describe a standard operating procedure (SOP) for estimating the concentration (expressed as weight percent) of amphibole asbestos found in soils in Libby, MT, by using scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS). Because confirmation of mineral type by x-ray diffraction pattern is not included, the method should be considered a screening technique. This SOP is specifically intended for use by employees of USEPA Region 8 and by contractors supporting USEPA Region 8 at the Libby, Montana, Superfund site.

2.0 RESPONSIBILITIES

Each laboratory performing analyses of soil from the Libby site for EPA shall have a Laboratory Supervisor who is responsible for ensuring that all analyses are performed in accord with this SOP, and for ensuring the accuracy and quality of the results.

It is the responsibility of the Laboratory Supervisor to communicate with the EPA's Laboratory Coordinator regarding specific analysis objectives and anticipated situations that may require any deviation from the SOP. Any sample-specific or SDG-specific deviations from the SOP shall be recorded on deviation sheets in accord with the process described in the Contaminant Screening Study Sampling and Analysis Plan (CDM 2002). It is also the responsibility of the Laboratory Coordinator to recommend any suggestions for improvement in this SOP to the CDM Project Manager and USEPA Region 8 personnel (Remedial Project Manager or Regional Chemist).

3.0 EQUIPMENT

Equipment required to perform the analyses described in this SOP includes the following:

- Negative Air Pressure, HEPA-filtered hood (used to capture airborne dust, fibers, and other potential contaminants)
- Analytical balance - accurate to 0.0001 g, range of 0.0001 g to 100 g.
- Porcelain crucible (for sample ashing).
- Muffle furnace - used for sample ashing.
- 500 to 800 mL glass beaker

- Teflon-coated 2-inch magnetic stir bar - used in sample mounting process
- Magnetic stirring plate - used in sample mounting used in sample mounting process
- Eppendorf pipette - capable of delivering 20 uL
- Eppendorf pipette tips - modified by cutting enough material off the tip to approximately double the opening
- SEM sample stubs with conductive carbon adhesive tabs
- Sample coater - carbon vacuum evaporator used to apply a conductive carbon layer to the SEM stub
- Desiccator and/or glass petri dish used for temporary storage of sample-mounted stubs.
- SEM fitted with EDS with the following attributes:

  For SEM:
  - Resolution: 5 nm or better[1]
  - Accelerating Voltage: 10 to 25 kV or greater
  - Minimum magnification range: 50x to 200,000x
  - SEI (secondary electron image)
  - BEI (backscattered electron image)

  For EDS:
  - Detector having the ability to resolve EDS spectral peaks for the elements of concern in LA, including Na, K, Mg, Al, Si, Ca, Cu, and Fe. EDS detector performance and accuracy should be assessed using the BIR-1G glass standard, as described in Section 8.1
  - Minimum resolution of 150 eV at Manganese Kα
  - Analyzer capability of background subtraction, peak overlap correction, correction for sum peaks and Si escape peaks.

4.0 METHOD SUMMARY

An aliquot of the soil sample is examined by SEM/EDS. In some cases, the material must be ashed to remove excessive organic matter. After ashing (if needed), a stub is prepared by suspending the sample in water, filtering an aliquot onto a polycarbonate filter, and mounting the filter on the stub.

[1] Based on the manufacturer's specifications. The resolution should be verified using gold on carbon at least once per year and documented as described in Section 8.

The stub is coated with carbon and is examined by SEM at several different magnifications. At each magnification, a number of different fields are selected either systematically or at random, and the area coverage (all particles) and the area fraction attributable to asbestos particles in each field are estimated or measured. These data are used to estimate the asbestos content of the soil sample.

5.0 SAMPLE STUB PREPARATION

5.1 Ashing (If necessary)

Substantial organic material in the sample can result in an outgassing of material under high vacuum such that carbon coating of the SEM stub or analysis in the SEM may be impaired. Therefore, if difficulty is encountered in coating the stubs or in the analysis, if the stub is otherwise of poor quality, that sample should be ashed and then re-prepared for examination. The procedure for sample ashing is as follows:

1. Mix the bulk sample by tipping the bag upside down, and then returning it to its original position. Use a clean sample thief to remove a uniform aliquot of the material, pushing the thief all the way through the sample. Place this sample into a clean glass beaker and mix thoroughly. After mixing, transfer about 2 grams of the sample into a pre-weighed crucible, being sure the sample does not fill more than about 1/3 of the crucible. Weigh the sample to the nearest 0.05 grams and record the weight of the crucible alone and the crucible plus sample in the Lab Data Sheet for the sample (Attachment 1).

2. Place a cover on the crucible, and place the crucible into a muffle furnace.

3. Turn the furnace on and set the temperature to 480°C. [Note: if the furnace can achieve this temperature faster than 2 hours, first turn the temperature to 250°C and hold at this temperature for 1 hour before increasing the temperature to 480°C.]

4. Hold the sample at 480°C for at least 4 hours. Shut off the furnace and allow to cool overnight.

5. When the sample has cooled, weigh the residue to the nearest 0.05 g and record the final mass in the Lab Data Sheet for the sample (Attachment 1).

Note: It is understood that this ashing procedure might result in some parts of the sample reaching temperatures that may begin to decompose chrysotile asbestos (about 500°C). However, this SOP

is intended for the analysis of amphibole asbestos, and amphibole asbestos is not expected to begin decomposition unless temperatures are substantially higher.

5.2 Mounting the Sample

Samples must be prepared in a clean HEPA hood. To prevent cross-contamination of samples, only one sample should be mounted onto a SEM stub at a time. The procedure to mount the soil sample onto the SEM stub is described in USGS (2002b), and is repeated below.

Set up a Millipore filter apparatus for use with 25 mm polycarbonate filters. Place a few drops of distilled water on the fritted glass surface and place a 25 mm polycarbonate filter (0.4 um or less pore size) on the water, shiny side up. Attach the top of the apparatus. Add a few milliliters of distilled water on the filter so that no part of the filter is exposed to air. Take a clean Eppendorf pipette tip and with a razor blade, cut enough material off the tip to increase the opening to approximately 1 mm. Transfer approximately 0.5 g of the sample prepared for SEM/EDS analysis to an 800 mL glass beaker containing 125 mL of distilled water and a 2-inch magnetic stirring bar, and place on a magnetic stirring plate. Slowly increase the speed of rotation until all the particles are suspended and a good vortex is achieved without excessive splashing. While the sample is mixing, and the particles are suspended, collect 20 uL of the mixture using the Eppendorf pipette. Transfer this to the polycarbonate filter by dropping the suspension from 3 to 4 cm above the water in the Millipore apparatus. Place two more 20 uL drops of the suspension onto the polycarbonate filter for a total of 60 uL. Allow the water to filter through the polycarbonate. If a hand-held vacuum pump is available, it may be used to decrease the time it takes for complete filtration. Do not use motorized pumps because sample will become too dry on the filter, increasing the possibility of sample loss.

Use forceps to pick up the filter just as the water has passed through but before it is dry and place it on a carbon adhesive tab on a standard SEM mount. The filter needs to be completely flat on the SEM stub. This is achieved by forming the wet filter into a gentle U-shape. Place the bottom curve of the filter onto the center of the carbon adhesive tab and slowly release the sides so they lay flat. Trim the edges of the polycarbonate filter using a razor blade. After drying, coat the stub with carbon using a carbon evaporator. The stub is then ready to be taken to the SEM in a clean, covered container.

Repeat the process above, using drop sizes of 15 uL and 10 uL. This will yield three polycarbonate filters with a range of particle loading. Begin by evaluating the filter loaded with 60 uL of

suspension. The SEM sample stub should have about 15-20% total coverage. If the loading on this filter is too high, select one of the filters with lower loading for evaluation .

6.0 ANALYSIS BY SEM

All analyses should be run at 15 kV. SEM analysis will be done at magnifications of 50x, 500x, and 2000x.

Counting Rules

At each magnification, record the occurrence of every structure that satisfies the following counting rules:

1. Particle diameter is within the range specified for the magnification:

50x	Diameter > 20 um
500x:	Diameter = 1-20 um
2000x	Diameter < 1 um

2. Aspect ratio ≥ 3:1

3. Aspect ratio is < 3:1, but particle is clearly fibrous

4. The EDS spectrum of the particle is characteristic of Libby amphibole asbestos, based on the EDS criteria presented in Attachment 3.

Data Recording

All data should be recorded using the electronic laboratory data recording sheet provided in Attachment 1.

For each field of view (FOV) observed, collect the following data:

1. The total area coverage (all particles). Record this in the Lab Data Sheet (Attachment 1). Whenever possible, this should be done by collecting a digital backscatter image and quantifying the particle coverage using appropriate software. When the instrument does not support this technique, the total coverage should be estimated visually using the visual reference aids provided in Attachment 2. The method used to estimate total coverage should be indicated in the Lab Data Sheet.

2. The length and thickness of all structures that satisfy the counting rules above. Record these values in the Lab Data Sheet.

3. Record and save an EDS spectrum and prepare a photomicrograph for the first structure recorded, and for every 10th structure thereafter. Identify these structures in the Lab Data Sheet.

Computations

All of the computations described below are performed automatically in the Lab Data Sheet.

The area of an LA particle is computed as the product of the length and width:

$$\text{Area} = \text{l (um)} \cdot \text{w (um)} \cdot \text{1E-06 mm}^2/\text{um}^2$$

The total area of LA in any FOV is simply the sum of the areas of the individual LA particles in that FOV:

$$\text{Area(total LA)} = \sum(l_{i,j,m} \cdot w_{i,j,m}) \cdot \text{1E-06 mm}^2/\text{um}^2$$

The Area Fraction (AF) of LA in each FOV "i" is calculated as:

$$AF(i) = \text{Area(total LA in FOV "i") / Total area of all particles in FOV "i"}$$

For each magnification "x", the average and standard deviation of AF across multiple FOVs are calculated as:

mean(x) = average of all FOV(i) values for mag x
stdev(x) = Standard deviation of all FOV(i) values for max x

Assuming that the distribution of area fractions can be approximated as a normal distribution, the average, standard deviation, and confidence limits of the total AF combined across all magnifications are given by:

$$AF(total) = mean(x1) + mean(x2) + mean(x3)$$
$$Stdev(total) = [stdev(x1)^2 + stdev(x2)^2 + stdev(x3)^2]^{0.5}$$
$$5\% \text{ Lower Confidence Limit (LCL)} = AF(total) - 1.645 \cdot Stdev(total)$$

95% Upper Confidence Limit (UCL) = AF(total) + 1.645·Stdev(total)

Stopping Rules (see Flow Chart in Figure 1):

The stopping rules require four input items to be specified by the data user, as follows:

1. UL (Upper limit). This is a concentration above which accurate quantification by SEM is not needed under this protocol. The choice of this value might be based on risk management strategy (e.g., any sample with a concentration that is determined to be greater than the UL is assumed to be unacceptable, and providing accurate quantitation above this values does not alter the risk management decision), or on analytical strategy (e.g., samples with concentrations above the UL will be assessed using a different analytical approach, more suited to quantification of relatively high concentrations). For the Libby Superfund site, the default value of UL is 1%, because at levels higher than this, an alternative method should be used for quantification. This value may be revised on a task-by-task basis, as deemed appropriate by decision makers.

2. LL (Lower limit). This is a concentration below which accurate quantification by SEM is not needed under this protocol. The choice of this value may be based either on risk management strategy (e.g., any sample with a concentration that is determined to be lower than the LL is assumed to be acceptable, and providing accurate quantitation below this above this values does not alter the risk management decision), or on analytical strategy (e.g., samples with concentrations lower than the LL will be assessed using a different analytical approach, more suited to quantification of very low concentrations). For the Libby Superfund site, the default value of LL is 0.01%, because it is not anticipated that SEM can effectively quantify values below this level. This value may be revised on a task-by-task basis, as deemed appropriate by decision makers.

3. DQO (Data Quality Objective). The ratio of the 95% UCL of the mean divided by the mean is an index of the relative uncertainty in the estimated value. For data with high uncertainty, the value of this ratio will be large, and as the quality of the data increase, the value of the ratio will decrease, ultimately approaching a value of 1.0. The DQO specifies the target maximum value for this ratio, with lower DQO values being associated with higher data confidence and lower uncertainty. In general, the value of the ratio can be decreased to the value of the DQO for any sample simply by counting more and more FOVs. The choice of the value for DQO is primarily a risk management decision, since the probability of Type I and Type II decision errors both tend to decrease as data quality increases. For the Libby Superfund site, the default value of DQO is 1.3, because this level of accuracy will usually be sufficient to support informed decision-making. This value may be revised on a task-by-task basis, as deemed appropriate by risk managers and decision makers.

4. Max (Maximum number of FOVs to be evaluated at medium and high magnification). As noted above, the analysis of any sample can be extended indefinitely by analysis of more and more FOVs, but this results in increased analytical cost and time per sample. The choice of the value for Max is primarily a risk management and cost-benefit decision. For the Libby Superfund site, the default value of Max is 60 FOVs, because it is expected that the DQO can usually be achieved within this number. This value may be revised on a project-by-project basis, as deemed appropriate by risk managers and decision makers.

In summary, the four inputs which must be provided by the data users and decision makers are:

Code	Description	Default
UL	Concentration above which reliable quantification is no longer required	1.0%
LL	Concentration below which reliable quantification is no longer required	0.01%
DQO	Ratio of 95% UCL / mean	1.3
Max	Maximum number of FOVs (excluding low mag)	60

Once these four inputs are specified, the stopping rules are as follows:

Step 1 (Low Magnification)

1. Count the full stub at low magnification (about 12 FOV)
2. Based on the data obtained in Step 1, compute the LCL. If LCL >= UL, then STOP. Otherwise, proceed to Step 2.

Step 2 (Medium Magnification)

1. Count 10 FOVs, selected using a systematic uniform random (SUR) approach. In this approach, the operator randomly chooses a starting field at the upper left corner of the sample stub, and then spaces the rest of the fields at grid points formed by evenly spaced horizontal and vertical lines, with the distance between fields being sufficient to prevent field overlap.
2. Based on the combined data from Step 1 and Step 2, compute the LCL. If the LCL >= UL, then STOP. Otherwise, proceed to Step 3.

Step 3. (High magnification)

1. Count 10 FOVs, selected using a systematic uniform random (SUR) approach. Using the combined data from steps 1, 2 and 3, compute the LCL and UCL. If LCL >= UL, then STOP. Else
2. If UCL <= LL, then STOP. Else
3. If UCL / Mean <= DQO, then STOP. Else proceed to Step 4.

Step 4. Additional FOVs

1 Count 10 additional FOVs. Press the macro button in the Lab Data Sheet to see the optimum number to be counted at medium and high magnification.
2. Using the combined data from steps 1, 2, 3 and 4, compute the LCL and UCL. If LCL >= UL, then STOP. Else
3. If UCL <= LL, then STOP. Else
4. If UCL / Mean <= DQO, the STOP. Else
5. If Total FOVs >= MAX, then STOP. Else repeat Step 4.

NOTE: All of these stopping rules are evaluated automatically as data are entered into the Lab Data Sheet and the outcome (continue of stop) is displayed in the sheet.

7.0 ESTIMATION OF MASS PERCENT

7.1 Area Fraction Approach

The mass of amphibole asbestos in the soil sample may be approximated as the area fraction of the sample that is occupied by amphibole asbestos structures. It is understood that mass fraction and area fraction are not identical, but this approximation is considered to be acceptable for the purposes of evaluating the potential utility of this method as a screening tool at the site.

7.2 Structure Count Approach

The structure count approach is based on the concept that the particle size-distribution of asbestos in fine-ground soil is approximately constant. If so, there will be an approximately linear relation between asbestos particle loading (average particles per field) of any specified size category and the mass percent concentration in the sample.

Calibration Curve

In this approach, quantification is achieved using an empiric calibration curve. That is, each laboratory should measure the average number of asbestos particles per field at 500x in a series of calibration standards of Libby Amphibole, plus a blank. The calibration curve should be composed of at least five independent preparations (stubs) of at least 5 calibration standards (blank plus four spiked samples) that span the range of observed field samples (e.g., 0.01% to 1%), and each preparation should be evaluated by counting asbestos structures in at least 20 fields at 500x. The calibration standards will be prepared and provided by USGS.

The results should be summarized in graphical format, in which the x-axis is the nominal concentration (mass percent) in the sample, and the y-axis is the average number of particles per field observed at 500x.

Sample Quantification

The concentration of a sample is obtained by counting the number of amphibole asbestos structures in each of 20 fields at 500x, and using the average value (i.e., the average count across the 20 fields) to estimate the mass percent from the empiric standard curve.

8.0 SEM/EDS INSTRUMENT CALIBRATION AND MAINTENANCE

8.1 Instrument Calibration

All instrument calibrations are performed under the same operating conditions as sample analysis.

Size (Magnification) Calibration

Calibration for size (magnification) is performed using magnification gratings (obtained from E. F. Fullam or other appropriate vendor) in accord with the manufacturer's instrument-specific instructions. Instrument calibration for size (magnification) must be performed at the following minimum frequency:

- prior to initial receipt of field samples
- monthly after the first calibration
- immediately after any maintenance activities are performed

EDS Calibration

The energy dispersive x-ray will be standardized at the beginning of each day, or at any time the instrument operating conditions change significantly. The US Geological Survey has provided a BIR-1G basalt glass as a reference material for this procedure. This material is provided to assist in evaluation of performance and inter-laboratory comparisons of energy dispersive x-ray analysis data obtained on a scanning electron microscope. The EDS standardization is essential for ensuring the instrument and software capability of distinguishing mineral species that have similar compositions and may have a fibrous or similar habit (for example, distinguish amphibole from pyroxene or biotite). The EDS spectrum of the BIR1-G sample should be provided as part of the standard data package for each set of analytical results.

The basalt glass is mounted in epoxy in a 1/4 inch polished mount that has been carbon coated in a high vacuum carbon evaporator. Detailed instructions for the analysis of this sample are provided in Attachment 4. In brief, always handle this sample with gloves in a dust free environment. Do not re-polish, re-coat, or touch the surface of this reference material mount, as this could result in damage to the material or a change in its composition. The following practices and analytical conditions must be used when analyzing this sample:

1) The diameter of the primary electron beam should be less than 50 um and greater than or equal to 20 um, or an equivalent raster size should be used. (Note: some raster patterns have prolonged dwell at the corners and/or the sides)
2) Primary beam current should not exceed 10 nA.
3) Avoid repeated analysis of the same spot on the sample.
4) Do not leave the beam on the sample for prolonged periods.
5) Do not examine the sample at a high magnification that would result in a beam exposure greater than or equivalent to that specified in item 1.
6) Do not expose the epoxy to the electron beam except at very low magnification and for very brief time periods.
7) Be sure that good electrical contact is made between the sides of the brass tube and the SEM stage during analysis.
8) Always store in a dry, dust-free environment.

Evaluate the analysis by comparing the values obtained with the recommended values for BIR-1G glass. Records will be kept each day of values obtained for the standard to determine the percent variation of accuracy and precision of the instrument. Acceptance criteria will be provided in an update to this SOP after results from several laboratories are available.

In the event that EDS calibration standard results do not meet the acceptance criteria provided above, analysis can not proceed or, if in progress, should cease until the problem is corrected. All EDS calibration data should be documented in the instrument logbook and should be traceable to each sample delivery group (SDG) associated with the calibration. A SDG is defined as a single set of samples submitted to the laboratory in one day or every 20 samples, whichever is more frequent.

8.2 Instrument Maintenance

Periodic instrument maintenance should be performed as necessary in accord with manufacturer's recommendations. All maintenance activities must be recorded in an instrument logbook dedicated to that particular instrument. As indicated above, if instrument maintenance is performed, the instrument should be re-calibrated and the calibration verified as described in Section 8.1.

9.0 LABORATORY QUALITY ASSURANCE/QUALITY CONTROL

Laboratory Quality Assurance/Quality Control activities are actions taken by the laboratory to obtain data that indicate if analyses are occurring within specified acceptable ranges of accuracy and precision. Any time that performance deviates from acceptable ranges, all analyses must be stopped until the source of the deviation is identified and remedied.

Accreditation and Training

All commercial laboratories involved in the analysis of site samples must be accredited by the National Voluntary Accreditation program (NVLAP), and all analysts must be properly trained by education or experience in identification and quantification of asbestos structures by SEM/EDS. In this regard, USEPA provides project-specific training and instruction to laboratories as described in the CSS SAP (USEPA 2002). The USGS has extensive experience in SEM analysis and has participated in the development of this SOP and is qualified to participate in the analysis of site samples.

Laboratory Blanks

Each laboratory shall prepare at least one laboratory blank SEM stub each day that samples are prepared for SEM analysis. This blank stub will be prepared exactly the same as all field sample stubs, except that no soil will be added to the water before application to the stub. This blank is intended to evaluate the general cleanliness of the sample preparation area and the potential for stub contamination during sample handing and preparation. If fibers are found on the laboratory blanks,

the laboratory should stop analysis immediately and discuss with EPA how to proceed. As a matter of organization, therefore, it is recommended that the laboratory blanks be analyzed prior any field sample stubs.

10.0 DECONTAMINATION

Decontamination (decon) procedures will be performed using standard laboratory decontamination procedures.

11.0 DOCUMENTATION

Instrument Logbook

An individual instrument logbook (hard copy and/or electronic) should be kept for each piece of equipment in use at the laboratory. All initial calibration, daily calibration results, and instrument maintenance activities must be recorded in the appropriate logbook. Copies (hard copy or electronic) of instrument logbooks should be provided along with all analytical reports.

Photomicrographs and EDS Spectra

Each EDS spectrum and each photomicrograph collected in accord with the SOP must be identified by the unique laboratory identification number and analysis date. Any opportunistic photographs taken are also numbered, but must accompany a brief explanation of the reason for taking the picture.

Electronic Data

Each day of data acquisition, all electronic files will be saved onto two separate media or devices. For example, the data may be saved onto a computer hard drive, but must also be backed up onto a type of portable media such as CD-ROM, floppy disc, or tape. Portable media will be maintained in a single location with limited access.

Hardcopy Data

All data sheets or logbooks, photographs, and spectrographs must be stored in a secured location with limited access (e.g., locking file cabinet) when not in use.

Copies (hardcopy and electronic) of the raw analytical data will be submitted to the oversight agency for archival.

12.0 REFERENCES

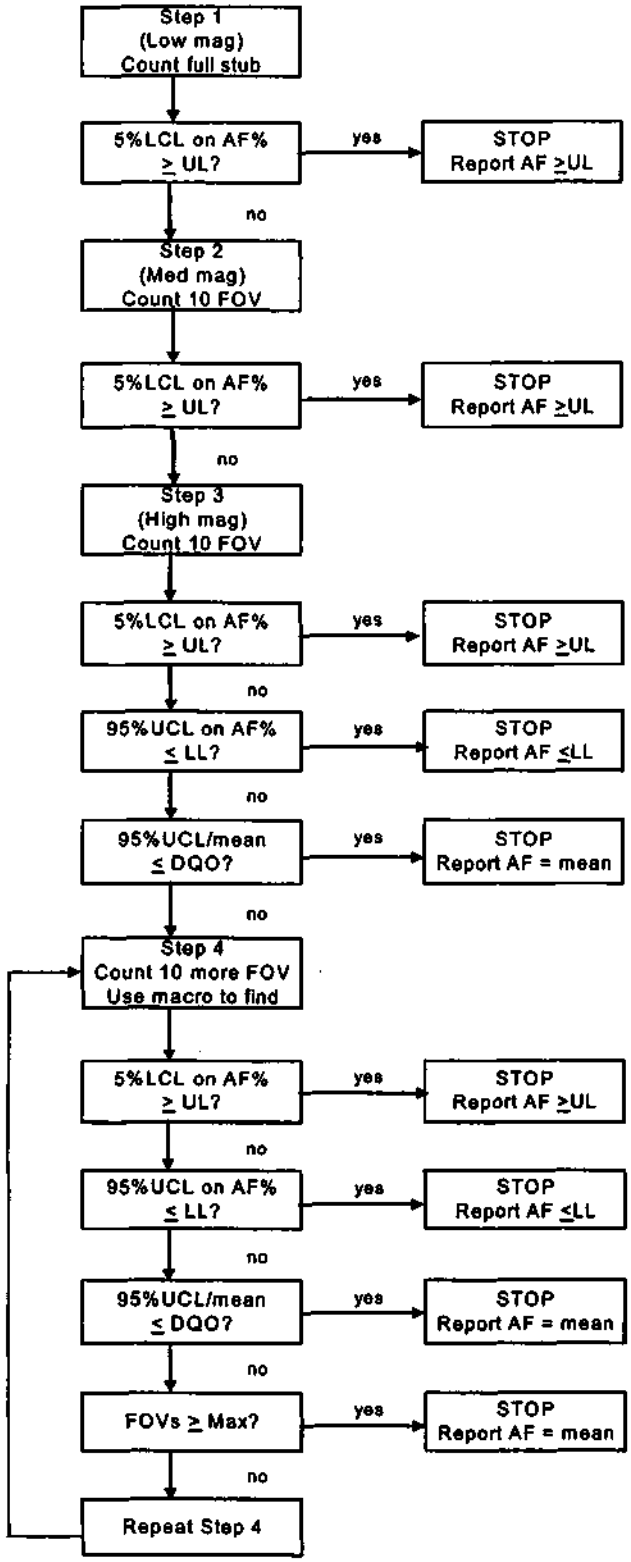
CDM. 2002. Sampling and Analysis Plan, Remedial Investigation, Contaminant Screening Study, Libby Asbestos Site, Operable Unit 4. 3282-116-PP-SAMP-14187. Camp, Dresser and McKee Denver, Colorado. April 2002.

EMSL. 2000. SOP EPA-LIBBY-01, Revision 0. Asbestos Analysis of Soil by SEM and EDS. Prepared by EMSL Analytical Inc. August 25, 2000.

USGS. 2002a. Analysis of Soil Samples for Asbestos Content by Scanning Electron Microscope and Energy Dispersive Spectroscopy. Prepared by A. Bern, G.P. Meeker, and I.K. Brownfield of the U.S. Geological Survey for the U.S. Environmental Protection Agency. October 4, 2002.

USGS. 2002b. Preparation and Analysis of Soil Samples For Amphibole Asbestos Content by Scanning Electron Microscopy And Energy Dispersive Spectrometry. U.S. Geological Survey Administrative Report prepared for the U.S. Environmental Protection Agency by A. Bern, I.K. Brownfield and G.P. Meeker, U.S. Geological Survey. December 18th, 2002.

FIGURE 1. STOPPING RULES FLOW CHART



ATTACHMENT 1

SEM LAB DATA SHEET

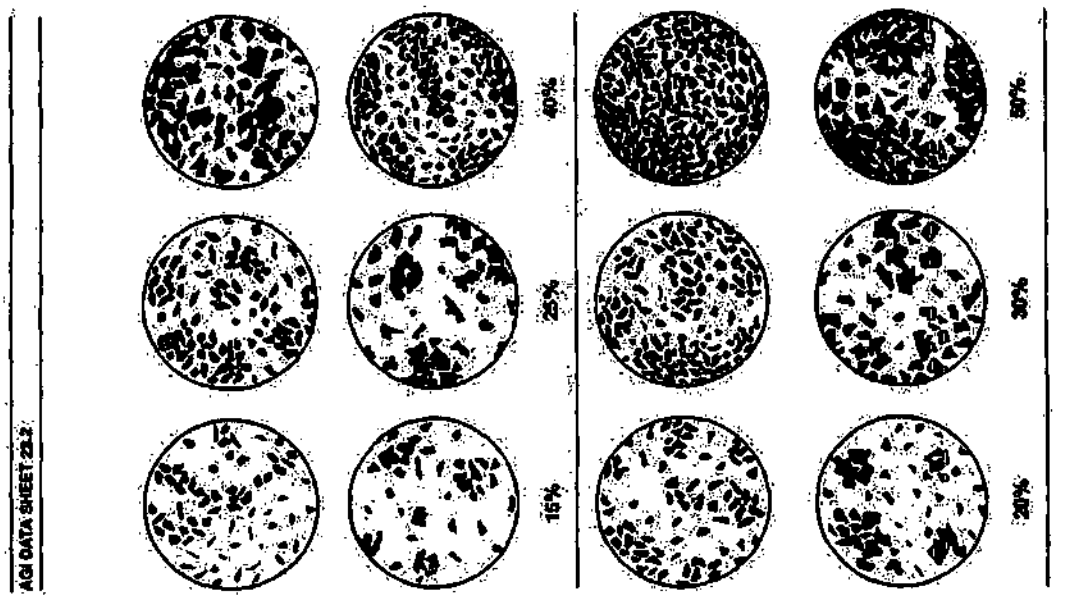
See electronic Excel file
"SEM Lab Data Sheet v4.xls"

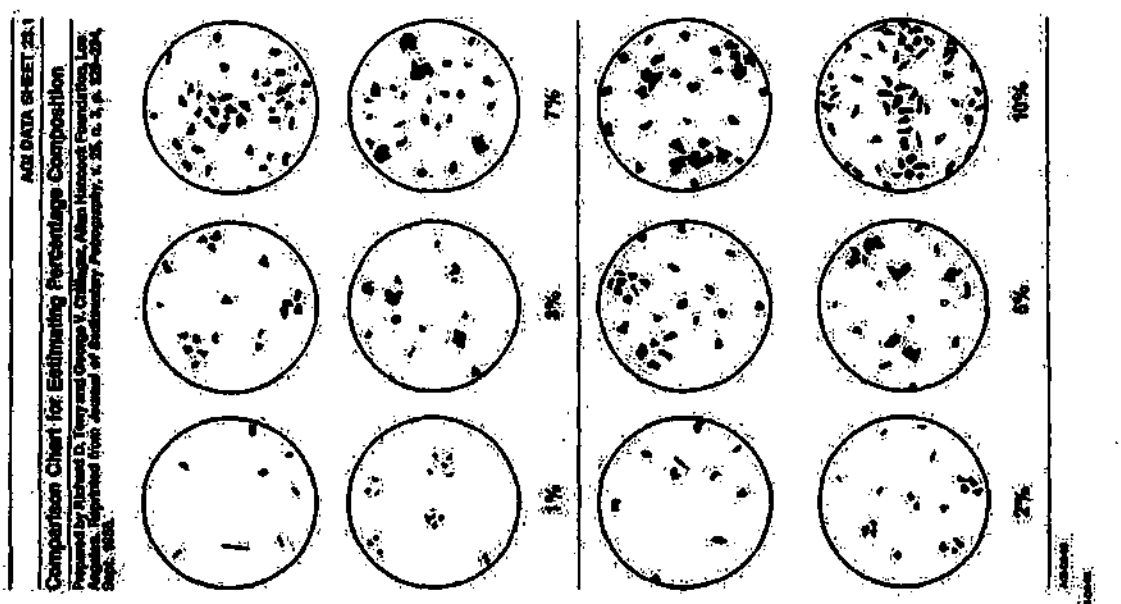
(Check with Volpe or SRC to determine the most recent version number)

ATTACHMENT 2

VISUAL AID FOR ESTIMATING TOTAL COVERAGE IN A FIELD OF VIEW

Source: Dutro Jr., J. T., Dietrich, R. V., Foose, R. M. 1989. AGI Data Sheets for Geology in the Field, Laboratory, and Office, Third Edition. American Geological Institute.


AGI DATA SHEET 23.2
40%
25%
15%
50%
30%
20%


AGI DATA SHEET 23.1
Comparison Chart for Estimating Percentage Composition
7%
3%
1%
10%
5%
2%

ATTACHMENT 3

GUIDANCE FOR IDENTIFYING LIBBY AMPHIBOLE USING EDS

USGS
science for a changing world

GUIDE TO ANALYSIS OF SOIL SAMPLES FROM LIBBY, MONTANA FOR ASBESTOS CONTENT BY SCANNING ELECTRON MICROSCOPE AND ENERGY DISPERSIVE SPECTROSCOPY

by

Amy Bern
Greg Meeker
Isabelle Brownfield

U.S. Geological Survey Administrative Report
For the U.S. Environmental Protection Agency

October 17, 2002

This report is preliminary and has not been reviewed for conformity with U.S. Geological Survey editorial standards. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Fibrous materials can not be reliably identified as amphibole by morphology alone. Other materials, such as calcite, pyroxene, or biotite, can also have cleavage that produces long thin particles or scrolls. Libby, Montana soils contain many of these minerals. To accurately identify amphibole fibers by SEM analysis, EDS spectra must be obtained to verify that mineral composition is consistent with an amphibole.

Two EDS spectra of amphiboles are illustrated in Figure 1a. The first is an example of an amphibole from Libby Montana. The second is an International Union Against Cancer (UICC) standard tremolite. Libby amphiboles contain the elements Na, Mg, Al, Si, K, Ca, Ti, Mn, and Fe in varying amounts, but similar to those shown in Figure 1a. F and Cl also occur, but usually not in quantities great enough to show on EDS spectra. Some diagnostic spectra of other minerals are presented for comparison.

Biotites contain many of the same elements as Libby amphiboles. The Al and Mg content is used to distinguish biotite from amphibole. Biotites contain much more aluminum than Libby amphiboles. EDS spectra of Libby micas are provided in Figure 1b for comparison.

Pyroxene and Libby amphibole can be difficult to tell apart, but usually can be distinguished by the absence of K and higher Al in pyroxene. An EDS spectrum for a typical pyroxene is given in Figure 1c.

The best way to determine if the chemical composition is consistent with an amphibole is to perform a "quantitative" analysis.[1] In most cases a "standardless" analysis will provide sufficient accuracy to give confidence that the material is compatible with an amphibole. For this analysis, oxygen should be determined by stoichiometry and cation ratios for Na, Mg, Al, Si, K, Ca, Ti, Mn, and Fe should be calculated based on 23 oxygens. The cation total should equal between 15 and 16. Examples of EDS analyses for Libby Amphibole weight percentages and cations are given in Table 1.

Table 1. EDS Analysis of Libby Amphibole Fibers.

Weight Percent									
	Na2O	MgO	Al2O3	SiO2	K2O	CaO	TiO2	MnO	Fe2O3
1	4.7	21	0.43	57	1.3	7.5	0.33	0.17	7.7
2	5.5	21	0	60	2.1	4.4	0	0.03	8.0
3	1.6	23	0.14	59	0.37	12	0.12	0.01	3.1

Cation Totals (all Fe is Fe3+)										
	Na	Mg	Al	Si	K	Ca	Ti	Mn	Fe3	Total
1	1.22	4.27	0.07	7.77	0.22	1.09	0.03	0.02	0.81	15.50
2	1.44	4.11	0.00	8.07	0.35	0.63	0.00	0.00	0.84	15.43
3	0.42	4.64	0.02	7.90	0.06	1.78	0.01	0.00	0.32	15.16

	T Site Total	C Site Total	B Site Total	A Site Total	Cation Total
1	7.87	5.00	2.00	0.63	15.50
2	8.00	5.02	2.00	0.42	15.43
3	7.93	4.96	2.00	0.26	15.16

T site-Si ≅ 8 C site-Al + Fe + Mg + Ti + Mn ≅ 5 A+B sites-Ca + Na + K >= 2

1. Quantitative analysis requires matrix corrections to correct for atomic number, absorption, and fluorescence effects. Matrix correction routines (e.g. ZAF or Phi Rho Z) assume that the sample is homogeneous in the volume analyzed, and has a flat polished surface. If these conditions are not met the results can have errors that are larger than what is normally expected. The magnitude of this error will depend on the size, shape and composition of the sample or particle and also on the analytical conditions, particularly accelerating voltage (Small and Armstrong, 2000). Our experience with the analysis of single structures of Libby amphibole using the analytical conditions specified in this procedure is that the errors for all elements are typically within +/-10 % relative concentration of the expected value, and often much better.

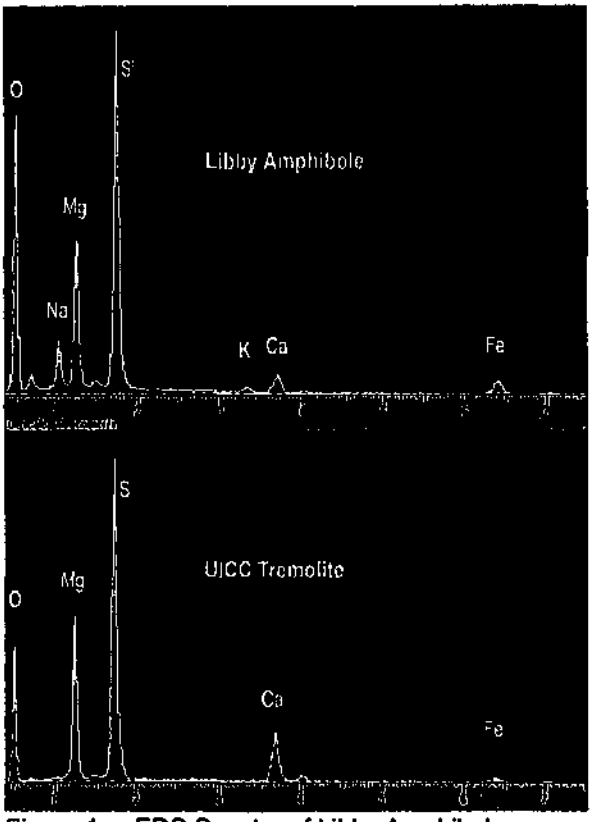


Figure 1a. EDS Spectra of Libby Amphibole and Tremolite.

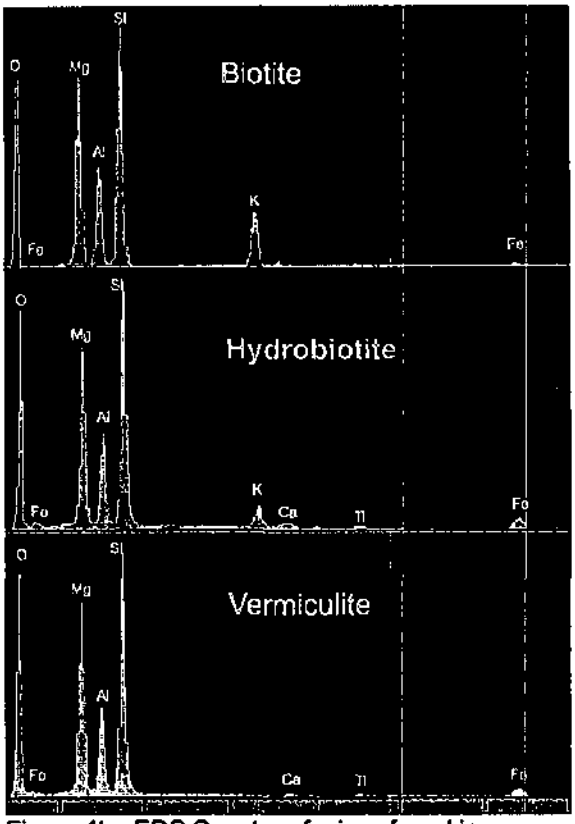


Figure 1b. EDS Spectra of micas found in Libby Samples.

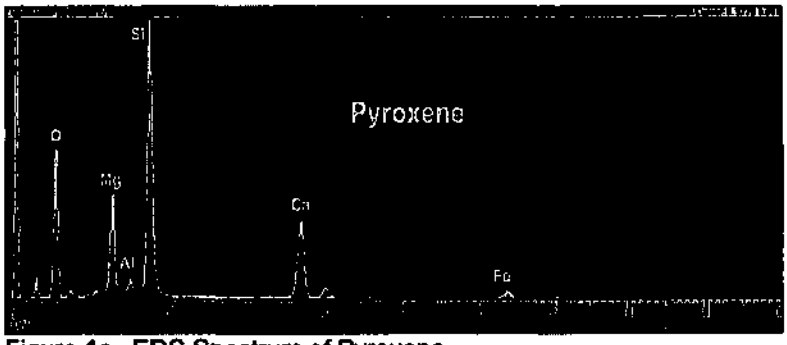


Figure 1c. EDS Spectrum of Pyroxene.

Reference

Small, J.A. and Armstrong, J.T., 2000, Improving the Analytical Accuracy in the Analysis of Particles by Employing Low Voltage Analysis. Microscopy and Microanalysis, vol. 6, supplement 2. Proceedings 2000, p.924-925.

ATTACHMENT 4

PROCEDURE FOR EVALUATION OF EDS USING GLASS STANDARD BIR1-G

Instructions for Analysis of BIR1-G Basalt Glass Reference Material

1. Follow all instructions on the handling and analysis sheet supplied with the reference material. A copy is included below.
2. Calibrate EDS system as per manufactures instructions at the proper working distance. The sample should be mounted with the surface normal to the beam unless your instrument is specifically designed otherwise. Locate basalt grains in epoxy at low magnification with the backscatter detector. Care should be taken to avoid using high current density on areas of epoxy to prevent contamination of the reference material.
3. Analyze the sample using a beam diameter (or equivalent raster) between 20 and 40 um. Do not use a beam size smaller than 20 um as this can damage the reference material.
4. Analyze the sample at 15 kV, approximately 20-40 % dead time, < 5 nA primary beam current.
5. Allow the spectrum to accumulate for at least 2 minutes (real time) or until the integrated Si peak contains approximately 50,000 counts (or about 16, 000 cts. peak height).
6. Perform a "quantitative" analysis of the spectrum using ZAF or Phi Rho Z, without standards, using the same software that you would use for Libby amphibole particles. Analyze for Na, Mg, Al, Si, K, Ca, Ti, Mn, Cr, and Fe (not all elements may be detectable). The analysis should include background subtraction, peak overlap correction, correction for sum peaks and Si escape peaks, and be normalized to 100 %.
7. Report results as weight percent oxide for Na_2O, MgO, Al_2O_3, SiO_2, K_2O, CaO, TiO_2, MnO, Cr_2O_3, Fe_2O_3 with oxygen calculated by stoichiometry. Report cation ratios based on 23 oxygen ions.
8. Please provide all spectra and numerical results including cation ratios.

Tabbed Page:
13

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

Date: May 6, 2004 SOP No. ISSI-LIBBY-01 (Rev. 8)

Title: SOIL SAMPLE PREPARATION

Author: William Brattin Syracuse Research Corporation(a)

SYNOPSIS: A standardized method for preparation of soil samples for asbestos analysis is described.

Received by QA Unit:

APPROVALS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
EPA Region 8	Jane Goldade	5/6/04
Syracuse Research Corp.	W J Brattin	5/6/04

Revision Number	Revision Date	Reason for Revision
1	1/7/00	Incorporation of sieving to the sample preparation.
2	7/12/00	Revision in sieve size, other minor edits.
3	5/7/02	Incorporate minor edits
4	8/1/02	Modify sieving procedure, add grinding step
5	3/6/03	Incorporate modifications to the procedure and documentation requirements

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Revision Number	Revision Date	Reason for Revision
6	3/24/03	Incorporate modifications to the logsheets to conform with electronic data storage requirements and add grinder blank requirements.
7	8/5/03	Incorporate modifications to drying and sample storage procedures
8	5/4/04	Incorporate modifications to drying batch size and recording of preparation information

(a) This SOP was originally prepared by ISSI Consulting Group. ISSI is no longer in existence, and finalization of the SOP was performed by Syracuse Research Corporation (SRC).

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1.0 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to provide a standardized method for preparation of soil samples for asbestos analysis. This procedure will be used by employees of United States Environmental Protection Agency (USEPA) Region 8 and by contractors/subcontractors supporting USEPA Region 8 projects and tasks for the Remedial Investigation work performed at the Libby, Montana site. Site-specific deviations from the procedures outlined in this document must be reviewed and approved within a Request for Modification by the Volpe Center Technical Lead or Libby Project Manager and the USEPA Region 8 Remedial Project Manager or Regional Chemist.

The contents of this SOP have been specifically designed for the Libby Asbestos site. For example, the particle size of 250 μm was selected in an attempt to balance two opposing goals: 1) grinding the sample to a small enough particle size to obtain homogeneous soil samples; and 2) keep the particle size distribution of sufficient size to accommodate analyses by several methods including polarized light microscopy-visual area estimation (PLM-VE), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). It is possible that for methods such as the TEM, further preparation at the laboratory may be necessary. If so, these additional steps will be addressed at the level of the TEM SOP.

Procedures outlined in this SOP have been designed with the intent to prepare soil samples having a target concentration greater or equal to approximately 0.1-0.2% (weight percent) total Libby amphibole (LA) material.

2.0 RESPONSIBILITIES

The Preparation Laboratory Project Leader (PL^2) may be an USEPA employee or contractor who is responsible for overseeing the soil sample preparation activities. The PL^2 is also responsible for checking all work performed and verifying that the work satisfies the specific tasks outlined by this SOP and the Close Support Facility Soil Preparation Plan, Libby Asbestos Site, Operable Unit 4, Libby, Montana (CSF SPP). It is the responsibility of the PL^2 to communicate with the Preparation Laboratory personnel regarding specific collection objectives and anticipated situations that require any deviation from the respective Project Plans. It is also the

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responsibility of the PL[2] to communicate and document the need for any deviations from the Project Plans with the appropriate USEPA Region 8 Remedial Project Manager or Regional Chemist.

Personnel preparing Libby soil samples are responsible for adhering to the applicable tasks outlined in this procedure and conducting all sample handling and preparation activities in the ventilation hood.

3.0 EQUIPMENT

- General purpose laboratory oven - must be capable of maintaining a constant temperature of approximately 89-91°C.
- Analytical balance - calibrated and accurate to tolerance limits indicated on Attachment 2, range of 0.1 g to at least 2000 g
- Riffle splitter - with 3/4 inch chutes to split samples
- Plate Grinder - capable of accepting soil particles of approximately 1/4 inch diameter and grinding to produce particle of approximately 250 µm
- Metal (other than plastic) scoop or spoon - for transferring samples
- 1/4 inch metal (other than plastic) sieve and catch pan - for coarse sieving samples
- 60 mesh (250 µm) and 200 mesh (74 µm) metal (other than plastic) sieves - for verification of the plate grinder settings
- Clean quartz sand - required for preparation of grinding and drying blank samples (Sections 6.2, 9.2, 12.1 and 12.3) and for decontamination of grinder (Section 9.4)
- Clean soil - sufficient aliquot required for calibration of grinder (Section 9.1)
- Drying Pans - pans used during the sample drying process

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- Sample containers - plastic ziplock bags (pint and gallon size)
- Gloves - for personal protection and to prevent cross-contamination of samples. May be plastic or latex. Disposable, powderless
- Field clothing and Personal Protective Equipment - as specified in the Health and Safety Plan (Appendix E of the CSF SPP, December 2003)
- Field notebook -used to record progress, any problems or observations and deviations
- Sample Drying Log Sheets - (Attachment 1) - used to record all sample drying information
- Sample Preparation Log Sheets (Attachment 1)- used to record all sample preparation information (splitting, sieving and grinding)
- Three-ring binder books - binders will contain:

    Analytical Balance Calibration and Maintenance Log (Attachment 2)
    Grinder Calibration and Maintenance Log (Attachment 3)
    Ventilation Hood Calibration and Maintenance Log (Attachment 4)
    Vacuum Maintenance Log (Attachment 5)
    Oven Temperature Calibration and Maintenance Log (Attachment 6)
    Sample labels

- Trash Bags - used to dispose of gloves, wipes and other investigation derived waste
- Indelible Marking Pen - used to record sample information onto plastic ziplock bags and to record logbook information
- Ballpoint Pen - used to record field logsheet information

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4.0 METHOD SUMMARY

Figure 1 provides an overview of the steps in this procedure. Soil samples are dried in a standard laboratory oven and split into a preparation sample and an archive sample. The preparation sample is sieved to separate coarse material (> 1/4 inch) from fine material (< 1/4 inch). The fine material is ground to a standard particle size of about 250 µm for subsequent asbestos analysis. The coarse material is examined by stereomicroscopy to determine if any large particles of asbestos are present (EPA SOP SRC-LIBBY-01).

5.0 SOIL STORAGE

Upon receipt of samples, samples will be grouped in an inventory batch of approximately 120 samples. Samples will be archived according to the inventory batch they are assigned to and filed by the inventory ID noted on the Sample Drying Log Sheet and Sample Preparation Log Sheet (Attachment 1). This box number will be automatically assigned by the electronic Libby Asbestos Sample Tracking Information System (eLASTIC) when the inventory batch is created in the database.

6.0 BULK SOIL DRYING

Prior to drying, samples will be grouped in a drying batch and assigned a drying batch number. The following sections detail all activities and procedures related to drying samples.

6.1 Calibration

Samples will be weighed prior to and following drying activities. The analytical balance used for drying activities will be calibrated on days when samples are loaded into, or unloaded from, the oven. Before weighing samples, calibrate the balance using S-1 class weights and record all measurements, any required maintenance, and the balance number on the Analytical Balance Calibration and Maintenance Log (Attachment 2).

All drying activities will be performed under a negative pressure HEPA filtered hood. Prior to loading the oven, the ventilation hood will be calibrated to ensure that the ventilation system is operating properly. Ventilation hood calibration and any required maintenance will be

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documented on the Ventilation Hood Calibration and Maintenance Log (Attachment 4).

A HEPA vacuum will be used to decontaminate the oven following the removal of dried samples. Vacuum calibration will be performed daily, prior to drying activities. All system checks, required maintenance and the vacuum number will be recorded on the Vacuum Maintenance Log (Attachment 5).

Oven temperature calibration will be performed on a weekly basis. Oven temperature calibration and any required maintenance will be documented on the Oven Temperature Calibration and Maintenance Log (Attachment 6).

6.2 Drying Blanks

A drying blank will be created and associated with each drying batch prior to loading samples into the oven. A drying batch will consist of approximately 15 samples. The drying blank will consist of approximately 100-200 grams of clean quartz sand, placed in a drying pan and assigned an index ID (see Section 6.1). Each drying blank will be identified in the notes section of the Sample Drying Log Sheet (Attachment 1) and will be prepared using the same methodology as other soil samples. Following preparation, whenever possible, each blank will be shipped with its associated batch samples. See Section 12.1 for more details regarding drying blanks.

6.3 Drying Procedure

Samples will be loaded into the drying oven using the following steps:

- Record the SOP and Revision Number used to prepare the samples on the Sample Drying Log Sheet (Attachment 1). Record the oven number used to dry the samples on the Sample Drying Log Sheet (Attachment 1).

- Prior to unsealing and drying each sample, record the sample mass to the nearest 0.1 g on the Sample Drying Log Sheet (Attachment 1), the technicians initials and the date. See Section 6.1 for calibration details.

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- Set the oven temperature to 90 ± 1°C. For every sample drying batch, check the oven temperature to verify that proper temperature has been reached and document the start date/time and temperature on the Sample Drying Log Sheet (Attachment 1).

- Transfer each sample to be dried from its zip top storage bag into a clean drying pan. Each sample should be transferred to its respective drying pan under the negative pressure HEPA filtered hood. Label each drying pan with its respective Index ID. Place each sample in the oven.

- Leave the samples in the oven for approximately 24-48 hours or until completely dry. Verify that each sample is dry, by squeezing a portion of the soil with a freshly gloved thumb and forefinger to test the cohesiveness. Once it is confirmed that samples are dry, record the technician's initials, and the date and time of completion, on the Sample Drying Log Sheet (Attachment 1).

- Turn off the oven and allow the samples to cool in the oven. Once the samples are cooled, unload each sample and transfer each sample volume to a clean zip top bag, re-bag the sample with another clean zip top bag and identify the dried sample with the index ID. All samples should be transferred to zip top bags under the negative pressure HEPA filtered hood to prevent potential exposure to fibers that might be released from the sample.

- Record the sample mass of each bagged sample to the nearest 0.1 g on the Sample Drying Log Sheet (Attachment 1), the technician's initials and the date.

6.4 Decontamination

Decontaminate the inside of the hood and the inside of the oven by HEPA vacuuming and wet wiping all surfaces before loading a new batch for drying.

Decontaminate all sample drying pans under the ventilation hood using compressed air or a HEPA vacuum to remove any residual organic material left on the pans. Wet wipe or brush off any visible material that is not removed from the air blast or vacuum. All pans will be decontaminated between samples.

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7.0 DIVISION OF ARCHIVE AND PREPARATION SAMPLES

Prior to sieving and grinding, samples will be divided into a portion for archive and a portion for preparation. The sections below describe the sample splitting procedure.

7.1 Calibration

Prior to any splitting, sieving, or grinding activities, calibrate the ventilation hood to ensure that the ventilation system is operating properly. Document ventilation hood calibration and any required maintenance on the Ventilation Hood Calibration and Maintenance Log (Attachment 4).

7.2 Procedure for Sample Division

Samples will be divided using the following steps:

- Place the cooled, re-bagged samples in the hood, and knead the contents of the bag to break up any soil clumps.
- Splitting must be performed in the hood to prevent potential exposure to fibers that might be released from the sample. Place one pan on each side of the riffle splitter. Divide the sample into two equal sub-parts by removing the sample from its original plastic bag and loading the dry material into the splitter.
- After splitting, set aside one part for sample preparation as described below (if the volume of the portion left for preparation is still too large for processing, split the sample again so that 3/4 of the original sample will be archived and 1/4 will be set aside for processing).
- Place the remaining split portion into a clean, zip top bag, re-bag the sample in another clean zip top bag, and store as an archive sample in the event additional analyses are required in the future. Identify the archive sample with the index ID and the suffix "A" (for archive fraction). Record the technician's initials and date on the Sample Preparation Log Sheet. Store the archive portion in the numbered inventory box noted on the Sample Preparation Log Sheet (Attachment 1).

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7.3 Duplicate Samples

One preparation duplicate sample will be processed for every 20 field samples prepared. A preparation duplicate is a sample split of material that is prepared in the same fashion as the parent sample (preparation split) and will be submitted to the laboratory blind. The preparation duplicate will be assigned a unique and random index identification number. For both samples, the corresponding index ID will be indicated in the notes section of the Sample Preparation Log Sheet (Attachment 1). If a preparation duplicate is not being prepared for a particular sample, proceed to Section 7.4.

Following the division of a sample for preparation and archive. Divide the designated sample into two equal sub-parts using a riffle splitter (as described in section 7.2). Retain one portion as the parent sample and assign the other portion the duplicate index ID. Record the technician's initials, and the date of creation on the Sample Preparation Log Sheet (Attachment 1), when the duplicate sample is prepared. Prepare each portion according to the processes outlined below. For further information on preparation duplicates, refer to Section 12.2.

7.4 Decontamination

The splitter will not be decontaminated following this step provided the fine ground sample will be split again into four fractions in Section 10.0. If for any reason the same sample is not immediately split further, the riffle splitter must be decontaminated as follows.

Use a HEPA vacuum and compressed air to decontaminate the splitter and brush or wipe off any visible material that is not removed by the air blast. The splitter is now ready to process the next sample.

8.0 PREPARATION SAMPLE SIEVING

All samples will be sieved prior to grinding to separate out the coarse and fine fractions. The sample sieving procedure is described in the sections below.

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8.1 Calibration

All sieving activities will take place in the hood. Refer to Section 6.1 for details regarding the frequency of ventilation hood calibration.

Samples will be weighed during sieving activities. The analytical balance will be calibrated daily with S-1 class weights before processing begins. All measurements, any required maintenance, and the analytical balance number will be recorded on the Analytical Balance Calibration and Maintenance Log (Attachment 2).

8.2 Sample Sieving Procedure

Conceptually, sample sieving may generate a coarse and a fine fraction sample. Samples will be sieved using the procedure outlined below.

Coarse Fraction

A 1/4 inch stainless steel screen with catch pan will be used to divide the fine and coarse fractions using the following procedure:

- Pour the sample through the 1/4 inch stainless steel sieve and give the screen a shake to ensure all particles < 1/4 inch in size are allowed to pass through the screen. In addition, a pestle may be used to break up any remaining soil clumps to ensure all particles <1/4 in size pass through the screen.

- Pour all material which does not pass through the screen (> 1/4 inch) into a new, tared, sample bag and identify the coarse sample with the index ID and the suffix "C" (for "coarse fraction").

- Record the mass of the coarse fraction to the nearest 0.1 g on the Sample Preparation Log Sheet (Attachment 1) and record the technician's initials and the date.

- Double-bag the coarse sample portion and identify the sample with the index ID and "C" suffix on the sample bag. Coarse fraction samples are now ready to be packaged for

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shipment to the analytical laboratory or archived as directed.

Fine Fraction

Tare an empty sieve pan, to account for the weight of the pan containing the fine sample, and weigh the fine material that passed through the sieve. Record the mass of the fine fraction to the nearest 0.1 g on the Sample Preparation Log Sheet (Attachment 1). If all of the material passes through the screen, such that there is no coarse fraction, record a mass of zero for the coarse fraction on the Sample Preparation Log Sheet.

Whenever possible, immediately process the fine material that passes through the screen in accord with the approach described in Section 9.3 (below). If processing cannot occur immediately, pour the fine material which passed through the sieve into a new plastic ziplock bag and identify the fine sample material with the index ID and the suffix "F" (for "fine fraction"). Double-bag the sample and identify the sample with the index ID and suffix on the outside of the bag.

8.3 Decontamination

Decontaminate all sieves, pans and the pestle under the ventilation hood using compressed air. Wipe or brush off any visible material that is not removed from the air blast. A HEPA vacuum may also be used to remove any residual organic material left on the sieve pans. All pans and sieves will be decontaminated between samples.

9.0 FINE SAMPLE GRINDING

The fine sieved sample will be ground to produce a material of about 250 µm. The final sample will be packaged and shipped to the laboratory for asbestos analysis. The procedure for grinding the fine sieved sample is outlined below.

9.1 Calibration

All grinding activities will take place in the hood. Refer to Section 7.1 for details regarding the frequency of ventilation hood calibration.

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A HEPA vacuum will be used to decontaminate the hood and processing equipment, following the preparation of each sample. Vacuum calibration will be performed daily, prior to grinding activities. All system checks, required maintenance and the vacuum number will be recorded on the Vacuum Maintenance Log (Attachment 5).

A standard BICO vertical plate grinder will be used to process samples. The grinder will be calibrated daily or after any adjustments are made to the plates. To verify proper particle size (approximately 250 µm), and demonstrate that samples will not be over-processed, grind a sample of clean soil (rather than quartz sand) and sieve using stacked sieves. Clean soil will be provided by the United States Geological Survey (USGS). Unlike the coarseness of quartz sand, soil will more accurately approximate the typical grain size and texture of the Libby samples being processed and will reduce the chance of over-processing. Note that the particle size is cited as "approximately 250 µm". This is due to the nature of grinding asbestos material. Some material that is longer than 250 µm may pass through the grinder if its longest side is parallel with the vertical grinder plates. The material that comes in contact more nearly perpendicular to the vertical grinder plates will be ground to <250 µm.

The grinder is adjusted acceptably if all material passes through a 60-mesh (250 µm) screen and is substantially retained by a 200-mesh (74 µm) sieve. If the appropriate amount of material does not pass through the stacked sieves, adjust the plates of the vertical grinder until all material processed passes through the aforementioned sieve sizes. Document the grinder number, verification of acceptable adjustment and any observations in the Grinder Calibration and Maintenance Log (Attachment 3).

Following the calibration activities, the stacked sieves will be decontaminated using a HEPA vacuum, compressed air and an aliquot of approximately 20 g of quartz sand will be passed through the grinder before the next sample is processed.

Samples will be weighed following grinding activities. The analytical balance will be calibrated daily with S-1 class weights before processing begins. All measurements, any required maintenance, and the analytical balance number will be recorded on the Analytical Balance Calibration and Maintenance Log (Attachment 2).

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9.2 Grinding Blanks

A grinding blank will be prepared daily, per grinder used, and will be associated with all samples prepared per day, per grinder. The grinding blank will consist of approximately 100-200 grams of clean quartz sand, and will be processed on days that field samples are ground. Each grinding blank will be identified in the notes section of the Sample Preparation Log Sheet (Attachment 1) and will be processed according to the direction of Section 9.3. Grinding blanks will be included with daily shipments to the laboratory. For further information on grinding blanks refer to Section 12.3.

9.3 Grinding of Fine Field Samples

The sample portion that was sieved to < 1/4 inch will be ground to a particle size of approximately 250 µm. Set up a catch pan under the grinder to collect all the ground material. Take the fine sample set aside in Section 8.2, load the grinder hopper, and allow the fine sample to pass through the plate grinder into the catch pan. Note the technician's initials, date of grinding, and grinder number on the Sample Preparation Log Sheet (Attachment 1).

9.4 Decontamination

When grinding is complete, do not move the plates for decontamination (this would require re-calibration). Decontaminate the hopper and catch pan by using a HEPA vacuum, followed by a blast of high pressure air. Set the catch pan aside and clean the grinder with several blasts of compressed air. Pay special attention to areas where dust from the grinding process is known to accumulate (e.g., between the plates and areas adjacent to the catch pan clamps). Reattach the catch pan to the grinder. Pass an aliquot of approximately 20 g of quartz sand through the grinder to clean out any residual soil. Discard the quartz sand and re-clean the grinder with the vacuum and another round of high pressure air blasts. After this decontamination procedure, the grinder is ready to process the next sample.

10.0 SPLITTING OF THE FINE GROUND SAMPLE

The fine ground soil sample should be distributed into four approximately equal subsamples using a splitter. All splitting activities will be performed in the hood. Refer to Section 7.1 for details regarding the frequency of ventilation hood calibration.

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10.1 Splitting Procedure for Fine Ground Sample

The following method for splitting a soil sample was adapted from EPA 540-R-97-028 (USEPA, 1997):

- Set up one receiving pan on each side of the splitter. Load the soil from the grinder catch pan (Section 9.3) into the splitter, collecting the sample in two receiving pans.
- Tap the catch pan vigorously several times to free any remaining material. Tap the splitter to facilitate the flow of all material through the chutes into the receiving pans.
- Empty each receiving pan into the grinder catch pan and sieve catch pan, respectively. Set the sieve pan aside; this portion of fine ground sample will be split again later.
- Replace the receiving pans under the splitter. Take the grinder catch pan, containing half of the fine ground sample and re-load the contents into the splitter as detailed above. Repeat the process of dispersing the sample material by shaking the catch pan and tapping the splitter to uniformly distribute the sample. The resulting splits are the "FG1" and "FG2" portions on the Sample Preparation Log Sheet (Attachment 1).
- Take these two portions and carefully transfer each into a clean, tared, zip top sample bag. Re-bag one sample portion in another clean zip top sample bag and identify this fine ground sample with the index ID, the suffix "FG" (for "fine fraction, ground") and the fraction number 1, (ex. CS-12345-FG1 for fine ground fraction #1). Identify the bagged second portion with the Index ID, the suffix "FG" and the fraction number 2 and set aside to be re-bagged with the following fine ground portions:
- Place the two empty receiving pans from the "FG1" and "FG2" portion next to the splitter. Repeat the splitting procedure using the other fine ground portion set aside in the sieve pan and split the remaining sample material to create the "FG3" and "FG4" portions.

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- Take the remaining "FG3" and "FG4" portions and carefully transfer each into a clean, tared, ziplock sample bag, identify each remaining fine ground sample with the index ID as noted above.

- Weigh each sample portion (FG1 through FG4), and record each mass along with the technician's initials and date on the Sample Preparation Log Sheet (Attachment 1).

- Combine all bagged portions (archive, coarse and fine) into one large clean, zip top sample bag.

Fine ground samples are now ready to be packaged for shipment to the analytical laboratory or archived as directed. When samples are requested for shipment, the "FG1" fraction will be sent first. If further analyses are required for the fine ground portion, the subsequent fractions will be double bagged and sent (i.e., FG-2 then FG-3, etc.). All archived fine ground portions will be filed in the appropriate inventory archive box noted on the Sample Preparation Log Sheet (Attachment 1).

10.2 Decontamination

Use the vacuum and compressed air to decontaminate the splitter and brush or wipe off any visible material that is not removed by the vacuum or air blast. The splitter is now ready to process the next sample.

11.0 DOCUMENTATION

Index ID numbers are recorded on the Sample Drying Sheet, Sample Preparation Log Sheet (Attachment 1) and on all sample containers. Sample Drying Sheets and Sample Preparation Log Sheets will be filed under their associated dry batch and preparation batch number. If revisions to the Sample Drying Sheet and/or Sample Preparation Log Sheet are necessary, the appropriate parties will be notified of the changes, however, these changes will not necessitate revision to the current standard operating procedure, a modification form will be filled out to document the revisions.

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As mentioned above, the following equipment calibration and maintenance logs will also be maintained:

- daily analytical balance calibration using S-1 class weights (Attachment 2)
- daily grinder setting verification for calibration check and/or post-adjustment verification, grinder maintenance as necessary (Attachment 3)
- daily ventilation hood operating condition verification (i.e., inline filter checks, changes) (Attachment 4)
- HEPA vacuum maintenance and bag changes (Attachment 5)
- weekly oven temperature calibration, oven maintenance as necessary (Attachment 6)

In addition, a field notebook will be maintained by each individual or team that is preparing samples. For each day that samples are processed, the following information should be collected:

- date
- time
- personnel
- PPE
- Governing Plan (CSF SPP, February 2004) and TSOP including revision number
- descriptions of any deviations to the SOP, the reason for the deviation and/or any modification forms being followed
- summary of laboratory activities (including number of samples prepared, and equipment calibrated and used)

12.0 QUALITY ASSURANCE

All quality control sample results will be monitored for potential contamination. If samples results indicate cross-contamination, the PL^2 will be notified. The PL^2 will then identify the affected samples and notify the appropriate parties of these samples. Laboratory procedures will also be re-assessed and appropriate changes will be made and documented accordingly by the PL^2.

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12.1 Drying Blanks

At least one drying blank will be processed with each batch (per oven) of approximately 15 field samples (Section 6.2). Results from each drying blank will determine if cross-contamination occurred during the drying process. The drying blank, consisting of clean quartz sand, will be assigned a random and unique index identification number and will be submitted to the laboratory blind. Detection of asbestos fibers in any drying blank (at the practical quantitation limit of about 0.1-0.2% LA) should be taken as a sign of potential cross-contamination, and steps should be taken to identify and address the source of the cross contamination.

12.2 Preparation Duplicates

One preparation duplicate sample (Section 7.3) will be processed for every 20 field samples prepared. Results from duplicate samples serve to evaluate the precision of the sample preparation process and of the laboratory analysis. A preparation duplicate is prepared by using a riffle splitter to divide the sample into two approximately equal portions, creating a parent and duplicate sample. Both samples are prepared in the same fashion. The preparation duplicate is assigned a unique and random index identification number, and is submitted to the laboratory blind. Inconsistent sample results should be taken as an indication of variability in sample preparation, and steps should be taken to identify and address the source of the variability in sample preparation.

12.3 Grinding Blanks

One grinding blank (Section 9.2), consisting of clean quartz sand, will be processed once per day, per grinder, on days that field samples are ground. Each grinder used in the lab will be assigned a number and all samples processed will be associated with the grinder used for preparation. The grinder number used for each sample will be noted on the Sample Preparation Log Sheet (Attachment 1). Grinding blanks will not be dried, split for archive, or sieved, a grinding blank will only be ground and split into four fine ground samples. Results from the grinding blank will determine if decontamination procedures of laboratory equipment are adequate in preventing cross-contamination of samples during sample grinding and fine ground sample splitting processes only. The grinding blank is assigned a random and unique index identification number and is submitted to the laboratory blind. If asbestos fibers are detected in

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any grinding blank the PL^2 will be notified. The PL^2 will identify all samples that were processed on the day the grinding blank was prepared, and the grinder that was used to process the grinding blank. Detection of asbestos fibers in any drying blank (at the practical quantitation limit of about 0.1-0.2% LA) should be taken as a sign of potential cross-contamination, and steps should be taken to identify and address the source of the cross contamination.

13.0 DECONTAMINATION

All non-disposable equipment used during sample preparation must be decontaminated prior to use. Scoops or spoons, splitters, sieves and drying pans that are re-used must be decontaminated with a HEPA vacuum, compressed air, wet-wiping and/or by brushing off any residual material. If soil particles are visible on any of the equipment, repeat the decontamination procedure until the equipment is clean.

Detailed decontamination procedures for specific equipment are noted in Sections 6.4, 7.4, 8.3, 9.4, and 10.2.

14.0 GLOSSARY

HEPA - High Efficiency Particulate Air

Project Plan - The written document that spells out the detailed site-specific procedures to be followed by the Project Leader and the Preparation Lab Personnel.

15.0 REFERENCES

American Society for Testing and Materials. 1998. Standard Practice for Reducing Samples of Aggregate to Testing Size, ASTM Designation: C 702 - 98, 4 p.

CDM. 2004. Close Support Facility Soil Preparation Plan, Libby Asbestos Site, Operable Unit 4, Libby, Montana. February.

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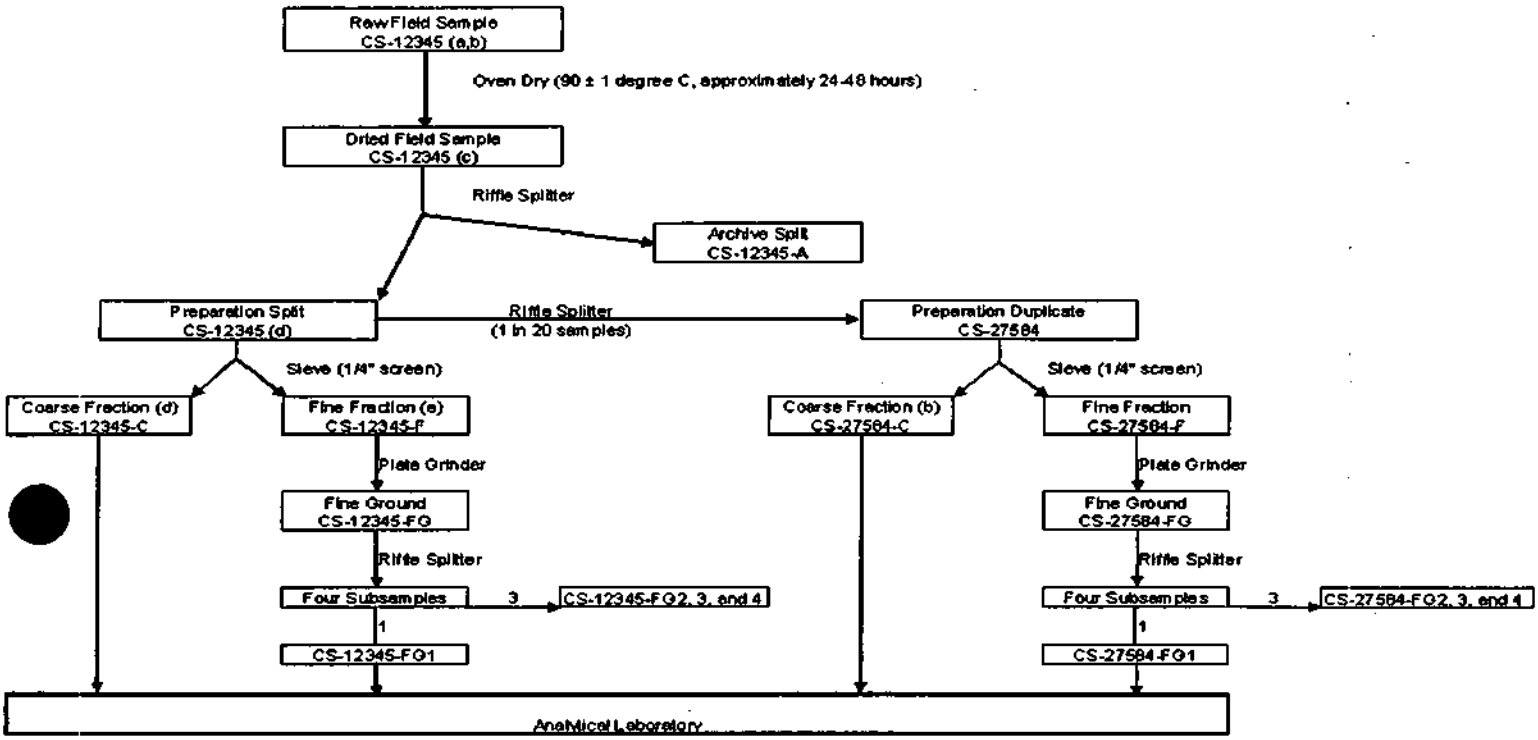
USEPA. 1997. Superfund Method for the Determination of Releasable Asbestos in Soils and Bulk Materials. EPA 540-R-97-028.

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

Figure 1 Soil Sample Preparation Flow Diagram



(a) Example sample number shown to illustrate naming conventions
(b) Drying blanks, created with clean quartz sand (Section 6.2) will be processed with each batch using the same sample processing procedures outlined in ISSI-Libby-01 (Rev 7)
(c) If the sample is designated as a duplicate, the sample will follow the duplicate splitting process defined below. If the sample is not a designated duplicate, it will proceed to the sieving step defined below.
(d) Coarse sample will be returned to CDM CSF for archive after laboratory analysis
(e) Grinding blanks (Section 6.3), created with clean quartz sand, will be ground and split into four fine ground samples using the same procedures outlined in ISSI-Libby-01 (Rev 7)

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

ATTACHMENT 1

SAMPLE DRYING AND SAMPLE PREPARATION LOG SHEETS

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

Sample Drying Log Sheet

[illegible]

Dry Batch ID ______________________ Sheet No.: CSF- ______________

Date/Time Drying Begun ______________________

Date/Time Drying Completed ______________________

Oven Number ______________________

Oven Temp (C) ______________________

Index ID	Inventory ID Number	SOP and Rev Number	Mass (g)			Original Sample Identification and Notes (indicate if dry blank sample)	QC
			Before Drying	After Drying	Initials and Date		Initials and Date

The following preparation steps require Technician Initials and Date to document activity: Sample Mass, Data Entry QC

Page 1 of 1

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

Sample Preparation Log Sheet

Prep Batch ID _____ Sheet No. CSF- _____

Index ID	SOP and Rev Number	Inventory ID	QA Batch ID	Archive Sample Splitting	Duplicate Sample Splitting	Sieving			Sample Grinding		Sample Splitting					Original Sample Identification and Notes (Indicate if grind blank, dry blank, or duplicate pair. For duplicate pair enter the parent/child ID)	QC
						Sample Mass (g)	Sample Mass (g)				Sample Mass (g)						
				Initials and Date	Initials and Date	Fraction >1/4"	Fraction <1/4"	Initials and Date	Initials and Date	Grinder #	FO1	FO2	FO3	FO4	Initials and Date		Initials and Date

The following preparation steps require Technician Initials and Date to document activity: Archive Sample Splitting, Preparation Duplicate Splitting, Sieving, Sample Homogenization, Sample Splitting, and Data Entry QC

Page 1 of 1

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

ATTACHMENT 2

ANALYTICAL BALANCE CALIBRATION AND MAINTENANCE LOG

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

Balance # = _____

Measurement Number		S - 1 Class Weight Measurements				Measurement within range? Yes or No	If "No" Recalibrate	Technician Initials	QC check Initials
	Calibration Weights	0.1 g	1 g	10 g	100 g				
	Tolerance Limit Range	0.05 - 0.15 g	0.90 - 1.10 g	9.75 - 10.25 g	99.00 - 101.00 g				
	Date								
1									
2									
3									
4									
5									
6									
7									
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9									
10									
11									
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15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									

The analytical balance calibration will be verified daily.
All tolerance limits are standard tolerance limits for Class S-1 weights.
After 20 measurements, the tolerance range will be evaluated for reasonableness.
Weights falling outside the range require that the balance be recalibrated using all S-class weights

Sheet No.: Balance - _____

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

ATTACHMENT 3

GRINDER CALIBRATION AND MAINTENANCE LOG

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

Grinder # = ________________

Date	Activity Type (D, A, M)	Sieve Test 60 mesh acceptable Y or N	 200 Mesh acceptable Y or N	Notes (Include description of action/maintenance)	Technician Initials	QC Check Initials

D = daily calibration
A= Adjustment Calibration Verification
M= Maintenance Only
For the 60 mesh sieve test to be acceptable all of the clean soil must pass through the sieve.
For the 200 mesh sieve test to be acceptable a substantial portion of the ground soil must be retained on the sieve.
Failure of either sieve test requires adjustment of the plates followed by adjustment verification prior to grinding samples.

Sheet No.: Grinder - ________________

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

ATTACHMENT 4

VENTILATION HOOD CALIBRATION AND MAINTENANCE LOG

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

Ventilation Hood # = ________________

Date	In-line HEPA indicators acceptable?	Notes (Include description of action/maintenance)	Technician Initials	QC Check Initials

Ventilation system operating conditions should be verified daily
In-line system should indicate the velocity/negative flow is 100 ft/min. If not, the problems with the system must be corrected prior to processing samples. Note actions or maintenance taken.

Sheet No.: Hood - ________________

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

ATTACHMENT 5

VACUUM MAINTENANCE LOG

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

Vacuum # = ____________

Date	System Check Acceptable? Yes or No	HEPA filter clean? Yes or No	Bag fill level acceptable? Yes or No	Notes (Include description of action/maintenance performed)	Technician Initials	QC Check Initials

Physically check the vacuum suction and note audible change in motor daily. If significant reduction in the vacuum suction or motor "strain" is audibly noted then the system check is unacceptable. If unacceptable, perform and document the HEPA filter and bag maintenance checks above and perform maintenance.

Sheet No.: Vacuum - ____________

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

ATTACHMENT 6

OVEN TEMPERATURE CALIBRATION AND MAINTENANCE LOG

NOTE: This SOP was designed for use on Libby Project Soils. For any other use the SOP needs to be re-evaluated based on specific project objectives

Oven # = _____

Date	Certified Temperature Reading (°C)	Thermometer ID	System Check Acceptable? 90°C (+/- 1°C) Yes or No	Notes (Include description of action/maintenance performed)	Technician Initials	QC Check Initials

Sheet No.:Oven - _____

Tabbed Page: 14

PB83-260471

ANALYTICAL METHOD FOR DETERMINATION OF ASBESTOS FIBERS IN WATER

by

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Contract 68-03-2717

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EPA 600/4-84-043

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16. ABSTRACT

An analytical method for measurement of asbestos fiber concentration in water samples is described. Initially, the water sample is treated with ozone gas and ultraviolet light to oxidize suspended organic materials. The water sample is then filtered through a 0.1 micrometer pore size capillary-pore polycarbonate filter, after which the filter is prepared by carbon extraction replication for examination in a transmission electron microscope (TEM). Fibers are classified using selected area electron diffraction and energy dispersive X-ray analysis. Measurement of characteristic features on a recorded and calibrated selected area electron diffraction pattern is specified for precise identification of chrysotile. Quantitative determination of the chemical composition, and quantitative interpretation of at least one calibrated zone axis selected area diffraction pattern are specified for precise identification of amphibole. Amphibole identification procedures and generation of the standard reporting format specified for the fiber count results are achieved using two computer programs that are integral to the analytical method.

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FOREWORD

Nearly every phase of environmental protection depends on a capability to identify and measure specific pollutants in the environment. As part of this Laboratory's research on the occurrence, movement, transformation, impact, and control of environmental contaminants, the Analytical Chemistry Branch develops and assesses new techniques for identifying and measuring chemical constituents of water and soil.

A 3-year study was conducted to develop improvements in the analytical method for determination of asbestos fiber concentrations in water samples. The research produced an improved sample preparation and analysis methodology, a rapid screening technique to reduce analysis cost, and a new reference analytical method for asbestos in water. The analytical method for determining asbestos fibers in water is perceived as representing the current state-of-the-art.

William T. Donaldson
Acting Director
Environmental Research Laboratory
Athens, Georgia

PREFACE

The Preliminary Interim Method for Determining Asbestos in Water was issued by the U.S. Environmental Protection Agency's Environmental Research Laboratory in Athens, Georgia. The method was based on filtration of the water sample through a sub-micrometer pore size membrane filter, followed by preparation of the filter for direct examination and counting of the fibers in a transmission electron microscope. Two alternative techniques were specified: one in which a cellulose ester filter was prepared by dissolution in a condensation washer; and another known as the carbon-coated Nuclepore® technique which used a polycarbonate filter. In January 1980 the method was revised (EPA-600/4-80-005) to eliminate the condensation washer approach, and a suggested statistical treatment of the fiber count data was incorporated.

The analytical method published here is a further refinement of the revised interim method. Major additions include the introduction of ozone-ultraviolet light oxidation prior to filtration, complete specification of techniques to be used for fiber identification and fiber counting rules, and incorporation of reference standard dispersions. A standardized reporting format has also been introduced. The major deletion is the low temperature ashing technique for samples high in organic material content; ashing is not required for the analysis of drinking water and drinking water supplies when samples are treated using the ozone-ultraviolet oxidation technique. The "field-of-view" approach for examination also has been deleted from the method. If a sample is too heavily loaded for examination of entire grid openings, a more reliable result is obtained by preparation of a new filter using a smaller volume of water.

ABSTRACT

An analytical method for measurement of asbestos fiber concentration in water samples is described. Initially, the water sample is treated with ozone gas and ultraviolet light to oxidize suspended organic materials. The water sample is then filtered through a 0.1 μm pore size capillary-pore polycarbonate filter, after which the filter is prepared by carbon extraction replication for examination in a transmission electron microscope (TEM). Fibers are classified using selected area electron diffraction (SAED) and energy dispersive X-ray analysis (EDXA). Measurement of characteristic features on a recorded and calibrated SAED pattern is specified for precise identification of chrysotile. Quantitative determination of the chemical composition, and quantitative interpretation of at least one calibrated zone axis SAED pattern are specified for precise identification of amphibole. Amphibole identification procedures and generation of the standard reporting format specified for the fiber count results are achieved using two computer programs which are integral to the analytical method.

This analytical method is a further development of the interim method issued in 1980, and incorporates results of research performed under Contract 68-03-2717 under sponsorship of the U.S. Environmental Protection Agency. This report covers a period from October 1978 to September 1981 and the work was completed as of September 1981.

CONTENTS

FOREWORD iii
PREFACE iv
ABSTRACT v
FIGURES x
TABLES xi

1. SCOPE AND APPLICATION 1
2. SUMMARY OF METHOD 1
3. DEFINITIONS, UNITS AND ABBREVIATIONS 2
   3.1 Definitions 2
   3.2 Units 4
   3.3 Abbreviations 5
4. EQUIPMENT AND APPARATUS 5
   4.1 Specimen Preparation Laboratory 5
   4.2 Instrumentation Requirements 6
      4.2.1 Transmission Electron Microscope 6
      4.2.2 Energy Dispersive X-ray Analyzer 8
      4.2.3 Computer 8
      4.2.4 Vacuum Evaporator 9
      4.2.5 Ozone Generator 9
   4.3 Apparatus, Supplies and Reagents 9
5. SAMPLE COLLECTION AND PRESERVATION 15
   5.1 Sample Container 15
   5.2 Sample Collection 16
   5.3 Quantity of Sample 16
   5.4 Sample Preservation and Storage 16
6. PROCEDURE 17
   6.1 Cleanliness and Contamination Control 17
   6.2 Oxidation of Organics 17
   6.3 Filtration 20
      6.3.1 General 20
      6.3.2 Filtration Procedure 22
   6.4 Preparation of Electron Microscope Grids 23
      6.4.1 Preparation of Jaffe Washer 24
      6.4.2 Selection of Filter Area for Carbon Coating. 24
      6.4.3 Carbon Coating of the Nuclepore Filter . . . 26
      6.4.4 Transfer of the Filter to Electron Microscope Grids 27
   6.5 Examination by Electron Microscopy 28
      6.5.1 Microscope Alignment and Magnification Calibration 28

6.5.2 Calibration of EDXA System 29
6.5.3 Grid Preparation Acceptability 30
6.5.4 Procedure for Fiber Counting 31
6.5.5 Estimation of Mass Concentration 34
6.6 Fiber Counting Criteria 35
6.6.1 Fiber Counting Method 35
6.6.2 Fibers Which Touch Grid Bars 35
6.6.3 Fibers Which Extend Outside the Field of View 36
6.6.4 Fibers with Stepped Sides 36
6.6.5 Fiber Bundles 37
6.6.6 Aggregates of Randomly Oriented Fibers . . . 37
6.6.7 Fibers Attached to Non-Fibrous Debris . . . 38
6.7 Fiber Identification Procedures 38
6.7.1 General 38
6.7.2 SAED and EDXA Techniques 39
6.7.3 Analysis of Fiber Identification Data . . . 43
6.7.4 Fiber Classification Categories 45
6.7.5 Procedure for Classification of Fibers With Tubular Morphology, Suspected to be Chrysotile 45
6.7.6 Procedure for Classification of Fibers Without Tubular Morphology, Suspected to be Amphibole 50
6.8 Blank and Control Determinations 54
6.8.1 Blank Determinations 54
6.8.2 Control Samples 54
7. CALCULATION OF RESULTS 55
7.1 Test for Uniformity of Fiber Deposit on Electron Microscope Grids 55
7.2 Calculation of the Mean and Confidence Interval of the Fiber Concentration 56
7.3 Estimated Mass Concentration 59
7.4 Fiber Length, Width, Mass and Aspect Ratio Distributions 61
7.4.1 Fiber Length Cumulative Number Distribution. 61
7.4.2 Fiber Width Cumulative Number Distribution . 62
7.4.3 Fiber Length Cumulative Mass Distribution . 62
7.4.4 Fiber Aspect Ratio Cumulative Number Distribution 62
7.4.5 Fiber Mass Cumulative Number Distribution . 63
7.5 Index of Fibrosity 63
8. REPORTING 63
9. LIMITATIONS OF ACCURACY 65
9.1 Errors and Limitations of Identification 65
9.2 Obscuration 66
9.3 Inadequate Dispersion 66
9.4 Contamination 66
9.5 Freezing 66
10. PRECISION AND ACCURACY 67
10.1 General 67
10.2 Precision 67

10.2.1 Intra-Laboratory Comparison Using Environmental Water Sources 67
10.2.2 Inter-Laboratory Comparison of Filters Prepared Using Standard Dispersions and Environmental Water Sources 67
10.3 Accuracy 71
10.3.1 Intra- and Inter-Laboratory Comparison of Standard Dispersions of Asbestos Fibers 71

SELECTED BIBLIOGRAPHY 74

APPENDIX A - TEST DATA AND COMPUTER LISTINGS FOR FIBER IDENTIFICATION 77

APPENDIX B - TEST DATA AND COMPUTER LISTINGS FOR DATA PROCESSING AND REPORTING. 176

FIGURES

Number		Page
1.	Calibration Markings on TEM Viewing Screen	7
2.	Diagram of Ozone-UV Equipment	18
3.	Ozone-UV Oxidation of Water Samples in Glass Bottles	19
4.	Nuclepore Dissolution Technique	24
5A.	Jaffe Washer Design	25
5B.	Jaffe Washer in Use	25
6.	Condensation Washer	28
7.	Sheet For Recording Water Sample Data	32
8.	Sheet For Recording Fiber Classification and Measurement Data	33
9.	Counting of Fibers Which Overlap Grid Bars	35
10.	Counting of Fibers Which Extend Outside the Field of View	36
11.	Counting and Measurement of Fiber Bundles	37
12.	Counting of Fiber Aggregates	37
13.	Counting and Measurement of Fibers Attached to Non-Fibrous Debris	38
14.	Measurement of Zone Axis SAED Patterns	41
15.	Classification Chart for Fiber With Tubular Morphology	47
16A.	TEM Micrograph of Chrysotile Fibril, showing Morphology	49
16B.	TEM Micrograph of UICC Canadian Chrysotile Fiber after Thermal Degradation by Electron Beam Irradiation	49
17.	SAED Pattern of Chrysotile Fiber with Diagnostic Features Labelled	50
18.	Classification Chart for Fiber Without Tubular Morphology	52

TABLES

Number		Page
1.	Limitation of Analytical Sensitivity by Volume of Water Sample Filtered	21
2.	Silicate Mineral Standards	29
3.	Classification of Fibers With Tubular Morphology	46
4.	Classification of Fibers Without Tubular Morphology	46
5.	Levels of Analysis for Amphibole	53
6.	Intra-Laboratory Comparison of Environmental Water Samples	68
7.	Inter-Laboratory Comparison: Standard Dispersions	69
8.	Inter-Laboratory Comparison: Environmental Water Samples	70
9.	Inter- and Intra-Laboratory Comparison: Chrysotile	72
10.	Inter- and Intra-Laboratory Comparison: Crocidolite	73

ANALYTICAL METHOD FOR DETERMINATION OF ASBESTOS FIBERS IN WATER

1. SCOPE AND APPLICATION

1.1 This method is applicable to drinking water and drinking water supplies, and should be used when the best available analytical procedure is required.

1.2 The method determines the numerical concentration of asbestos fibers, the length and width of each fiber, and the estimated mass concentration of asbestos in the water. Fiber size and aspect ratio distributions are also determined.

1.3 The method permits, if required, identification of all mineral fibers found in water. In particular, chrysotile can be distinguished from the amphiboles, and fibers of specific amphiboles can be identified.

1.4 The analytical sensitivity which can be achieved depends primarily on the amount of other particulate matter which is present in the sample. This limits the proportion of the sample which can be mounted for examination in the electron microscope. In drinking water which meets the AWWA turbidity criterion of 0.1 NTU, an asbestos concentration of 0.01 million fibers per liter (MFL) can be detected. The contamination level in the laboratory environment may degrade the sensitivity. The analytical sensitivity for the determination of mass concentration is a function of the preceding parameters and also depends on the size distribution of the fibers. In low turbidity drinking water the analytical sensitivity is usually of the order of 0.1 nanogram per liter (ng/L).

1.5 It is beyond the scope of this document to provide detailed instruction in electron microscopy, electron diffraction, crystallography or X-ray fluorescence techniques. It is assumed that those performing this analysis will be sufficiently knowledgeable in these fields to understand the specialized techniques involved.

2. SUMMARY OF METHOD

Water collected in a polyethylene or glass container is treated with ozone and ultraviolet light to oxidize organic matter. After mild ultrasound treatment to disperse the fibers uniformly, a known volume of the water is filtered through a 0.1 micrometer (μm) pore size Nuclepore® polycarbonate filter. A carbon coating is then applied in

vacuum to the active surface of the filter. The carbon layer coats and retains in position the material which has been collected on the filter surface. A small portion of the carbon-coated filter is placed on an electron microscope grid and the polycarbonate filter material is removed by dissolution in an organic solvent. The carbon film containing the original particulate, supported on the electron microscope grid, is then examined in a transmission electron microscope (TEM) at a magnification of about 20,000. In the TEM, selected area electron diffraction (SAED) is used to examine the crystal structure of a fiber, and its elemental composition is determined by energy dispersive X-ray analysis (EDXA).

Fibers are classified according to the techniques which have been used to identify them. A simple code is used to record for each fiber the degree to which the identification attempt was successful. The fiber classification procedure is based on successive inspection of the morphology, the selected area electron diffraction pattern, and the qualitative and quantitative energy dispersive X-ray analyses. Confirmation of the identification of chrysotile is only by quantitative SAED, and confirmation of amphibole is only by quantitative EDXA and quantitative zone axis SAED.

Several levels of analysis are specified, three for chrysotile and four for amphibole, defined by the most specific fiber classification to be attempted for all fibers. The procedure permits this target classification to be defined on the basis of previous knowledge, or lack of it, about the particular sample. Attempts are then made to raise the classification of all fibers to this target classification, and to record the degree of success in each case. The lengths and widths of all identified fibers are recorded. The number of fibers found on a known area of the microscope sample, together with the equivalent volume of water filtered through this area, are used to calculate the fiber concentration in MFL. The mass concentration is calculated in a similar manner by summation of the volume of the identified fibers, assuming their density to be that of the bulk material.

3. DEFINITIONS, UNITS AND ABBREVIATIONS

3.1 Definitions

Acicular - The shape shown by an extremely slender crystal with small cross-sectional dimensions.

Amphibole - A group of rock-forming ferromagnesian silicate minerals, closely related in crystal form and composition and having the general formula: $A_{2-3}B_5(Si,Al)_8O_{22}(OH)_2$, where A = Mg, Fe^{+2}, Ca, Na or K, and B = Mg, Fe^{+2}, Fe^{+3} or Al. Some of these elements may also be substituted by Mn, Cr, Li, Pb, Ti or Zn. It is characterized by a cross-linked double chain of Si-O tetrahedra with a silicon:oxygen ratio of 4:11, by columnar or fibrous prismatic crystals and by good prismatic cleavage in

two directions parallel to the crystal faces and intersecting at angles of about 56^0 and 124^0.

Amphibole Asbestos - Amphibole in an asbestiform habit.

Analytical Sensitivity - The calculated concentration in MFL equivalent to counting of one fiber.

Asbestos - A commercial term applied to a group of silicate minerals that readily separate into thin, strong fibers that are flexible, heat resistant and chemically inert.

Aspect Ratio - The ratio of length to width in a particle.

Camera Length - The equivalent projection length between the sample and its electron diffraction pattern, in the absence of lens action.

Chrysotile - A mineral of the serpentine group: $Mg_3Si_2O_5(OH)_4$. It is a highly fibrous, silky variety of serpentine, and constitutes the most important type of asbestos.

Cleavage - The breaking of a mineral along its crystallographic planes, thus reflecting crystal structure.

Cleavage Fragment - A fragment of a crystal that is bounded by cleavage faces.

d-Spacing - The separation between identical adjacent and parallel planes of atoms in a crystal.

Diatom - A microscopic, single-celled plant of the class Bacillariophyceae, which grows in both marine and fresh water. Diatoms secrete walls of silica, called frustules, in a great variety of forms.

Electron Scattering Power - The extent to which a thin layer of a substance scatters electrons from their original path directions.

Energy Dispersive X-ray Analysis - Measurement of the energies and intensities of X-rays by use of a solid state detector and multichannel analyzer system.

Eucentric - The condition when an object is placed with its center on a rotation or tilting axis.

Fibril - A single fiber, which cannot be separated into smaller components without losing its fibrous properties or appearances.

Fiber - A particle which has parallel or stepped sides, an aspect ratio equal to or greater than 3:1, and is greater than 0.5 μm in length.

Fiber Aggregate - An assembly of randomly oriented fibers.

Fiber Bundle - A fiber composed of parallel, smaller diameter fibers attached along their lengths.

Habit - The characteristic crystal form or combination of forms of a mineral, including characteristic irregularities.

Miller Index - A set of three or four integer numbers used to specify the orientation of a crystallographic plane in relation to the crystal axes.

Replication - A procedure in electron microscopy specimen preparation in which a thin copy, or replica, of a surface is made.

Selected Area Electron Diffraction - A technique in electron microscopy in which the crystal structure of a small area of a sample may be examined.

Serpentine - A group of common rock-forming minerals having the formula: $(Mg,Fe)_3\ Si_2O_5(OH)_4$.

Unopened Fiber - Large diameter asbestos fiber which has not been separated into its constituent fibrils.

Zone Axis - That line or crystallographic direction through the center of a crystal which is parallel to the intersection edges of the crystal faces defining the crystal zone.

3.2 Units

eV - electron volt

g/cm^3 - grams per cubic centimeter

kV - kilovolt

μg/L - micrograms per liter (10^{-6} grams per liter)

μm - micrometer (10^{-6} meter)

MFL - Million Fibers per Liter

ng/L - nanograms per liter (10^{-9} grams per liter)

nm - nanometer (10^{-9} meter)

NTU - Nephelometric Turbidity Unit

ppm - parts per million

3.3 Abbreviations

AWWA - American Water Works Association

EDXA - Energy Dispersive X-ray Analysis

HEPA - High Efficiency Particle Absolute

SAED - Selected Area Electron Diffraction

SEM - Scanning Electron Microscope

STEM - Scanning Transmission Electron Microscope

TEM - Transmission Electron Microscope

UICC - Union Internationale Contre le Cancer (International Union Against Cancer)

UV - Ultraviolet

4. EQUIPMENT AND APPARATUS

4.1 Specimen Preparation Laboratory

Asbestos, particularly chrysotile, is present in small quantities in practically all laboratory reagents. Many building materials also contain significant amounts of asbestos or other mineral fibers which may interfere with analysis. It is therefore essential that all specimen preparation steps be performed in an environment where contamination of the sample is minimized. The primary requirement of the sample preparation laboratory is that a blank determination using known fiber-free water must yield a result which will meet the requirements specified in Section 6.8.1. Preparation of samples should be carried out only after acceptable blank values have been demonstrated.

The sample preparation areas should be a separate clean room with no asbestos-containing materials such as flooring, ceiling tiles, insulation and heat-resistant products. The work surfaces should be stainless steel or plastic-laminate. The room should be operated under positive pressure and have absolute (HEPA) filters, electrostatic precipitation, or equivalent, in the air supply. A laminar flow hood is recommended for sample manipulation. It is recommended that a supply of disposable laboratory coats and disposable overshoes be obtained to be worn in the clean room. This will reduce the levels of dust, and particularly asbestos, which might be transferred inadvertently by the operator into the

clean area. Normal electrical and water services are required. An air extract (fume hood) is required to remove surplus ozone from the area near the ozone generator.

4.2 Instrumentation Requirements

4.2.1 Transmission Electron Microscope

A transmission electron microscope having an accelerating potential of a minimum of 80 kV, a resolution better than 1.0 nm, and a magnification range of 300 to 100,000 is required. The ability to obtain a direct screen magnification of at least 20,000 is necessary. An overall magnification of about 100,000 is necessary for inspection of fiber morphology; this magnification may be obtained by supplementary optical enlargement of the screen image by use of a binocular if it cannot be obtained directly. It is also required that the viewing screen be calibrated (as shown in Figure 1) with concentric circles and a millimeter scale such that the lengths and widths of fiber images down to 1 mm width can be measured in increments of 1 mm.

For Bragg angles less than 0.01 radians the instrument must be capable of performing selected area electron diffraction from an area of 0.6 μm^2 or less, selected from an in-focus image at a screen magnification of 20,000. This performance requirement defines the minimum separation between particles at which independent diffraction patterns can be obtained from each. The capability of a particular instrument may normally be calculated using the following relationship:

$$A = \frac{\pi}{4}\left(\frac{D}{M} + 2000\ C_s\theta^3\right)^2$$

where: A = Effective SAED area in μm^2

D = Diameter of SAED aperture in μm

M = Magnification of objective lens

C_s = Objective lens spherical aberration coefficient in mm

θ = Maximum Bragg angle in radians

Although almost all instruments of current manufacture meet these requirements, many older instruments which are still in service do not. It is obviously not possible to reduce the area of analysis indefinitely by use of apertures

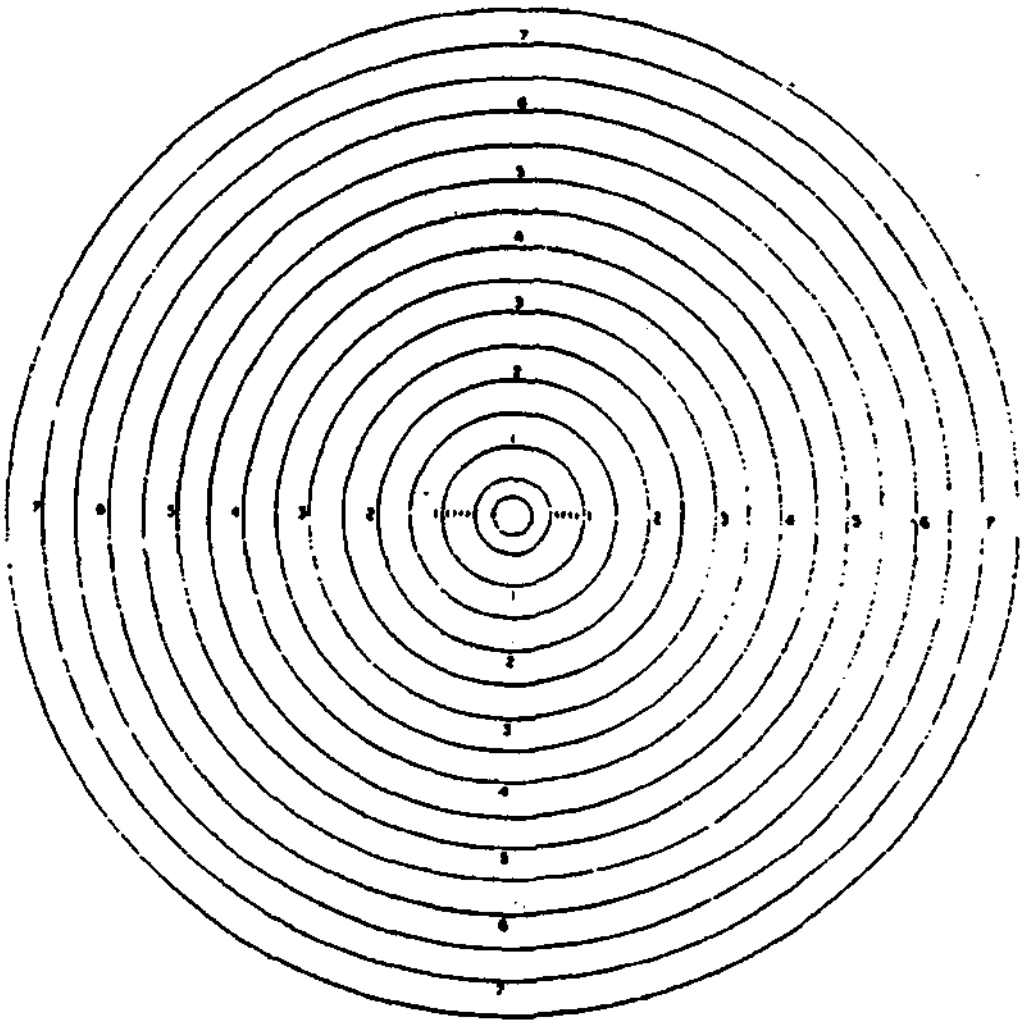


Figure 1. Calibration Markings on TEM Viewing Screen.

smaller in diameter than those specified by the manufacturer, since there is a fundamental limitation imposed by the spherical aberration coefficient of the objective lens.

If zone axis SAED analyses are to be performed, it is required that the electron microscope be fitted with a goniometer stage which permits either a 360^0 rotation combined with tilting through at least $+30^0$ to -30^0, or tilting through at least $+30^0$ to -30^0 around two perpendicular axes in the plane of the sample. The work is greatly facilitated if the goniometer permits eucentric tilting.

It is also essential that the electron microscope have an illumination and condenser lens system capable of forming an electron probe smaller than 100 nm in diameter.

Use of an anti-contamination trap around the specimen is recommended if the required instrumental performance is to be obtained.

4.2.2 Energy Dispersive X-ray Analyzer

An energy dispersive X-ray analyzer is required. Since the performance of individual combinations of equipment is critically dependent on a number of geometrical factors, the required performance of the combination of electron microscope and X-ray analyzer is specified in terms of the measured X-ray intensity from a small diameter fiber, using a known electron beam diameter. X-ray detectors are generally least sensitive in the low energy region, and so measurement of sodium in crocidolite is selected as the performance criterion. The combination of electron microscope and X-ray analyzer must yield a background-subtracted NaKα peak integral count rate of more than 1 count per second (cps) from a 50 nm diameter fiber of UICC crocidolite irradiated by a 100 nm diameter electron probe at an accelerating potential of 80 kV. The equivalent peak/background ratio should exceed 1.0.

The EDXA equipment must provide the means for subtraction of the background, identification of elemental peaks, and calculation of net peak areas.

4.2.3 Computer

Many repetitive numerical calculations are necessary, and these can be performed conveniently by relatively simple computer programs. For analyses of zone axis diffraction pattern measurements, a computer facility with minimum available memory of 64K words is required to accommodate

the more complex programs involved. Suggested program listings for standardized data reporting and fiber identification routines are included as part of this analytical procedure. (Appendices A and B).

4.2.4 Vacuum Evaporator

A vacuum evaporator capable of producing a vacuum better than 10^{-4} Torr (0.013 Pa) is required for vacuum deposition of carbon on to the polycarbonate filters. A sample holder is desirable which allows a 51 x 75 mm glass microscope slide to be tilted and rotated during the coating procedure. Use of a liquid nitrogen cold trap above the diffusion pump will minimize the possibility of contamination of the filter surfaces by oil from the pumping system. The vacuum evaporator may also be used for deposition of the thin film of gold, or other reference material, required on electron microscope samples for calibration of electron diffraction patterns. For gold deposition, a sputter coater may allow better control of the process, and is therefore recommended.

4.2.5 Ozone Generator

An ozone generator, in combination with ultraviolet light irradiation, is used for the oxidation of organic material in water samples. This procedure is necessary on all water samples. The generator should be capable of generating at least 400 g of ozone per day at a concentration of at least 1% by weight when supplied with dry oxygen. The ozone generator Model GL-1 (PCI Ozone Corporation, 1 Fairfield Crescent, West Caldwell, New Jersey 07006) or equivalent has been found to meet the requirements of this analytical technique.

4.3 Apparatus, Supplies and Reagents

4.3.1 Gas Supply to Ozone Generator

The ozone generator can be supplied by either compressed air or oxygen. The input gas must be regulated to the pressure specified by the generator manufacturer. It is recommended that oxygen be provided in order to reduce the possibility of acid formation in the sample.

4.3.2 Gas-Line Drying Tube

The ozone generator operates more efficiently when supplied with dry oxygen. An in-line drying tube, filled with a desiccant, followed by a 0.2 μm pore size polytetrafluoroethylene filter to prevent particulate from the desiccant entering the ozone generator is recommended.

A stainless steel pressure filtration assembly (Millipore Corporation, Bedford MA 01730, Cat. No. XX40 047 00) with a 0.2 μm pore size Fluoropore® filter (Millipore Corporation, Cat. No. FGLP 047 00) in the normal filter position and silica gel in the reservoir have been found to be satisfactory for this purpose.

4.3.3 In-Line Gas Filtration Assembly

A filter is placed in the ozone line immediately before the gas enters the sample. A 25 mm stainless steel gas line filter holder (Millipore Corporation, Cat. No. XX40 025 00) or equivalent with a 0.2 um pore size Fluoropore filter (Millipore Corporation, Cat. No. FGLP 025 00) or equivalent is used in each ozone supply line to ensure that the ozone entering the sample is particle-free.

4.3.4 Ultraviolet Lamp

A submersible short wavelength (254 nm) ultraviolet lamp is required for the ozone-UV oxidation treatment of water samples. A 6 inch Pen-Ray® ultraviolet lamp (Part No. 90-0004-11) and power supply model SCT-4 (Ultra-Violet Products Inc., 5100 Walnut Grove Avenue, San Gabriel, California 91778) or equivalent have been found to meet the requirements of this analytical technique.

4.3.5 Source of Known Fiber-Free Water

For blank determinations, final washing of analytical equipment, and dilution of some samples, a source of water which is free of both particles and fibers is required. Fresh double-distilled water from a glass distillation apparatus (MEGA-PURE™ manufactured by Corning and available from all authorized Corning Laboratory Supply Dealers) or equivalent is preferable, and has been found to meet this requirement. De-ionized water, filtered through a 0.1 um pore size Nuclepore polycarbonate filter has also been found to be satisfactory, but the filtration assembly itself tends to contribute some particles to the filtrate.

4.3.6 Filtration Apparatus

The water sample is filtered through a membrane filter of either 47 mm diameter or 25 mm diameter. The filtration assembly should be chosen to suit the size of filter in use. A glass frit support is required in order to obtain a uniform deposit on the filter. The reservoir must be easily cleaned in order to prevent sample cross-contamination. A 47 mm analytical filter holder (Millipore Corporation, Cat. no. XX10 047 00) or a 25 mm analytical filter holder (Millipore Corporation, Cat. No. XX10 025 00)

or equivalent has been found to be suitable. When using the larger diameter equipment it is necessary to filter proportionately larger volumes of water.

4.3.7 Filtration Manifold

When a number of samples are to be filtered, several filtration units can be operated simultaneously from a single vacuum source by using a multiple port filtration manifold (Millipore Corporation, Cat. No. XX26 047 35) or equivalent. The manifold should include valves to permit each port to be opened or closed independently.

4.3.8 Vacuum Pump

A pump is required to provide a vacuum of 20 kPa for the filtration of water samples. A water jet pump (Edwards High Vacuum Inc., Grand Island, NY 14072, Cat. No. 01-C046-01-000-female connection or 01-C039-01-000-male connection) or equivalent has been found to provide sufficient vacuum for a 3-port filtration manifold and also incorporates a non-return valve to prevent back-streaming.

4.3.9 Membrane Filters

The diameters of the membrane filters should be matched to the diameters of the filtration apparatus in use. For filtration of water samples, two types of filters are required:

- polycarbonate capillary-pore membrane filters, 0.1 µm pore size (Nuclepore Corporation, 7035 Commerce Circle, Pleasanton, California 94566) or equivalent, are used to collect the suspended material from a water sample.

- mixed esters of cellulose membrane filters, 0.45 µm pore size Type HA (Millipore Corporation, Bedford, MA 01730) or equivalent, are used as a support filter placed between the glass frit of the filtration apparatus and the polycarbonate filter.

4.3.10 Jaffe Washer

A Jaffe Washer is used for dissolution of Nuclepore filters. Several designs of Jaffe Washer have been used which are modifications of the original design. Provided that the polycarbonate filter can be completely dissolved, and that the materials used in the different designs of washer are demonstrably free of mineral fiber contamination, the precise design is not considered

important. Because of recent changes in the formulation of Nuclepore polycarbonate filters which have degraded their solubility in chloroform, a more complex dissolution procedure may be required. The additional steps in the preparation are more easily completed if the original washer design is followed. This original design is illustrated in Figure 5A. Figure 5B shows samples being placed on a Jaffe Washer of this design. Alternatively, methylene chloride may be substituted for chloroform, but because this has a higher vapor pressure it is then necessary to ensure that the Jaffe Washer is tightly sealed to avoid excessive evaporation.

4.3.11 Condensation Washer

A condensation washer may be useful if TEM specimens are required more quickly than is possible if the Jaffe Washer is used alone to dissolve some batches of Nuclepore polycarbonate filters. A condensation washer consists of a system with controlled heating, controlled refluxing, and a cold finger for holding the electron microscope sample grids. Figure 6 shows one model of the condensation washer (Cat. No. 16950, Ladd Research Industries, Inc., P.O. Box 901, Burlington, Vermont 05401) which has been found satisfactory.

4.3.12 Electron Microscope Grids

Specimen grids of 200 mesh and 3 mm diameter are required in both copper and gold. The grid openings should be approximately 80 μm square. The fiber count result obtained is proportional to the mean area of the openings examined. Therefore, it is important that an accurate measurement of the dimensions of each grid opening can be obtained. Since there is a wide range of quality in the available copper specimen grids, these should be examined carefully to establish the degree of uniformity of both the grid openings and the grid bars. Copper specimen grids Cat. No. SPI #3020C and 3020T, SPI Supplies Division of Structure Probe, Inc., P.O. Box 342, West Chester, PA 19380, or equivalent, have been found to meet the requirements. In addition, these grids have a mark at the center opening. This reference can be used to indicate the location of openings which have been examined. Alternatively, finder grids may be substituted if re-examination of specific grid openings is to be required. Gold specimen grids Cat. No. 21612, Ernest F. Fullam, Inc., P.O. Box 444, Schenectady, N.Y. 12301, or equivalent, have been found to meet the requirements for gold grids.

4.3.13 Ultrasonic Bath

An ultrasonic bath is required for dispersing particulate in sample containers and for general cleaning of equipment. The size of unit selected is unimportant, and should be related to the volume of work in progress. Bransonic Model B-52 (Branson Cleaning Equipment Company, Parrott Drive, Shelton Connecticut 06484) has a power of 200 watts at a frequency of 50 kHz and has been found to meet the requirements.

4.3.14 Carbon Rod Electrodes

Spectrochemically pure carbon rods are required for use in the vacuum evaporator during carbon coating of filters. Type AGKSP, National Spectroscopic Electrodes, manufactured by Union Carbide, or equivalent, have been found to meet the requirements.

4.3.15 Carbon Rod Sharpener

This device is used to sharpen the carbon rods to a neck of 3.6 mm long and 1.0 mm diameter. The use of necked rods, or equivalent, allows the carbon layer to be applied with a minimum of heating of the polycarbonate membrane. The sharpener, Cat. No. 1204, Ernest F. Fullam, Inc., Schenectady, N.Y. 12301, or equivalent, meets the requirements.

4.3.16 Standards

a) Reference Standard Fiber Suspensions. Glass ampoules of stable concentrated chrysotile or amphibole fiber dispersions, (Electron Optical Laboratory, Ontario Research Foundation, Sheridan Park, Mississauga, Ontario, Canada L5K 1B3) can be used to establish quality assurance in analytical programs. The reference suspensions of known mass and numerical fiber concentrations are used to generate control samples for inclusion in analytical programs.

b) Reference Silicate Mineral Standards on TEM Grids. For calibration of the EDXA system, reference silicate mineral standards are required (Electron Optical Laboratory, Ontario Research Foundation, Sheridan Park, Mississauga, Ontario, Canada L5K 1B3).

c) Asbestos Bulk Material. Chrysotile (Canadian), Chrysotile (Rhodesian), Crocidolite, Amosite. UICC (Union Internationale Contre le Cancer) Standards. Available from Duke Standards Company, 445 Sherman Avenue, Palo Alto, CA 94306.

4.3.17 Carbon Grating Replica

A carbon grating replica with about 2000 parallel lines per mm (Cat. No. 10020, Ernest F. Fullam, Inc., Schenectady, N.Y. 12301) or equivalent is required for calibration of the magnification of the TEM.

4.3.18 Chloroform

Spectrograde chloroform, distilled in glass (preserved with 1% (v/v) ethanol, Burdick & Jackson Laboratories Inc., Muskegon, Michigan 49442) or equivalent, is required for the dissolution of the polycarbonate filters.

4.3.19 Petri Dishes

Disposable plastic petri dishes (Millipore Corp. Cat. No. PD 10 047 00) or equivalent, are useful for storage of sample filters and specimen grids. If charge build-up on these dishes is experienced, it has been found that rinsing them with a weak detergent solution will reduce the problem.

4.3.20 Quartz Pipets

Quartz pipets are used to bubble ozone through the liquid sample. These pipets are formed by heating quartz tubing and drawing it to a tip of approximately 0.35 mm inside diameter. The pipet should be sufficiently long to reach within 1 inch of the bottom of the sample bottle, to create good mixing of the liquid during oxidation.

4.3.21 Mercuric Chloride Solution

A 0.01 molar solution of mercuric chloride may be required for preservation of water samples. This is prepared by dissolving 2.71 g of reagent grade mercuric chloride in 100 mL of fiber-free water. The solution is then filtered twice through the same 0.1 um pore size Nuclepore filter, using the filtration apparatus described in Section 4.3.6 and a conventional filtration flask.

4.3.22 Routine Electron Microscopy Preparation Supplies

Electron microscopy preparation supplies such as scalpels, disposable scalpel blades (curved cutting edge), double-sided adhesive tape, sharp point tweezers and specimen scissors are required. These items are available from most EM supply houses.

4.3.23 Routine Laboratory Supplies

Routine laboratory supplies and labware are required. The general supplies include a detergent for cleaning apparatus, marking pens for labelling glass and plastic apparatus, glass microscope slides, lens paper (for preparation of Jaffe Washer and lining of TEM grid storage dishes), lint free tissues. General labware includes such items as graduated cylinders, beakers of several sizes, pipets. Whenever possible, disposable plastic labware is recommended to avoid the problems of contamination from new glassware and cross-contamination between samples.

5. SAMPLE COLLECTION AND PRESERVATION

5.1 Sample Container

The sample container will be an unused, pre-cleaned, screw-capped bottle of glass or low density (conventional) polyethylene and capable of holding at least 1 liter. It is recommended that the use of polypropylene bottles be avoided since problems of particulate being released into water samples have been observed.

Ideally, water samples are best collected in glass bottles. However, glass can have significant levels of asbestos on the surfaces and therefore requires careful cleaning before use. Glass is also difficult to ship because of possible breakage through drooping or freezing. Because of these disadvantages, polyethylene bottles are more convenient to use and therefore are recommended.

The bottles should first be rinsed twice by filling approximately one third full with fiber-free water and shaking vigorously for 30 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.

It is recommended that blank determinations be made on the bottles before sample collection. The following method has been found satisfactory for these determinations. A pre-washed bottle containing approximately 800 milliliters of fiber-free water is processed as described for preparation of samples, including ozone-UV and ultrasonic treatments. When using polyethylene bottles, 1 bottle in each batch or a minimum of 1 bottle in each 24 is tested for background level. When using glass bottles, the risk of asbestos contamination from the bottle is greater and a minimum of 4 bottles in each 24 are examined for background level. Additional blanks may be desirable when sampling waters suspected of containing very low levels of asbestos, or when additional confidence in the bottle blanks is desired.

5.2 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. However, some specific considerations apply to asbestos fibers because they are a special type of particulate matter. These fibers are small, and in water range in length from 0.1 μm to 20 μm or more.

Because of the range of sizes there may be a vertical distribution of particle sizes in large bodies of water. This distribution may vary with depth depending upon the vertical distribution of temperature, the water current pattern and the local meteorological conditions. Sampling should take place according to the objective of the analysis. If a representative sample of a water supply is required, a carefully designated set of samples should be taken representing the vertical as well as the horizontal distribution and these samples should be composited for analysis.

When sampling from a faucet, remove all hoses or fittings and allow the water to run to waste for a sufficiently long period to ensure that the sample collected is representative of fresh water. Faucets or valves should not be adjusted until all samples have been collected. If possible, sampling at hydrants and at the ends of distribution systems should be avoided.

As an additional precaution against contamination, before collection of the sample, each bottle may be rinsed several times in the source water being sampled. In the case of depth sampling in bodies of water, this rinsing may compromise the results and should be omitted.

5.3 Quantity of Sample

Two separate samples of approximately 800 milliliters each are required. An air space must be left in the bottle to allow efficient redispersal of settled material before analysis. The second bottle is stored for analysis if confirmation of the results obtained from the analysis of the first bottle is required.

5.4 Sample Preservation and Storage

Samples must be transported to the analytical laboratory as soon as possible after collection. No preservatives should be added during sampling; the addition of acids should be particularly avoided.

If the sample cannot be given ozone-UV treatment and filtered within 48 hours after arrival at the analytical laboratory, amounts (1 milliliter per liter of sample) of a pre-filtered 2.71% solution of mercuric chloride sufficient to give a final concentration of 20 ppm of mercury may be added, to prevent bacterial growth. Appropriate care should be taken when handling mercury compounds.

At all times after collection, it is recommended that the samples should be stored in the dark and refrigerated at about 5°C in order to minimize bacterial and algal growth. The samples should not be allowed to freeze, since the effects on asbestos fiber dispersions are not known.

Before the sample bottles are opened, the exterior surfaces should be thoroughly washed and then rinsed in fiber-free water to avoid inadvertent contamination of the sample by material which may be attached to the bottles.

6. PROCEDURE

6.1 Cleanliness and Contamination Control

It is most important that all glassware and apparatus be cleaned thoroughly in order to minimize the possibility of specimen contamination. All phases of the specimen preparation should be conducted in the clean room facilities or in a laminar flow hood. Glassware should be cleaned in an ultrasonic bath using a detergent solution. After this, it should be rinsed three times using fiber-free water. After drying, equipment should be stored in clean containers and covered using aluminum foil or parafilm. All glassware must be washed by the above procedure before each use.

6.2 Oxidation of Organics

Oxidation of the high molecular weight organic components in water samples prior to filtration has been found necessary if precise results are to be obtained. Asbestos fibers have an affinity for these organic materials. Three separate effects have been identified which result from this affinity and which give rise to serious errors if this oxidation is not carried out:

a) asbestos fibers associated with organic materials tend to adhere to the container walls;

b) asbestos fibers tend to aggregate with organic materials;

c) fibers embedded in organic material are not transferred to the TEM specimen.

All three effects give rise to low results. Before sub-samples are taken from the bottle it is necessary to ensure that all the particulate material is in suspension. The organic material and associated fibers must be released from the container walls. This can be achieved by treating the water sample in the original collection container using the ozone-ultraviolet (ozone-UV) technique to oxidize the organic materials. However, if a sample is known to be free of organic interferences the ozone-UV oxidation may not be required.

The equipment should be assembled as shown in Figures 2 and 3.

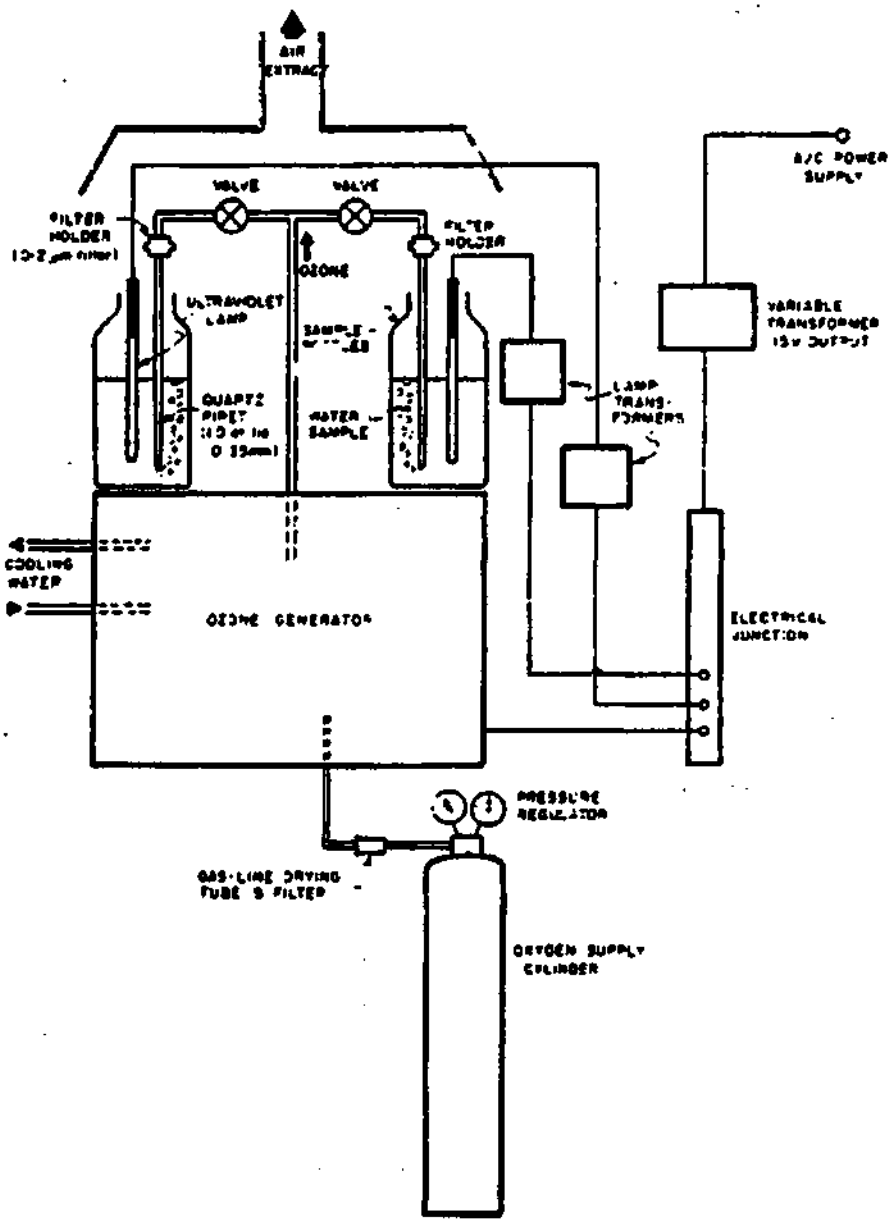


Figure 2. Diagram of Ozone-UV Equipment.

Figure 3. Ozone-UV Oxidation of Water Samples in Glass Bottles. The ozone supply line has been split into two lines to permit simultaneous oxidation of two samples. A valve and a filter holder are incorporated in each of the supply lines to the samples.

An air extract to remove surplus ozone is required. If it is necessary to check that the ozone generator is functioning within the specifications, the output can be verified by normal chemical methods. A suitable technique is to bubble the ozone through a solution of potassium iodide and to titrate the displaced iodine with sodium thiosulfate solution, using starch as an indicator.

Before the ozone-UV treatment, place each polyethylene or glass bottle containing the water sample in the ultrasonic bath for a period of 15 minutes. Mark the level of the liquid in the sample bottle using a waterproof felt marker. The quartz pipets should be thoroughly washed before each use, and installed on the ozone supply as indicated so that the tip is close to the bottom of the sample bottle. The UV lamp is also thoroughly washed and then immersed in the sample and switched on.

At an ozone concentration of 4% in oxygen, treat each sample with about 1 liter/minute of gas for approximately 3 hours. At other ozone concentrations, adjust the oxidation time so that each sample receives about 10 grams of ozone. The gas flow rate should be

sufficient to produce a mixing action in the liquid but should not splash sample out of the container. It is not easy to indicate when oxidation is complete, but this treatment as described has been found to be adequate for all water samples so far handled. When oxidation is complete, remove the UV lamp and quartz pipet, re-cap the bottle and place it in the ultrasonic bath for a period of 15 minutes. This allows particulate released from the oxidized organic materials and the container surfaces to be uniformly dispersed throughout the sample.

The water level in the bottle may have fallen, due to evaporation during the oxidation procedure. The loss of volume should be noted and can be accounted for if it is significant. The sample should be filtered immediately after it is removed from the ultrasonic bath.

6.3 Filtration

6.3.1 General

The separation of suspended particulate by filtration of the sample through a membrane filter is a critical step in the analytical procedure. The objective is to produce a Nuclepore filter on which the suspended solids from the sample are distributed uniformly, with a minimum of overlapping of particles. The volume to be filtered depends on the diameter of the filtration equipment in use, the total suspended solids content of the sample, and in some samples the volume depends on the fiber concentration present.

Table 1 shows the limitation of the analytical sensitivity as a function of the volume of water filtered. In practice, it is usually found that the concentration of suspended solids limits the filtration volume. The maximum particulate loading on the filter which can be tolerated is about 20 $\mu g/cm^2$, with an optimum value of about 5 $\mu g/cm^2$. Where the concentration of suspended solids is known, the maximum volume which can be used may be estimated. Usually, however, nothing is known about the sample and the best procedure is to prepare several filters using different volumes of the sample. It has been found that suitable filter samples display a faint coloration of the surface, and with experience over-loaded filters usually can be recognized. The determination of a suitable volume to filter is usually a matter of trial and error in the analysis of samples of relatively low total suspended solids but high asbestos concentration.

No attempt should be made to filter sample volumes less than 10 mL for 25 mm diameter equipment, and 50 mL for 47 mm diameter equipment. If smaller volumes are filtered

TABLE 1. LIMITATION OF ANALYTICAL SENSITIVITY BY VOLUME OF WATER SAMPLE FILTERED

Volume Filtered (mL)		Analytical Sensitivity[1] (Fibers/Liter)
Using 25 mm Diameter Filter[2]	Using 47 mm Diameter Filter[3]	
0.1	0.6	1.5×10^7
0.5	2.8	3.0×10^6
1.0	5.7	1.5×10^6
2.0	11	0.8×10^6
5.0	28	3.0×10^5
10	57	1.5×10^5
25	142	6.0×10^4
50	285	3.0×10^4
100	570	1.5×10^4

[1]Concentration corresponding to 1 fiber detected in 20 grid openings of nominal 200 mesh grid (approximately 80 μm square grid openings)

[2]Assuming Active Filter Area of 1.99 cm^2

[3]Assuming Active Filter Area of 11.34 cm^2

It is difficult to ensure that a uniform deposit of particulate will be obtained on the filter. Samples of high solids content, or of high fiber content, may require filtration of volumes less than these. Such samples should be diluted with fiber-free water so that the volumes filtered exceed the minima specified. Dilutions should be made by transferring a known volume of the sample to a disposable plastic beaker and making up to a known volume with fiber-free water. The mixture should be stirred vigorously before sub-sampling takes place.

6.3.2 Filtration Procedure

a) The sample must be filtered immediately after the ozone-UV and ultrasonic bath treatment. If for any reason the sample has been stored for more than a few hours after these treatments, it is recommended that ozone-UV oxidation be repeated for a short period of about 15 minutes, followed by an additional 15 minutes in the ultrasonic bath.

b) Assemble the filtration base and turn on the vacuum. The upper surface of the filtration base (both the glass frit and the ground mating surface) must be dry before the membrane filters are installed. Place a 0.45 um pore size type HA Millipore filter on the glass frit. If the filter appears to become wet by capillary action on residual water in the glass frit it must be discarded and replaced by another filter. Place a 0.1 um pore size Nuclepore filter, shiny side up, on top of the Millipore filter. If the Nuclepore filter becomes folded it must be discarded and replaced. The mating surface of the reservoir component of the filtration apparatus (the funnel) should be dried by shaking off any surplus water and draining on paper towel or tissue. The funnel should be positioned on the filters and firmly clamped, taking care not to disturb the filters. The vacuum should not be released until the filtration has been completed.

It is necessary to comment on the use of filtration equipment which is still wet after washing, since improper procedures at this point can very seriously compromise the results. If the glass frit is wet when the Millipore filter is applied to it, capillary action will result in some areas of the Millipore filter structure being filled by water. When the Nuclepore filter is applied to the surface of the Millipore filter

and the vacuum is applied, the differential pressure across the Millipore filter will be insufficient to overcome the surface tension of the water in the filled areas. Thus no filtration will take place through the corresponding areas of the Nuclepore filter, and a grossly non-uniform deposit of particulate will be obtained.

c) Add the required volume of sample water to the filtration funnel. Disposable plastic beakers and pipets provide a means of measuring the required sample volume without introducing problems of sample cross-contamination. The reservoir may not be sufficiently large to accommodate the total volume to be filtered. In this case more of the sample may be added during the filtration, but this should be done carefully and only when the reservoir is more than half full. In this way the addition will not disturb or affect the uniformity of particulate already deposited on the Nuclepore filter. Do not rinse the sides of the funnel, and avoid other manipulations which may disturb the particulate deposit on the filter.

d) Disassemble the filtration unit, and transfer the Nuclepore filter to a labelled, clean petri dish. Since the Nuclepore filters are more easily handled while they are still wet, it is recommended that the strip of filter to be used for TEM sample preparation should be cut as described in Section 6.4.2 before the filter is dried. Place the cover loosely over the dish to limit any deposition of dust onto the filter. Dry the filter under an infra-red heat lamp for a short time before closing the petri dish completely. Discard the Millipore filter.

6.4 Preparation of Electron Microscope Grids

Preparation of the grid for examination in the electron microscope requires a high degree of manual dexterity and is a critical step in the procedure. The objective is to replicate the filter surface by deposition of a carbon film and then to dissolve away the filter itself with a minimum of particle movement and breakage of the carbon film. The filter dissolution procedure is illustrated in Figure 4.

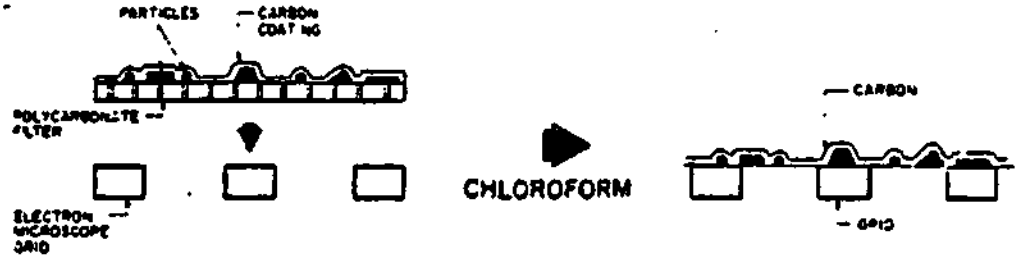


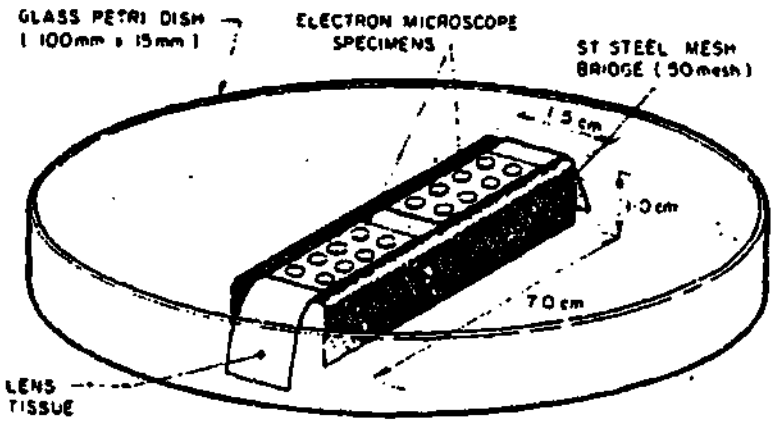
Figure 4. Nuclepore Dissolution Technique

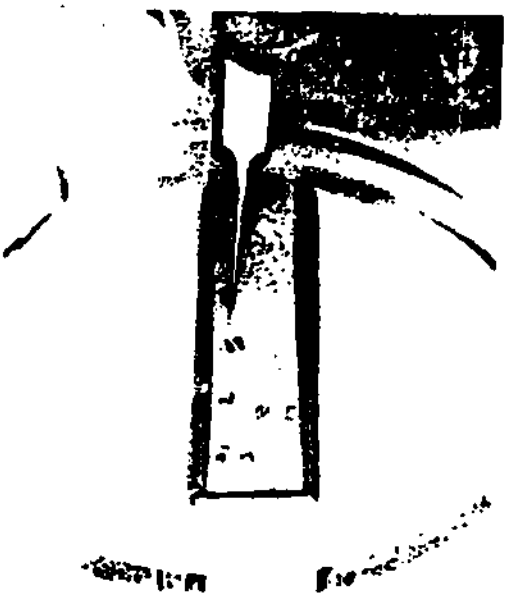
6.4.1 Preparation of Jaffe Washer

Prepare the Jaffe Washer as illustrated in Figure 5A. The stainless steel mesh is formed into a bridge slightly less than 1 cm high, and placed in a 10 cm diameter glass petri dish with a tight fitting lid. A narrow strip of lens cleaning tissue is placed over the bridge with each end of the tissue extending beyond the bridge to the base of the petri dish. The other dimensions of the stainless steel bridge and the length of the lens tissue are not critical, but those specified in Figure 5A have been found to be satisfactory. After the assembly is complete, fill the petri dish with chloroform to a level just below that of the horizontal surface of the stainless steel bridge. It may be found that the chloroform contacts the underside surface of the stainless steel mesh; this is not critical. Cover the petri dish with the lid and the Jaffe Washer is ready for use. Each time the Jaffe Washer is used, the lens tissue and solvent should be discarded and replaced with new lens tissue and fresh solvent. Appropriate precautions should be taken when handling chloroform.

6.4.2 Selection of Filter Area for Carbon Coating

Polycarbonate filters are easily stretched during handling, and cutting of areas for further preparation must be performed with great care. The best method is to use a curved edge scalpel blade to cut the filter while it is in


GLASS PETRI DISH
(100mm x 15mm)
ELECTRON MICROSCOPE
SPECIMENS
ST STEEL MESH
BRIDGE (50mesh)
~1.5cm
1.0cm
7.0cm
LENS
TISSUE



the plastic petri dish. Press the scalpel point on the filter at the beginning of the desired cut, and rock the blade downwards while maintaining pressure. It will be found that a clean cut is obtained without stressing of the filter. The process should be repeated along all four directions to remove a rectangular portion from the active filtration area of the filter. This filter portion should be selected from along a diameter of the filter, and should be about 3 mm wide by a minimum of 15 mm long. Areas close to the perimeter of the active filtration area should be avoided.

6.4.3 Carbon Coating of the Nuclepore Filter

The ends of the selected filter strips should be attached to a glass microscope slide using double-sided adhesive tape. This must be performed carefully to ensure that the filter strips lie flat on the slide and are not stretched. The filter strips can be identified by using a wax pencil on the glass slide. After inserting the necked carbon rods into the vacuum evaporator, place the glass slide on the sample rotation and tilting device. The separation between the sample and the tips of the carbon rods should be about 7.5 cm to 10 cm.

If desired, the amount of carbon to be evaporated can be monitored instrumentally so that a thickness of about 30 nm to 50 nm is deposited on the filter strips. Alternatively, a porcelain fragment will serve as a simple carbon deposition monitor. Place a small drop of silicone diffusion pump oil on the surface of a clean fragment of white glazed porcelain. Locate the porcelain in the evaporation chamber with the oil droplet towards the carbon rods and at a distance from the carbon rods equal to that separating the rods from the filter strips. Carbon will not deposit on the oil drop whereas it does on the other areas of the porcelain. With experience, the correct thickness can be monitored visually by observation of the contrast between the darkened areas of the porcelain and the uncoated areas under the oil drop.

Pump down the evaporation chamber to a vacuum better than 10^{-4} Torr (0.013 Pa). Use of a liquid nitrogen cold trap above the diffusion pump will minimize the possibility of contamination of the filter surfaces by oil from the pumping system. Continuously rotate and tilt the glass slide holding the filter strips, while the carbon is evaporated in intermittent bursts, allowing the rods to cool between each evaporation. This procedure is necessary to avoid overheating of the filter strips. Overheating tends to cross-link the polycarbonate which then becomes difficult to dissolve in chloroform.

6.4.4 Transfer of the Filter to Electron Microscope Grids

Remove the glass slide carrying the filter strips from the evaporator, and using the technique described in 6.4.2 cut four pieces slightly less than about 3 mm x 3 mm in size from each filter strip. The square of filter should fit within the circumference of an electron microscope grid. Three of the filter pieces are to be prepared on 200 mesh copper grids, and unless the analysis is to be for chrysotile only, a fourth piece should be prepared on a 200 mesh gold grid. The specimens prepared on copper grids are used for fiber counting and most EDXA examinations. The preparation on the gold grid is intended for EDXA work on fibers containing sodium.

Place a piece of the carbon-coated filter, carbon side up, on to the shiny side of an electron microscope grid. Using fine tweezers, pick up the grid and filter together and place quickly on to the chloroform-saturated lens tissue in the Jaffe Washer, as shown in Figure 5B. It is important that the sample be placed on the lens tissue quickly, since hesitation while the sample is exposed to chloroform vapor will cause it to curl. This is a simplified technique which does not involve dropping of chloroform on to the samples.

Some components of the polycarbonate filters now available dissolve in chloroform only very slowly. Consequently, the grids must be left in the Jaffe Washer for longer than 4 days, and the solvent must be replaced every day. Depending on the particular lot number of the filters, even this period may be insufficient to yield satisfactory grids clear of undissolved plastic. In this event, or if a more rapid sample preparation is desired, after a minimum period of 30 minutes in the Jaffe Washer the lens paper supporting the grids may be transferred to the condensation washer as illustrated in Figure 6. The condensation washer should then be operated for a period of between 30 and 60 minutes, after which the grids will have been cleared of residual plastic. The rate of condensation in the washer is not critical, provided that chloroform drips rapidly from the cold finger for the whole of the washing period and the condensation level is above the samples.

During the dissolution, it is recommended that the grids not be allowed to dry since this has been found to greatly increase the time required for complete dissolution of the polycarbonate.

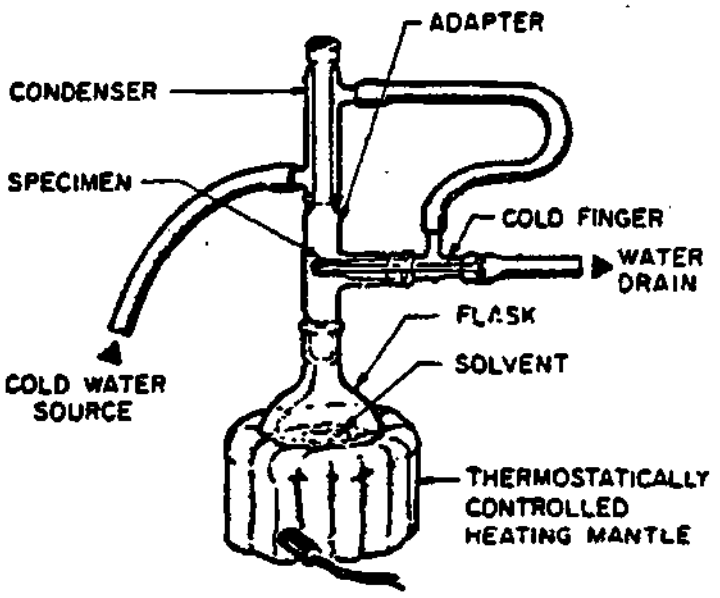


Figure 6. Condensation Washer.

6.5 Examination by Electron Microscopy

6.5.1 Microscope Alignment and Magnification Calibration

Align the electron microscope according to the specifications of the manufacturer. Initially, and at regular intervals, carry out a calibration of the two magnifications used for the analysis (approximately 20,000 and 2,000) using a diffraction grating replica. The calibration should always be repeated after any instrumental maintenance or change of operating conditions. The magnification of the screen image is not the same as that obtained on photographic plates or film. The ratio between these is usually a constant value for the instrument. It is most important that before the magnification calibration is carried out the sample height is adjusted so that the sample is in the eucentric position.

6.5.2 Calibration of EDXA System

The purpose of the calibration is to enable quantitative composition data, at an accuracy of about 10% of the elemental concentration, to be obtained from EDXA spectra of silicate minerals involving the elements sodium, magnesium, aluminum, silicon, potassium, calcium, manganese and iron. If quantitative determinations are required for minerals containing other elements, suitable calibration information may be incorporated in the computer analysis. The well-characterized standards recommended permit calibration of any TEM-EDXA combination which meets the instrumental specifications of Section 4.2, so that data from different instruments can be compared. The standards used for calibration, and the elements which they represent, are shown in Table 2.

TABLE 2. SILICATE MINERAL STANDARDS

Elements	Mineral Standard
Na, Fe, Si	Riebeckite
Mg, Si	Chrysotile
Al, Si	Halloysite
K, Si	Phlogopite
Ca, Si	Wollastonite
Mn, Si	Bustamite

The compositions of these standards have been determined by microprobe analysis, and the TEM grids were prepared from fragments of the same selected mineral specimens. They permit the computer program of Appendix A to be used with any TEM-EDXA system.

Place the first grid into the microscope, form an image at the calibrated higher magnification of about 20,000, and adjust the specimen height to the eucentric point. Tilt the specimen towards the X-ray detector as required by the instrument geometry. Select an isolated fiber or particle less than 0.5 um in width, and accumulate an EDXA spectrum using an electron probe of suitable diameter. When a well defined spectrum has been obtained, perform an appropriate background subtraction and obtain the net peak areas for each element listed, using energy windows centered on the

peaks and about 130 eV wide. Compute the ratio of the peak area for each specified element relative to the peak area for silicon. Repeat the procedure for about 20 particles of each mineral standard. Analyses of any obvious foreign particles should be rejected, and the data from any one standard should be reasonably self-consistent. Calculate the arithmetic mean peak area ratios for each specified element of each mineral standard. These values are required initially as input for the fiber identification program, and apart from occasional routine checks to ensure that there has been no degradation of the detector resolution, the calibration need not be repeated unless there has been a change of instrumental operating conditions.

6.5.3 Grid Preparation Acceptability

Insert the specimen grid into the electron microscope and adjust the magnification to a value sufficiently low (300 - 1000) so that complete grid openings can be inspected. Examine at least 10 grid openings to evaluate the fiber and total particulate loadings, the uniformity of the particulate deposit, and the extent to which the carbon film is unbroken. The grid must be rejected from further analysis if:

a) the grid is too heavily loaded with fibers to perform an accurate count. Accurate counts cannot be performed if the grid has more than about 50 fibers per grid opening. A new grid preparation must be made using either a smaller volume of water or a suitable volume of the water diluted with fiber-free water;

b) the overall distribution of the deposited debris is noticeably non-uniform. A new grid preparation must be made, paying particular attention to proper particulate dispersal and filtration procedures;

c) the grid is too heavily loaded with debris to allow examination of individual particles by SAED and EDXA. A new grid preparation must be made using either a smaller volume of water or a dilution of the original water sample;

d) a large proportion of the grid openings have broken carbon film. Since the breakage is usually more frequent in areas of heavy deposit, counting of the intact openings could lead to biased results. Therefore, a new grid preparation must be made from a more completely dispersed sample, a reduced volume of sample, or alternatively, a thicker carbon film may be necessary to support the larger particles.

6.5.4 Procedure for Fiber Counting

The number of fibers to be counted depends on the statistical precision desired. In the absence of fibers, the area of the electron microscope grids which must be examined depends on the analytical sensitivity required. For statistical reasons, discussed in Section 7.2, the fibers on a minimum of 4 grid openings must be counted. The precision of the fiber count depends not only on the total number of fibers counted, but also on their uniformity from one grid opening to the next. In practice, it has been found that termination of the fiber count at a minimum of 100 fibers or 20 grid openings, whichever occurs first, yields results which usually require no further refinement. Additional fiber counting will be necessary if greater precision is required.

At least three grids prepared from the filter must be used in the fiber count. Several grid openings are to be selected from each grid, and the data are all incorporated in the calculation of the results. This permits the measurements to be spread across a diameter of the original filter, so that any gross deviations from a uniform deposition of fibers should be detected.

Figures 7 and 8 show specimen fiber counting raw data sheets which represent the minimum standard of data reporting for this analytical procedure. Figure 7 shows page 1 of the raw data tabulation, which contains all specimen preparation details. Figure 8 is a continuation sheet for the fiber classification and measurement data; several of these sheets may be required for analysis of a sample.

Select a typical grid opening from one of the grids. Set the magnification to the calibrated higher value (about 20,000). Adjust the sample height until the features in the center of the screen are at the eucentric point. Check that the goniometer tilt is set at zero. Reduce the magnification to the lower calibrated value of about 2,000. Measure both dimensions of the grid opening image in millimeters, using the markings on the fluorescent screen. In columns 1 and 2 specify the sequential number of the grid opening, and its dimensions. These two columns are not used again until fiber counting is commenced in the next grid opening to be examined. Adjust the magnification to the upper calibrated value, close to 20,000, and position the grid opening so that one corner is visible on the screen. Move the image by adjustment of only one translation control, carefully examining the sample for fibers, until the opposite side of the opening is encountered. Move the image by one screen width using the

ASBESTOS ANALYSIS - WATER SAMPLE DATA

SEQ: _____ JOB: | SAMPLE: | CODE

PREP: By ____ Date - - | COUNT: By ____ Date - - | PROCESS: By ____ Date - -

INSTRUMENT: | MAGNIFICATIONS: Grid _____ Count _____

DILUTIONS: 0

1 Volume Taken (mL) _____ Final Volume (mL) _____

2 Volume Taken (mL) _____ Final Volume (mL) _____

FINAL PREPARATION FILTRATION: Vol. Filtered (mL) _____ Active Area (cm^2) _____

COMMENTS: (for inclusion in computer print-out; format in 5 lines of 50 characters)

FIBER CLASSIFICATIONS:

COUNT. NAM TM CM CO CQ CMQ CDQ UF AQ AX ADX AQ ADQ AZQ AZZ

PROCESS

FIBER TYPE	CLASSIFICATION	FIBER TYPE	CLASSIFICATION

NOTES: Preparation: _____

Examination: _____

Figure 7. Sheet for Recording Water Sample Data.

ASBESTOS ANALYSIS - FIBER CLASSIFICATION

SAMPLE:

Page ____ of ____

Grid Opening No.	Grid Opening Dimensions (mm)	Fiber Classification: NAN	TH	CH	CD	CU	CHQ	CDQ	UF	AD	AX	ADX	AQ	ADQ	AZQ	AZZ	Fiber Size: Length (mm)	Fiber Size: Width (mm)	EDXA Peak Areas	Comments
	x																			
	x																			
	x																			
	x																			
	x																			
	x																			
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Figure 8. Sheet for Recording Fiber Classification and Measurement Data.

Continued on Page ____

other translation control, and then scan the image in the reverse direction. Continue in this manner until the entire grid opening has been inspected. When a fiber is detected, classify it according to the procedures described in Section 6.7, and then insert the appropriate classification on the data sheet. Measure the length and width of the fiber image in millimeters and record these in the appropriate columns of the data sheet. Do not record fibers of obvious biological origin or diatom fragments. Continue the examination until 100 fibers have been recorded in all classification categories of interest, or until 20 grid openings have been inspected. The data should be drawn approximately equally from the three grids. In all samples, fibers on a minimum of 4 grid openings must be counted. Fibers less than 0.5 μm in length will not be incorporated in the fiber concentration calculation.

6.5.5 Estimation of Mass Concentration

If the primary objective of the analysis is to determine the mass concentration, the fiber counting should be approached in a different manner. The number of fibers which must be counted in order to achieve a reliable estimate of the mass concentration depends primarily upon the range of the fiber diameter distribution. The mass concentration measurement is most sensitive to fibers of large diameter, which unfortunately are among those which occur infrequently. When the diameter distribution is narrow, such as that found in the case of chrysotile fibrils, then the mass concentration has approximately the same precision as that of the number concentration. However, the mass concentration may be actually meaningless when calculated from a low number of fibers observed during a routine fiber count, if these fibers have a broad distribution of widths.

If the mass concentration is the primary interest, and the precision required is greater than is possible from the normal fiber count, a different approach to the fiber count must be used. Initially, establish the largest width of fiber which can be detected on the grid by a cursory survey, at a reduced magnification, of a large number of grid openings (about 50). Calculate the volume of this fiber. Adjust the magnification to a value such that a width of 1 mm on the screen corresponds to 10% of the width of the previously selected large fiber. Carry out a routine fiber count for a minimum of 100 fibers, recording only fiber images greater than 1 mm in width. Continue counting until the total volume of fibers is at least 10 times the volume of the originally selected large fiber. The precision and accuracy of this technique has not been

investigated fully, but for samples with broad width distributions it is capable of yielding significantly more precise mass determinations than are obtainable by the conventional fiber count.

The remaining problem concerns the assumption that the widths also represent the thicknesses of the fibers. Measurements of particle thicknesses can be made separately, using the shadow casting technique. Before the filter is carbon coated, apply a vacuum coating of platinum-carbon or gold to the active surface of the Nuclepore filter at an angle of 45°. In the TEM, the fibers will then display shadows on the carbon film which approximate to their thicknesses. Suitable techniques for shadowing are described in the paper by D.E. Bradley included in the Selected Bibliography.

6.6 Fiber Counting Criteria

6.6.1 Fiber Counting Method

Fiber counting with this analytical method will be performed only by the grid opening technique. If a specimen grid is too heavily loaded for examination of entire grid openings, a more reliable result is obtained by preparation of a new filter, using a smaller volume of sample.

6.6.2 Fibers Which Touch Grid Bars

A fiber which intersects a grid bar will be counted only for two sides of the grid opening, as illustrated in Figure 9. The length of the fiber will be recorded as twice the visible length. Fibers intersecting either of the other two sides will not be included in the count.

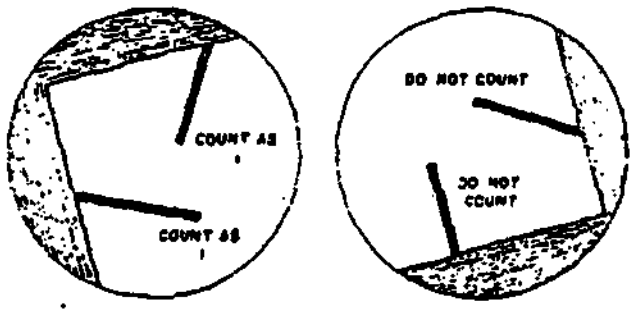

Figure 9. Counting of Fibers Which Overlap Grid Bars.

This procedure ensures that the numerical count will be accurate, and that the best average estimate of length has been made.

6.6.3 Fibers Which Extend Outside the Field of View

During scanning of a grid opening, fibers which extend outside of the field of view must be counted systematically to avoid double-counting. In general, a rule must be established so that fibers extending outside the field of view in only two quadrants are counted. Fibers without terminations in the field of view must not be counted. The procedure is illustrated by Figure 10. The length of each fiber counted is established by moving the sample, and then returning to the original field of view before scanning is continued.

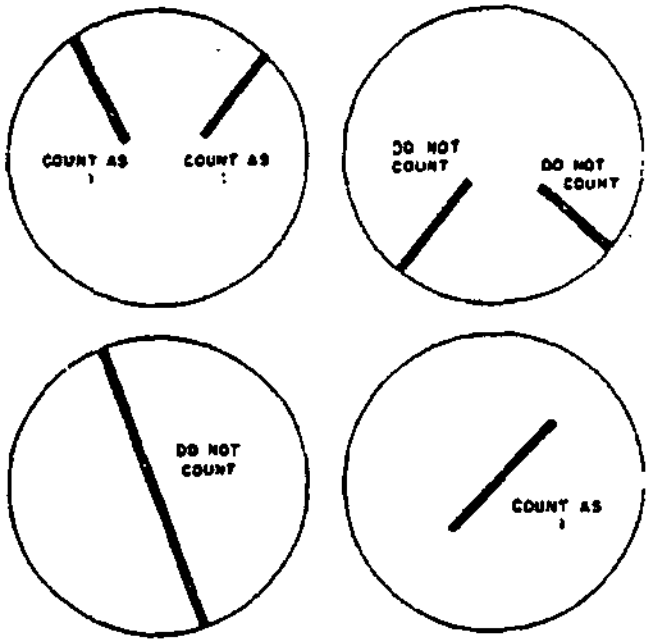

Figure 10. Counting of Fibers Which Extend Outside the Field of View.

6.6.4 Fibers with Stepped Sides

A fiber with stepped sides will be assigned a width mid-way between the minimum and maximum widths.

6.6.5 Fiber Bundles

A fiber bundle composed of many parallel fibers will be counted as a single fiber of a width equal to an estimate of the mean bundle width. Figure 11 shows examples of the procedure.

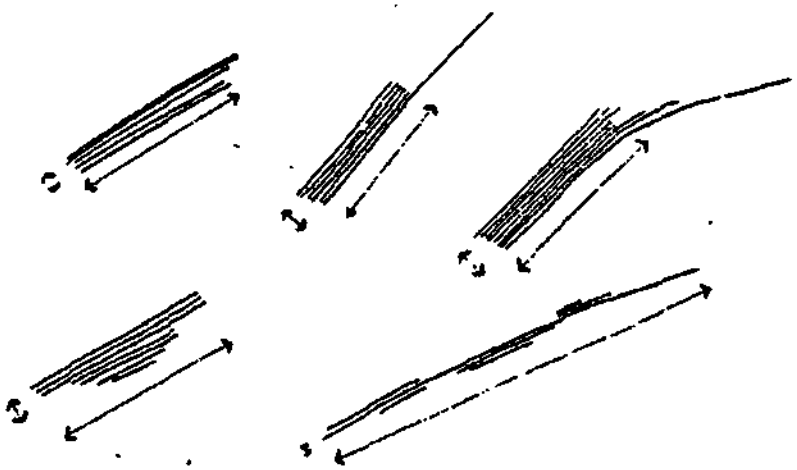

Figure 11. Counting and Measurement of Fiber Bundles. Each bundle to be counted as one fiber with dimensions as indicated by arrows.

6.6.6 Aggregates of Randomly Oriented Fibers

The structure of an aggregate of randomly oriented fibers may be sufficiently visible that the constituent fibers can be counted. This is illustrated in Figure 12. In this

Figure 12. Counting of Fiber Aggregates.

case individual fibers will be recorded. Where the fiber aggregate is too large and complex to count each individual fiber, the identification and aggregate dimensions will be recorded, but it will not be incorporated in the fiber count and mass calculations.

6.6.7 Fibers Attached to Non-Fibrous Debris

A fiber may be attached to, or partially concealed by, a particle of non-fibrous debris. If two ends are visible which appear to be the ends of a single fiber, the fiber will be counted. Where only one end of a fiber is visible, the fiber will be counted as a single fiber having a length equal to twice the 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 extension of it to the opposite side of the particle. Examples of the procedure are shown in Figure 13. There may be more than one fiber attached to a single particle of debris; each one should be counted. If an assembly of fibers and particles is too complex to treat in this way, the overall dimensions should be recorded, but the assembly should not be incorporated in the fiber count and mass calculations.

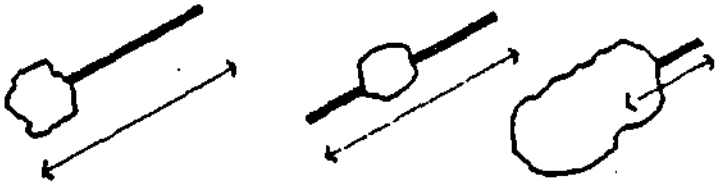

Figure 13. Counting and Measurement of Fibers Attached to Non-Fibrous Debris.

6.7 Fiber Identification Procedures

6.7.1 General

Before it is incorporated into the fiber count, each particle with an aspect ratio of 3 to 1 or greater and not of obviously biological origin must be identified according to defined criteria. It is recognized that economic considerations usually preclude unequivocal identification of every fiber reported. In this analytical method, the

requirement for unequivocal identification is limited to a small proportion of the fibers in order to demonstrate the presence of the particular species. The proportion of fibers examined for unequivocal identification will be stated in the analytical result. The remainder of the fibers are then classified on the basis of crystallographic or chemical similarity, or both, to the identified fibers. If on later examination it is considered necessary to perform a more complete and rigorous identification, additional fibers may be examined in more detail to confirm conclusions based on the fiber classification data.

In general, it will be found that for various instrumental reasons it may be impossible to identify a specific fiber completely, even though the fiber may be of a well-characterized variety. It is, nevertheless, important to record the degree to which the procedures were successful in classification or identification of a particular fiber.

6.7.2 SAED and EDXA Techniques

Fibers are initially classified into two categories on the basis of morphology: those fibers with tubular morphology, and those fibers without tubular morphology. Further analysis of each fiber is conducted using SAED and EDXA methods. Although the precise techniques and classification procedures are specified in Sections 6.7.4 and 6.7.5, some general guidance on the use of SAED and EDXA methods is given here.

The crystal structure of some mineral fibers, such as chrysotile, is easily degraded by the high current densities required for EDXA examination. Therefore, SAED investigation of these sensitive fibers must be completed before attempts are made to obtain EDXA spectra. When examining more stable fibers, such as the amphiboles, the order of work is unimportant.

The SAED technique can be either qualitative or quantitative. Qualitative SAED consists of visual examination of the pattern obtained on the microscope screen from a randomly oriented fiber. SAED patterns obtained from fibers with cylindrical symmetry, such as chrysotile, are an exception since they are not sensitive to axial tilt, and patterns from randomly oriented fibers can be interpreted quantitatively. For non-cylindrical fibers, quantitative (zone axis) SAED requires alignment of the fiber so that a principal crystallographic axis is parallel to the electron beam. The pattern is then recorded and its consistency with known mineral structures is checked by a computer program. The SAED pattern obtained from one zone axis may not be sufficiently

specific to identify the mineral fiber, but it is often possible to tilt the fiber to another angle and to record a different zone axis pattern. The angle between the two axes can also be checked for consistency with the structure of a suspected mineral.

For visual examination of the SAED pattern, the camera length of the TEM should be set to a low value and the SAED pattern then should be viewed through the binoculars. This procedure minimizes the irradiation and possible degradation of the fiber. However, the pattern is distorted by the tilt angle of the viewing screen. For recording purposes, a camera length of at least 2 meters must be used if accurate measurement of the pattern is to be possible. It is of extreme importance that, when obtaining an SAED pattern for either recording or visual evaluation, the sample height be properly adjusted to the eucentric point and the image be focussed in the plane of the selected area aperture. If this is not done there may be some components of the SAED pattern which do not originate from the selected area. It will be found in general that the smallest SAED aperture will be necessary.

For accurate measurements of the SAED pattern, an internal calibration standard is required. A thin coating of gold, or other calibration material, must be applied to the underside of the TEM specimen. This coating can be applied either by vacuum evaporation or, more conveniently, by sputtering. The polycrystalline gold film yields diffraction rings on every SAED pattern and these rings provide the required calibration information.

To form an SAED pattern, move the image of the fiber to the center of the screen and insert a suitable selected area aperture into the electron beam so that the fiber, or a portion of it, is in the illuminated area. The size of the aperture and the portion of the fiber should be such that particles other than the one to be examined are excluded from the selected area. Observe the diffraction pattern with the binocular attachment. If an incomplete diffraction pattern is obtained, move the particle around in the selected area to attempt to get a clearer diffraction pattern or to eliminate possible interferences from neighboring particles.

If a zone axis SAED analysis is to be attempted on the fiber, the sample must be in the appropriate holder. The most convenient holder allows complete rotation of the sample and single axis tilting. Rotate the sample until the fiber image indicates that the fiber is oriented with its length coincident with the tilt axis of the goniometer, and adjust the sample height until the fiber is at the

eucentric position. Tilt the fiber until a pattern appears which is a symmetrical, two dimensional array of spots. The recognition of zone axis alignment conditions requires some experience on the part of the operator. During tilting of the fiber to obtain zone axis conditions, the manner in which the intensities of the spots vary should be observed. If weak reflections occur at some points on a matrix of strong reflections, the possibility of multiple diffraction exists, and some caution should be exercised in selection of diffraction spots for measurement. A full discussion of electron diffraction and multiple diffraction can be found in the references by J.A. Gard, P.B. Hirsch et al, and H.R. Wenk, included in the Selected Bibliography. Not all zone axis patterns which can be obtained are useful or definitive. Only those which have closely-spaced reflections corresponding to low indices in at least one direction should be recorded. Patterns in which all d-spacings are less than about 0.3 nm are not useful and are usually very wasteful in computer time. A useful guideline is that the lowest angle reflections should be within the radius of the first gold diffraction ring (111), and that patterns with smaller distances between reflections are usually the most definitive.

Five spots, closest to the center spot, along two intersecting lines of the zone axis pattern must be selected for measurement, as illustrated in Figure 14.

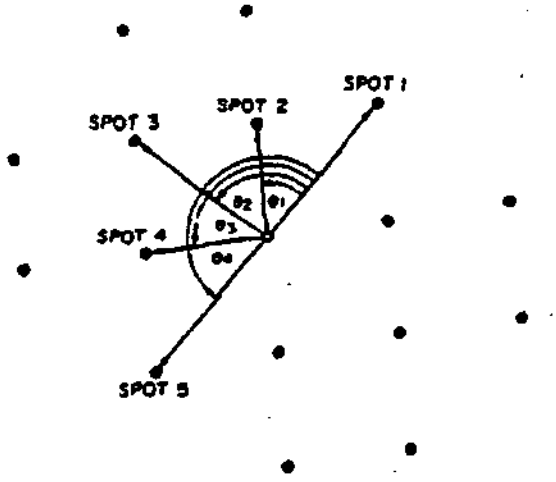

Figure 14. Measurement of Zone Axis SAED Patterns.

The distances of these spots from the center spot and the four angles shown are the input for the computer program. Since the center spot is usually very over-exposed, it does not form a suitable origin for measurement. The required distances must therefore be obtained by measuring between pairs of spots symmetrically disposed about the center spot, preferably separated by several repeat distances. The distances must be measured with a precision of better than 0.3 mm, and the angles better than 2.5°. The diameter of the first or second ring of the calibration pattern (111 and 200) must also be measured with the same precision.

The camera constant (λL) required for the computer program is given by:

$$\lambda L = \frac{aD}{\sqrt{h^2 + k^2 + l^2}}$$

where:

λ = Wavelength of the incident electrons

L = Effective camera length in mm

a = Unit cell dimension in Ångstroms

D = Diameter of the (h, k, l) diffraction rings in millimeters

h, k, l = Miller indices of the scattering plane of the crystal.

Using gold, the camera constant is given by:

λL = 2.3548 D (first ring)

λL = 2.0393 D (second ring)

Analysis of a fiber by EDXA is required in this analytical procedure. Interpretation of the EDXA spectrum may be either qualitative or quantitative. For qualitative interpretation of a spectrum, the elements originating from the fiber are recorded. For quantitative interpretation, the net peak areas, after background subtraction, are obtained for the elements originating from the fiber. As discussed in Section 6.5.2, this method provides for quantitative interpretation for those minerals which contain silicon.

To obtain an EDXA spectrum move the image of the fiber to the center of the screen and remove the objective aperture. Select an appropriate electron beam diameter and deflect the spot to impinge on the fiber. Depending on the instrumentation, it may be necessary to tilt the sample and in some instruments to use Scanning Transmission Electron Microscopy (STEM) mode of operation.

The time for acquisition of a suitable spectrum varies with the fiber diameter, and also with instrumental factors. For quantitative interpretation, spectra should have a statistically valid number of counts in each peak. Analyses of small diameter fibers which contain sodium are the most critical, since it is in the low energy range that the X-ray detector is least sensitive. Accordingly, it is necessary to acquire a spectrum for a sufficiently long period that the presence of sodium can be detected in such fibers. It has been found that satisfactory quantitative analyses can be obtained if acquisition is continued until the background-subtracted silicon Ka peak integral exceeds 10000 counts. The spectrum should then be manipulated to subtract the background and to obtain the net areas of the elemental peaks.

After quantitative EDXA classification of some fibers by computer analysis of the net peak areas, it may be possible to classify further fibers in the same sample on the basis of comparison of spectra at the instrument. Frequently, visual comparisons can be made after somewhat shorter acquisition times.

6.7.3 Analysis of Fiber Identification Data

Since the fiber identification procedure can be involved and time-consuming, a Fortran computer program has been provided, the listing of which is given in Appendix A. This program permits the EDXA and zone axis SAED measurements to be compared against a library of compositional and structural data for 226 minerals. The mineral library includes fibrous species which have been listed by several authors, together with other minerals which are known to be similar to amphibole in either their compositions or some aspects of their crystallography. Additional minerals may be added to the library if they are thought to be of concern in particular situations. Rejection of a mineral by the program indicates that either the compositional or crystallographic data for the mineral in the library are inconsistent with the measurements made on the unknown fiber. Demonstration that the measurements are consistent with the data for a particular test mineral does not uniquely identify the unknown, since the possibility exists that data from other minerals may also

be consistent. It is, however, very unlikely that a mineral of another structural class could yield data consistent with that from an amphibole fiber identified uniquely by quantitative EDXA and two zone axis SAED patterns.

The computer program classifies fibers initially on the basis of chemical composition. Either qualitative or quantitative EDXA information may be entered. The procedure using qualitative EDXA consists of entering the list of elements which originate from the particle. For quantitative EDXA (silicon-containing minerals only), the list of elements and the areas under the corresponding X-ray emission peaks, after background correction, form the input data for the computer program. The width of the fiber is also required as input into the program. The program will select from the file a list of minerals which are consistent in composition with that measured for the unknown fiber. To proceed further, it is necessary to obtain the first zone axis SAED pattern, according to the instructions in Section 6.7.2.

It would be attractive to specify a particular zone axis pattern to be obtained for confirmation of amphibole, particularly if such a pattern could be considered characteristic. Unfortunately, for a fiber with random orientation on the grid, no specimen holder and goniometer currently available will permit convenient and rapid location of two pre-selected zone axes. The most practical approach has been adopted, which is to accept those low index patterns which are easily obtained, and then to test their consistency with the structures of the minerals already pre-selected on the basis of the EDXA data. Even the structures of non-amphibole minerals in this pre-selected list must be tested against the zone axis data obtained for the unknown fiber, since non-amphibole minerals may yield similar patterns consistent with amphibole structures in some orientations.

The zone axis SAED interpretation part of the program will consider all minerals previously selected from the file as being chemically compatible with the EDXA data. It will then return a second and usually reduced list of minerals for which solutions have been found. A second set of zone axis data from another pattern obtained on the same fiber can then be processed either as further confirmation or to attempt elimination of an ambiguity. In addition, the angle measured between the orientations of the two zone axes can be entered into the computer to be checked for consistency with the structures of minerals. Caution should be exercised in rationalizing the inter-zone axis angle, since if the fiber contains c-axis twinning, the two zone axis SAED patterns may originate from the separate twin crystals.

In practice, the full program will normally be applied to very few fibers, unless precise identification of all fibers is required.

6.7.4 Fiber Classification Categories

It is not always possible to proceed to a definitive identification of a fiber; this may be due to instrumental limitations or to the actual nature of the fiber. In many analyses a definitive identification of each fiber may not actually be necessary if there is other knowledge available about the sample, or if the concentration is below a level of interest. The analytical procedure must therefore take account of both instrumental limitations and varied analytical requirements. Accordingly, a system of fiber classification has been devised to permit accurate recording of data. The classifications are shown in Tables 3 and 4, and are directed towards identification of chrysotile and amphibole respectively. Fibers will be reported in these categories.

The general principle to be followed in this analytical procedure is first to define the most specific fiber classification (target classification) which is to be attempted. Then, for each fiber examined, the classification which is actually achieved is recorded. Depending on the intended use of the results, criteria for acceptance of fibers as "identified" can then be established at any time after completion of the analysis.

In an unknown sample, chrysotile will be regarded as confirmed only if a recorded, calibrated SAED pattern from one fiber in the CO category is obtained. Amphibole will be regarded as confirmed only by obtaining recorded data which yields exclusively amphibole solutions for fibers classified in the AZQ, AZZ or AZZQ categories.

6.7.5 Procedure for Classification of Fibers With Tubular Morphology, Suspected to be Chrysotile

Many fibers are encountered which have tubular morphology similar to that of chrysotile, but which defy further attempts at characterization by either SAED or EDXA. They may be non-crystalline, in which case SAED techniques are not useful, or they may be in a position on the grid which does not permit an EDXA spectrum to be obtained. Alternatively, the fiber may be of organic origin, but not sufficiently definitive that it can be disregarded.

Classification attempts will meet with various degrees of success. Figure 15 shows the classification procedure to be used for fibers which display any tubular morphology.

TABLE 3. CLASSIFICATION OF FIBERS WITH TUBULAR MORPHOLOGY

TM	-	Tubular Morphology not sufficiently characteristic for classification as chrysotile
CM	-	Characteristic Chrysotile Morphology
CD	-	Chrysotile SAED pattern
CQ	-	Chrysotile composition by Quantitative EDXA
CMQ	-	Chrysotile Morphology and composition by Quantitative EDXA
CDQ	-	Chrysotile SAED pattern and composition by Quantitative EDXA
NAM	-	Non-Asbestos Mineral

TABLE 4. CLASSIFICATION OF FIBERS WITHOUT TUBULAR MORPHOLOGY

UF	-	Unidentified Fiber
AD	-	Amphibole by random orientation SAED (shows layer pattern of 0.53 nm spacing)
AX	-	Amphibole by qualitative EDXA. Spectrum has elemental components consistent with amphibole
ADX	-	Amphibole by random orientation SAED and Qualitative EDXA
AQ	-	Amphibole by Quantitative EDXA
AZ	-	Amphibole by one Zone Axis SAED
ADQ	-	Amphibole by random orientation SAED and Quantitative EDXA
AZQ	-	Amphibole by one Zone Axis SAED pattern and Quantitative EDXA
AZZ	-	Amphibole by two Zone Axis SAED patterns with consistent inter-axial angle
AZZQ	-	Amphibole by two Zone Axis SAED patterns, consistent inter-axial angle and Quantitative EDXA
NAM	-	Non-Asbestos Mineral

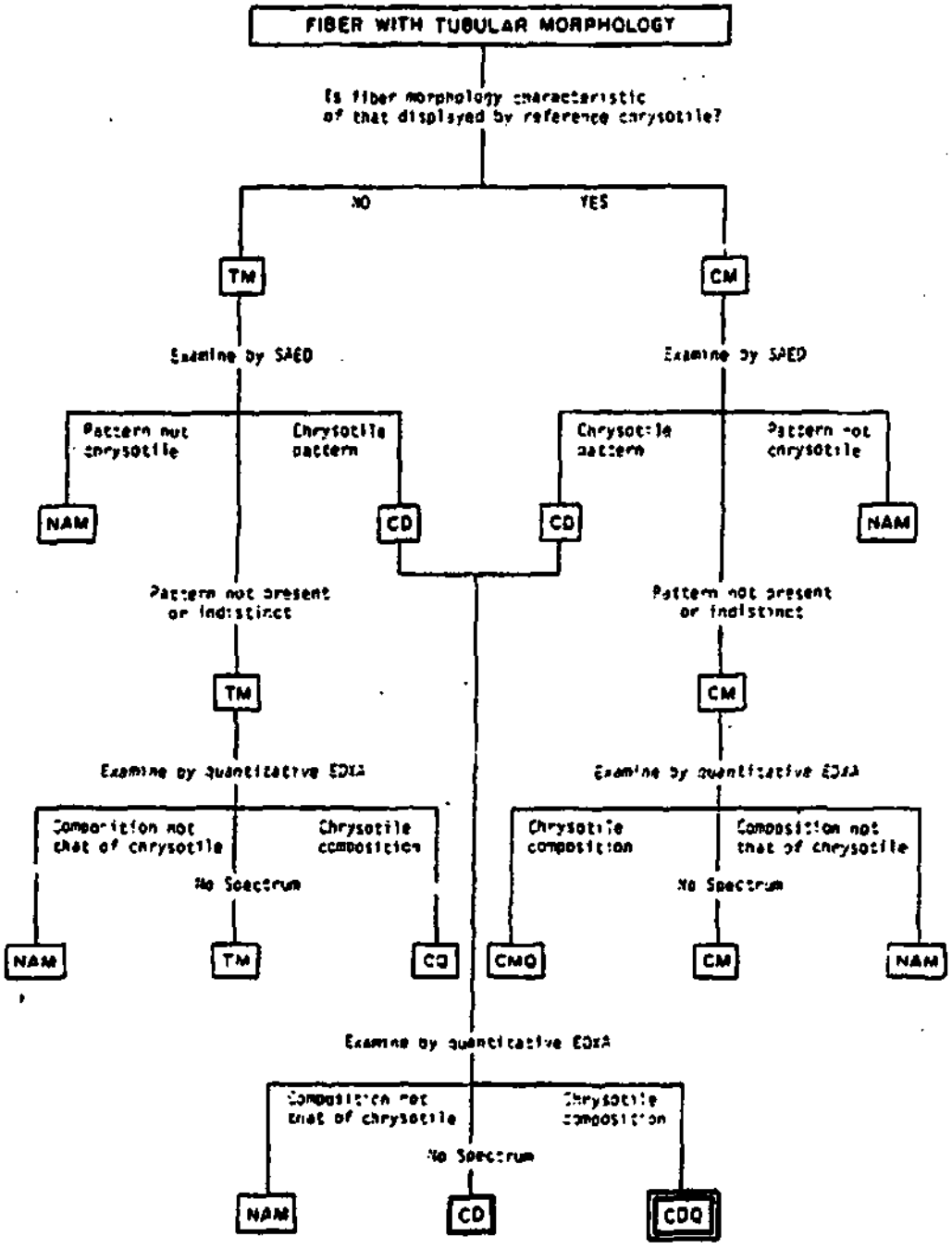

Figure 15. Classification Chart for Fiber With Tubular Morphology.

The chart is self explanatory, and essentially every fiber is either rejected as a non-asbestos mineral (NAM), or classified in some way which could still contribute to the chrysotile fiber count.

Morphology is the first consideration, and if this is not similar to that usually seen in chrysotile standard samples, the initial classification is TM. Regardless of the doubtful morphology, the fiber will still be examined by SAED and EDXA methods according to Figure 15. Where the morphology is more definitive, it may be possible to classify the fiber as having chrysotile morphology (CM).

The morphological characteristics required will be:

a) the individual fibrils should have high aspect ratios exceeding 10:1 and be about 40 nm in diameter;

b) the electron scattering power of the fiber at 60 to 100 kV accelerating potential should be sufficiently low for internal structure to be visible; and

c) there should be some evidence of internal structure suggesting a tubular appearance similar to that shown in Figure 16A, which may degrade in the electron beam to the appearance shown in Figure 16B.

Every fiber having these morphological characteristics will be examined by the SAED technique, and only those which give diffraction patterns with the precise characteristics of Figure 17 will be classified as chrysotile by SAED (CD). The relevant features in this pattern for identification of chrysotile are indicated. The (002) reflections should be examined to determine that they correspond approximately to a spacing of 0.73 nm, and the layer line repeat distance should correspond to 0.53 nm. There should also be "streaking" of the (110) and (130) reflections. Using the millimeter calibrations on the microscope viewing screen, these observations can readily be made at the instrument. A TEM micrograph of at least one representative fiber will be recorded, and its SAED pattern will also be recorded on a separate film or plate. This plate will also carry calibration rings from a known polycrystalline substance such as gold. This calibrated pattern is the only documentary proof that the particular fiber is chrysotile and not some other tubular or scrolled species such as halloysite, palygorskite, talc or vermiculite. The proportion of fibers which can be successfully identified as chrysotile by SAED is variable, and to some extent dependent on both the instrument and the procedures of the operator. The fibers that fail to yield an identifiable SAED pattern will remain in the TM or CM categories unless they are examined by EDXA.

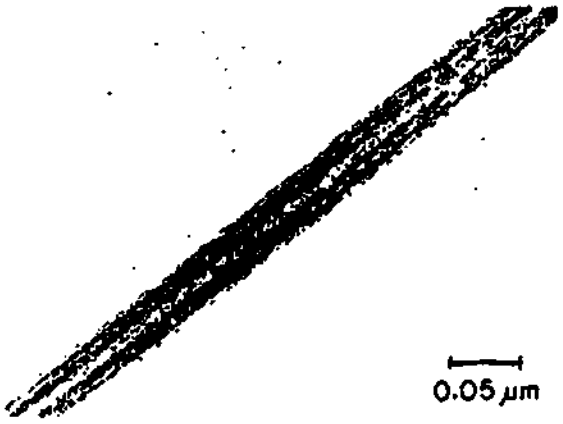

Figure 16A. TEM Micrograph of Chrysotile Fibril, showing Morphology.

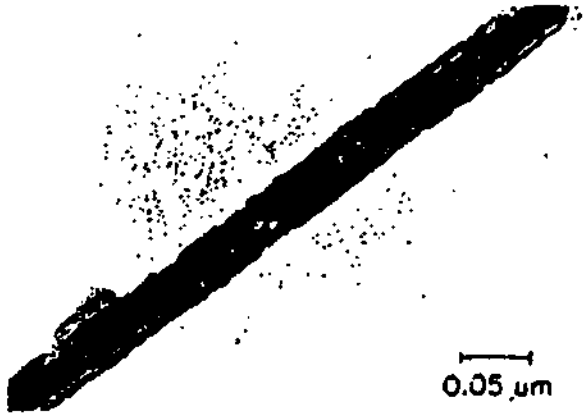

Figure 16B. TEM Micrograph of UICC Canadian Chrysotile Fiber after Thermal Degradation by Electron Beam Irradiation.

Figure 17. SAED Pattern of Chrysotile Fiber with Diagnostic Features Labelled. Necessary criteria are the presence of 0.73 nm spacing for the 002 reflections, 0.53 nm spacing for the layer line repeat and characteristic streaking of the 110 and 130 reflections.

In the EDXA analysis of chrysotile there are only two elements which are relevant. For fiber classification, the EDXA analysis must be quantitative. If the spectrum displays prominent peaks from magnesium and silicon, with their areas in the appropriate ratio, and with only minor peaks from other elements, the fiber will be classified as chrysotile by quantitative EDXA, in the categories CQ, CMQ or CDQ, as appropriate.

For chrysotile analyses there are essentially three possible levels of analysis:

1. morphological and SAED discrimination only (Target classification CD);

2. in addition, EDXA of only those fibers unclassified by SAED (Target classification CD);

3. EDXA in addition to SAED on all fibers (Target classification CDQ).

6.7.6 Procedure for Classification of Fibers Without Tubular Morphology, Suspected to be Amphibole

Every particle without tubular morphology and which is not obviously of biological origin, with an aspect ratio of 3 to 1 or greater and having parallel or stepped sides, will be considered as a suspected amphibole fiber. Further examination of the fiber by SAED and EDXA techniques will

meet with a variable degree of success, depending on the nature of the fiber and on a number of instrumental limitations. It will not be possible to identify every fiber completely, even if time and cost were of no concern. Moreover, confirmation of the presence of amphibole can be achieved only by quantitative interpretation of zone axis SAED patterns, a very time-consuming procedure. Accordingly, for routine samples from unknown sources, this analytical procedure limits the requirement for zone axis SAED work to a minimum of one fiber representative of each compositional class reported. In some samples, it may be necessary to identify more fibers by the zone axis technique. When analyzing samples from well-characterized sources, the cost of identification by zone axis methods may not be justified.

The 0.53 nm layer spacing of the random orientation SAED pattern is not by itself diagnostic for amphibole. However, the presence of c-axis twinning in many fibers leads to contributions to the layers in the patterns by several individual parallel crystals of different axial orientations. This apparently random positioning of the spots along the layer lines, if also associated with a high fiber aspect ratio, is a characteristic of amphibole asbestos, and thus has some limited diagnostic value. If a pattern of this type is not obtained, the identity of the fiber is still ambiguous, since the absence of a recognizable pattern may be a consequence of an unsuitable orientation relative to the electron beam, or the fiber may be some other mineral species.

Figure 18 shows the fiber classification chart for suspected amphibole fibers. This chart shows all the classification paths possible in analysis of a suspected amphibole fiber, when examined systematically by SAED and EDXA. Initially two routes are possible, depending on whether an attempt to obtain an EDXA spectrum or a random orientation SAED pattern is made first. The normal procedure for analysis of a sample of unknown origin will be to examine the fiber by random orientation SAED, qualitative EDXA, quantitative EDXA, and zone axis SAED, in this sequence. The final fiber classification assigned will be defined either by successful analysis at the target level or by the instrumental limitations. The maximum classification achieved for each fiber will be recorded on the counting sheet in the appropriate column. The various classification categories can then be combined in any desired way for calculation of the fiber concentration, and a complete record of the results from each fiber is maintained for reassessment of the data if necessary.

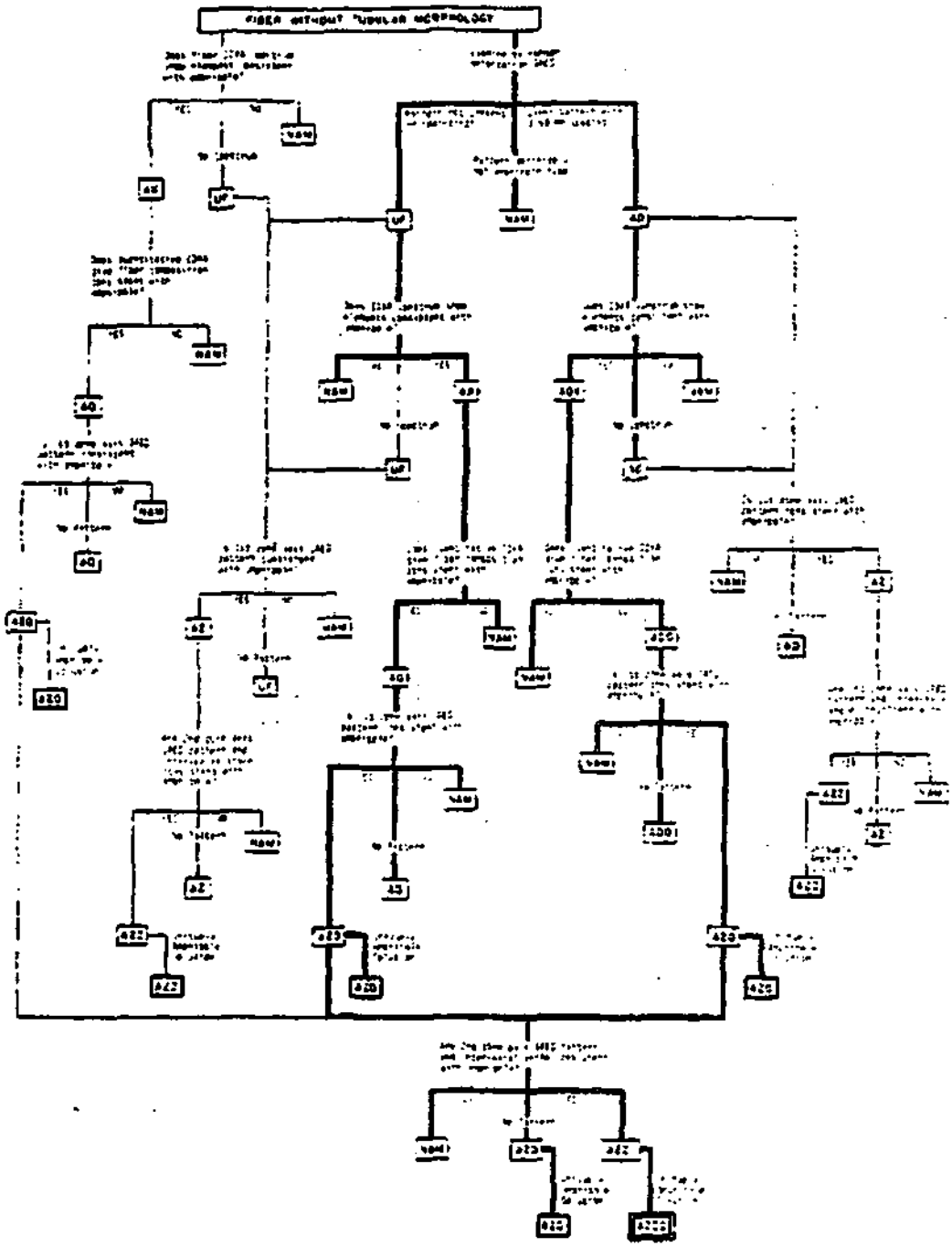

Figure 18. Classification Chart for Fiber Without Tubular Morphology. Bold Lines indicate the Preferred Paths.

Depending on the particular situation, four levels of analysis can be defined in this analytical procedure, and these are shown in Table 5.

In the routine unknown sample, a level 3 analysis will be required if the presence of amphibole is to be confirmed. For this level of analysis, attempts will be made to raise the classification of every fiber to the ADQ category. In addition, at least one fiber from each type of suspected amphibole found will be examined by zone axis SAED methods to confirm the identification.

TABLE 5. LEVELS OF ANALYSIS FOR AMPHIBOLE

Level of Analysis	Application	Target Classification for all Fibers	Required Classification for Confirmation of Amphibole in a Proportion of the Fibers
1	Routine monitoring of known and well-characterized sources for one mineral fiber type.	ADX	Not Applicable
2	Routine monitoring of known and well-characterized sources where discrimination between two or more amphibole fiber types is required.	ADQ	Not Applicable
3	Routine samples from uncharacterized sources in which presence or absence of amphibole is to be confirmed.	ADQ	AZZ, AZQ or AZZQ - Solutions must include only amphiboles.
4	Samples where precise identification of all amphibole fibers is an important issue.	AZQ	AZZQ - Solutions must include only amphiboles.

6.8 Blank and Control Determinations

To ensure that contamination by extraneous fibers during sample preparation is insignificant compared with the results reported on samples, it is necessary to establish a continuous program of blank measurements. Initially, and at intervals during an analytical program, it is also necessary to ensure that samples of known fiber concentrations can be analyzed satisfactorily.

6.8.1 Blank Determinations

At least one blank determination will be made along with every group of samples prepared at any one time. For the blank determination, a 0.1 μm Nuclepore filter will be prepared by filtration of 100 mL of ozone-UV treated fiber-free water if using 25 mm diameter equipment, and 500 mL treated water if using 47 mm diameter equipment. If the samples have been preserved with mercuric chloride, an equivalent amount of the solution should be added to the water used for the preparation of the blank. This blank filter will be carbon coated at the same time as the group of samples, and solvent extracted in the same Jaffe Washer. All aspects of the sample preparation will then be identical to those for the actual samples. All fibers on 20 grid openings of the blank sample will be recorded. The mean fiber concentration for the blank must be less than 0.05 MFL or less than 1% of the lowest individual value reported in the samples concerned, whichever is the greater value. If a value higher than these criteria is encountered, satisfactory blank values must be demonstrated before further analyses are carried out. If it is suspected that samples could have been contaminated during the original preparation, the duplicate bottles should be used for the repreparation of the samples concerned.

6.8.2 Control Samples

Control samples must be incorporated into sample analysis programs in order to demonstrate that the expected results can be produced from samples of known fiber concentration. Such reference suspensions can be prepared using ampoules of stable fiber dispersions listed in Section 4.3.16. It is recommended that the range of fiber concentrations found in the real samples should be simulated using the reference suspensions. The sealed ampoules of fiber dispersions become unstable when they are opened, and the fiber concentration value should not be relied upon for more than 8 hours after opening. Accordingly, it is recommended that, upon opening a dispersion concentrate ampoule, several reference suspensions of different fiber concentrations be prepared in sample bottles. These

bottles can then be stored for preparation and analysis along with water samples of unknown fiber concentrations.

7. CALCULATION OF RESULTS

The results are conveniently calculated using a computer program, the listing of which is provided in Appendix B. The methods by which the calculations are made are described below.

7.1 Test for Uniformity of Fiber Deposit on Electron Microscope Grids

A check must be made using the chi-square test, to determine whether the fibers found on individual grid openings are randomly and uniformly distributed among the grid openings. If the total number of fibers found in k grid openings is n, and the areas of the k individual grid openings are designated A_1 to A_k, then the total area examined is

$$A = \sum_{i=1}^{i=k} A_i$$

The fraction of the total area examined which is represented by the individual grid opening area, a_i, is given by A_i/A. If the fibers are randomly and uniformly dispersed over the k grid openings counted, the expected number of fibers falling in one grid opening with area A_i is np_i. If the observed number found on that grid opening is n_i, then:

$$\chi^2 = \sum_{i=1}^{i=k} \frac{(n_i - np_i)^2}{np_i}$$

This value is compared with significance points of the χ^2 distribution, having $(k - 1)$ degrees of freedom. Significance levels lower than 0.1% are cause for the sample analysis to be rejected, since this corresponds to a very inhomogeneous deposit. If this occurs, a new filter should be prepared, paying more attention to both uniform dispersal of the suspension and the filtration procedure as described in Section 6.3.2.

7.2 Calculation of the Mean and Confidence Interval of the Fiber Concentration

In the fiber count, a maximum of 20 grid openings have been sampled from a population of grid openings, and it is required to determine the mean grid opening fiber count for the population on the basis of this sampling. The interval about the sample mean, which, with 95% confidence, contains the population mean, is also required.

The distribution of fibers on the grid openings should theoretically approximate a Poisson distribution. Because of fiber aggregation and size-dependent identification effects, the fiber count data often do not conform to the Poisson distribution, particularly at high fiber counts. Simple assumption of a Poisson distribution may therefore lead to confidence intervals narrower than are justified by the data. Moreover, if a Poisson distribution is assumed, the variance is fixed in relation to the total number of fibers counted. Thus a particular fiber count conducted on one grid opening is considered to have the same confidence interval as that for the same number of fibers found on many grid openings. However, the area of sample actually counted is very small in relation to the total area of the filter, and for this reason fibers must be counted on a minimum of 4 grid openings taken from different areas of the filter in order to ensure representative evaluation of the deposit.

At high fiber counts, where there are adequate numbers of fibers per grid opening to allow a sample estimate of the variance to be made, the distribution can be approximated to Gaussian, with independent values for the mean and variance. Where the sample estimate of variance exceeds that implicit in the Poissonian assumption, use of Gaussian statistics with the variance defined by the actual data is the most conservative approach to calculation of confidence intervals.

At low fiber counts, it is not possible to obtain a reliable sample estimate of the variance, and the distribution also becomes asymmetric, but not necessarily Poissonian. For 30 fibers and below, the distribution becomes sufficiently asymmetric that the Gaussian fit is no longer a reasonable one, and sample variance estimates are unreliable. Accordingly, for fiber counts below 30 fibers, the assumption of a Poisson distribution must be made for calculation of the confidence intervals.

For total fiber counts less than 5, the lower 95% confidence value corresponds to one fiber or less, and in addition, the upper 95% confidence value corresponding to a fiber count of zero is 3.69 fibers. Therefore, it is not meaningful to quote lower confidence interval points for fiber counts of less than 5, and the result should be specified as "less than" the corresponding Poisson upper 95% confidence value.

For fiber counts higher than 30, the sample estimate of variance is also calculated, and the larger of the two confidence intervals is selected. For calculation of Poisson 95% confidence intervals, Table 40 of the reference by E.S. Pearson and H.O. Hartley should be used, with an extension to an expectation of 100. For more than 100 fibers, the Poisson distribution can be accurately approximated by a Gaussian distribution, still using the Poisson variance estimate. For counts of more than 30 fibers, the 95% confidence interval based on a sample estimate of variance is calculated using the Student's "t" distribution. For the two-sided Student's "t" calculation, k values of grid opening fiber count are compared with the expected values for the areas of the grid openings concerned.

In summary, fiber counting data will be reported as follows:

No fibers detected

The value will be reported as less than 369/ of the concentration equivalent to one fiber.

1 to 4 fibers

When 1 to 4 fibers are counted, the result will be reported as less than the corresponding upper 95% confidence limit (Poisson).

5 to 30 fibers

Mean and 95% confidence intervals will be reported on the basis of the Poisson assumption.

More than 30 fibers

When more than 30 fibers are counted, both the Gaussian 95% confidence interval and the Poisson 95% confidence interval will be calculated. The larger of these 2 intervals will be selected for data reporting. When the Gaussian 95% confidence interval is selected for data reporting, the Poisson interval will also be noted.

Fiber counts performed on less than 4 grid openings yield very wide 95% confidence intervals when using Gaussian statistics. This is because the value of Student's "t" is very large for 1 and 2 degrees of freedom. Accordingly, fiber counts must not be made on less than 4 grid openings.

The sample estimate of variance S^2 is first calculated:

$$S^2 = \frac{\sum_{i=1}^{i=k} (n_i - np_i)^2}{(k-1)}$$

where:

n_i = Number of fibers on the i'th grid opening

n = Total number of fibers found in k grid openings

p_i = Fraction of the total area examined represented by the i'th grid opening

k = Number of grid openings

For the 95% confidence interval, the value of $t_{0.975}$ is obtained from tables for $(k - 1)$ degrees of freedom. If the mean value of fiber count is calculated to be $\bar{n}$, the upper and lower values of the 95% confidence interval are given by:

$$n_U = \bar{n} + \frac{ts}{\sqrt{k}}$$

$$n_L = \bar{n} - \frac{ts}{\sqrt{k}}$$

where:

n_U = Upper 95% confidence limit

n_L = Lower 95% confidence limit

$\bar{n}$ = Mean number of fibers per grid opening

s = Standard deviation (square root of sample estimate of variance)

k = Number of grid openings

The fiber concentration in MFL which corresponds to counting of one fiber is given by:

$$C = \frac{A_f \times R_D}{A \times V \times 1000}$$

where:

A_f = Effective filtration area of filter membrane in mm^2 used for filtration of liquid sample

A = Total area examined in mm^2

V = Original volume of sample filtered (mL)

R_D = Dilution ratio of original sample

The mean concentration in MFL is obtained by multiplying the mean number of fibers per grid opening by kC. To obtain the upper and lower 95% confidence limits for the concentration (in MFL) multiply the values n_U and n_L by kC.

7.3 Estimated Mass Concentration

The mass of each amphibole fiber in micrograms is calculated using the relationship:

$$M = L \times W^2 \times D \times 10^{-6}$$

where:

M = Mass in micrograms

L = Length in um

W = Width in um

D = Density of fiber in g/cm^3

For chrysotile, the mass may be calculated using the relationship for a cylinder:

$$M = \frac{\pi}{4} \times L \times W^2 \times D \times 10^{-6}$$

The estimated mass concentration is then given by:

$$M_c = C \times \sum_{i=1}^{i=n} M_i \times 10^6$$

where:

M_c = Mass concentration in ng/L

C = Fiber concentration in MFL, which corresponds to counting of one fiber

M_i = Mass of the i'th fiber, in micrograms

n = Total number of fibers found in k grid openings

The densities to be assumed are as follows:

Chrysotile	2.55	g/cm^3
Crocidolite	3.37	g/cm^3
Cummingtonite	3.28	g/cm^3
Grunerite	3.52	g/cm^3
Amosite	3.43	g/cm^3
Anthophyllite	3.00	g/cm^3
Tremolite	3.00	g/cm^3
Actinolite	3.10	g/cm^3
Unknown Amphibole	3.20	g/cm^3

7.4 Fiber Length, Width, Mass and Aspect Ratio Distributions

The distributions all approximate to logarithmic-normal, and so the size range intervals for calculation of the distribution must be spaced logarithmically. The other characteristics required for the choice of size intervals are that they should allow for a sufficient number of size classes, while still retaining a statistically-valid number of fibers in each class. Interpretation is also facilitated if each size class repeats at decade intervals. A ratio from one class to the next of 1.468 satisfies all of these requirements. The other constraints are that the length distribution should include 0.5 μm as one interval point, since this is the minimum length to be counted in the method, and the minimum aspect ratio is by definition 3.0. The resulting size classes for the various distributions can be seen in the example shown in Appendix B. The distributions, being approximately logarithmic-normal, must be plotted using a logarithmic ordinate scale and a Gaussian abscissa.

7.4.1 Fiber Length Cumulative Number Distribution

This distribution allows the fraction of the total number of fibers either shorter or longer than a given length to be determined. It is calculated using the relationship:

$$C_{(N)k} = \frac{\sum_{i=1}^{i=k} n_i}{\sum_{i=1}^{i=N} n_i} \times 100$$

where:

$C_{(N)k}$ = Cumulative number percentage of fibers which have lengths less than the upper bound of the k'th class

n_i = Number of fibers in the i'th length class

N = Total number of length classes

7.4.2 Fiber Width Cumulative Number Distribution

This distribution allows the fraction of the total number of fibers either narrower or wider than a given width to be determined. It is calculated in a similar way to that used in 7.4.1 for the length distribution.

7.4.3 Fiber Length Cumulative Mass Distribution

This distribution allows the fraction of the total mass incorporated in fibers either shorter or longer than a given length to be determined. It is computed using the relationship:

$$C_{(M)k} = \frac{\sum_{i=1}^{i=k} \sum_{j=1}^{j=n_i} l_j w_j^2}{\sum_{i=1}^{i=N} \sum_{j=1}^{j=n_i} l_j w_j^2} \times 100$$

where:

$C_{(M)k}$ = Cumulative mass percentage of fibers which have lengths less than the upper bound of the k'th class

n_i = Number of fibers in the i'th length class

l_j = Length of the j'th fiber in the i'th length class

w_j = Width of the j'th fiber in the i'th length class

N = Total number of length classes

7.4.4 Fiber Aspect Ratio Cumulative Number Distribution

This distribution allows the fraction of the total number of fibers which have aspect ratios either smaller or larger than a given aspect ratio to be determined. It is

calculated in a similar way to that used in 7.4.1 for the length distribution.

7.4.5 Fiber Mass Cumulative Number Distribution

This distribution allows the fraction of the total number of fibers which have masses either smaller or larger than a given mass to be determined. It is calculated by placing the fibers into logarithmically-spaced mass categories, after which the cumulative frequency distribution is obtained in a similar way to that used in 7.4.1 for the length distribution.

7.5 Index of Fibrosity

It is possible to discriminate between amphibole asbestos fibers and amphibole cleavage fragments on the basis of the distribution of their aspect ratios. The concept of fibrosity in a mineral embodies a high median aspect ratio, together with a large spread of aspect ratios above the median value. A single number can be used to describe the fibrosity of a mineral fiber dispersion, and in many cases the value can be used to state if the material is or is not asbestos. The fibrosity index can be defined thus:

$$F = R^g$$

where R is the median of the aspect ratio distribution and g is the geometric standard deviation of the aspect ratio distribution above the median. The value of g is obtained from that portion of the distribution lying between one and two geometric standard deviations above the median. Meaningful values of the index of fibrosity can be obtained for most waterborne fiber dispersions if more than 50 fibers have been measured.

The fibrosity index as defined above has values exceeding 100 for waterborne dispersions of asbestos. Values below 50 indicate a distribution characteristic of cleavage fragments, or one from which the high aspect ratio fibers have been selectively removed.

8. REPORTING

The computer program provided in Appendix B satisfies all of the reporting requirements for this analytical method, and it is recommended that this format be used. The size classifications used must be the same as those in Appendix B.

8.1 Before the fiber count data can be processed to give concentration values, a decision must be made as to which fiber classifications are to be considered adequate as identification of the fiber species in question. This decision will depend on how much is known about the particular source from which the sample was collected.

For a sample from a completely uncharacterized source, the following procedure will be used to accumulate the classified fibers:

a) Confirmed Amphibole: AZZQ + AZQ + AZZ
(solutions must include only amphiboles)

b) Amphibole Best Estimate*: AZZQ + AZQ + AZZ + AZ + ADQ + AQ

c) Suspected Amphibole: ADX + AX + AD

d) Confirmed Chrysotile: CDQ + CD

e) Chrysctile Best Estimate*: CDQ + CD + CMQ + CQ

f) Suspected Chrysotile: CM

*NOTE: Best estimate can be reported only if some fibers are also reported in the confirmed category, otherwise all fiber classifications must be reported as suspected amphibole or chrysotile.

8.2 The concentration in MFL, together with 95% confidence intervals, will be reported for the groupings in Section 8.1 (a) to (f).

8.3 Two significant figures will normally be used for concentrations greater than 1 MFL, and one significant figure for concentrations less than 1 MFL.

8.4 For confirmation of chrysotile, a micrograph and a calibrated diffraction pattern will be provided from a typical fiber. The identification features in Figure 17 must be visible on the diffraction pattern.

For confirmation of amphibole, either (1) or (2) or (3) below must be provided for a typical fiber of each amphibole variety reported. The data provided must yield solutions which include only amphibole.

1) A micrograph, a calibrated zone axis SAED pattern, and an EDXA spectrum together with peak area measurements and EDXA calibration data;

2) A micrograph, and two calibrated zone axis SAED patterns with a measurement of the angular rotation between the two patterns;

3) A micrograph, two calibrated zone axis SAED patterns with a measurement of the angular rotation between the two patterns, and an EDXA spectrum together with peak area measurements and EDXA calibration data.

8.5 Tabulate the length, width and aspect ratio distributions.

8.6 Report the estimated mass concentration in ng/L for each of the groupings in Section 8.1 (a) to (f).

8.7 One significant figure will normally be used for reporting mass concentration.

8.8 Report the concentration in MFL corresponding to one fiber detected.

8.9 Report the total number of fibers counted in each of the groupings in Section 8.1 (a) to (f).

8.10 Report the χ^2 value for each of the groupings in Section 8.1 (a) to (f).

8.11 Report the number of fiber aggregates not included in the fiber count

8.12 Report any special circumstances or observations such as aggregation, presence of organic materials, amount of debris, presence of other fibers and their probable identity if known.

9. LIMITATIONS OF ACCURACY

9.1 Errors and Limitations of Identification

Complete identification of every chrysotile fiber is not possible, due to both instrumental limitations and the nature of some of the fibers. The requirement for a calibrated SAED pattern eliminates the possibility of an incorrect identification of the fiber selected. However, there is a possibility of misidentification of other chrysotile fibers for which both morphology and SAED pattern are reported on the basis of visual inspection only. 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.

As in the case of chrysotile fibers, complete identification of every amphibole fiber is not possible due to instrumental

limitations and the nature of some of the fibers. Moreover, complete identification of every amphibole fiber is usually not practical due to limitations of both time and cost. Particles of a number of other minerals having compositions similar to those of some amphiboles could be erroneously classified as amphibole when the classification criteria do not include zone axis SAED techniques. However, the requirement for quantitative EDXA measurements on all fibers as support for the random orientation SAED technique makes misidentification very unlikely, particularly when other similar fibers in the same sample have been identified as amphibole by zone axis methods. 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.

9.2 Obscuration

If large amounts of other materials are present, some asbestos fibers may not be observed because of physical overlapping. This will result in low values for the reported asbestos content.

9.3 Inadequate Dispersion

If the initial water sample contains organic material which is incompletely oxidized in the ozone-UV treatment, it will not be possible to disperse any fibers associated with the organic material. This may lead to adhesion of some fibers to the container walls and aliquots taken during filtration will then not be representative. It may also lead to a large proportion of fiber aggregates which are either not transferred during the replication and filter dissolution step or which cannot be counted during the sample examination. The result obtained from such an analysis will be low. The sample will also be inadequately dispersed if it is not treated in an ultrasonic bath prior to filtration, and therefore instructions regarding this treatment must be followed closely.

9.4 Contamination

Contamination by introduction of extraneous fibers during the analysis is an important source of erroneous results, particularly for chrysotile. The possibility of contamination, therefore, should always be a consideration.

9.5 Freezing

The effect of freezing on asbestos fibers is not known but there is reason to suspect that fiber breakdown could occur and result in a higher fiber count than was present in the original sample. Therefore, the sample should be transported to the laboratory and stored under conditions that will avoid freezing.

10. PRECISION AND ACCURACY

10.1 General

The precision that can be obtained is dependent upon the number of fibers counted, and on the uniformity of particulate deposit on the original filter. If 100 fibers are counted and the loading is at least 3.5 fibers/grid square, computer modeling of the counting procedure shows that a relative standard deviation of about 10% can be expected. As the number of fibers counted decreases, the precision will also decrease approximately as $\sqrt{N}$ where N is the number of fibers counted. In actual practice, some degradation from this precision will be observed. This degradation is a consequence of sample preparation errors, non-uniformity of the filtered particulate deposit, and fiber identification variability between operators and between instruments. The 95% confidence interval about the mean for a single fiber concentration measurement using this analytical method should be about ±25% when about 100 fibers are counted over 20 grid openings. For these conditions the precision of the computed mass concentration is generally lower than the precision for the fiber number concentration. The precision to be expected for a single determination of mass concentration is critically dependent on the fiber width distribution. For a result based on measurement of a minimum of about 100 fibers, the 95% confidence interval about the mean computed mass concentration may vary between ±25% and ±60%. If better precision is required for a mass determination, the alternative counting method described in Section 6.5.5 should be used.

10.2 Precision

10.2.1 Intra-Laboratory Comparison Using Environmental Water Sources

Table 6 shows the results obtained from analysis of 10 replicate samples from each of 8 water sampling locations. Four of these locations were associated with a source of chrysotile and four associated with a source of amphibole. It can be seen that the relative standard deviations of the number concentrations range between 13% and 22%. The corresponding relative standard deviations for the mass concentrations range between 29% and 69%.

10.2.2 Inter-Laboratory Comparison of Filters Prepared Using Standard Dispersions and Environmental Water Sources

Tables 7 and 8 show the fiber counting results obtained when sectors of filters prepared in the ORF Laboratory were distributed to six laboratories considered experienced in asbestos analysis by the identification and counting techniques incorporated in this manual. The samples as

TABLE 6. INTRA-LABORATORY COMPARISON OF ENVIRONMENTAL WATER SAMPLES

Type of Fiber in Water Source	Sampling Location	Number of Samples	Number Concentration Results			Mass Concentration Results			Mean Number of Fibers Counted per Sample
			Fiber Concentration, MFL		Relative Standard Deviation	Mass Concentration, ng/L		Relative Standard Deviation	
			Mean	95% Confidence Interval		Mean	95% Confidence Interval		
Chrysotile	Domestic Faucet	10	48.3	39.7 - 48.9	15%	0.27	0.19 - 0.35	41%	140
	Domestic Faucet	10	39.7	30.6 - 46.8	18%	0.25	0.19 - 0.32	37%	144
	River	10	15.7	14.0 - 17.3	15%	0.14	0.07 - 0.20	68%	29
	Treatment Plant	10	24.9	22.5 - 27.3	14%	0.17	0.13 - 0.20	29%	93
Amphibole	Domestic Hose	10	10.0	15.1 - 18.1	13%	12.4	9.0 - 15.8	38%	190
	Domestic Hose	10	9.73	8.93 - 10.5	12%	7.8	5.21 - 10.4	46%	110
	Lakeside	10	5.25	4.72 - 5.79	14%	6.55	3.32 - 9.78	69%	38
	Treatment Plant	10	3.42	2.88 - 3.97	22%	2.18	1.45 - 2.92	67%	44

TABLE 7. INTER-LABORATORY COMPARISON: STANDARD DISPERSIONS

Laboratory	Sample 1			Sample 2			Sample 3		
	Concentration, f/cc			Concentration, f/cc			Concentration, f/cc		
	Mean	95% Confidence Interval	Number of Fibers Counted	Mean	95% Confidence Interval	Number of Fibers Counted	Mean	95% Confidence Interval	Number of Fibers Counted
1	9.16	7.02 - 11.3	81	28.2	20.1 - 36.3	100	1.58	1.17 - 1.99	69
2	9.48	6.74 - 14.2	66	32.3	Not Available	75	2.67	1.82 - 3.52	56
3	12.1	9.20 - 15.0	86	23.0	19.6 - 34.4	186	1.86	1.53 - 2.19	55
4	9.26	7.12 - 11.4	96	20.1	11.2 - 29.0	150	1.76	0.97 - 2.55	32
5	9.66	7.17 - 11.9	85	36.0	28.9 - 43.1	494	1.18	0.85 - 1.51	42
6	8.99	3.22 - 14.7	55	16.2	3.27 - 29.1	202	1.90	1.14 - 2.65	1
Mean Concentration	9.74			26.3			1.79		
Standard Deviation	0.52			6.89			0.49		
Relative Standard Deviation	8.4%			26%			27%		
Fiber Species Reported	Chrysotile			Chrysotile			Amphibole		

TABLE 8. INTER-LABORATORY COMPARISON: ENVIRONMENTAL WATER SAMPLES

Laboratory	Sample 4			Sample 5		
	Concentration, MFL		Number of Fibers Counted	Concentration, MFL		Number of Fibers Counted
	Mean	95% Confidence Interval		Mean	95% Confidence Interval	
1	1.71	1.19 - 2.23	48	10.6	9.06 - 12.1	113
2	1.47	0.61 - 2.33	27	5.87	3.30 - 8.44	41
3	2.11	1.36 - 2.86	29	6.10	4.92 - 7.28	76
4	2.50	2.01 - 2.99	35	6.74	5.22 - 8.26	79
5	1.31	0.63 - 1.99	19	5.17	4.14 - 6.20	72
6	1.53	0.62 - 2.44	30	6.27	0.72 - 11.8	78
Mean Concentration	1.77			6.79		
Standard Deviation	0.45			1.94		
Relative Standard Deviation	25%			29%		
Fiber Species Reported	Amphibole			Chrysotile		

distributed were identified by number only. In Table 7 it can be seen that the relative standard deviations for the six results on each of the standard dispersion filters did not exceed 27%. In Table 8, the environmental water sources used to prepare the filter samples contained similar types of suspended materials as those used to generate the intra-laboratory results in Table 6. The relative standard deviations do not exceed 29%, which appears higher than the values obtained for the intra-laboratory results. However, when the 6 inter-laboratory results are compared with the 10 intra-laboratory values, there is no statistically significant difference to indicate that there has been any degradation of precision.

10.3 Accuracy

10.3.1 Intra- and Inter-Laboratory Comparison of Standard Dispersions of Asbestos Fibers

Tables 9 and 10 show the results obtained between two laboratories when stable aqueous fiber dispersions of known mass concentrations were analyzed. The fiber concentrations reported displayed no significant difference between values from the two laboratories. The relative standard deviation of the mean fiber concentration was 17% for chrysotile and 16% for crocidolite. The corresponding relative standard deviations for the mass concentration were 16% for chrysotile, and 37% for crocidolite. The higher variability for crocidolite is a consequence of the low statistical reliability of the large diameter fiber counts. The computed mean mass concentration for chrysotile was about 46% higher than the known mass concentration. This may be a consequence of the difficulty of diameter measurement for single chrysotile fibrils or the assumption of the bulk value for the density. The computed mean value for mass concentration for the crocidolite sample was 67.8 µg/L, which is very close to the known concentration of 50 µg/L.

TABLE 9. INTER- AND INTRA-LABORATORY COMPARISON: CHRYSOTILE

ANALYSIS OF REPLICATES OF A 1120 ng/L CHRYSOTILE FIBER DISPERSION

Laboratory	Sample	Fiber Concentration, MFL		Estimated Mass Concentration, ng/L	Number of Fibers Counted
		Mean	95% Confidence Interval		
1	1C	210	165 - 255	1792	147
	2C	199	149 - 248	1435	147
	3C	196	166 - 226	1352	141
2	4C	132	89 - 176	1600	50
	5C	172	146 - 198	1980	79
Mean Concentration		182		1632	
Standard Deviation		31		257	
Relative Standard Deviation		17%		16%	

TABLE 10. INTER- AND INTRA-LABORATORY COMPARISON: CROCIDOLITE

ANALYSIS OF REPLICATES OF A 50 µg/L CROCIDOLITE FIBER DISPERSION

Laboratory	Sample	Fiber Concentration, MFL		Estimated Mass Concentration, µg/L	Number of Fibers Counted
		Mean	95% Confidence Interval		
1	1A	148	112 - 183	94.0	218
	2A	143	123 - 163	89.4	195
	3A	136	98 - 175	66.4	195
2	4A	188	132 - 243	33.0	73
	5A	189	149 - 229	54.1	178
Mean Concentration		161		67.4	
Standard Deviation		26		25.3	
Relative Standard Deviation		16%		37%	

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Tabbed Page: 15

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40 CFR - CHAPTER I - PART 763

View Part

Appendix A to Subpart E -- Interim Transmission Electron Microscopy Analytical Methods -- Mandatory and Nonmandatory -- and Mandatory Section to Determine Completion of Response Actions

I. Introduction

The following appendix contains three units. The first unit is the mandatory transmission electron microscopy (TEM) method which all laboratories must follow; it is the minimum requirement for analysis of air samples for asbestos by TEM. The mandatory method contains the essential elements of the TEM method. The second unit contains the complete non-mandatory method. The non-mandatory method supplements the mandatory method by including additional steps to improve the analysis. EPA recommends that the non-mandatory method be employed for analyzing air filters; however, the laboratory may choose to employ the mandatory method. The non-mandatory method contains the same minimum requirements as are outlined in the mandatory method. Hence, laboratories may choose either of the two methods for analyzing air samples by TEM.

The final unit of this Appendix A to subpart E defines the steps which must be taken to determine completion of response actions. This unit is mandatory.

II. Mandatory Transmission Electron Microscopy Method

A. Definitions of Terms

1. *Analytical sensitivity* -- Airborne asbestos concentration represented by each fiber counted under the

electron microscope. It is determined by the air volume collected and the proportion of the filter examined. This method requires that the analytical sensitivity be no greater than 0.005 structures/cm 3.

2. *Asbestiform* -- A specific type of mineral fibrosity in which the fibers and fibrils possess high tensile strength and flexibility.

3. *Aspect ratio* -- A ratio of the length to the width of a particle. Minimum aspect ratio as defined by this method is equal to or greater than 5:1.

4. *Bundle* -- A structure composed of three or more fibers in a parallel arrangement with each fiber closer than one fiber diameter.

5. *Clean area* -- A controlled environment which is maintained and monitored to assure a low probability of asbestos contamination to materials in that space. Clean areas used in this method have HEPA filtered air under positive pressure and are capable of sustained operation with an open laboratory blank which on subsequent analysis has an average of less than 18 structures/mm 2 in an area of 0.057 mm 2 (nominally 10 200-mesh grid openings) and a maximum of 53 structures/mm 2 for any single preparation for that same area.

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. Groupings must have more than two intersections.

7. *ED* -- Electron diffraction.

8. *EDXA* -- Energy dispersive X-ray analysis.

9. *Fiber* -- A structure greater than or equal to 0.5 •m in length with an aspect ratio (length to width) of 5:1 or greater and having substantially parallel sides.

10. *Grid* -- An open structure for mounting on the sample to aid in its examination in the TEM. The term is used here to denote a 200-mesh copper lattice approximately 3 mm in diameter.

11. *Intersection* -- Nonparallel touching or crossing of fibers, with the projection having an aspect ratio of 5:1 or greater.

12. *Laboratory sample coordinator* -- That person responsible for the conduct of sample handling and the certification of the testing procedures.

13. *Filter background level* -- The concentration of structures per square millimeter of filter that is considered indistinguishable from the concentration measured on a blank (filters through which no air has been drawn). For this method the filter background level is defined as 70 structures/mm 2.

14. *Matrix* -- Fiber or fibers with one end free and the other end embedded in or hidden by a particulate. The exposed fiber must meet the fiber definition.

15. *NSD* -- No structure detected.

16. *Operator* -- A person responsible for the TEM instrumental analysis of the sample.

17. *PCM* -- Phase contrast microscopy.

18. *SAED* -- Selected area electron diffraction.

19. *SEM* -- Scanning electron microscope.

20. *STEM* -- Scanning transmission electron microscope.

21. *Structure* -- a microscopic bundle, cluster, fiber, or matrix which may contain asbestos.

22. *S/cm 3* -- Structures per cubic centimeter.

23. *S/mm 2* -- Structures per square millimeter.

24. *TEM* -- Transmission electron microscope. B. Sampling

1. The sampling agency must have written quality control procedures and documents which verify compliance.

2. Sampling operations must be performed by qualified individuals completely independent of the abatement contractor to avoid possible conflict of interest (References 1, 2, 3, and 5 of Unit II.J.).

3. Sampling for airborne asbestos following an abatement action must use commercially available cassettes.

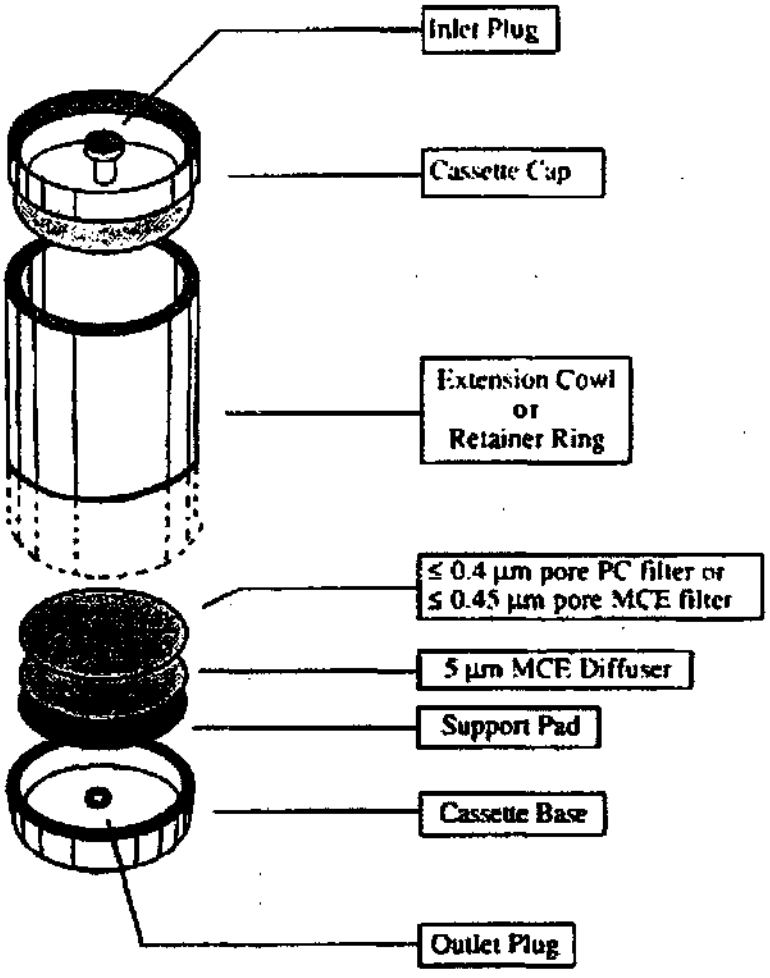
4. Prescreen the loaded cassette collection filters to assure that they do not contain concentrations of asbestos which may interfere with the analysis of the sample. A filter blank average of less than 18 s/mm 2 in an area of 0.057 mm 2 (nominally 10 200-mesh grid openings) and a single preparation with a maximum of 53 s/mm 2 for that same area is acceptable for this method.

5. Use sample collection filters which are either polycarbonate having a pore size less than or equal to 0.4 •m or mixed cellulose ester having a pore size less than or equal to 0.45 •m.

6. Place these filters in series with a 5.0 •m backup filter (to serve as a diffuser) and a support pad. See

the following Figure 1:

FIGURE 1--SAMPLING CASSETTE CONFIGURATION



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7. Reloading of used cassettes is not permitted.

8. Orient the cassette downward at approximately 45 degrees from the horizontal.

9. Maintain a log of all pertinent sampling information.

10. Calibrate sampling pumps and their flow indicators over the range of their intended use with a recognized standard. Assemble the sampling system with a representative filter (not the filter which will be used in sampling) before and after the sampling operation.

11. Record all calibration information.

12. Ensure that the mechanical vibrations from the pump will be minimized to prevent transferral of vibration to the cassette.

13. Ensure that a continuous smooth flow of negative pressure is delivered by the pump by damping out any pump action fluctuations if necessary.

14. The final plastic barrier around the abatement area remains in place for the sampling period.

15. After the area has passed a thorough visual inspection, use aggressive sampling conditions to dislodge any remaining dust. (See suggested protocol in Unit III.B.7.d.)

16. Select an appropriate flow rate equal to or greater than 1 liter per minute (L/min) or less than 10 L/min for 25 mm cassettes. Larger filters may be operated at proportionally higher flow rates.

17. A minimum of 13 samples are to be collected for each testing site consisting of the following:

a. A minimum of five samples per abatement area.

b. A minimum of five samples per ambient area positioned at locations representative of the air entering the abatement site.

c. Two field blanks are to be taken by removing the cap for not more than 30 seconds and replacing it at the time of sampling before sampling is initiated at the following places:

i. Near the entrance to each abatement area.

ii. At one of the ambient sites. (DO NOT leave the field blanks open during the sampling period.)

d. A sealed blank is to be carried with each sample set. This representative cassette is not to be opened in the field.

18. Perform a leak check of the sampling system at each indoor and outdoor sampling site by activating the pump with the closed sampling cassette in line. Any flow indicates a leak which must be eliminated before initiating the sampling operation.

19. The following Table I specifies volume ranges to be used:

TABLE 1--NUMBER OF 200 MESH EM GRID OPENINGS (0.0057 MM^2) THAT NEED TO BE ANALYZED TO MAINTAIN SENSITIVITY OF 0.005 STRUCTURES/CC BASED ON VOLUME AND EFFECTIVE FILTER AREA

Effective Filter Area 385 sq mm		Effective Filter Area 855 sq mm	
Volume (liters)	# of grid openings	Volume (liters)	# of grid openings
560	24	1,250	24
600	23	1,300	23
700	19	1,400	21
800	17	1,600	19
900	15	1,800	17
1,000	14	2,000	15
1,100	12	2,200	14
1,200	11	2,400	13
1,300	10	2,600	12
1,400	10	2,800	11
1,500	9	3,000	10
1,600	8	3,200	9
1,700	8	3,400	9
1,800	8	3,600	8
1,900	7	3,800	8
2,000	7	4,000	8
2,100	6	4,200	7
2,200	6	4,400	7
2,300	6	4,600	7
2,400	6	4,800	6
2,500	5	5,000	6
2,600	5	5,200	6
2,700	5	5,400	6
2,800	5	5,600	5
2,900	5	5,800	5
3,000	5	6,000	5
3,100	4	6,200	5
3,200	4	6,400	5
3,300	4	6,600	5
3,400	4	6,800	4
3,500	4	7,000	4
3,600	4	7,200	4
3,700	4	7,400	4
3,800	4	7,600	4

Recommended Volume Range

Recommended Volume Range

Note minimum volumes required:
25 mm : 500 liters
37 mm : 1250 liters

Filter diameter of 25 mm = effective area of 385 sq mm
Filter diameter of 37 mm = effective area of 855 sq mm

20. Ensure that the sampler is turned upright before interrupting the pump flow.

21. Check that all samples are clearly labeled and that all pertinent information has been enclosed before transfer of the samples to the laboratory.

22. Ensure that the samples are stored in a secure and representative location.

23. Do not change containers if portions of these filters are taken for other purposes.

24. A summary of Sample Data Quality Objectives is shown in the following Table II:

TABLE II--SUMMARY OF SAMPLING AGENCY DATA QUALITY OBJECTIVES

This table summarizes the data quality objectives from the performance of this method in terms of precision, accuracy, completeness, representativeness, and comparability. These objectives are assured by the periodic control checks and reference checks listed here and described in the rest of the method.

Unit Operation	QC Check	Frequency	Confidence Estimation
Sampling materials	Sealed blank	1 per lot no.	95%
Sample procedure	Field blanks	2 per lot no.	95%
	Pump calibration	Before and after each field series	90%
Sample custody	Review of chain-of-custody record	Each sample	95% complete
Sample shipment	Review of mailing report	Each sample	95% complete

C. Sample Shipment

Ship bulk samples to the analytical laboratory in a separate container from air samples. D. Sample Receiving

1. Designate one individual as sample coordinator at the laboratory. While that individual will normally be available to receive samples, the coordinator may train and supervise others in receiving procedures for those times when he/she is not available.

2. Bulk samples and air samples delivered to the analytical laboratory in the same container shall be rejected. E. Sample Preparation

1. All sample preparation and analysis shall be performed by a laboratory independent of the abatement contractor.

2. Wet-wipe the exterior of the cassettes to minimize contamination possibilities before taking them into the clean room facility.

3. Perform sample preparation in a well-equipped clean facility.

>**Note:** The clean area is required to have the following minimum characteristics. The area or hood must be capable of maintaining a positive pressure with make-up air being HEPA-filtered. The cumulative analytical blank concentration must average less than 18 s/mm 2 in an area of 0.057 mm 2 (nominally 10 200-mesh grid openings) and a single preparation with a maximum of 53 s/mm 2 for that same area.

4. Preparation areas for air samples must not only be separated from preparation areas for bulk samples, but they must be prepared in separate rooms.

5. Direct preparation techniques are required. The object is to produce an intact film containing the particulates of the filter surface which is sufficiently clear for TEM analysis.

a. TEM Grid Opening Area measurement must be done as follows:

i. The filter portion being used for sample preparation must have the surface collapsed using an acetone vapor technique.

ii. Measure 20 grid openings on each of 20 random 200-mesh copper grids by placing a grid on a glass and examining it under the PCM. Use a calibrated graticule to measure the average field diameters. From the data, calculate the field area for an average grid opening.

iii. Measurements can also be made on the TEM at a properly calibrated low magnification or on an optical microscope at a magnification of approximately 400X by using an eyepiece fitted with a scale that has been calibrated against a stage micrometer. Optical microscopy utilizing manual or automated procedures may be used providing instrument calibration can be verified.

b. TEM specimen preparation from polycarbonate (PC) filters. Procedures as described in Unit III.G. or other equivalent methods may be used.

c. TEM specimen preparation from mixed cellulose ester (MCE) filters.

i. Filter portion being used for sample preparation must have the surface collapsed using an acetone vapor technique or the Burdette procedure (Ref. 7 of Unit II.J.)

ii. Plasma etching of the collapsed filter is required. The microscope slide to which the collapsed filter pieces are attached is placed in a plasma asher. Because plasma ashers vary greatly in their performance, both from unit to unit and between different positions in the asher chamber, it is difficult to specify the conditions that should be used. Insufficient etching will result in a failure to expose embedded filters, and too much etching may result in loss of particulate from the surface. As an interim measure, it is recommended that the time for ashing of a known weight of a collapsed filter be established and that the etching rate be calculated in terms of micrometers per second. The actual etching time used for the particulate asher and operating conditions will then be set such that a 1-2 •m (10 percent) layer of collapsed surface will be removed.

iii. Procedures as described in Unit III. or other equivalent methods may be used to prepare samples. F. TEM Method

1. An 80-120 kV TEM capable of performing electron diffraction with a fluorescent screen inscribed with calibrated gradations is required. If the TEM is equipped with EDXA it must either have a STEM attachment or be capable of producing a spot less than 250 nm in diameter at crossover. The microscope shall be calibrated routinely for magnification and camera constant.

2. *Determination of Camera Constant and ED Pattern Analysis.* The camera length of the TEM in ED operating mode must be calibrated before ED patterns on 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 is evaporated on the specimen TEM grid to obtain zone-axis ED patterns superimposed with a 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 film would normally give multiple gold rings, but it will tend to mask weaker diffraction spots from the unknown fibrous particulate. Since the unknown d-spacings of most interest in asbestos analysis are those which lie closest to the transmitted beam, multiple gold rings are unnecessary on zone-axis ED patterns. An average camera constant using multiple gold rings can be determined. The camera constant is one-half the diameter of the rings times the interplanar spacing of the ring being measured.

3. *Magnification Calibration.* The magnification calibration must be done at the fluorescent screen. The TEM must be calibrated at the grid opening magnification (if used) and also at the magnification used for fiber counting. This is performed with a cross grating replica (e.g., one containing 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). A logbook must be maintained, and the dates of calibration and the values obtained must be recorded. The frequency of calibration depends on the past history of the particular microscope. After any maintenance of the microscope that involved adjustment of the power supplied to the lenses or the high-voltage system or the mechanical disassembly of the electron optical column apart from filament exchange, the magnification must be recalibrated. Before the TEM calibration is performed, the analyst must ensure that the cross grating replica is placed at the same distance from the objective lens as the specimens are. For instruments that incorporate a eucentric tilting specimen stage, all specimens and the cross grating replica must be placed at the eucentric position.

4. While not required on every microscope in the laboratory, the laboratory must have either one microscope equipped with energy dispersive X-ray analysis or access to an equivalent system on a TEM in another laboratory.

5. Microscope settings: 80-120 kV, grid assessment 250-1,000X, then 15,000-20,000X screen magnification for analysis.

6. Approximately one-half (0.5) of the predetermined sample area to be analyzed shall be performed on one sample grid preparation and the remaining half on a second sample grid preparation.

7. Individual grid openings with greater than 5 percent openings (holes) or covered with greater than 25 percent particulate matter or obviously having nonuniform loading must not be analyzed.

8. Reject the grid if:

a. Less than 50 percent of the grid openings covered by the replica are intact.

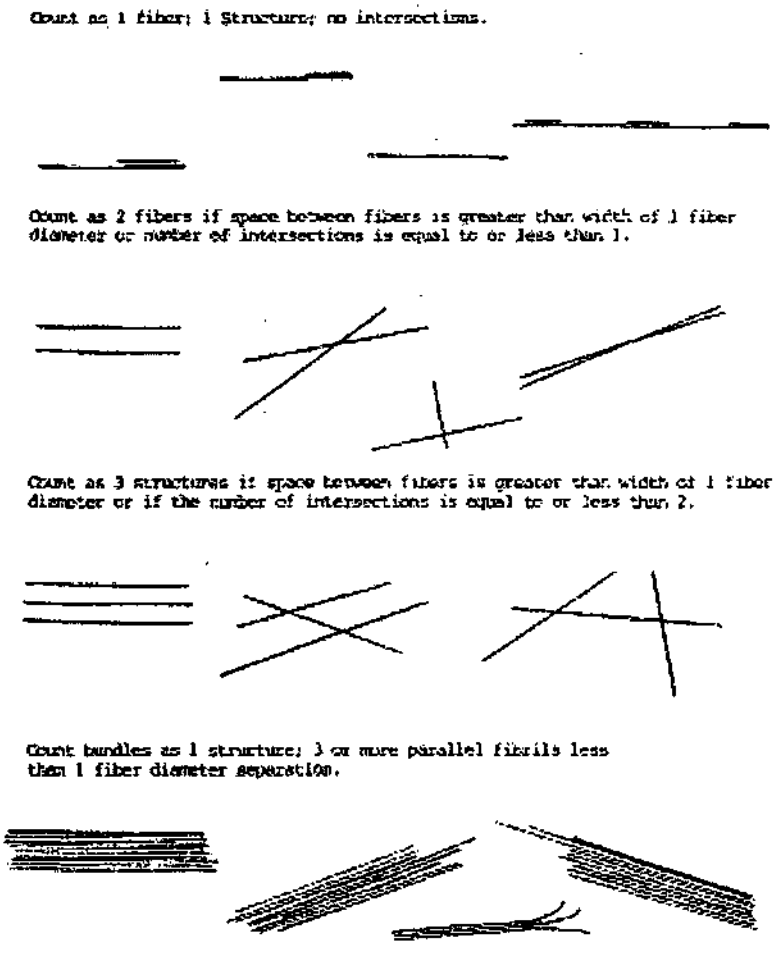
b. The replica is doubled or folded.

c. The replica is too dark because of incomplete dissolution of the filter.

9. *Recording Rules.*

a. Any continuous grouping of particles in which an asbestos fiber with an aspect ratio greater than or equal to 5:1 and a length greater than or equal to 0.5 •m is detected shall be recorded on the count sheet. These will be designated asbestos structures and will be classified as fibers, bundles, clusters, or matrices. Record as individual fibers any contiguous grouping having 0, 1, or 2 definable intersections. Groupings having more than 2 intersections are to be described as cluster or matrix. An intersection is a nonparallel touching or crossing of fibers, with the projection having an aspect ratio of 5:1 or greater. See the following Figure 2:

FIGURE 2--COUNTING GUIDELINES USED IN DETERMINING ASBESTOS STRUCTURES



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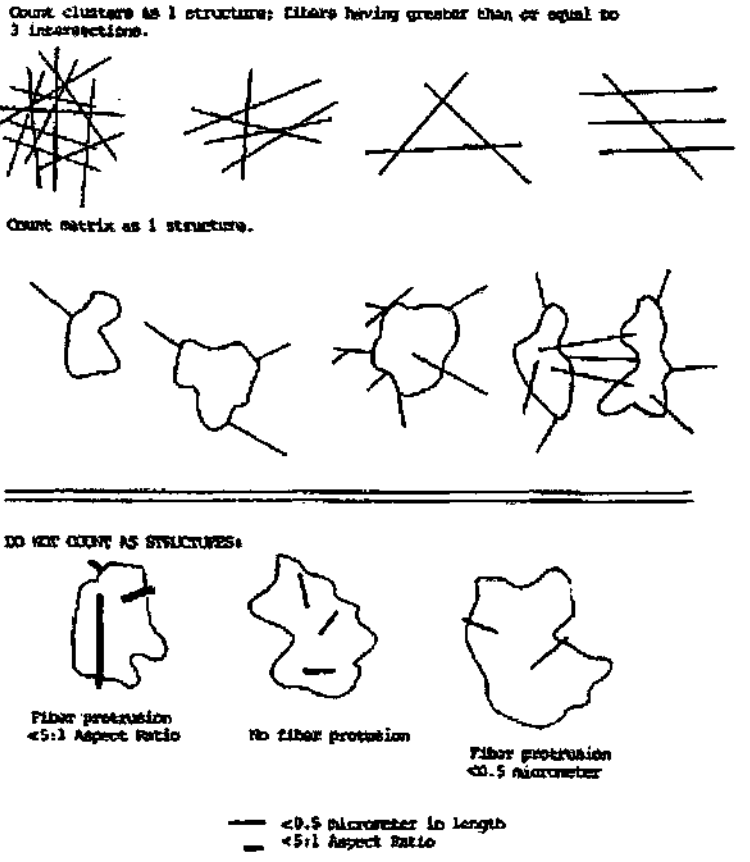

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i. *Fiber.* A structure having a minimum length greater than or equal to 0.5 •m and an aspect ratio (length to width) of 5:1 or greater and substantially parallel sides. Note the appearance of the end of the fiber, i.e., whether it is flat, rounded or dovetailed.

ii. *Bundle.* A structure composed of three or more fibers in a parallel arrangement with each fiber closer than one fiber diameter.

iii. *Cluster.* A structure with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group. Groupings must have more than two intersections.

iv. *Matrix.* Fiber or fibers with one end free and the other end embedded in or hidden by a particulate. The exposed fiber must meet the fiber definition.

b. Separate categories will be maintained for fibers less than 5 •m and for fibers equal to or greater than 5 •m in length.

c. Record NSD when no structures are detected in the field.

d. Visual identification of electron diffraction (ED) patterns is required for each asbestos structure counted which would cause the analysis to exceed the 70 s/mm 2 concentration. (Generally this means the first four fibers identified as asbestos must exhibit an identifiable diffraction pattern for chrysotile or amphibole.)

e. The micrograph number of the recorded diffraction patterns must be reported to the client and maintained in the laboratory's quality assurance records. In the event that examination of the pattern by a qualified individual indicates that the pattern has been misidentified visually, the client shall be contacted.

f. Energy Dispersive X-ray Analysis (EDXA) is required of all amphiboles which would cause the analysis results to exceed the 70 s/mm 2 concentration. (Generally speaking, the first 4 amphiboles would require EDXA.)

g. If the number of fibers in the nonasbestos class would cause the analysis to exceed the 70 s/mm 2 concentration, the fact that they are not asbestos must be confirmed by EDXA or measurement of a zone axis diffraction pattern.

h. Fibers classified as chrysotile must be identified by diffraction or X-ray analysis and recorded on a count sheet. X-ray analysis alone can be used only after 70 s/mm 2 have been exceeded for a particular sample.

i. Fibers classified as amphiboles must be identified by X-ray analysis and electron diffraction and recorded on the count sheet. (X-ray analysis alone can be used only after 70 s/mm 2 have been exceeded for a particular sample.)

j. If a diffraction pattern was recorded on film, record the micrograph number on the count sheet.

k. If an electron diffraction was attempted but no pattern was observed, record N on the count sheet.

l. If an EDXA spectrum was attempted but not observed, record N on the count sheet.

m. If an X-ray analysis spectrum is stored, record the file and disk number on the count sheet.

10. Classification Rules.

a. *Fiber.* A structure having a minimum length greater than or equal to 0.5 •m and an aspect ratio (length to width) of 5:1 or greater and substantially parallel sides. Note the appearance of the end of the fiber, i.e., whether it is flat, rounded or dovetailed.

b. *Bundle.* A structure composed of three or more fibers in a parallel arrangement with each fiber closer than one fiber diameter.

c. *Cluster.* A structure with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group. Groupings must have more than two intersections.

d. *Matrix.* Fiber or fibers with one end free and the other end embedded in or hidden by a particulate. The exposed fiber must meet the fiber definition.

11. After finishing with a grid, remove it from the microscope, and replace it in the appropriate grid holder. Sample grids must be stored for a minimum of 1 year from the date of the analysis; the sample cassette must be retained for a minimum of 30 days by the laboratory or returned at the client's request.
G. Sample Analytical Sequence

1. Under the present sampling requirements a minimum of 13 samples is to be collected for the clearance testing of an abatement site. These include five abatement area samples, five ambient samples, two field blanks, and one sealed blank.

2. Carry out visual inspection of work site prior to air monitoring.

3. Collect a minimum of 5 air samples inside the work site and 5 samples outside the work site. The indoor and outdoor samples shall be taken during the same time period.

4. Remaining steps in the analytical sequence are contained in Unit IV of this Appendix. H. Reporting

1. The following information must be reported to the client for each sample analyzed:

a. Concentration in structures per square millimeter and structures per cubic centimeter.

b. Analytical sensitivity used for the analysis.

c. Number of asbestos structures.

d. Area analyzed.

e. Volume of air sampled (which must be initially supplied to lab by client).

f. Copy of the count sheet must be included with the report.

g. Signature of laboratory official to indicate that the laboratory met specifications of the method.

h. Report form must contain official laboratory identification (e.g., letterhead).

i. Type of asbestos. I. Quality Control/Quality Assurance Procedures (Data Quality Indicators)

Monitoring the environment for airborne asbestos requires the use of sensitive sampling and analysis procedures. Because the test is sensitive, it may be influenced by a variety of factors. These include the supplies used in the sampling operation, the performance of the sampling, the preparation of the grid from the filter and the actual examination of this grid in the microscope. Each of these unit operations must produce a product of defined quality if the analytical result is to be a reliable and meaningful test result. Accordingly, a series of control checks and reference standards are to be performed along with the sample analysis as indicators that the materials used are adequate and the operations are within acceptable limits. In this way, the quality of the data is defined and the results are of known value. These checks and tests also provide timely and specific warning of any problems which might develop within the sampling and analysis operations. A description of these quality control/quality assurance procedures is summarized in the following Table III:

TABLE III--SUMMARY OF LABORATORY DATA QUALITY OBJECTIVES

Unit Operation	QC Check	Frequency	Conformance Expectation
Sample receiving	Review of receiving report	Each sample	95% complete
Sample custody	Review of chain-of-custody record	Each sample	99% complete
Sample preparation	Supplies and reagents	On receipt	Meet specs. or reject
	Grid opening size	20 openings/20 grids/lot of 1000 or 1 opening/sample	100%
	Special clean area monitoring	After cleaning or service	Meet specs or reclean
	Laboratory blank	1 per prep series or 10%	Meet specs. or reanalyze series
	Plasma etch blank	1 per 20 samples	75%
	Multiple preps (2 per sample)	Each sample	One with count of 15 complete grid sqs.
Sample analysis	System check	Each day	Each day
	Alignment check	Each day	Each day
	Magnification calibration with low and high standards	Each month or after service	95%
	ED calibration by gold standard	Weekly	95%
	EDS calibration by copper line	Daily	95%
Performance check	Laboratory blank (measure of cleanliness)	Prep 1 per series or 10% read 1 per 25 samples	Meet specs or reanalyze series
	Replicate counting (measure of precision)	1 per 100 samples	1.5 x Poisson Std. Dev.
	Duplicate analysis (measure of reproducibility)	1 per 100 samples	2 x Poisson Std. Dev.
	Known Samples of typical materials (working standards)	Training and for comparison with unknowns	100%
	Analysis of NBS SRM 1876 and/or RM 8410 (Methods of accuracy and comparability)	1 per analyst per year	1.5 x Poisson Std. Dev.
	Data entry review (data validation and measure of completeness)	Each sample	95%
	Record and verify ED electron diffraction pattern of structure	1 per 3 samples	80% accuracy
Calculation and data reduction	Hand calculation of automated data reduction procedure or independent recalculation of hand-calculated data	1 per 100 samples	85%

1. When the samples arrive at the laboratory, check the samples and documentation for completeness and requirements before initiating the analysis.

2. Check all laboratory reagents and supplies for acceptable asbestos background levels.

3. Conduct all sample preparation in a clean room environment monitored by laboratory blanks. Testing with blanks must also be done after cleaning or servicing the room.

4. Prepare multiple grids of each sample.

5. Provide laboratory blanks with each sample batch. Maintain a cumulative average of these results. If there are more than 53 fibers/mm 2 per 10 200-mesh grid openings, the system must be checked for possible sources of contamination.

6. Perform a system check on the transmission electron microscope daily.

7. Make periodic performance checks of magnification, electron diffraction and energy dispersive X-ray systems as set forth in Table III under Unit II.I.

8. Ensure qualified operator performance by evaluation of replicate analysis and standard sample comparisons as set forth in Table III under Unit II.I.

9. Validate all data entries.

10. Recalculate a percentage of all computations and automatic data reduction steps as specified in Table III under Unit II.I.

11. Record an electron diffraction pattern of one asbestos structure from every five samples that contain asbestos. Verify the identification of the pattern by measurement or comparison of the pattern with patterns collected from standards under the same conditions. The records must also demonstrate that the identification of the pattern has been verified by a qualified individual and that the operator who made the identification is maintaining at least an 80 percent correct visual identification based on his measured patterns.

12. Appropriate logs or records must be maintained by the analytical laboratory verifying that it is in compliance with the mandatory quality assurance procedures. J. References

For additional background information on this method, the following references should be consulted.

1. "Guidance for Controlling Asbestos-Containing Materials in Buildings," EPA 560/5-85-024, June 1985.

2. "Measuring Airborne Asbestos Following an Abatement Action," USEPA, Office of Pollution Prevention and Toxics, EPA 600/4-85-049, 1985.

3. Small, John and E. Steel. Asbestos Standards: Materials and Analytical Methods. N.B.S. Special Publication 619, 1982.

4. Campbell, W.J., R.L. Blake, L.L. Brown, E.E. Cather, and J.J. Sjoberg. Selected Silicate Minerals and Their Asbestiform Varieties. Information Circular 8751, U.S. Bureau of Mines, 1977.

5. Quality Assurance Handbook for Air Pollution Measurement System. Ambient Air Methods, EPA 600/4-77-027a, USEPA, Office of Research and Development, 1977.

6. Method 2A: Direct Measurement of Gas Volume through Pipes and Small Ducts. 40 CFR Part 60 Appendix A.

7. Burdette, G.J., Health & Safety Exec. Research & Lab. Services Div., London, "Proposed Analytical Method for Determination of Asbestos in Air."

8. Chatfield, E.J., Chatfield Tech. Cons., Ltd., Clark, T., PEI Assoc., "Standard Operating Procedure for Determination of Airborne Asbestos Fibers by Transmission Electron Microscopy Using Polycarbonate Membrane Filters," WERL SOP 87-1, March 5, 1987.

9. NIOSH Method 7402 for Asbestos Fibers, 12-11-86 Draft.

10. Yamate, G., Agarwall, S.C., Gibbons, R.D., IIT Research Institute, "Methodology for the Measurement of Airborne Asbestos by Electron Microscopy," Draft report, USEPA Contract 68-02-3266, July 1984.

11. "Guidance to the Preparation of Quality Assurance Project Plans," USEPA, Office of Pollution Prevention and Toxics, 1984.

III. Nonmandatory Transmission Electron Microscopy Method

A. Definitions of Terms

1. *Analytical sensitivity* -- Airborne asbestos concentration represented by each fiber counted under the electron microscope. It is determined by the air volume collected and the proportion of the filter examined. This method requires that the analytical sensitivity be no greater than 0.005 s/cm 3.

2. *Asbestiform* -- A specific type of mineral fibrosity in which the fibers and fibrils possess high tensile strength and flexibility.

3. *Aspect ratio* -- A ratio of the length to the width of a particle. Minimum aspect ratio as defined by this method is equal to or greater than 5:1.

4. *Bundle* -- A structure composed of three or more fibers in a parallel arrangement with each fiber closer than one fiber diameter.

5. *Clean area* -- A controlled environment which is maintained and monitored to assure a low probability of asbestos contamination to materials in that space. Clean areas used in this method have HEPA filtered air under positive pressure and are capable of sustained operation with an open laboratory blank which on subsequent analysis has an average of less than 18 structures/mm 2 in an area of 0.057 mm 2 (nominally 10 200 mesh grid openings) and a maximum of 53 structures/mm 2 for no more than one single preparation for that same area.

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. Groupings must have more than two intersections.

7. *ED* -- Electron diffraction.

8. *EDXA* -- Energy dispersive X-ray analysis.

9. *Fiber* -- A structure greater than or equal to 0.5 •m in length with an aspect ratio (length to width) of 5:1 or greater and having substantially parallel sides.

10. *Grid* -- An open structure for mounting on the sample to aid in its examination in the TEM. The term is used here to denote a 200-mesh copper lattice approximately 3 mm in diameter.

11. *Intersection* -- Nonparallel touching or crossing of fibers, with the projection having an aspect ratio of 5:1 or greater.

12. *Laboratory sample coordinator* -- That person responsible for the conduct of sample handling and the certification of the testing procedures.

13. *Filter background level* -- The concentration of structures per square millimeter of filter that is considered indistinguishable from the concentration measured on blanks (filters through which no air has been drawn). For this method the filter background level is defined as 70 structures/mm 2.

14. *Matrix* -- Fiber or fibers with one end free and the other end embedded in or hidden by a particulate. The exposed fiber must meet the fiber definition.

15. *NSD* -- No structure detected.

16. *Operator* -- A person responsible for the TEM instrumental analysis of the sample.

17. *PCM* -- Phase contrast microscopy.

18. *SAED* -- Selected area electron diffraction.

19. *SEM* -- Scanning electron microscope.

20. *STEM* -- Scanning transmission electron microscope.

21. *Structure* -- a microscopic bundle, cluster, fiber, or matrix which may contain asbestos.

22. *S/cm 3* -- Structures per cubic centimeter.

23. *S/mm 2* -- Structures per square millimeter.

24. *TEM* -- Transmission electron microscope. B. Sampling

1. Sampling operations must be performed by qualified individuals completely independent of the abatement contractor to avoid possible conflict of interest (See References 1, 2, and 5 of Unit III.L.) Special precautions should be taken to avoid contamination of the sample. For example, materials that have not been prescreened for their asbestos background content should not be used; also, sample handling procedures which do not take cross contamination possibilities into account should not be used.

2. Material and supply checks for asbestos contamination should be made on all critical supplies, reagents, and procedures before their use in a monitoring study.

3. Quality control and quality assurance steps are needed to identify problem areas and isolate the cause of the contamination (see Reference 5 of Unit III.L.). Control checks shall be permanently recorded to document the quality of the information produced. The sampling firm must have written quality control procedures and documents which verify compliance. Independent audits by a qualified consultant or firm should be performed once a year. All documentation of compliance should be retained indefinitely to provide a guarantee of quality. A summary of Sample Data Quality Objectives is shown in Table II of Unit II.B.

4. Sampling materials.

a. Sample for airborne asbestos following an abatement action using commercially available cassettes.

b. Use either a cowling or a filter-retaining middle piece. Conductive material may reduce the potential for particulates to adhere to the walls of the cowl.

c. Cassettes must be verified as "clean" prior to use in the field. If packaged filters are used for loading or preloaded cassettes are purchased from the manufacturer or a distributor, the manufacturer's name and lot number should be entered on all field data sheets provided to the laboratory, and are required to be listed on all reports from the laboratory.

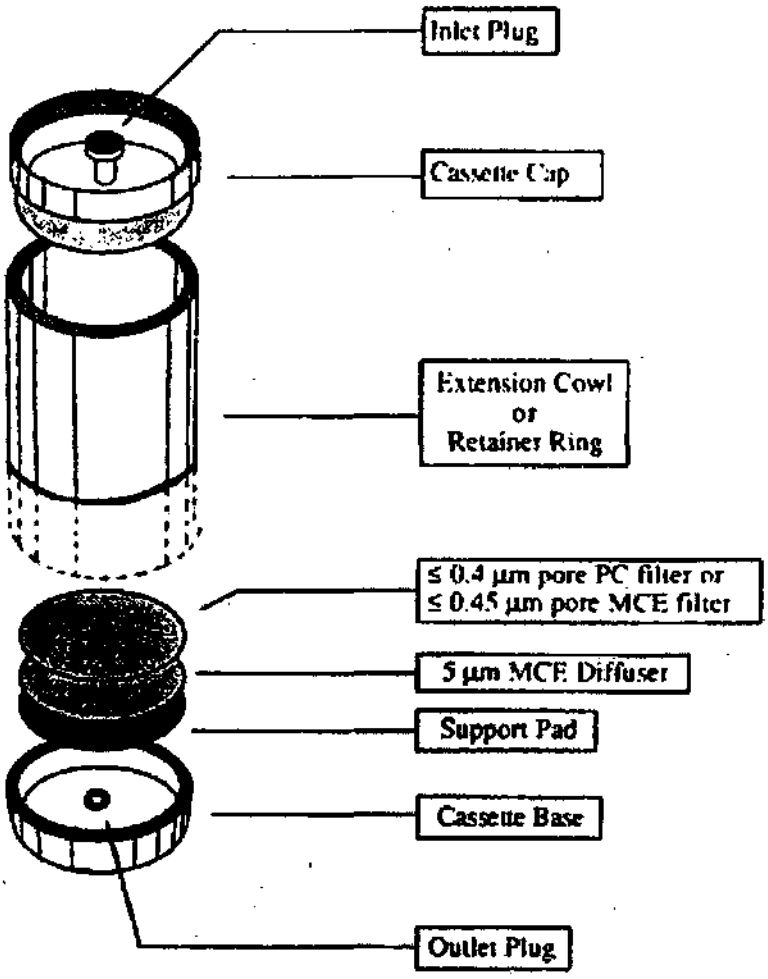
d. Assemble the cassettes in a clean facility (See definition of clean area under Unit III.A.).

e. Reloading of used cassettes is not permitted.

f. Use sample collection filters which are either polycarbonate having a pore size of less than or equal to 0.4 •m or mixed cellulose ester having a pore size of less than or equal to 0.45 •m.

g. Place these filters in series with a backup filter with a pore size of 5.0 •m (to serve as a diffuser) and a support pad. See the following Figure 1:

FIGURE I--SAMPLING CASSETTE CONFIGURATION



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h. When polycarbonate filters are used, position the highly reflective face such that the incoming particulate is received on this surface.

i. Seal the cassettes to prevent leakage around the filter edges or between cassette part joints. A mechanical press may be useful to achieve a reproducible leak-free seal. Shrink fit gel-bands may be used for this purpose and are available from filter manufacturers and their authorized distributors.

j. Use wrinkle-free loaded cassettes in the sampling operation.

5. Pump setup.

a. Calibrate the sampling pump over the range of flow rates and loads anticipated for the monitoring period with this flow measuring device in series. Perform this calibration using guidance from EPA Method 2A each time the unit is sent to the field (See Reference 6 of Unit III.L.).

b. Configure the sampling system to preclude pump vibrations from being transmitted to the cassette by using a sampling stand separate from the pump station and making connections with flexible tubing.

c. Maintain continuous smooth flow conditions by damping out any pump action fluctuations if necessary.

d. Check the sampling system for leaks with the end cap still in place and the pump operating before initiating sample collection. Trace and stop the source of any flow indicated by the flowmeter under these conditions.

e. Select an appropriate flow rate equal to or greater than 1 L/min or less than 10 L/min for 25 mm cassettes. Larger filters may be operated at proportionally higher flow rates.

f. Orient the cassette downward at approximately 45 degrees from the horizontal.

g. Maintain a log of all pertinent sampling information, such as pump identification number, calibration data, sample location, date, sample identification number, flow rates at the beginning, middle, and end, start and stop times, and other useful information or comments. Use of a sampling log form is recommended. See the following Figure 2:

FIGURE 2--SAMPLING LOG FORM

Sample Number	Location of Sample	Pump I.D.	Start Time	Middle Time	End Time	Flow Rate

Inspector: _____ Date: _____

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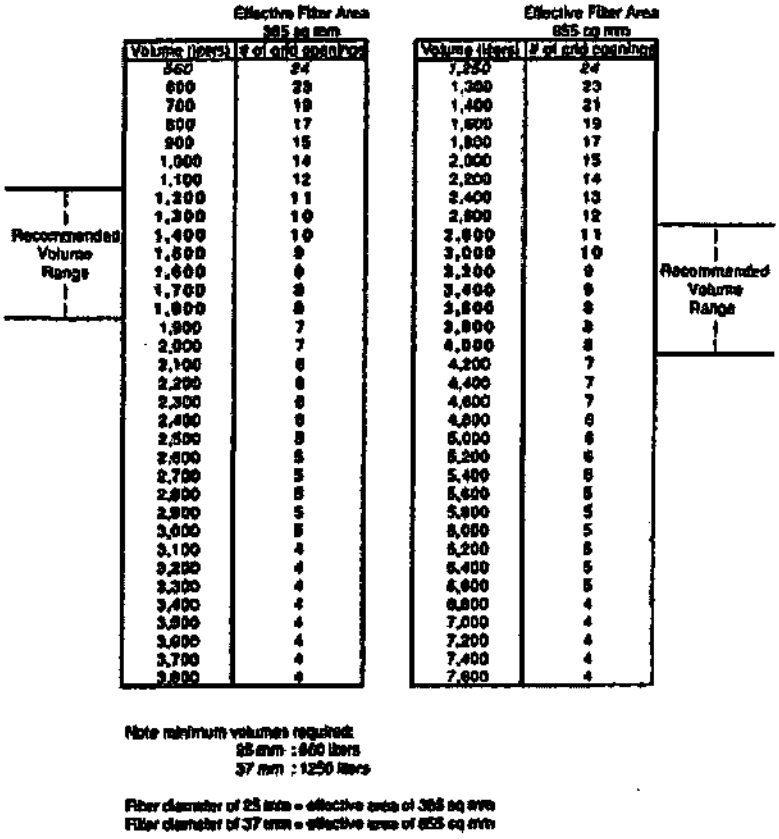
h. Initiate a chain of custody procedure at the start of each sampling, if this is requested by the client.

i. Maintain a close check of all aspects of the sampling operation on a regular basis.

j. Continue sampling until at least the minimum volume is collected, as specified in the following Table I:

TABLE 1--NUMBER OF 200 MESH EM GRID OPENINGS ($0.0057\ MM^2$) THAT NEED TO BE ANALYZED TO MAINTAIN SENSITIVITY OF 0.005 STRUCTURES/CC BASED ON VOLUME AND EFFECTIVE FILTER AREA



k. At the conclusion of sampling, turn the cassette upward before stopping the flow to minimize possible particle loss. If the sampling is resumed, restart the flow before reorienting the cassette downward. Note the condition of the filter at the conclusion of sampling.

l. Double check to see that all information has been recorded on the data collection forms and that the cassette is securely closed and appropriately identified using a waterproof label. Protect cassettes in individual clean resealed polyethylene bags. Bags are to be used for storing cassette caps when they are removed for sampling purposes. Caps and plugs should only be removed or replaced using clean hands or clean disposable plastic gloves.

m. Do not change containers if portions of these filters are taken for other purposes.

6. Minimum sample number per site. A minimum of 13 samples are to be collected for each testing consisting of the following:

a. A minimum of five samples per abatement area.

b. A minimum of five samples per ambient area positioned at locations representative of the air entering the abatement site.

c. Two field blanks are to be taken by removing the cap for not more than 30 sec and replacing it at the time of sampling before sampling is initiated at the following places:

i. Near the entrance to each ambient area.

ii. At one of the ambient sites.

(**Note:** Do not leave the blank open during the sampling period.)

d. A sealed blank is to be carried with each sample set. This representative cassette is not to be opened in the field.

7. Abatement area sampling.

a. Conduct final clearance sampling only after the primary containment barriers have been removed; the abatement area has been thoroughly dried; and, it has passed visual inspection tests by qualified personnel. (See Reference 1 of Unit III.L.)

b. Containment barriers over windows, doors, and air passageways must remain in place until the TEM clearance sampling and analysis is completed and results meet clearance test criteria. The final plastic barrier remains in place for the sampling period.

c. Select sampling sites in the abatement area on a random basis to provide unbiased and representative samples.

d. After the area has passed a thorough visual inspection, use aggressive sampling conditions to dislodge any remaining dust.

i. Equipment used in aggressive sampling such as a leaf blower and/or fan should be properly cleaned and decontaminated before use.

ii. Air filtration units shall remain on during the air monitoring period.

iii. Prior to air monitoring, floors, ceiling and walls shall be swept with the exhaust of a minimum one (1) horsepower leaf blower.

iv. Stationary fans are placed in locations which will not interfere with air monitoring equipment. Fan air

is directed toward the ceiling. One fan shall be used for each 10,000 ft 3 of worksite.

v. Monitoring of an abatement work area with high-volume pumps and the use of circulating fans will require electrical power. Electrical outlets in the abatement area may be used if available. If no such outlets are available, the equipment must be supplied with electricity by the use of extension cords and strip plug units. All electrical power supply equipment of this type must be approved Underwriter Laboratory equipment that has not been modified. All wiring must be grounded. Ground fault interrupters should be used. Extreme care must be taken to clean up any residual water and ensure that electrical equipment does not become wet while operational.

vi. Low volume pumps may be carefully wrapped in 6-mil polyethylene to insulate the pump from the air. High volume pumps cannot be sealed in this manner since the heat of the motor may melt the plastic. The pump exhausts should be kept free.

vii. If recleaning is necessary, removal of this equipment from the work area must be handled with care. It is not possible to completely decontaminate the pump motor and parts since these areas cannot be wetted. To minimize any problems in this area, all equipment such as fans and pumps should be carefully wet wiped prior to removal from the abatement area. Wrapping and sealing low volume pumps in 6-mil polyethylene will provide easier decontamination of this equipment. Use of clean water and disposable wipes should be available for this purpose.

e. Pump flow rate equal to or greater than 1 L/min or less than 10 L/min may be used for 25 mm cassettes. The larger cassette diameters may have comparably increased flow.

f. Sample a volume of air sufficient to ensure the minimum quantitation limits. (See Table I of Unit III.B.5.j.)

8. Ambient sampling.

a. Position ambient samplers at locations representative of the air entering the abatement site. If makeup air entering the abatement site is drawn from another area of the building which is outside of the abatement area, place the pumps in the building, pumps should be placed out of doors located near the building and away from any obstructions that may influence wind patterns. If construction is in progress immediately outside the enclosure, it may be necessary to select another ambient site. Samples should be representative of any air entering the work site.

b. Locate the ambient samplers at least 3 ft apart and protect them from adverse weather conditions.

c. Sample same volume of air as samples taken inside the abatement site. C. Sample Shipment

1. Ship bulk samples in a separate container from air samples. Bulk samples and air samples delivered to the analytical laboratory in the same container shall be rejected.

2. Select a rigid shipping container and pack the cassettes upright in a noncontaminating nonfibrous medium such as a bubble pack. The use of resealable polyethylene bags may help to prevent jostling of individual cassettes.

3. Avoid using expanded polystyrene because of its static charge potential. Also avoid using particle-based packaging materials because of possible contamination.

4. Include a shipping bill and a detailed listing of samples shipped, their descriptions and all identifying numbers or marks, sampling data, shipper's name, and contact information. For each sample set, designate which are the ambient samples, which are the abatement area samples, which are the field blanks, and which is the sealed blank if sequential analysis is to be performed.

5. Hand-carry samples to the laboratory in an upright position if possible; otherwise choose that mode of transportation least likely to jar the samples in transit.

6. Address the package to the laboratory sample coordinator by name when known and alert him or her of the package description, shipment mode, and anticipated arrival as part of the chain of custody and sample tracking procedures. This will also help the laboratory schedule timely analysis for the samples when they are received. D. Quality Control/Quality Assurance Procedures (Data Quality Indicators)

Monitoring the environment for airborne asbestos requires the use of sensitive sampling and analysis procedures. Because the test is sensitive, it may be influenced by a variety of factors. These include the supplies used in the sampling operation, the performance of the sampling, the preparation of the grid from the filter and the actual examination of this grid in the microscope. Each of these unit operations must produce a product of defined quality if the analytical result is to be a reliable and meaningful test result. Accordingly, a series of control checks and reference standards is performed along with the sample analysis as indicators that the materials used are adequate and the operations are within acceptable limits. In this way, the quality of the data is defined, and the results are of known value. These checks and tests also provide timely and specific warning of any problems which might develop within the sampling and analysis operations. A description of these quality control/quality assurance procedures is summarized in the text below.

1. Prescreen the loaded cassette collection filters to assure that they do not contain concentrations of asbestos which may interfere with the analysis of the sample. A filter blank average of less than 18 s/mm 2 in an area of 0.057 mm 2 (nominally 10 200-mesh grid openings) and a maximum of 53 s/mm 2 for that same area for any single preparation is acceptable for this method.

2. Calibrate sampling pumps and their flow indicators over the range of their intended use with a recognized standard. Assemble the sampling system with a representative filter -- not the filter which will be used in sampling -- before and after the sampling operation.

3. Record all calibration information with the data to be used on a standard sampling form.

4. Ensure that the samples are stored in a secure and representative location.

5. Ensure that mechanical calibrations from the pump will be minimized to prevent transferral of vibration to the cassette.

6. Ensure that a continuous smooth flow of negative pressure is delivered by the pump by installing a damping chamber if necessary.

7. Open a loaded cassette momentarily at one of the indoor sampling sites when sampling is initiated. This sample will serve as an indoor field blank.

8. Open a loaded cassette momentarily at one of the outdoor sampling sites when sampling is initiated. This sample will serve as an outdoor field blank.

9. Carry a sealed blank into the field with each sample series. Do not open this cassette in the field.

10. Perform a leak check of the sampling system at each indoor and outdoor sampling site by activating the pump with the closed sampling cassette in line. Any flow indicates a leak which must be eliminated before initiating the sampling operation.

11. Ensure that the sampler is turned upright before interrupting the pump flow.

12. Check that all samples are clearly labeled and that all pertinent information has been enclosed before transfer of the samples to the laboratory. E. Sample Receiving

1. Designate one individual as sample coordinator at the laboratory. While that individual will normally be available to receive samples, the coordinator may train and supervise others in receiving procedures for those times when he/she is not available.

2. Adhere to the following procedures to ensure both the continued chain-of-custody and the accountability of all samples passing through the laboratory:

a. Note the condition of the shipping package and data written on it upon receipt.

b. Retain all bills of lading or shipping slips to document the shipper and delivery time.

c. Examine the chain-of-custody seal, if any, and the package for its integrity.

d. If there has been a break in the seal or substantive damage to the package, the sample coordinator shall

immediately notify the shipper and a responsible laboratory manager before any action is taken to unpack the shipment.

e. Packages with significant damage shall be accepted only by the responsible laboratory manager after discussions with the client.

3. Unwrap the shipment in a clean, uncluttered facility. The sample coordinator or his or her designee will record the contents, including a description of each item and all identifying numbers or marks. A Sample Receiving Form to document this information is attached for use when necessary. (See the following Figure 3.)

FIGURE 3--SAMPLE RECEIVING FORM

Date of package delivery _____ Package shipped from _____

Carrier _____ Shipping bill received _____

*Condition of package on receipt _____

*Condition of custody seal _____

Number of samples received _____ Shipping manifest attached _____

Purchase Order No. _____ Project I.D. _____

Comments _____

No.	Description	Sampling Medium PC	Sampling Medium MCE	Sampled Volume Liters	Receiving ID #	Assigned #
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						

(Use as many additional sheets as needed.)

Comments _____

Date of acceptance into sample bank _____

Signature of chain-of-custody recipient _____

Disposition of samples _____

*Note: If the package has sustained substantial damage or the custody seal is broken, stop and contact the project manager and the shipper.

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Note: The person breaking the chain-of-custody seal and itemizing the contents assumes responsibility for the shipment and signs documents accordingly.

4. Assign a laboratory number and schedule an analysis sequence.

5. Manage all chain-of-custody samples within the laboratory such that their integrity can be ensured and documented. F. Sample Preparation

1. Personnel not affiliated with the Abatement Contractor shall be used to prepare samples and conduct TEM analysis. Wet-wipe the exterior of the cassettes to minimize contamination possibilities before taking them to the clean sample preparation facility.

2. Perform sample preparation in a well-equipped clean facility.

Note: The clean area is required to have the following minimum characteristics. The area or hood must be capable of maintaining a positive pressure with make-up air being HEPA filtered. The cumulative analytical blank concentration must average less than 18 s/mm 2 in an area of 0.057 s/mm 2 (nominally 10 200-mesh grid openings) with no more than one single preparation to exceed 53 s/mm 2 for that same area.

3. Preparation areas for air samples must be separated from preparation areas for bulk samples. Personnel must not prepare air samples if they have previously been preparing bulk samples without performing appropriate personal hygiene procedures, i.e., clothing change, showering, etc.

4. *Preparation.* Direct preparation techniques are required. The objective is to produce an intact carbon film containing the particulates from the filter surface which is sufficiently clear for TEM analysis. Currently recommended direct preparation procedures for polycarbonate (PC) and mixed cellulose ester (MCE) filters are described in Unit III.F.7. and 8. Sample preparation is a subject requiring additional research. Variation on those steps which do not substantively change the procedure, which improve filter clearing or which reduce contamination problems in a laboratory are permitted.

a. Use only TEM grids that have had grid opening areas measured according to directions in Unit III.J.

b. Remove the inlet and outlet plugs prior to opening the cassette to minimize any pressure differential that may be present.

c. Examples of techniques used to prepare polycarbonate filters are described in Unit III.F.7.

d. Examples of techniques used to prepare mixed cellulose ester filters are described in Unit III.F.8.

e. Prepare multiple grids for each sample.

f. Store the three grids to be measured in appropriately labeled grid holders or polyethylene capsules.

5. Equipment.

a. Clean area.

b. Tweezers. Fine-point tweezers for handling of filters and TEM grids.

c. Scalpel Holder and Curved No. 10 Surgical Blades.

d. Microscope slides.

e. Double-coated adhesive tape.

f. Gummed page reinforcements.

g. Micro-pipet with disposal tips 10 to 100 •L variable volume.

h. Vacuum coating unit with facilities for evaporation of carbon. Use of a liquid nitrogen cold trap above the diffusion pump will minimize the possibility of contamination of the filter surface by oil from the pumping system. The vacuum-coating unit can also be used for deposition of a thin film of gold.

i. *Carbon rod electrodes.* Spectrochemically pure carbon rods are required for use in the vacuum evaporator for carbon coating of filters.

j. *Carbon rod sharpener.* This is used to sharpen carbon rods to a neck. The use of necked carbon rods (or equivalent) allows the carbon to be applied to the filters with a minimum of heating.

k. *Low-temperature plasma asher.* This is used to etch the surface of collapsed mixed cellulose ester (MCE) filters. The asher should be supplied with oxygen, and should be modified as necessary to provide a throttle or bleed valve to control the speed of the vacuum to minimize disturbance of the filter. Some early models of ashers admit air too rapidly, which may disturb particulates on the surface of the filter during the etching step.

l. *Glass petri dishes, 10 cm in diameter, 1 cm high.* For prevention of excessive evaporation of solvent when these are in use, a good seal must be provided between the base and the lid. The seal can be improved by grinding the base and lid together with an abrasive grinding material.

m. Stainless steel mesh.

n. Lens tissue.

o. Copper 200-mesh TEM grids, 3 mm in diameter, or equivalent.

p. Gold 200-mesh TEM grids, 3 mm in diameter, or equivalent.

q. Condensation washer.

r. Carbon-coated, 200-mesh TEM grids, or equivalent.

s. Analytical balance, 0.1 mg sensitivity.

t. Filter paper, 9 cm in diameter.

u. Oven or slide warmer. Must be capable of maintaining a temperature of 65-70 °C.

v. Polyurethane foam, 6 mm thickness.

w. Gold wire for evaporation.

6. Reagents.

a. *General.* A supply of ultra-clean, fiber-free water must be available for washing of all components used in the analysis. Water that has been distilled in glass or filtered or deionized water is satisfactory for this purpose. Reagents must be fiber-free.

b. Polycarbonate preparation method -- chloroform.

c. Mixed Cellulose Ester (MCE) preparation method -- acetone or the Burdette procedure (Ref. 7 of Unit III.L.).

7. TEM specimen preparation from polycarbonate filters.

a. *Specimen preparation laboratory.* It is most important to ensure that contamination of TEM specimens by extraneous asbestos fibers is minimized during preparation.

b. Cleaning of sample cassettes. Upon receipt at the analytical laboratory and before they are taken into the clean facility or laminar flow hood, the sample cassettes must be cleaned of any contamination adhering to the outside surfaces.

c. Preparation of the carbon evaporator. If the polycarbonate filter has already been carbon-coated prior to receipt, the carbon coating step will be omitted, unless the analyst believes the carbon film is too thin. If there is a need to apply more carbon, the filter will be treated in the same way as an uncoated filter. Carbon coating must be performed with a high-vacuum coating unit. Units that are 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 to 12 cm from the surface of a microscope slide held in the rotating and tilting device.

d. Selection of filter area for carbon coating. Before preparation of the filters, a 75 mm x 50 mm microscope slide is washed and dried. This slide is used to support strips of filter during the carbon evaporation. Two parallel strips of double-sided adhesive tape are applied along the length of the slide. Polycarbonate filters are easily stretched during handling, and cutting of areas for further preparation must be performed with great care. The filter and the MCE backing filter are removed together from the cassette and placed on a cleaned glass microscope slide. The filter can be cut with a curved scalpel blade by rocking the blade from the point placed in contact with the filter. The process can be repeated to cut a strip approximately 3 mm wide across the diameter of the filter. The strip of polycarbonate filter is separated from the corresponding strip of backing filter and carefully placed so that it bridges the gap between the adhesive tape strips on the microscope slide. The filter strip can be held with fine-point tweezers and supported underneath by the scalpel blade during placement on the microscope slide. The analyst can place several such strips on the same microscope slide, taking care to rinse and wet-wipe the scalpel blade and tweezers before handling a new sample. The filter strips should be identified by etching the glass slide or marking the slide using a marker insoluble in water and solvents. After the filter strip has been cut from each filter, the residual parts of the filter must be returned to the cassette and held in position by reassembly of the cassette. The cassette will then be archived for a period of 30 days or returned to the client upon request.

e. Carbon coating of filter strips. The glass slide holding the filter strips is placed on the rotation-tilting device, and the evaporator chamber is evacuated. The evaporation must be performed in very short bursts, separated by some seconds to allow the electrodes to cool. If evaporation is too rapid, the strips of polycarbonate filter will begin to curl, which will lead to cross-linking of the surface material and make it relatively insoluble in chloroform. An experienced analyst can judge the thickness of carbon film to be applied, and some test should be made first on unused filters. If the film is too thin, large particles will be lost from the TEM specimen, and there will be few complete and undamaged grid openings on the specimen. If the coating is too thick, the filter will tend to curl when exposed to chloroform vapor and the carbon film may not adhere to the support mesh. Too thick a carbon film will also lead to a TEM image that is lacking in contrast, and the ability to obtain ED patterns will be compromised. The carbon film should be as thin as possible and remain intact on most of the grid openings of the TEM specimen intact.

f. Preparation of the Jaffe washer. The precise design of the Jaffe washer is not considered important, so any one of the published designs may be used. A washer consisting of a simple stainless steel bridge is recommended. Several pieces of lens tissue approximately 1.0 cm x 0.5 cm are placed on the stainless

steel bridge, and the washer is filled with chloroform to a level where the meniscus contacts the underside of the mesh, which results in saturation of the lens tissue. See References 8 and 10 of Unit III.L.

g. Placing of specimens into the Jaffe washer. The TEM grids are first placed on a piece of lens tissue so that individual grids can be picked up with tweezers. Using a curved scalpel blade, the analyst excises three 3 mm square pieces of the carbon-coated polycarbonate filter from the filter strip. The three squares are selected from the center of the strip and from two points between the outer periphery of the active surface and the center. The piece of filter is placed on a TEM specimen grid with the shiny side of the TEM grid facing upwards, and the whole assembly is placed boldly onto the saturated lens tissue in the Jaffe washer. If carbon-coated grids are used, the filter should be placed carbon-coated side down. The three excised squares of filters are placed on the same piece of lens tissue. Any number of separate pieces of lens tissue may be placed in the same Jaffe washer. The lid is then placed on the Jaffe washer, and the system is allowed to stand for several hours, preferably overnight.

h. *Condensation washing*. It has been found that many polycarbonate filters will not dissolve completely in the Jaffe washer, even after being exposed to chloroform for as long as 3 days. This problem becomes more serious if the surface of the filter was overheated during the carbon evaporation. The presence of undissolved filter medium on the TEM preparation leads to partial or complete obscuration of areas of the sample, and fibers that may be present in these areas of the specimen will be overlooked; this will lead to a low result. Undissolved filter medium also compromises the ability to obtain ED patterns. Before they are counted, TEM grids must be examined critically to determine whether they are adequately cleared of residual filter medium. It has been found that condensation washing of the grids after the initial Jaffe washer treatment, with chloroform as the solvent, clears all residual filter medium in a period of approximately 1 hour. In practice, the piece of lens tissue supporting the specimen grids is transferred to the cold finger of the condensation washer, and the washer is operated for about 1 hour. If the specimens are cleared satisfactorily by the Jaffe washer alone, the condensation washer step may be unnecessary.

8. TEM specimen preparation from MCE filters.

a. This method of preparing TEM specimens from MCE filters is similar to that specified in NIOSH Method 7402. See References 7, 8, and 9 of Unit III.L.

b. Upon receipt at the analytical laboratory, the sample cassettes must be cleaned of any contamination adhering to the outside surfaces before entering the clean sample preparation area.

c. Remove a section from any quadrant of the sample and blank filters.

d. Place the section on a clean microscope slide. Affix the filter section to the slide with a gummed paged reinforcement or other suitable means. Label the slide with a water and solvent-proof marking pen.

e. Place the slide in a petri dish which contains several paper filters soaked with 2 to 3 mL acetone. Cover the dish. Wait 2 to 4 minutes for the sample filter to fuse and clear.

f. Plasma etching of the collapsed filter is required.

i. The microscope slide to which the collapsed filter pieces are attached is placed in a plasma asher. Because plasma ashers vary greatly in their performance, both from unit to unit and between different positions in the asher chamber, it is difficult to specify the conditions that should be used. This is one area of the method that requires further evaluation. Insufficient etching will result in a failure to expose embedded filters, and too much etching may result in loss of particulate from the surface. As an interim measure, it is recommended that the time for ashing of a known weight of a collapsed filter be established and that the etching rate be calculated in terms of micrometers per second. The actual etching time used for a particular asher and operating conditions will then be set such that a 1-2 •m (10 percent) layer of collapsed surface will be removed.

ii. Place the slide containing the collapsed filters into a low-temperature plasma asher, and etch the filter.

g. Transfer the slide to a rotating stage inside the bell jar of a vacuum evaporator. Evaporate a 1 mm x 5 mm section of graphite rod onto the cleared filter. Remove the slide to a clean, dry, covered petri dish.

h. Prepare a second petri dish as a Jaffe washer with the wicking substrate prepared from filter or lens paper placed on top of a 6 mm thick disk of clean spongy polyurethane foam. Cut a V-notch on the edge of the foam and filter paper. Use the V-notch as a reservoir for adding solvent. The wicking substrate should be thin enough to fit into the petri dish without touching the lid.

i. Place carbon-coated TEM grids face up on the filter or lens paper. Label the grids by marking with a pencil on the filter paper or by putting registration marks on the petri dish lid and marking with a waterproof marker on the dish lid. In a fume hood, fill the dish with acetone until the wicking substrate is saturated. The level of acetone should be just high enough to saturate the filter paper without creating puddles.

j. Remove about a quarter section of the carbon-coated filter samples from the glass slides using a surgical knife and tweezers. Carefully place the section of the filter, carbon side down, on the appropriately labeled grid in the acetone-saturated petri dish. When all filter sections have been transferred, slowly add more solvent to the wedge-shaped trough to bring the acetone level up to the highest possible level without disturbing the sample preparations. Cover the petri dish. Elevate one side of the petri dish by placing a slide under it. This allows drops of condensed solvent vapors to form near the edge rather than in the center where they would drip onto the grid preparation. G. TEM Method

1. *Instrumentation.*

a. Use an 80-120 kV TEM capable of performing electron diffraction with a fluorescent screen inscribed

with calibrated gradations. If the TEM is equipped with EDXA it must either have a STEM attachment or be capable of producing a spot less than 250 nm in diameter at crossover. The microscope shall be calibrated routinely (see Unit III.J.) for magnification and camera constant.

b. While not required on every microscope in the laboratory, the laboratory must have either one microscope equipped with energy dispersive X-ray analysis or access to an equivalent system on a TEM in another laboratory. This must be an Energy Dispersive X-ray Detector mounted on TEM column and associated hardware/software to collect, save, and read out spectral information. Calibration of Multi-Channel Analyzer shall be checked regularly for A1 at 1.48 KeV and Cu at 8.04 KeV, as well as the manufacturer's procedures.

i. Standard replica grating may be used to determine magnification (e.g., 2160 lines/mm).

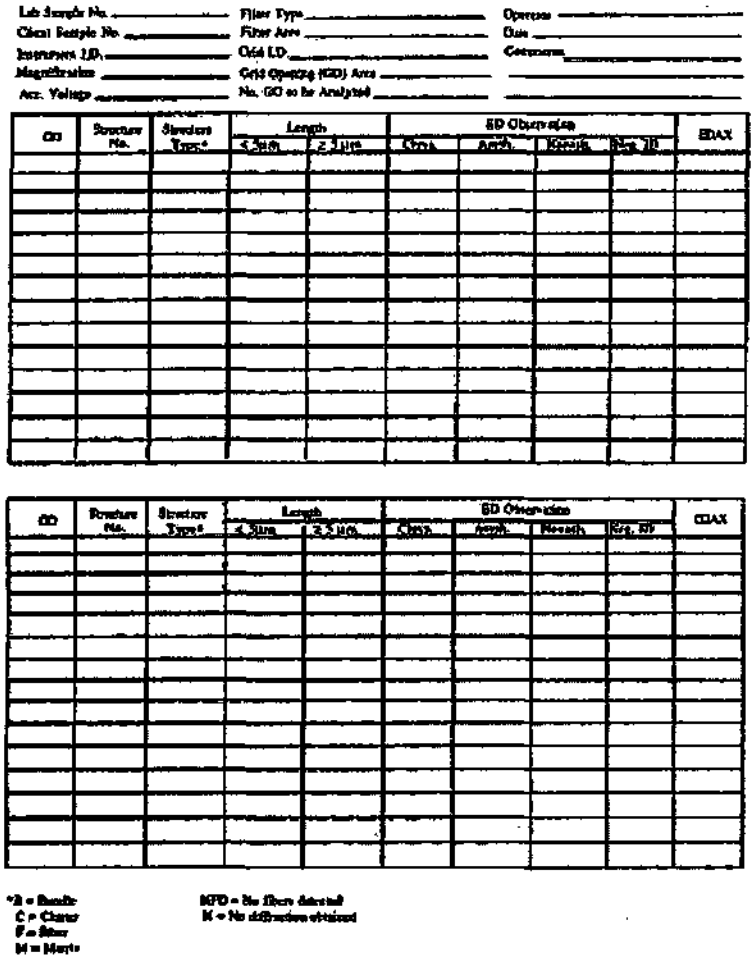
ii. Gold standard may be used to determine camera constant.

c. Use a specimen holder with single tilt and/or double tilt capabilities.

2. Procedure.

a. Start a new Count Sheet for each sample to be analyzed. Record on count sheet: analyst's initials and date; lab sample number; client sample number microscope identification; magnification for analysis; number of predetermined grid openings to be analyzed; and grid identification. See the following Figure 4:

FIGURE 4--COUNT SHEET



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b. Check that the microscope is properly aligned and calibrated according to the manufacturer's specifications and instructions.

c. Microscope settings: 80-120 kV, grid assessment 250-1000X, then 15,000-20,000X screen magnification for analysis.

d. Approximately one-half (0.5) of the predetermined sample area to be analyzed shall be performed on one sample grid preparation and the remaining half on a second sample grid preparation.

e. Determine the suitability of the grid.

i. Individual grid openings with greater than 5 percent openings (holes) or covered with greater than 25 percent particulate matter or obviously having nonuniform loading shall not be analyzed.

ii. Examine the grid at low magnification (<1000X) to determine its suitability for detailed study at higher magnifications.

iii. Reject the grid if:

(1) Less than 50 percent of the grid openings covered by the replica are intact.

(2) It is doubled or folded.

(3) It is too dark because of incomplete dissolution of the filter.

iv. If the grid is rejected, load the next sample grid.

v. If the grid is acceptable, continue on to Step 6 if mapping is to be used; otherwise proceed to Step 7.

f. Grid Map (Optional).

i. Set the TEM to the low magnification mode.

ii. Use flat edge or finder grids for mapping.

iii. Index the grid openings (fields) to be counted by marking the acceptable fields for one-half (0.5) of the area needed for analysis on each of the two grids to be analyzed. These may be marked just before examining each grid opening (field), if desired.

iv. Draw in any details which will allow the grid to be properly oriented if it is reloaded into the microscope and a particular field is to be reliably identified.

g. Scan the grid.

i. Select a field to start the examination.

ii. Choose the appropriate magnification (15,000 to 20,000X screen magnification).

iii. Scan the grid as follows.

(1) At the selected magnification, make a series of parallel traverses across the field. On reaching the end

of one traverse, move the image one window and reverse the traverse.

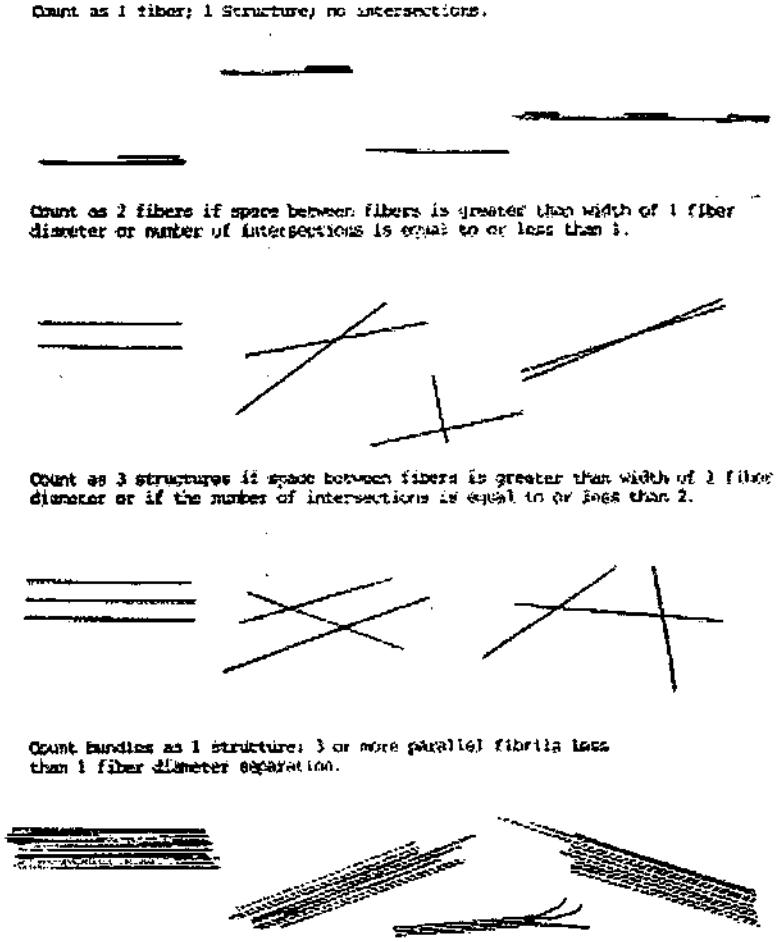
Note: A slight overlap should be used so as not to miss any part of the grid opening (field).

(2) Make parallel traverses until the entire grid opening (field) has been scanned.

h. Identify each structure for appearance and size.

i. Appearance and size: Any continuous grouping of particles in which an asbestos fiber within aspect ratio greater than or equal to 5:1 and a length greater than or equal to 0.5 •m is detected shall be recorded on the count sheet. These will be designated asbestos structures and will be classified as fibers, bundles, clusters, or matrices. Record as individual fibers any contiguous grouping having 0, 1, or 2 definable intersections. Groupings having more than 2 intersections are to be described as cluster or matrix. See the following Figure 5:

FIGURE 5--COUNTING GUIDELINES USED IN DETERMINING ASBESTOS STRUCTURES



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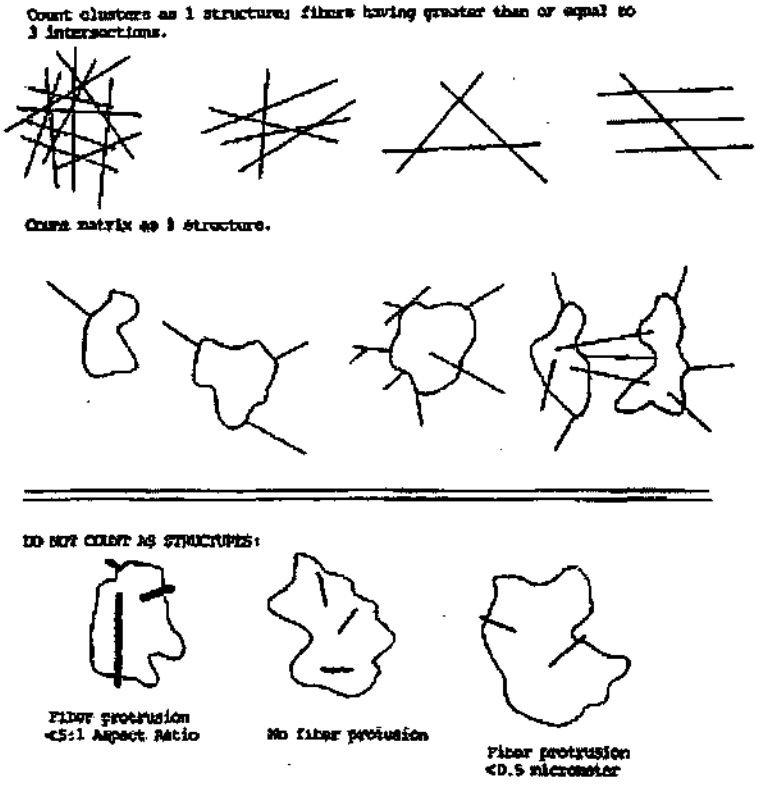

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An intersection is a non-parallel touching or crossing of fibers, with the projection having an aspect ratio of 5:1 or greater. Combinations such as a matrix and cluster, matrix and bundle, or bundle and cluster are categorized by the dominant fiber quality -- cluster, bundle, and matrix, respectively. Separate categories will be maintained for fibers less than 5 •m and for fibers greater than or equal to 5 •m in length. Not required, but useful, may be to record the fiber length in 1 •m intervals. (Identify each structure morphologically and analyze it as it enters the "window".)

(1) *Fiber.* A structure having a minimum length greater than 0.5 •m and an aspect ratio (length to width) of 5:1 or greater and substantially parallel sides. Note the appearance of the end of the fiber, i.e., whether it is flat, rounded or dovetailed, no intersections.

(2) *Bundle.* A structure composed of 3 or more fibers in a parallel arrangement with each fiber closer than one fiber diameter.

(3) *Cluster.* A structure with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group; groupings must have more than 2 intersections.

(4) *Matrix.* Fiber or fibers with one end free and the other end embedded in or hidden by a particulate. The exposed fiber must meet the fiber definition.

(5) *NSD.* Record NSD when no structures are detected in the field.

(6) *Intersection.* Non-parallel touching or crossing of fibers, with the projection having an aspect ratio 5:1 or greater.

ii. Structure Measurement.

(1) Recognize the structure that is to be sized.

(2) Memorize its location in the "window" relative to the sides, inscribed square and to other particulates in the field so this exact location can be found again when scanning is resumed.

(3) Measure the structure using the scale on the screen.

(4) Record the length category and structure type classification on the count sheet after the field number and fiber number.

(5) Return the fiber to its original location in the window and scan the rest of the field for other fibers; if the direction of travel is not remembered, return to the right side of the field and begin the traverse again.

i. Visual identification of Electron Diffraction (ED) patterns is required for each asbestos structure counted which would cause the analysis to exceed the 70 s/mm 2 concentration. (Generally this means the first four fibers identified as asbestos must exhibit an identifiable diffraction pattern for chrysotile or amphibole.)

i. Center the structure, focus, and obtain an ED pattern. (See Microscope Instruction Manual for more detailed instructions.)

ii. From a visual examination of the ED pattern, obtained with a short camera length, classify the observed structure as belonging to one of the following classifications: chrysotile, amphibole, or nonasbestos.

(1) Chrysotile: The chrysotile asbestos pattern has characteristic streaks on the layer lines other than the central line and some streaking also on the central line. There will be spots of normal sharpness on the central layer line and on alternate lines (2nd, 4th, etc.). The repeat distance between layer lines is 0.53 nm and the center doublet is at 0.73 nm. The pattern should display (002), (110), (130) diffraction maxima; distances and geometry should match a chrysotile pattern and be measured semiquantitatively.

(2) Amphibole Group [includes grunerite (amosite), crocidolite, anthophyllite, tremolite, and actinolite]: Amphibole asbestos fiber patterns show layer lines formed by very closely spaced dots, and the repeat distance between layer lines is also about 0.53 nm. Streaking in layer lines is occasionally present due to crystal structure defects.

(3) Nonasbestos: Incomplete or unobtainable ED patterns, a nonasbestos EDXA, or a nonasbestos morphology.

iii. The micrograph number of the recorded diffraction patterns must be reported to the client and maintained in the laboratory's quality assurance records. The records must also demonstrate that the identification of the pattern has been verified by a qualified individual and that the operator who made the identification is maintaining at least an 80 percent correct visual identification based on his measured patterns. In the event that examination of the pattern by the qualified individual indicates that the pattern had been misidentified visually, the client shall be contacted. If the pattern is a suspected chrysotile, take a photograph of the diffraction pattern at 0 degrees tilt. If the structure is suspected to be amphibole, the sample may have to be tilted to obtain a simple geometric array of spots.

j. Energy Dispersive X-Ray Analysis (EDXA).

i. Required of all amphiboles which would cause the analysis results to exceed the 70 s/mm 2 concentration. (Generally speaking, the first 4 amphiboles would require EDXA.)

ii. Can be used alone to confirm chrysotile after the 70 s/mm 2 concentration has been exceeded.

iii. Can be used alone to confirm all nonasbestos.

iv. Compare spectrum profiles with profiles obtained from asbestos standards. The closest match identifies and categorizes the structure.

v. If the EDXA is used for confirmation, record the properly labeled spectrum on a computer disk, or if a hard copy, file with analysis data.

vi. If the number of fibers in the nonasbestos class would cause the analysis to exceed the 70 s/mm 2 concentration, their identities must be confirmed by EDXA or measurement of a zone axis diffraction pattern to establish that the particles are nonasbestos.

k. Stopping Rules.

i. If more than 50 asbestiform structures are counted in a particular grid opening, the analysis may be terminated.

ii. After having counted 50 asbestiform structures in a minimum of 4 grid openings, the analysis may be terminated. The grid opening in which the 50th fiber was counted must be completed.

iii. For blank samples, the analysis is always continued until 10 grid openings have been analyzed.

iv. In all other samples the analysis shall be continued until an analytical sensitivity of 0.005 s/cm 3 is reached.

l. Recording Rules. The count sheet should contain the following information:

i. Field (grid opening): List field number.

ii. Record "NSD" if no structures are detected.

iii. Structure information.

(1) If fibers, bundles, clusters, and/or matrices are found, list them in consecutive numerical order, starting over with each field.

(2) Length. Record length category of asbestos fibers examined. Indicate if less than 5 •m or greater than or equal to 5 •m.

(3) Structure Type. Positive identification of asbestos fibers is required by the method. At least one diffraction pattern of each fiber type from every five samples must be recorded and compared with a standard diffraction pattern. For each asbestos fiber reported, both a morphological descriptor and an identification descriptor shall be specified on the count sheet.

(4) Fibers classified as chrysotile must be identified by diffraction and/or X-ray analysis and recorded on the count sheet. X-ray analysis alone can be used as sole identification only after 70s/mm 2 have been exceeded for a particular sample.

(5) Fibers classified as amphiboles must be identified by X-ray analysis and electron diffraction and

recorded on the count sheet. (X-ray analysis alone can be used as sole identification only after 70s/mm 2 have been exceeded for a particular sample.)

(6) If a diffraction pattern was recorded on film, the micrograph number must be indicated on the count sheet.

(7) If an electron diffraction was attempted and an appropriate spectra is not observed, N should be recorded on the count sheet.

(8) If an X-ray analysis is attempted but not observed, N should be recorded on the count sheet.

(9) If an X-ray analysis spectrum is stored, the file and disk number must be recorded on the count sheet.

m. Classification Rules.

i. *Fiber.* A structure having a minimum length greater than or equal to 0.5 •m and an aspect ratio (length to width) of 5:1 or greater and substantially parallel sides. Note the appearance of the end of the fiber, i.e., whether it is flat, rounded or dovetailed.

ii. *Bundle.* A structure composed of three or more fibers in a parallel arrangement with each fiber closer than one fiber diameter.

iii. *Cluster.* A structure with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group. Groupings must have more than two intersections.

iv. *Matrix.* Fiber or fibers with one end free and the other end embedded in or hidden by a particulate. The exposed fiber must meet the fiber definition.

v. *NSD.* Record NSD when no structures are detected in the field.

n. After all necessary analyses of a particle structure have been completed, return the goniometer stage to 0 degrees, and return the structure to its original location by recall of the original location.

o. Continue scanning until all the structures are identified, classified and sized in the field.

p. Select additional fields (grid openings) at low magnification; scan at a chosen magnification (15,000 to 20,000X screen magnification); and analyze until the stopping rule becomes applicable.

q. Carefully record all data as they are being collected, and check for accuracy.

r. After finishing with a grid, remove it from the microscope, and replace it in the appropriate grid hold.

Sample grids must be stored for a minimum of 1 year from the date of the analysis; the sample cassette must be retained for a minimum of 30 days by the laboratory or returned at the client's request. H. Sample Analytical Sequence

1. Carry out visual inspection of work site prior to air monitoring.

2. Collect a minimum of five air samples inside the work site and five samples outside the work site. The indoor and outdoor samples shall be taken during the same time period.

3. Analyze the abatement area samples according to this protocol. The analysis must meet the 0.005 s/cm 3 analytical sensitivity.

4. Remaining steps in the analytical sequence are contained in Unit IV. of this Appendix. I. Reporting

The following information must be reported to the client. See the following Table II:

TABLE I1--EXAMPLE LABORATORY LETTERHEAD

Laboratory I.D.	Client I.D.	FILTER MEDIA DATA				Analyzed[4] Area, mm²	Sample Volume, cc
		Type	Diameter, mm	Effective Area, mm²	Pore Size, µm		

INDIVIDUAL ANALYTICAL RESULTS

Laboratory I.D.	Client I.D.	# Asbestos Structures	Analytical Sensitivity, s/cc	CONCENTRATION	
				Structures/mm²[1]	Structures/cc

The analysis was carried out to the approved TEM method. This laboratory is in compliance with the quality specified by the method.

Authorized Signature

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1. Concentration in structures per square millimeter and structures per cubic centimeter.

2. Analytical sensitivity used for the analysis.

3. Number of asbestos structures.

4. Area analyzed.

5. Volume of air samples (which was initially provided by client).

6. Average grid size opening.

7. Number of grids analyzed.

8. Copy of the count sheet must be included with the report.

9. Signature of laboratory official to indicate that the laboratory met specifications of the AHERA method.

10. Report form must contain official laboratory identification (e.g., letterhead).

11. Type of asbestos. J. Calibration Methodology

Note: Appropriate implementation of the method requires a person knowledgeable in electron diffraction and mineral identification by ED and EDXA. Those inexperienced laboratories wishing to develop capabilities may acquire necessary knowledge through analysis of appropriate standards and by following detailed methods as described in References 8 and 10 of Unit III.L.

1. *Equipment Calibration.* In this method, calibration is required for the air-sampling equipment and the transmission electron microscope (TEM).

a. *TEM Magnification.* The magnification at the fluorescent screen of the TEM must be calibrated at the grid opening magnification (if used) and also at the magnification used for fiber counting. This is performed with a cross grating replica. A logbook must be maintained, and the dates of calibration depend on the past history of the particular microscope; no frequency is specified. After any maintenance of the microscope that involved adjustment of the power supplied to the lenses or the high-voltage system or the mechanical disassembly of the electron optical column apart from filament exchange, the magnification must be recalibrated. Before the TEM calibration is performed, the analyst must ensure that the cross grating replica is placed at the same distance from the objective lens as the specimens are. For instruments that incorporate an eucentric tilting specimen stage, all speciments and the cross grating replica must be placed at the eucentric position.

b. Determination of the TEM magnification on the fluorescent screen.

i. 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).

ii. Insert a diffraction grating replica (for example a grating containing 2,160 lines/mm) into the specimen holder and place into the microscope. Orient the replica so that the grating lines fall perpendicular to the scale on the TEM fluorescent screen. Ensure that the goniometer stage tilt is 0 degrees.

iii. Adjust microscope magnification to 10,000X or 20,000X. Measure the distance (mm) between two widely separated lines on the grating replica. Note the number of spaces between the lines. Take care to measure between the same relative positions on the lines (e.g., between left edges of lines).

Note: The more spaces included in the measurement, the more accurate the final calculation. On most microscopes, however, the magnification is substantially constant only within the central 8-10 cm diameter region of the fluorescent screen.

iv. Calculate the true magnification (M) on the fluorescent screen:

$$M=XG/Y$$

where:

X=total distance (mm) between the designated grating lines;

G=calibration constant of the grating replica (lines/mm):

Y=number of grating replica spaces counted along X.

c. Calibration of the EDXA System. Initially, the EDXA system must be calibrated by using two reference elements to calibrate the energy scale of the instrument. When this has been completed in accordance with the manufacturer's instructions, calibration in terms of the different types of asbestos can proceed. The EDXA detectors vary in both solid angle of detection and in window thickness. Therefore, at a particular accelerating voltage in use on the TEM, the count rate obtained from specific dimensions of fiber will vary both in absolute X-ray count rate and in the relative X-ray peak heights for different elements. Only a few minerals are relevant for asbestos abatement work, and in this procedure the calibration is specified in terms of a "fingerprint" technique. The EDXA spectra must be recorded from individual fibers of the relevant minerals, and identifications are made on the basis of semiquantitative comparisons with these reference spectra.

d. Calibration of Grid Openings.

i. Measure 20 grid openings on each of 20 random 200-mesh copper grids by placing a grid on a glass slide and examining it under the PCM. Use a calibrated graticule to measure the average field diameter and use this number to calculate the field area for an average grid opening. Grids are to be randomly selected from batches up to 1,000.

Note: A grid opening is considered as one field.

ii. The mean grid opening area must be measured for the type of specimen grids in use. This can be accomplished on the TEM at a properly calibrated low magnification or on an optical microscope at a magnification of approximately 400X by using an eyepiece fitted with a scale that has been calibrated against a stage micrometer. Optical microscopy utilizing manual or automated procedures may be used providing instrument calibration can be verified.

e. Determination of Camera Constant and ED Pattern Analysis.

i. The camera length of the TEM in ED operating mode must be calibrated before ED patterns on 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 is evaporated on the specimen TEM grid to obtain zone-axis ED patterns superimposed with a ring pattern from the polycrystalline gold film.

ii. 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 film would normally give multiple gold rings, but it will tend to mask weaker diffraction spots from the unknown 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 are unnecessary on zone-axis ED patterns. An average camera constant using multiple gold rings can be determined. The camera constant is one-half the diameter, D, of the rings times the interplanar spacing, d, of the ring being measured. K. Quality Control/Quality Assurance Procedures (Data Quality Indicators)

Monitoring the environment for airborne asbestos requires the use of sensitive sampling and analysis procedures. Because the test is sensitive, it may be influenced by a variety of factors. These include the supplies used in the sampling operation, the performance of the sampling, the preparation of the grid from the filter and the actual examination of this grid in the microscope. Each of these unit operations must produce a product of defined quality if the analytical result is to be a reliable and meaningful test result. Accordingly, a series of control checks and reference standards is performed along with the sample analysis as indicators that the materials used are adequate and the operations are within acceptable limits. In this way, the quality of the data is defined and the results are of known value. These checks and tests also provide timely and specific warning of any problems which might develop within the sampling and analysis operations. A description of these quality control/quality assurance procedures is summarized in the following Table III:

TABLE III--SUMMARY OF LABORATORY DATA QUALITY OBJECTIVES

Unit Operation	QC Check	Frequency	Conformance Percentage
Sample receiving	Review of receiving report	Each sample	95% complete
Sample custody	Review of chain-of-custody record	Each sample	95% complete
Sample preparation	Supplies and reagents	On receipt	Meet specs. or reject
	Grid opening size	25 openings/20 grids/or of 1000 or 1 opening/sample	100%
	Spatial clean area monitoring	After cleaning or service	Meet specs or reclean
	Laboratory blank	1 per prep series or 10%	Meet specs. or reanalyze series
	Plasma ash blank	1 per 20 samples	15%
	Multiple preps (2) per sample	Each sample	One with cover of 15 complete grid sqs.
Sample analysis	System check	Each day	Each day
	Alignment check	Each day	Each day
	Magnification calibration with low and high standards	Each month or after service	95%
	ED calibration by gold standard	Weekly	95%
	EDS calibration by copper line	Daily	95%
Performance check	Laboratory blank (measure of cleanliness)	Prep 2 per series or 10% and 1 per 20 samples	Meet specs or reanalyze series
	Replicate counting (measure of precision)	1 per 100 samples	1.5 x Poisson Std. Dev.
	Duplicate analysis (measure of reproducibility)	1 per 100 samples	2 x Poisson Std. Dev.
	Known samples of typical materials (working standards)	Training and for comparison with unknowns	100%
	Analysis of NBS SRM 1878 and/or RM 8410 (measure of accuracy and comparability)	1 per analyst per year	1.5 x Poisson Std. Dev.
	Data entry review (data validation and measure of completeness)	Each sample	95%
	Record and verify ID electron diffraction pattern of structure	1 per 5 samples	80% accuracy
Calculations and data reduction	Hand calculation of automated data reduction procedure or independent recalculation of hand-calculated data	1 per 100 samples	85%

1. When the samples arrive at the laboratory, check the samples and documentation for completeness and requirements before initiating the analysis.

2. Check all laboratory reagents and supplies for acceptable asbestos background levels.

3. Conduct all sample preparation in a clean room environment monitored by laboratory blanks and special testing after cleaning or servicing the room.

4. Prepare multiple grids of each sample.

5. Provide laboratory blanks with each sample batch. Maintain a cumulative average of these results. If this average is greater than 53 f/mm 2 per 10 200-mesh grid openings, check the system for possible sources of contamination.

6. Check for recovery of asbestos from cellulose ester filters submitted to plasma asher.

7. Check for asbestos carryover in the plasma asher by including a blank alongside the positive control sample.

8. Perform a systems check on the transmission electron microscope daily.

9. Make periodic performance checks of magnification, electron diffraction and energy dispersive X-ray systems as set forth in Table III of Unit III.K.

10. Ensure qualified operator performance by evaluation of replicate counting, duplicate analysis, and standard sample comparisons as set forth in Table III of Unit III.K.

11. Validate all data entries.

12. Recalculate a percentage of all computations and automatic data reduction steps as specified in Table III.

13. Record an electron diffraction pattern of one asbestos structure from every five samples that contain asbestos. Verify the identification of the pattern by measurement or comparison of the pattern with patterns collected from standards under the same conditions. The outline of quality control procedures presented above is viewed as the minimum required to assure that quality data is produced for clearance testing of an asbestos abated area. Additional information may be gained by other control tests. Specifics on those control procedures and options available for environmental testing can be obtained by consulting References 6, 7, and 11 of Unit III.L. L. References

For additional background information on this method the following references should be consulted.

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IV. Mandatory Interpretation of Transmission Electron Microscopy Results to Determine Completion of Response Actions

A. Introduction

A response action is determined to be completed by TEM when the abatement area has been cleaned and the airborne asbestos concentration inside the abatement area is no higher than concentrations at locations outside the abatement area. "Outside" means outside the abatement area, but not necessarily outside the building. EPA reasons that an asbestos removal contractor cannot be expected to clean an abatement area to an airborne asbestos concentration that is lower than the concentration of air entering the abatement area from outdoors or from other parts of the building. After the abatement area has passed a thorough visual inspection, and before the outer containment barrier is removed, a minimum of five air samples inside the abatement area and a minimum of five air samples outside the abatement area must be collected. Hence, the response action is determined to be completed when the average airborne asbestos concentration measured inside the abatement area is not statistically different from the average airborne asbestos concentration measured outside the abatement area.

The inside and outside concentrations are compared by the Z-test, a statistical test that takes into account the variability in the measurement process. A minimum of five samples inside the abatement area and five samples outside the abatement area are required to control the false negative error rate, i.e., the probability of declaring the removal complete when, in fact, the air concentration inside the abatement area is significantly higher than outside the abatement area. Additional quality control is provided by requiring three blanks (filters through which no air has been drawn) to be analyzed to check for unusually high filter contamination that would distort the test results.

When volumes greater than or equal to 1,199 L for a 25 mm filter and 2,799 L for a 37 mm filter have

been collected and the average number of asbestos structures on samples inside the abatement area is no greater than 70 s/mm 2 of filter, the response action may be considered complete without comparing the inside samples to the outside samples. EPA is permitting this initial screening test to save analysis costs in situations where the airborne asbestos concentration is sufficiently low so that it cannot be distinguished from the filter contamination/background level (fibers deposited on the filter that are unrelated to the air being sampled). The screening test cannot be used when volumes of less than 1,199 L for 25 mm filter or 2,799 L for a 37 mm filter are collected because the ability to distinguish levels significantly different from filter background is reduced at low volumes.

The initial screening test is expressed in structures per square millimeter of filter because filter background levels come from sources other than the air being sampled and cannot be meaningfully expressed as a concentration per cubic centimeter of air. The value of 70 s/mm 2 is based on the experience of the panel of microscopists who consider one structure in 10 grid openings (each grid opening with an area of 0.0057 mm 2) to be comparable with contamination/background levels of blank filters. The decision is based, in part, on Poisson statistics which indicate that four structures must be counted on a filter before the fiber count is statistically distinguishable from the count for one structure. As more information on the performance of the method is collected, this criterion may be modified. Since different combinations of the number and size of grid openings are permitted under the TEM protocol, the criterion is expressed in structures per square millimeter of filter to be consistent across all combinations. Four structures per 10 grid openings corresponds to approximately 70 s/mm 2. B. Sample Collection and Analysis

1. A minimum of 13 samples is required: five samples collected inside the abatement area, five samples collected outside the abatement area, two field blanks, and one sealed blank.

2. Sampling and TEM analysis must be done according to either the mandatory or nonmandatory protocols in Appendix A. At least 0.057 mm 2 of filter must be examined on blank filters. C. Interpretation of Results

1. The response action shall be considered complete if either:

a. Each sample collected inside the abatement area consists of at least 1,199 L of air for a 25 mm filter, or 2,799 L of air for a 37 mm filter, and the arithmetic mean of their asbestos structure concentrations per square millimeter of filter is less than or equal to 70 s/mm 2; or

b. The three blank samples have an arithmetic mean of the asbestos structure concentration on the blank filters that is less than or equal to 70 s/mm 2 and the average airborne asbestos concentration measured inside the abatement area is not statistically higher than the average airborne asbestos concentration measured outside the abatement area as determined by the Z-test. The Z-test is carried out by calculating

$$Z = \frac{\bar{Y}_I - \bar{Y}_O}{0.8(1/n_I + 1/n_O)^{1/2}}$$

where YI is the average of the natural logarithms of the inside samples and YO is the average of the natural logarithms of the outside samples, nI is the number of inside samples and nO is the number of outside samples. The response action is considered complete if Z is less than or equal to 1.65.

Note: When no fibers are counted, the calculated detection limit for that analysis is inserted for the concentration.

2. If the abatement site does not satisfy either (1) or (2) of this Section C, the site must be recleaned and a new set of samples collected. D. Sequence for Analyzing Samples

It is possible to determine completion of the response action without analyzing all samples. Also, at any point in the process, a decision may be made to terminate the analysis of existing samples, reclean the abatement site, and collect a new set of samples. The following sequence is outlined to minimize the number of analyses needed to reach a decision.

1. Analyze the inside samples.

2. If at least 1,199 L of air for a 25 mm filter or 2,799 L of air for a 37 mm filter is collected for each inside sample and the arithmetic mean concentration of structures per square millimeter of filter is less than or equal to 70 s/mm 2, the response action is complete and no further analysis is needed.

3. If less than 1,199 L of air for a 25 mm filter or 2,799 L of air for a 37 mm filter is collected for any of the inside samples, or the arithmetic mean concentration of structures per square millimeter of filter is greater than 70 s/mm 2, analyze the three blanks.

4. If the arithmetic mean concentration of structures per square millimeter on the blank filters is greater than 70 s/mm 2, terminate the analysis, identify and correct the source of blank contamination, and collect a new set of samples.

5. If the arithmetic mean concentration of structures per square millimeter on the blank filters is less than or equal to 70 s/mm 2, analyze the outside samples and perform the Z-test.

6. If the Z-statistic is less than or equal to 1.65, the response action is complete. If the Z-statistic is greater than 1.65, reclean the abatement site and collect a new set of samples.

[52 FR 41857, Oct. 30, 1987]

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Tabbed Page:
16

EPA/540/2-90/005a
OERR Directive 9285.5-02-1
February 1990

Environmental Asbestos Assessment Manual

Superfund Method for the Determination of Asbestos in Ambient Air

Part 1: Method

INTERIM VERSION

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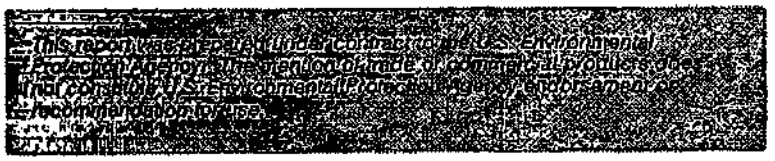

Contents

Figures vi
Tables vii
Acknowledgements viii

1. Introduction 1
2. Background 3
    2.1. Sensitivity 5
    2.2. Sampled Air Volumes 6
    2.3. Filter Selection 7
    2.4. Precision 9
    2.5. Asbestos Characteristics 10
    2.6. Cost Considerations 10

3. Overview of Method 12
    3.1. Sample Collection 12
    3.2. Sample Preparation 12
        3.2.1 Indirect TEM Specimen Preparation 12
        3.2.2 Direct TEM Specimen Preparation 13
    3.3. Analysis 13

4. Scope and Field of Application 15
    4.1. Substance Determined 15
    4.2. Range 15
        4.2.1 Upper Limit of Range 15
        4.2.2 Lower Limit of Detection 16
    4.3. Analytical Sensitivities 17
    4.4. Dimensional Detection Limits 17

5. Definitions 19

6. Symbols and Abbreviations 22
    6.1. Symbols 22
    6.2. Abbreviations 22

7. Equipment and Apparatus 24
    7.1. Air Sampling - Equipment and Consumable Supplies 24
        7.1.1. Filter Cassette 24
        7.1.2. Sampling Pump 24
        7.1.3 Stand 24
        7.1.4 Rotameter 24
    7.2 Specimen Preparation Laboratory 25
    7.3 Laboratory Equipment 25
        7.3.1. Transmission Electron Microscope 25
        7.3.2. Energy Dispersive X-ray Analyzer 27
        7.3.3. Computer 28
        7.3.4. Plasma Asher 28
        7.3.5. Filtration Apparatus 28
        7.3.6. Filtration Manifold 28
        7.3.7. Vacuum Pump 29

7.3.8. Vacuum Coating Unit 29
7.3.9. Sputter Coater 29
7.3.10. Solvent Washer (Jaffe Washer) 29
7.3.11. Condensation Washer 31
7.3.12. Slide Warmer or Oven 31
7.3.13. Ultrasonic Bath 31
7.3.14. Carbon Grating Replica 31
7.3.15. Calibration Grids for EDXA 31
7.3.16. Carbon Rod Sharpener 31
7.3.17. Disposable-Tip Micropipettes 31
7.4. Consumable Laboratory Supplies 33
7.4.1. Glass Beakers 33
7.4.2. Membrane Filters 33
7.4.3. Copper Electron Microscope Grids 33
7.4.4. Gold Electron Microscope Grids 33
7.4.5. Carbon Rod Electrodes 33
7.4.6. Disposable Tips for Micropipette 33
7.4.7. Routine Electron Microscopy Tools and Supplies 34
7.4.8. Reference Asbestos Samples 34

8 Reagents 35
8.1. Freshly-Distilled Water 35
8.2. Dimethyl Formamide, Analytical Grade 35
8.3 Glacial Acetic Acid, Analytical Grade 35
8.4. Acetone, Analytical Grade 36
8.5. Hydrochloric Acid, Analytical Grade 35

9. Air Sample Collection 38
9.1. Required Sensitivity 38
9.2. Air Volume 38
9.3. Flow Rate 39
9.4. Sampling Procedures 39

10. Procedure for Analysis 41
10.1. Introduction 41
10.1.1. Indirect TEM Specimen Preparation Method 41
10.1.2. Direct TEM Specimen Preparation Method 42
10.2. Preparation of TEM Specimen Grids by the Indirect Method 42
10.2.1. Cleaning of Sample Cassettes 42
10.2.2. Ashing of MCE Filter 42
10.2.3. Re-dispersal of Ashed Residues 42
10.2.4. Filtration of the Aqueous Suspension 43
10.2.5 Selection of Area of Filter for Preparation 45
10.2.6. Preparation of Solution for Collapsing MCE Filters 45
10.2.7. Filter Collapsing Procedure 45
10.2.8. Carbon Coating of Filter Sectors 45
10.2.9 Preparation of the Jaffe Washer 46
10.2.10. Placing of Specimens into the Jaffe Washer 46
10.2.11. Rapid Preparation of TEM Specimens from MCE Filters 46

10.3. Preparation of TEM Specimen Grids by the Direct Method 46
10.3.1. Cleaning of Sample Cassettes 46
10.3.2. Selection of Area of Filter for Preparation 47
10.3.3. Filter Collapsing Procedure 47
10.3.4. Plasma Etching of the Filter Surface 47
10.3.5. Carbon Coating of Filters 47
10.3.6. Preparation of the Jaffe Washer 47
10.3.7. Placing of Specimens into the Jaffe Washer 47
10.3.8. Rapid Preparation of TEM Specimens from MCE Filters 47

10.4. Criteria for Acceptable TEM Specimen Grids 47

10.5. Procedure for Structure Counting by TEM 48
10.5.1 Introduction 48
10.5.2. Measurement of Mean Grid Opening Area 49
10.5.3. TEM Alignment and Calibration Procedures 49
10.5.4. Determination of Stopping Point 49
10.5.5. General Procedure for Structure Counting and Size Analysis 50
10.5.6. Measurement of Concentration for Asbestos Structures Longer than 5 µm 54

10.6. Blank and Quality Control Determinations 57
10.7. Calculation of Results 58

11. Performance Characteristics 59
11.1. Interferences and Limitations of Structure Identification 59
11.2. Precision and Accuracy 59
11.2.1. Precision 59
11.2.2. Accuracy 59
11.3. Analytical Sensitivity 60
11.4. Limit of Detection 61

12. Reporting Requirements 62
12.1. Sample Analysis Report 62
12.2. Sample Batch Report 63
12.3. Data Review Report 64

Appendix A - Determination of Operating Conditions for Plasma Asher 71
Appendix B - Calibration Procedures 72
Appendix C - Structure Counting Criteria 76
Appendix D - Fiber Identification Procedure 84
Appendix E - Calculation of Results 99
Appendix F - Bibliography 107

Figures

7.1	Calibration Markings on the TEM Viewing Screen
7.2	Design of a Solvent Washer (Jaffe Washer).
7.3	Design of a Condensation Washer
10.1	Structure Counting Form
10.2	Scanning Procedure for a TEM Specimen Examination
12.1A	Format for Reporting of Structure Counting Data, Page 1
12.1B	Format for Reporting of Structure Counting Data, Page 2
12.1C	Format for Reporting of Structure Counting Data, Page 3
12.2	Format for Summary Laboratory Report
12.3	Format for Data Review Summary Report
C.1	Fundamental Morphological Structure Types
C.2	Examples of Recording of Complex Asbestos Clusters
C.3	Examples of Recording of Complex Asbestos Matrices
C.4	Counting of Structures that Intersect Grid Bars
C.5	Counting of Structures that Extend Outside the field of View
D.1	Measurement of Zone Axis SAED Patterns
D.2	Classification Chart for Fiber With Tubular Morphology
D.3	Chrysotile SAED Pattern
D.4	Classification Chart for Fibers Without Tubular Morphology

Tables

9.1	Examples of the Minimum Number of Grid Openings Required to Achieve a Particular Analytical Sensitivity and Detection Limit
10.1	Specifications for Phase 1 and 2 Sampling and Analysis
D.1	Classification of Fibers With Tubular Morphology
D.2	Classification of Fibers Without Tubular Morphology

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1. INTRODUCTION

This is a sampling and analysis method for the determination of asbestos in air. Samples are analyzed by transmission electron microscopy (TEM). Although a small subset of samples are to be prepared using a direct procedure, the majority of samples analyzed using this method will be prepared using an indirect technique. The method allows for the determination of the mineralogical type(s) of asbestos present and for distinguishing asbestos from non-asbestos minerals. In the method, asbestos structures are characterized as fibers, bundles, clusters, or matrices and the length and width of each asbestos structure are measured. Although the method is designed specifically to provide results suitable for supporting risk assessments at Superfund sites, it is applicable to a wide range of ambient air situations.

To support a risk assessment, this method addresses two objectives:

(a) to provide increased precision at the low concentrations of asbestos typically found in the environment;

(b) to provide measurements that can be compared with risk factors derived from existing epidemiology studies.

An additional consideration addressed in this method is the need to control sampling and analysis costs.

The method focuses on sampling requirements for individual sampling stations and the analysis of sample filters collected at such stations. During a site investigation, sampling stations would be arranged in an array designed to distinguish spatial trends in airborne asbestos concentrations. Sampling schedules would be fashioned to establish temporal trends. Thus proper design of a comprehensive sampling strategy, detailing the design of the array of sampling locations and the schedule for sample collection, is also critical to the success of an investigation. However, design of a sampling strategy is necessarily site specific and site-specific considerations are beyond the scope of this document.

Satisfying the two method objectives listed above requires innovations that tax the limits of available technology. Consequently, several variations were considered during development and this method represents a workable compromise among several technical constraints. Although the method has not been validated as a whole and the feasibility of a few procedures needs to be better documented, many of the component procedures of this method have been performed in the laboratory. Thus, the principle features of the method are well enough established that the method can be profitably employed in current field investigations. There is no better way to acquire the necessary experience and data for completing method validation. At the same time, until a validation study and appropriate pilot studies are completed, this should be considered an interim method.

Details of the considerations addressed during the development of this method are provided in a companion Technical Background Document, Part 2 of this report, under separate cover. A summary is presented in Chapter 2 (Background) of this document.

NOTE

This document is intended to serve several audiences including site project managers, field sampling teams, data reviewers, and laboratory analysts. The document may be separated into segments so that individuals may focus on the sections of most interest to their particular roles in a project.

2. BACKGROUND

During the development of this method, existing sampling and analysis technologies were considered to determine an appropriate approach for achieving defined method objectives while remaining within the known constraints associated with the measurement of airborne asbestos. The method is designed to provide the analytical sensitivity and precision necessary to distinguish background from asbestos concentrations typical of environmental contamination. The method is also designed to provide results recorded in a format that allows comparison with published risk factors or other proposed representations of the biological activity of asbestos.

Recent developments toward understanding the relationship between asbestos exposure and risk indicate that, to assess risks, modern field measurements should be tailored to quantify asbestos characteristics that best relate to biological activity. This is true despite the fact that existing risk factors are derived from epidemiology studies in which asbestos concentrations were derived primarily by phase contrast microscopy (PCM). A broader range of asbestos structures potentially contribute to biological activity than can be detected by PCM.

To provide the needed capabilities for distinguishing the broadest range of asbestos characteristics, transmission electron microscopy (TEM) was selected as the analytical technique employed in this method. Although considered, both PCM and scanning electron microscopy (SEM) were rejected for use in this method due to their inherent limitations.

The method is designed to provide the best description possible of the nature, numerical concentration, and sizes of asbestos-containing particles found in an air sample. The method of data recording specified in the method is designed to allow re-evaluation of the structure characterization and counting data without the necessity for re-examination of the specimens.

Currently-recognized methods require collection of airborne particulate on a membrane filter, followed by preparation of TEM specimens from the filter. Airborne asbestos is collected either on mixed cellulose ester (MCE) filters or polycarbonate filters. TEM specimens can be prepared from such membrane filters either by a direct-transfer technique or by an indirect method.

Direct-transfer TEM specimen preparation methods have the following advantages:

(a) the particulate and fiber size distributions are undisturbed during specimen preparation;

(b) the limited amount of specimen manipulation reduces the possibility of fiber loss or introduction of extraneous contamination.

However, the direct-transfer TEM preparation methods also have some significant disadvantages:

(a) the achievable detection limit is restricted by the particulate density on the filter, which in turn is controlled by the sampled air volume and the total suspended particulate concentration in the atmosphere being sampled;

(b) the precision of the result is dependent on the uniformity of the deposit of asbestos structures on the sample collection filter;

(c) air samples must be collected that have particulate and fiber loadings within narrow ranges. If too high a particulate loading occurs on the filter, it is not possible to prepare satisfactory TEM specimens by a direct-transfer method. If too high a fiber loading occurs on the filter, even if satisfactory TEM specimens can be prepared, accurate fiber counting will not be possible.

Indirect TEM specimen preparation techniques permit some of the disadvantages of direct-preparation to be overcome:

(a) air samples can be collected without regard to the amount of deposit on the filter surface. The filter loading can be adjusted in the laboratory to provide satisfactory TEM specimens;

(b) interfering particulate material can be completely or partially removed through a combination of dissolution and oxidation (ashing);

(c) the uniformity of distribution of asbestos structures on the filters to be analyzed is improved.

Indirect TEM specimen preparation methods have the following disadvantages:

(a) the size distribution of asbestos structures is modified;

(b) there is increased opportunity for fiber loss or introduction of extraneous contamination;

(c) when sample collection filters are ashed, any fiber contamination in the filter medium is concentrated on the TEM specimen grid.

The question as to whether direct or indirect specimen preparation yields the "correct" result (in terms of representing biological activity) is currently unresolved. It can be argued that the direct methods yield an under-estimate of the asbestos structure concentration because many of the asbestos fibers present are concealed by other particulate material with which they are associated. Conversely, the indirect methods can be considered to yield an over-estimate because some types of complex asbestos structures disintegrate during the preparation, resulting in an increase in the numbers of structures counted.

2.1. SENSITIVITY

To provide the required sensitivity at asbestos levels typically found in the environment, it is necessary either to selectively concentrate asbestos, or to count structures over a much greater area of a TEM specimen grid than has traditionally been required. To the extent that it can be employed, selective concentration is the least costly of the alternatives for increasing sensitivity. The optimal asbestos concentration on a filter requires filter loadings of total particulate matter in excess of what can generally be tolerated for analysis using direct-transfer methods for TEM specimen preparation. Consequently, an indirect technique for TEM specimen preparation is employed in this method, which incorporates steps allowing for the selective concentration of asbestos.

In most ambient environments, a proportion of the suspended particulate is organic, consisting of soot, spores, pollens and other debris from vegetation. Organic materials such as these can be removed from the analysis by low-temperature ashing.

It is common to find substantial numbers of calcium sulfate fibers (gypsum) in airborne particulate collected in urban environments. These can arise from various sources, and they can also be generated either in the atmosphere or on the sample collection filter by reaction of airborne calcite or dolomite particles with atmospheric sulfur dioxide. Gypsum can be removed by dissolution in water or dilute acids.

Carbonates are another major component of most exterior atmospheres. Calcite and dolomite are commonly found in urban atmospheres; these originate from various sources, including erosion of concrete and cement, local geology, and in some cases from industrial operations. Such carbonates can be removed by extraction with hydrochloric acid. If acid extraction procedures are carried out correctly, no chemical or crystallographic degradation of asbestos can be detected by routine methods of TEM analysis.

Removal of a large proportion of the suspended particulate by use of these techniques permits the asbestos present in the sample to be concentrated on to a smaller area of the TEM specimen. Consequently, the area of the TEM specimen that must be examined to achieve a particular analytical sensitivity is proportionately reduced. Also, many of the non-asbestos fibrous structures normally found in a directly-prepared TEM specimen, each of which must be identified and rejected from the asbestos structure count, do not appear on the indirectly-prepared TEM specimen.

For many environmental samples, a selective concentration of the asbestos structures, incorporating low temperature ashing, re-suspension in water, and acidification with HCl is capable of removing substantial amounts of interfering particulate from the analysis, and, for a

particular analytical sensitivity, reducing the area of the TEM specimens that must be examined.

The concentration steps included in this method, together with the requirement to measure very low airborne asbestos concentrations, make control of asbestos contamination more critical than for specimens prepared by direct-transfer techniques. Accordingly, the procedures in this method have been designed to minimize the effects of contamination.

2.2. SAMPLED AIR VOLUMES

This method incorporates an indirect preparation procedure to provide flexibility in the amount of deposit that can be tolerated on the sample filter and to allow for the selective concentration of asbestos prior to analysis. To minimize contributions to background contamination from asbestos present in the plastic matrices (polycarbonate or mixed cellulose ester) of membrane filters while allowing for sufficient quantities of asbestos to be collected, this method also requires the collection of a larger volume of air per unit area of filter than has traditionally been collected for asbestos analysis.

Due to the need to collect large volumes of air, higher sampling flow rates are recommended in this method than have generally been employed for asbestos sampling in the past. As an alternative, samples may be collected over longer time intervals but this restricts the flexibility required to allow samples to be collected while uniform meteorological conditions prevail. Higher flow rates through a 25 mm filter result in increased face velocities at the filter surface. Potential problems associated with the higher flow rates and increased face velocities include:

(a) destruction of the filter due to failure of its physical support under force from the increased pressure drop;

(b) leakage of air around the filter mount so that the filter is bypassed;

(c) damage to the asbestos structures due to increased impact velocities.

The recommended flow rates and face velocities have been employed in air sampling using membrane filters in the past. Based on such studies, (c) is not likely to be a problem. Using the filters and flow-rates specified, the first two concerns (a and b) are also unlikely to impose limitations. However, documented experience at the increased flow rates with the types of filter cassettes to be used in this method is very limited. Consequently, a pilot study is recommended to confirm that the structural integrity of a 25 mm filter cassette is not altered at the increased flow rates recommended. There is also some question

whether available pumps can maintain such flow rates throughout the collection of large volumes of air while the filter loading and, correspondingly, the resistance to flow steadily increase.

2.3. FILTER SELECTION

Selection of the type of filter to be used in this method was determined by considering the performance of the filter during sample collection, sample handling, and TEM specimen preparation (by an indirect technique). Concerns include the degree of asbestos loss that may occur during sample transport or handling, the efficiency with which asbestos is transferred from the filter to the TEM specimen, and the level of asbestos contamination potentially introduced to the TEM specimen from the filter material itself. Both mixed cellulose ester (MCE) and polycarbonate filters were evaluated.

It is generally accepted that deposited particles may move on the surface of polycarbonate filters when these filters are heavily loaded. Such movement may occur either during sample transport or handling in the laboratory. Such movement may potentially lead to asbestos losses between the time samples are collected and the time they are prepared for analysis.

Unlike the smooth surface of a polycarbonate filter, MCE filters exhibit sponge-like surfaces that trap filters within layers of porous material. The potential for movement of deposits on the surface of this type of filter is generally considered minimal. Correspondingly, the potential for loss of asbestos during transport or handling is also reduced. Thus, MCE filters are preferred over polycarbonate filters in terms of ease of handling.

Two methods are available for removal of sampled particulate from the sample collection filter in an indirect preparation: complete ashing of the filter and the particulate deposit, or washing of the particulate deposit from the filter surface followed by ashing of the wash-suspended deposit only. A number of problems have been experienced with the ashing of filters during indirect-transfer procedures. The efficiency with which deposited asbestos is transferred by washing has not been thoroughly investigated.

Complete ashing of a filter causes any asbestos contamination that may be present within the polymer matrix of the filter to be transferred to the final TEM preparation. Direct-transfer preparations from polycarbonate filters are known to yield a measurable background of asbestos contamination and current knowledge indicates that ashing of polycarbonate filters will yield an even higher value. Direct-transfer preparations of MCE filters generally yield low background asbestos contamination. However, ashing of either filter may yield higher levels of background asbestos contamination due to sources of contamination associated with the preparation itself.

Problems that have been experienced in association with low temperature ashing include both potential sources of additional contamination and filter behavior potentially leading to asbestos losses. During filter ashing, background contamination can arise from within the asher chamber, the containers in which the filters are ashed, the distilled water supply used for re-dispersing the ash, the pipettes used for transfer of dispersion for filtration, and the filtration funnel.

During ashing, both MCE and polycarbonate filters have occasionally been observed to curl up and give the appearance of being completely ashed, yet asbestos is trapped in a few very large, incompletely ashed particles that do not disperse. Consequently, such asbestos is lost and does not appear on the final TEM preparation. Procedures for avoiding this problem need to be refined.

Due to the problems associated with ashing the filters, washing of the particulate from the filter surface into ethanol is considered a superior approach. It is considered that washing of particulate from the surface of a polycarbonate filter can be accomplished with high removal efficiency. However, contamination of polycarbonate filters by asbestos has been measured by various laboratories, and currently appears to be within the range of 50-200 asbestos structures/sq.mm. on the active filter surface.[1]

During the washing of the filter surface, asbestos contamination on the surface of a polycarbonate filter (observed on preparations from direct-transfer procedures) may be detached along with the collected particulate and contribute to the background against which the concentrations of any collected asbestos must be measured. If the filter is removed from the cassette and the washing of the filter is performed ultrasonically in a beaker, contamination from both sides of the filter may be detached, leading to higher background counts. Therefore, potential but unquantified problems may be associated with the washing of polycarbonate filters.

Currently, it is not known whether collected particulate can be efficiently removed by washing the surfaces of 0.45 μm or 0.8 μm pore size MCE filters. The MCE filter has a sponge-like texture, and many of the fibers collected are embedded or entrapped within this structure. It has been shown that any attempt to use ultrasonic techniques results in disintegration of the filter surface and release of fragments of

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[1] Certain laboratories have consistently reported lower values for polycarbonate filter contamination than other laboratories. Although this is well known and the matter has been addressed by numerous investigators, the source of the apparent discrepancy has not been elucidated.

filter material. It is possible that this disintegration of the filter surface may also release most of the collected particulate, but this has not been demonstrated. On the basis of current knowledge, it is unlikely that the small amount of polymer removed from the surface of the filter would contribute a significant level of contamination to the analysis, because contamination levels on the surface of MCE filters have been demonstrated to be very low.

Based on the above considerations, MCE filters were selected for use in this method. Although available data suggest that the optimum combination of filter and preparation technique would be for samples to be collected on MCE filters and to re-disperse the particulate in water by washing of the filters, little is known concerning the efficiency with which asbestos can be removed from the surface of an MCE filter by washing. It is known that some of the filter polymer is detached from the filter surface during washing and particles of this polymer can interfere with the analysis. Thus, ashing of the wash suspension would be necessary in order to remove the filter polymer fragments. Because the efficacy of the filter washing procedure has not been adequately substantiated, the preparation procedure incorporated into this method is ashing of the filter followed by re-dispersion of the ash in water.

2.4. PRECISION

To maximize precision, potential sources of variation within the method must be controlled. To minimize systematic variation during sampling and analysis, the method specifies detailed procedures. Random variation is minimized by maximizing the number of structures to be identified and counted, which includes increasing the probability of encountering asbestos structures during analysis, as discussed in Section 2.1. In addition, detailed and unambiguous counting rules and rules for identification are specified in the method to minimize variation due to subjective interpretation.

Airborne asbestos is often found, not as single fibers, but as very complex structures that may or may not be aggregated with equant (non-asbestos) particles. The mineralogy of structures found suspended in an ambient atmosphere must be identified to confirm a structure as asbestos. Such structures can often be identified unequivocally, if sufficient measurement effort is expended. However, if each structure were to be identified in this way, the analysis becomes prohibitively expensive. Because of instrumental deficiencies or because of the nature of the particulate, some structures cannot be positively identified as asbestos, even though the measurements all indicate that they could be asbestos. Subjective factors therefore contribute to this measurement, and consequently a very precise procedure for identification and enumeration of asbestos is incorporated in this method.

Despite the imposition of systematic counting and identification criteria, there are large potential errors in characterizing asbestos in ambient atmospheres, associated with the variability between filter samples and the performance of individual microscopists. For this reason, a replicate sampling and analysis scheme is also incorporated in order to determine the accuracy and precision of the method.

2.5. ASBESTOS CHARACTERISTICS

To analyze asbestos samples so that results can be related to potential risks, it is necessary to quantify the characteristics of asbestos that relate to biological activity. This involves enumeration of individual structures within certain size categories with particular emphasis on the longest and thinnest structures. Although the range of dimensions over which asbestos structures contribute to biological activity has yet to be precisely defined, this method is designed to provide a detailed characterization of structures encompassing the entire range of potential importance.

When evaluating detailed asbestos size characterizations, it is important to consider the effects of sample preparation. The appearance of the size distribution representing a sample of asbestos may vary depending on whether the sample was prepared by a direct or an indirect technique. Existing risk factors are based largely on studies incorporating direct transfer techniques.[2] Because indirect preparation is preferred to achieve the desired analytical sensitivity for environmental analysis (see Section 2.1), a procedure for evaluating the relationship between structure counts on samples prepared by an indirect technique and by a direct transfer technique is included in the method.

2.6. COST CONSIDERATIONS

In environmental samples, the amount of airborne asbestos is usually low relative to the total suspended particulate concentration. Therefore, when using a direct-transfer preparation, large areas of the TEM specimen grids must be examined to obtain a statistically valid measurement for asbestos. Consequently, the analysis is expensive. It is therefore not feasible to perform this type of analysis on all of the filters collected as part of a site investigation.

A cost-effective compromise has been incorporated into this method. A two phase sampling and analysis scheme has been adopted. In Phase 1, the majority of samples collected at a site will be prepared by

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[1] PCM analyses are performed directly on sample filters where the filter material has been rendered transparent by a suitable solvent. Since the fibers are observed as originally deposited, this corresponds closely to a direct transfer technique for TEM analysis.

the indirect-transfer technique (to maximize precision) and analysis will be performed using an efficient evaluation procedure. Air volumes collected on Phase 1 samples may be maximized subject to the limitations of sampling equipment.

In Phase 2 a small subset of samples (5 to 10%) will be collected and analyzed using an extended TEM examination to derive a relationship between counts from samples prepared by a direct technique and counts from samples prepared by an indirect technique for each of the various structure size fractions of interest that are observed in Phase 1 and Phase 2 samples. Filters from Phase 2 samples will be split so that half may be prepared by the direct-transfer procedure and half by the indirect-transfer procedure. Because half of each filter to be analyzed under Phase 2 will be prepared by the direct-transfer technique, the volume of air collected for each sample is limited by the loading that can be tolerated during analysis of a specimen prepared using a direct-transfer technique. Phase 1 and 2 samples shall be collected simultaneously.

3. OVERVIEW OF METHOD

Samples are collected and prepared for TEM examination by one of two techniques depending on the purpose for the sample. The majority of air samples (denoted Phase 1 samples) will be analyzed using an indirect procedure for preparation of TEM specimens, optimized to provide low detection limits and high precision. A small number of samples (denoted Phase 2 samples) will be collected in such a way that they can be analyzed using both indirect and direct procedures for preparation of TEM specimens, to allow comparisons to be made between the results from the two specimen preparation procedures. TEM examination procedures used for the two sets of samples also differ.

3.1. SAMPLE COLLECTION

A sample of airborne particulate is collected by drawing a measured volume of air through a 25 mm diameter, 0.45 μm pore size MCE membrane filter by means of a pump. Air volumes collected on Phase 1 samples will be maximized. Air volumes collected on Phase 2 samples will be limited to provide optimum loadings for filters to be prepared by a direct-transfer procedure.

3.2. SAMPLE PREPARATION

TEM grids will be prepared according to either 3.2.1 or 3.2.2 below.

3.2.1. Indirect TEM Specimen Preparation

This preparation will be applied to all Phase 1 and Phase 2 samples. The filter is split and half of the filter is stored for future use. The remaining half of the filter is ashed in a low-temperature plasma asher. The residual ash is ultrasonically dispersed in freshly-distilled water. The suspension is acidified using hydrochloric acid, and immediately filtered through a 25 mm diameter, 0.1 μm pore size MCE filter. The filter is dried and the filter structure is collapsed using a mixture of dimethyl formamide, acetic acid and water. A thin film of carbon is evaporated onto the collapsed filter surface and small areas are cut from the filter. These areas of filter are supported on TEM specimen grids and the filter medium is dissolved away by a solvent extraction procedure.

NOTE

An alternate procedure for indirect preparation in which filter deposits are washed off of MCE filters followed by the ashing of the wash-suspended deposit may be substituted into this method upon completion of validation in a pilot study.

3.2.2. Direct TEM Specimen Preparation

The remaining one-half of the filters from all Phase 2 samples will be prepared by this procedure. One quarter of the filter is collapsed using a mixture of dimethyl formamide, acetic acid and water. The collapsed filter is etched for a short time in a low temperature plasma asher to remove the surface layer of filter polymer which may have encapsulated asbestos structures during the collapsing procedure. A thin film of carbon is evaporated onto the collapsed filter surface and small areas are cut from the filter. These areas of filter are supported on TEM specimen grids and the filter medium is dissolved away by a solvent extraction procedure.

3.3. ANALYSIS

The TEM specimen grids are examined at both low and high magnifications to check that they are suitable for analysis before carrying out a quantitative examination on randomly-selected grid openings. In addition to isolated fibers, ambient air samples often contain more complex aggregates of fibers, with or without equant particles. Some particles are composites of asbestos fibers with other materials. Individual fibers and these more complex structures are referred to as "asbestos structures". A coding system is used to record the type of fibrous structure, and to provide the optimum description of each of these complex structures.

The method requires that separate examinations be made for asbestos structures of all sizes (incorporating asbestos fibers with lengths greater than 0.5 μm) and for long asbestos structures (incorporating structures longer than 5 μm). In both cases, asbestos structures are defined as those exhibiting mean aspect ratios equal to or greater than 5:1. This TEM examination procedure allows for specification of a lower analytical sensitivity for the measurement of the concentration of asbestos structures longer than 5 μm.

In the TEM analysis, electron diffraction (ED) is used to examine the crystal structure of a fiber, or fibrous components of complex structures, and the elemental composition is determined by energy dispersive X-ray analysis (EDXA). For a number of reasons, it is not possible to identify (determine the mineralogy of) each structure unequivocally, and structures are classified according to the techniques that have been used to identify them. A simple code is used to record the manner in which each structure is classified.

The classification procedure is based on successive inspection of the morphology, the electron diffraction pattern, and the energy dispersive X-ray spectrum. Confirmation of the identification of chrysotile is only by quantitative ED, and confirmation of amphibole is only by quantitative EDXA and quantitative zone-axis ED.

Several levels of analysis to confirm mineralogy are specified, the higher levels providing a more rigorous approach to the identification of structures. The procedure permits a minimum required asbestos identification procedure to be defined on the basis of previous knowledge, or lack of it, about the particular sample. Attempts are then made to achieve this defined minimum procedure for each asbestos structure, and the degree of success is recorded for each. The two codes remove from the microscopist the requirement to interpret observations made during the TEM examination, and allow this evaluation to be made later without the requirement for re-examination of the TEM specimens.

The lengths and widths of all classified asbestos structures are recorded. The number of asbestos structures found on a known area of the TEM specimen grids, together with the equivalent volume of air filtered through this area, are used to calculate the airborne concentration in asbestos structures/liter of air.

This method requires that the desired analytical sensitivities for the measurements of:

(a) asbestos structures of all sizes (incorporating structures longer than 0.5 μm); and,

(b) asbestos structures longer than 5 μm,

shall be specified before air samples are collected. In both cases, structures to be counted are defined as those exhibiting aspect ratios equal to or greater than 5:1. It will not always be possible to achieve these analytical sensitivities, because the volume of air that can be sampled is dictated by the nature and concentration of the suspended particulate in the atmosphere being sampled. To some degree, this limitation can be overcome by selective concentration of asbestos structures during the specimen preparation procedures and by examination of a larger area of the TEM specimens. However, the ease and cost of achieving a specific value for the analytical sensitivity will vary from sample to sample.

4. SCOPE AND FIELD OF APPLICATION

This method defines procedures for analyzing of airborne asbestos collected by drawing a known volume of air through mixed cellulose ester (MCE) filters. The method is suitable for the determination of asbestos in ambient air.

4.1. SUBSTANCE DETERMINED

The method defined uses transmission electron microscopy for determination of the concentration of asbestos in ambient atmospheres. This method includes measurement of the lengths and widths of asbestos structures and a calculation of their aspect ratios. In the method, asbestos structures are characterized as fibers, bundles, clusters, or matrices. The method allows for the determination of the mineralogical type(s) of asbestos present. The method, however, cannot discriminate between individual fibers of the asbestos and non-asbestos analogues of the same amphibole mineral.

4.2. RANGE

The range of concentrations that can be determined on a TEM specimen grid is approximately 50 to 7000 asbestos structures/mm^2. The lower limit is generally defined by the requirement that the concentration of asbestos collected from the air be distinguishable from background contamination contributed by the method and the filter. The upper limit is defined by the concentration at which asbestos structures will significantly overlap and interfere with proper detection.

4.2.1. Upper Limit of Range

The upper limit of the range for detecting asbestos on a filter may be limited both by excessive overlap of asbestos structures and by the presence of other interfering particles. In practice, if obscuration of asbestos structures is not to be a significant factor in a method's precision, the final TEM specimen grid should not exhibit more than approximately a 10% coverage by particulate. For the indirect TEM specimen preparation method, this maximum permissible particulate loading is determined by the concentration of that portion of the total suspended particulate concentration that cannot be removed by procedures such as oxidation or chemical dissolution.

Using the direct-transfer preparation method, it has been found that if the total particulate loading of the sample collection filter exceeds approximately 10 $\mu g/cm^2$ of filter surface, corresponding to approximately 10% coverage of the collection filter by particulate, the TEM specimens are loaded to a point that obscuration of asbestos structures by particulate becomes a significant source of error. If the particulate loading

exceeds approximately 25 $\mu g/cm^2$ of filter surface, the specimens are seriously overloaded and it is often not possible to prepare TEM specimens that have grid openings with intact carbon film.

The degree that non-asbestos particulates may interfere with asbestos analysis is a function of the concentration of total suspended particulate (TSP) present in the atmosphere in addition to the asbestos being sampled. Concentrations of the TSP vary over several orders of magnitude depending on location, time of day, and weather. Urban and agricultural sites tend to have significantly higher concentrations of the TSP than rural locations. Consequently, higher volumes of air may be collected at rural locations before interference by the TSP becomes a limiting factor.

In addition to variation in overall concentration, the composition of the TSP varies significantly as a function of location. At urban sites and specific rural locations, the TSP tends to be composed principally of organic matter that can be ashed or inorganic substances that are soluble in acidified media. Agricultural locations and other rural locations frequently exhibit higher concentrations of refractory silicate particles.

Due to the wide spatial and temporal variation in the TSP concentrations, a general rule for estimating levels can not be provided. However, a review of available environmental studies indicates that air volumes of 1 m^3 per cm^2 of filter area may reasonably be collected for direct preparation samples in most urban and rural environments. Subject to the types of the TSP present, samples collected for indirect preparation can be loaded to levels up to an order of magnitude higher. Data on the range of local levels of TSP should be obtained and evaluated prior to finalizing a sampling plan for a site.

4.2.2. Lower Limit of Detection

The detection limit theoretically can be lowered indefinitely by:

(a) filtration of progressively larger volumes of air;

(b) use of intermediate specimen preparation procedures to concentrate the collected particulate on to smaller areas of the TEM specimen grids; and,

(c) examination of greater areas of the TEM specimens in the electron microscope.

Although it might appear that the detection limit can be lowered indefinitely by examination of a greater area of the TEM

specimens, this is not actually the case if unused filters (blanks), or the analytical procedure itself, contribute background asbestos. Although it is considered that the background asbestos structure counts of TEM specimens prepared by the collapsed MCE filter technique (direct preparation) are very low, there are few data currently available concerning the asbestos content of the MCE filter polymers themselves, which might contribute to background during an indirect preparation.

In a technique that incorporates complete ashing of MCE filter membranes, any asbestos contained in the filter polymer will be concentrated on the final TEM specimens. Accordingly, this effect should be minimized by collection of the maximum volumes of air possible, and a continuous program of blank measurements must form part of the quality assurance procedures.

4.3. ANALYTICAL SENSITIVITIES

Based on a review of data from past environmental asbestos investigations and published studies of rural and urban background, the lower end of the ranges of concentrations typical of these types of studies center on 5 structures/liter (s/L) for asbestos structures of all sizes and 0.2 s/L for structures longer than 5 μm. The significance of the asbestos structures longer than 5 μm is discussed in Section 2.5. To distinguish among such concentrations at acceptable levels of statistical significance, it is assumed that a minimum of 10 asbestos structures should be identified and counted (see Section 4.2 of the Technical Background Document, Part 2 of this report). Thus, analytical sensitivities (defined as the concentration represented by the detection of 1 structure) required by this method are 0.5 s/L and 0.02 s/L for asbestos structures of all sizes and asbestos structures longer than 5μm, respectively.

4.4. DIMENSIONAL DETECTION LIMITS

Microscopists vary in their ability to detect very small asbestos structures. Therefore, a minimum length of 0.5 μm has been defined as the shortest fiber to be incorporated in the reported results.

The minimum detectable width for asbestos structures counted in this method is determined by the ability of an operator to detect them in a routine examination at the specified magnification of the method. The minimum magnification specified in this method (10,000) is more than sufficient to ensure visibility of the thinnest chrysotile asbestos fibrils.

5. DEFINITIONS

Acicular: The shape shown by an extremely slender crystal with cross-sectional dimensions which are small relative to its length.

Amphibole: A group of rock-forming ferromagnesium silicate minerals, closely related in crystal form and composition, and having the nominal formula:

$$A_{0-1}B_2C_5T_8O_{22}(OH,F,Cl)_2$$

where:

A = K, Na;

B = Fe^{2+}, Mn, Mg, Ca, Na;

C = Al, Cr, Ti, Fe^{3+}, Mg, Fe^{2+};

T = Si, Al, Cr, Fe^{3+}, Ti.

In some varieties of amphibole, these elements can be partially substituted by Li, Pb, or Zn. Amphibole is characterized by a cross-linked double chain of Si-O tetrahedra with a silicon:oxygen ratio of 4:11, by columnar or fibrous prismatic crystals and by good prismatic cleavage in two directions parallel to the crystal faces and intersecting at angles of about 56° and 124°.

Amphibole asbestos: Amphibole in an asbestiform habit.

Analytical sensitivity: The calculated airborne concentration, in asbestos structures/liter, equivalent to counting of one asbestos structure in the analysis.

Asbestiform: A specific type of mineral fibrosity in which the fibers and fibrils possess high tensile strength and flexibility.

Asbestos: A term applied to a group of fibrous silicate minerals that readily separate into thin, strong fibers that are flexible, heat resistant and chemically inert.

Asbestos component: A term applied to any individually identifiable asbestos sub-structure that is part of a larger asbestos structure.

Asbestos structure: A term applied to any contiguous grouping of asbestos fibers, with or without equant particles.

Aspect ratio: The ratio of length to width of a particle.

Blank: A fiber count made on TEM specimens prepared from an unused filter, to determine the background measurement. Blanks consist of filter blanks, field blanks and laboratory blanks.

Bragg Angle: The angle between a crystal surface and an incident ray of incoming x-radiation.

Bundle: A fiber composed of parallel, smaller diameter fibers attached along their lengths.

Camera length: The equivalent projection length between the specimen and its electron diffraction pattern, in the absence of lens action.

Chrysotile: A mineral of the serpentine group which has the nominal composition:

$$Mg_3Si_2O_5(OH)_4$$

In some varieties of chrysotile, the silicon may be partially substituted by Al or less commonly by Fe. The magnesium may be partially substituted by Fe, Ni, Mn or Co. Some varieties contain Na, Cl or both. Chrysotile is a highly fibrous, silky variety, and constitutes the most prevalent type of asbestos.

Cleavage: The breaking of a mineral along one of its crystallographic directions.

Cleavage fragment: A fragment of a crystal that is bounded by cleavage faces.

Cluster: An assembly of randomly oriented fibers.

Component count: For any sample, a tally that includes the individually identified components of complex asbestos structures and each single asbestos structure with no identifiable components.

d-spacing: The distance between identical adjacent and parallel planes of atoms in a crystal.

Detection limit: The calculated airborne fiber concentration in fibers/liter, equivalent to counting of 3.69 fibers in the analysis.

Electron diffraction: A technique in electron microscopy by which the crystal structure of a specimen is examined.

Electron scattering power: The extent to which a thin layer of substance scatters electrons from their original directions.

Energy dispersive X-ray analysis: Measurement of the energies and intensities of X-rays by use of a solid state detector and multi-channel analyzer system.

Eucentric: The condition when an object is placed with its center on a rotation or tilting axis.

Fiber: An elongated particle which has parallel or stepped sides. In this method, a fiber is defined to have an aspect ratio equal to or greater than 5:1.

Fibril: A single fiber of asbestos, which cannot be further separated longitudinally into smaller components without losing its fibrous properties or appearances.

Fibrous structure: A contiguous grouping of fibers, with or without equant particles.

Field blank: A filter cassette which has been taken to the sampling site, opened, and then closed. Such a filter is analyzed to determine the background asbestos structure count for the measurement.

Filter Blank: An unused filter which is analyzed to determine the background asbestos structure count.

Habit: The characteristic crystal form or combination of forms of a mineral, including characteristic irregularities.

Identify: During analysis, the use of a sequential set of procedures to determine and confirm the mineralogy of a structure.

Laboratory Blank: An unused filter which is analyzed along with sample filters to determine the background asbestos structure count.

Matrix: A connected assembly of asbestos fibers with particles of another species (non-asbestos).

Miller Index: A set of either three or four integer numbers used to specify the orientation of a crystallographic plane in relation to the crystal axes.

PCM equivalent structure: A structure of aspect ratio greater than or equal to 3:1, longer than 5 μm, and which has a mean diameter between 0.2 μm and 3.0 μm for a part of its length greater than 5 μm. In this method, PCME structures also must contain at least one asbestos component.

Replication: A procedure in electron microscopy specimen preparation in which a thin copy, or replica, of a surface is made.

Selected area electron diffraction: A technique in electron microscopy in which the crystal structure of a small area of a sample is examined.

Serpentine: A group of common rock-forming minerals having the nominal formula:

$$Mg_3Si_2O_5(OH)_4$$

Structure Count: For any sample, a tally of each individually identified asbestos structure regardless of whether the structure contains identifiable components. This is equivalent to a count of the total number of separate asbestos entities encountered on the sample.

Twinning: The occurrence of crystals of the same species joined together at a particular mutual orientation, and such that the relative orientations are related by a definite law.

Unopened Fiber: A large diameter asbestos fiber bundle which has not been separated into its constituent fibrils or fibers.

Zone-axis: The line or crystallographic direction through the center of a crystal which is parallel to the intersection edges of the crystal faces defining the crystal zone.

6. SYMBOLS AND ABBREVIATIONS

6.1. SYMBOLS

Å	-	Ångström unit (10^{-10} meter)
eV	-	electron volt
kV	-	kilovolt
L/min	-	liters per minute
µg	-	microgram (10^{-6} grams)
µm	-	micrometer (10^{-6} meter)
nm	-	nanometer (10^{-9} meter)
Pa	-	Pascal
s/L	-	structures per liter
W	-	watt

6.2. ABBREVIATIONS

DMF	-	Dimethyl formamide
ED	-	Electron diffraction
EDXA	-	Energy dispersive X-ray analysis
FWHM	-	Full width, half maximum
HEPA	-	High efficiency particle absolute
MCE	-	Mixed cellulose ester
PCM	-	Phase contrast optical microscopy
PCME	-	Phase contrast microscopy equivalent
SAED	-	Selected area electron diffraction
SEM	-	Scanning electron microscope

STEM	-	Scanning transmission electron microscope
TEM	-	Transmission electron microscope
TSP	-	Total suspended particulate
UICC	-	Union Internationale Contre le Cancer

7. EQUIPMENT AND APPARATUS

7.1. AIR SAMPLING - EQUIPMENT AND CONSUMABLE SUPPLIES

7.1.1. Filter Cassette

Commercially available field monitors, comprising 25 mm diameter three-piece cassettes, with conductive extension cowls shall be used for sample collection. The cassette must be new and not previously used. The cassette shall be loaded with an MCE filter of pore size 0.45 µm, and supplied from a lot number which has been qualified as low background for asbestos determination. The filter shall be backed by a 5 µm pore size MCE filter, and supported adequately so that distortion of the filter by the differential pressure across it does not occur during sampling. The cassettes shall be purchased with the required filters in position, or shall be assembled in a laminar flow hood or clean area such that the filter blank determinations meet the criteria of Section 10.6. When the filters are in position, a shrink cellulose band or adhesive tape should be applied to cassette joints to prevent air leakage. Suitable precautions shall be taken to ensure that the filters are tightly clamped in the assembly so that significant air leakage around the filter cannot occur.

7.1.2. Sampling Pump

The sampling pump shall be capable of a flow-rate and pumping time sufficient to achieve the desired volume of air sampled. The face velocity through the filter shall be between 4.0 cm/s and 65.0 cm/s. The sampling pump used shall provide a non-fluctuating air-flow through the filter, and shall maintain the initial volume flow-rate to within ±10% throughout the sampling period. A constant flow or critical orifice controlled pump meets these requirements. Flexible tubing shall be used to connect the filter cassette to the sampling pump. A means for calibration of the flow-rate of each pump is also required.

7.1.3. Stand

A stand shall be used to hold the filter cassette at the desired height for sampling and the filter cassette shall be isolated from the vibrations of the pump.

7.1.4. Rotameter

A rotameter or other flow measuring device calibrated such that the operator can measure flow rates to ± 5% accuracy at the expected sampling flow rate.

7.2. SPECIMEN PREPARATION LABORATORY

Asbestos, particularly chrysotile, is present in varying quantities in many laboratory reagents. Many building materials also contain significant amounts of asbestos or other mineral fibers that may interfere with the analysis if they are inadvertently introduced during preparation of specimens. It is most important to ensure that during preparation, contamination of TEM specimens by any extraneous asbestos fibers is minimized. All specimen preparation steps shall therefore be performed in an environment where contamination of the sample is minimized. The primary requirement of the sample preparation laboratory is that a blank determination shall yield a result which will meet the specifications in 10.6. A minimum facility considered suitable for preparation of TEM specimens is a laminar flow hood. However, it has been established that work practices in specimen preparation appear to be more important than the type of clean handling facilities in use. Preparation of samples shall be carried out only after acceptable blank values have been demonstrated.

NOTE

Activities involving manipulation of bulk asbestos shall not be performed in the same area as TEM specimen preparation, because of the possibilities of contaminating the TEM specimens.

7.3. LABORATORY EQUIPMENT

7.3.1. Transmission Electron Microscope

A TEM operating at an accelerating potential of 80-120 kV, with a resolution better than 1.0 nm, and a magnification range of approximately 300 to 100,000 shall be used. The ability to obtain a direct screen magnification of about 100,000 is necessary for inspection of fiber morphology; this magnification may be obtained by supplementary optical enlargement of the screen image by use of a binocular if it cannot be obtained directly. It is also required that the viewing screen be calibrated (as shown in Figure 7.1) with concentric circles and a millimeter scale such that the lengths and widths of fiber images down to 1 mm width can be measured in increments of 1 mm.

For Bragg angles less than 0.01 radians, the TEM shall be capable of performing ED from an area of 0.6 μm^2 or less, selected from an in-focus image at a screen magnification of 20,000. This performance requirement defines the minimum separation between particles at which independent ED patterns can be obtained from

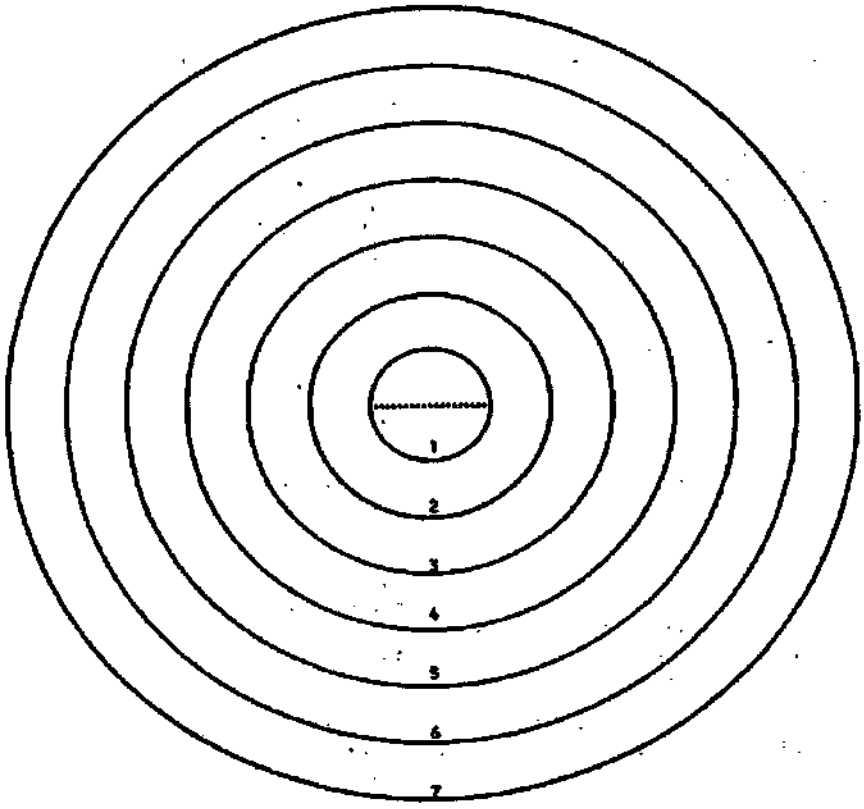

Figure 7.1: Calibration Markings on the TEM Viewing Screen

each particle. If SAED is used, the performance of a particular instrument may normally be calculated using the following relationship:

$$A = 0.7854 \times (D/M + 2000 \times C_s\theta^3)^2$$

Where: A = the effective SAED area in μm^2
D = the diameter of the SAED aperture in μm
M = the magnification of the objective lens
C_s = the spherical aberration coefficient of the objective lens in mm
θ = maximum required Bragg angle in radians

It is not possible to reduce the effective SAED area indefinitely by the use of progressively smaller SAED apertures, because there is a fundamental limitation imposed by the spherical aberration coefficient of the objective lens.

If zone-axis ED analyses are to be performed, the TEM shall incorporate a goniometer stage which permits the TEM specimen to be either:

(a) rotated through 360°, combined with tilting through at least +30° to -30° about an axis in the plane of the specimen; or,

(b) tilted through at least +30° to -30° about two perpendicular axes in the plane of the specimen.

The analysis is greatly facilitated if the goniometer permits eucentric tilting, although this is not essential. If EDXA and zone-axis ED are required on the same fiber, the goniometer shall be of a type which permits tilting of the specimen and acquisition of EDXA spectra without change of specimen holder.

The TEM shall have an illumination and condenser lens system capable of forming an electron probe smaller than 250 nm in diameter.

Use of an anti-contamination trap around the specimen is recommended if the required instrumental performance is to be maintained.

7.3.2. Energy Dispersive X-ray Analyzer

The TEM shall be equipped with an energy dispersive X-ray analyzer capable of achieving a resolution better than 175 eV (FWHM) on the MnK$_\alpha$ peak. Since the performance of individual combinations of TEM and EDXA equipment is dependent on a number of

geometrical factors, the required performance of the combination of the TEM and X-ray analyzer is specified in terms of the measured X-ray intensity obtained from a fibre of small diameter, using a known electron beam diameter. Solid state X-ray detectors are least sensitive in the low energy region, and so measurement of sodium in crocidolite shall be the performance criterion. The combination of electron microscope and X-ray analyzer shall yield, under routine analytical conditions, a background-subtracted NaK_α integrated peak count rate of more than 1 count per second (cps) from a 50 nm diameter fiber of UICC crocidolite irradiated by a 250 nm diameter electron probe at an accelerating potential of 80 kV. The peak/background ratio for this performance test shall exceed 1.0.

The EDXA unit shall provide the means for subtraction of the background, identification of elemental peaks, and calculation of background-subtracted peak areas.

7.3.3. Computer

Many repetitive numerical calculations are necessary, and these may be performed conveniently by relatively simple computer programs. For analyses of zone-axis ED pattern measurements, a computer with adequate memory is required to accommodate the more complex programs involved.

7.3.4. Plasma Asher

For ashing of filters or particulate deposits washed from filters, a plasma asher, with a radio frequency power rating of 100 W or higher, shall be used. The asher shall be supplied with a controlled oxygen flow, and shall be modified, if necessary, to provide a valve to control the speed of air admission so that rapid air admission does not disturb the ashed particulate. It is recommended that filters be fitted to the oxygen supply and the air admission line.

7.3.5. Filtration Apparatus

After re-dispersal of the ashed particulate in water, the particulate suspension is filtered through a membrane filter of 25 mm diameter. A glass frit support is required in order to obtain a uniform deposit on the filter. The reservoir must be easily cleaned in order to prevent sample cross-contamination. A 25 mm analytical filter holder (Millipore Corporation, Cat. No. XX10 025 00) or equivalent has been found to be suitable.

7.3.6. Filtration Manifold

When a number of samples are to be filtered, several

filtration units can be operated simultaneously from a single vacuum source by using a multiple port filtration manifold (Millipore Corporation, Cat. No. XX26 047 35 or equivalent has been found to be suitable). The manifold should include valves to permit each port to be opened or closed independently.

7.3.7. Vacuum Pump

A pump is required to provide a vacuum of approximately 20 kPa for the filtration of water samples. A water jet pump (Edwards High Vacuum Inc., Grand Island, NY 14072, Cat. No. 01-C046-01-000-female connection or 01-C039-01-000-male connection or equivalent) has been found to provide sufficient vacuum for a 3-port filtration mani-fold and also incorporates a non-return valve to prevent back-streaming.

7.3.8. Vacuum Coating Unit

A vacuum coating unit capable of producing a vacuum better than 0.013 Pa shall be used for vacuum deposition of carbon on the membrane filters. A sample holder is required which will allow a glass microscope slide to be continuously rotated during the coating procedure. A mechanism which allows the rotating slide also to be tilted through an angle of approximately 45° during the coating procedure is recommended. A liquid nitrogen cold trap above the diffusion pump may be used to minimize the possibility of contamination of the filter surfaces by oil from the pumping system. The vacuum coating unit may also be used for deposition of the thin film of gold, or other calibration material, when it is required on TEM specimens as an internal calibration of ED patterns.

7.3.9. Sputter Coater

A sputter coater with a gold target may be used for deposition of gold on to TEM specimens as an internal calibration of ED patterns. Experience has shown that a sputter coater allows better control of the thickness of the calibration material.

7.3.10. Solvent Washer (Jaffe Washer)

The purpose of the Jaffe washer is to allow dissolution of the filter polymer while leaving an intact evaporated carbon film supporting the fibers and other particles from the filter surface. One design of a washer that has been found satisfactory for various solvents and filter media is shown in Figure 7.2. Several acceptable variations have been described in the scientific literature. When specimens are not being inserted or removed, the petri dish lid should be in place. The washer should be cleaned before it is used for each batch of specimens.

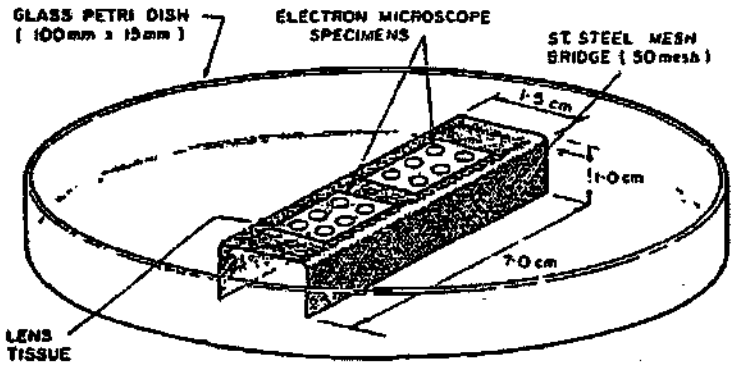

Figure 7.2: Design of a Solvent Washer (Jaffe Washer). Solvent is added until the meniscus contacts the underside of the stainless steel mesh

7.3.11. Condensation Washer

For more rapid dissolution of the filter polymer, a condensation washer, consisting of a flask, condenser and cold finger assembly, with a heating mantle and means for controlling the temperature may be used. A suitable assembly is shown in Figure 7.3, and acetone should be used as the solvent for MCE filters.

7.3.12. Slide Warmer or Oven

Either a slide warmer or an oven shall be used for heating slides during the preparation of TEM specimens. It is required to maintain a temperature of 65-70°C.

7.3.13. Ultrasonic Bath

An ultrasonic bath is required for cleaning of apparatus used for TEM specimen preparation, and for re-dispersal of ashed residues from MCE filters.

7.3.14. Carbon Grating Replica

A carbon grating replica with about 2000 parallel lines per mm shall be used to calibrate the magnification of the TEM.

7.3.15. Calibration Grids for EDXA

For calibration of the efficiency of the EDXA system, reference silicate standard material must be used. The National Institute for Standards and Technology (NIST) Standard Reference Material (SRM) 2063 is a sputtered thin film containing known concentrations of magnesium, silicon, calcium and iron. Other suitable calibration materials include riebeckite, chrysotile, halloysite, phlogopite, wollastonite and bustamite. The mineral used for calibration of the EDXA system for sodium must be prepared using a gold TEM grid.

7.3.16. Carbon Rod Sharpener

The use of necked carbon rods, or equivalent, allows the carbon to be evaporated on to the filters without creating sufficient heat to damage the filters.

7.3.17. Disposable-Tip Micropipettes

A disposable tip micropipette, capable of transferring a volume of approximately 30 µL, is required for the preparation of TEM specimen grids.

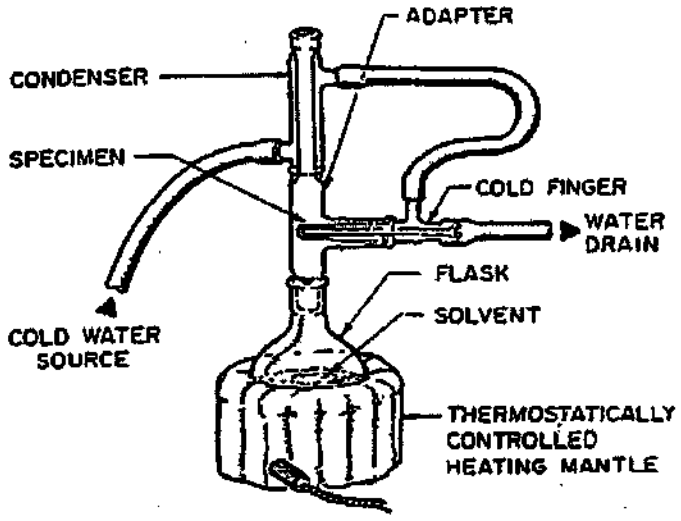

Figure 7.3: **Design of a Condensation Washer**

7.4. CONSUMABLE LABORATORY SUPPLIES

7.4.1. Glass Beakers

Borosilicate glass beakers of 100 mL capacity are required for ashing of filters and re-dispersal of ashed residues.

7.4.2. Membrane Filters

For filtration of the particulate suspensions, two types of filter are required:

(a) MCE filters of 0.1 μm pore size (Millipore Corporation VCWP 025 00 or equivalent) are used to collect the particles from the liquid suspension;

(b) MCE filters of 5 μm pore size (Millipore Corporation SMWP 025 00 or equivalent). One of these filters is placed between the glass frit of the filtration apparatus and the 0.1 μm pore size filter, in order to ensure a uniform particulate deposit.

7.4.3. Copper Electron Microscope Grids

Two-hundred mesh TEM grids are required. Grids which have grid openings of uniform size such that they meet the requirement of 10.4.2 should be chosen.

7.4.4. Gold Electron Microscope Grids

Two-hundred mesh gold TEM grids are required to mount TEM specimens when sodium measurements are required in the fiber identification procedure. Grids that have grid openings of uniform size such that they meet the requirement of 10.4.2 should be chosen.

7.4.5. Carbon Rod Electrodes

Spectrochemically pure carbon rods are required for use in the vacuum evaporator during carbon coating of filters.

7.4.6. Disposable Tips for Micropipette

Disposable tips for a micropipette are required for measurement of 30 μL volumes of the filter collapsing solution.

7.4.7. Routine Electron Microscopy Tools and Supplies

Fine-point tweezers, scalpel holders and blades, microscope slides, double-coated adhesive tape, lens tissue, gold wire, tungsten filaments, liquid nitrogen, and other routine supplies are required.

7.4.8. Reference Asbestos Samples

Asbestos samples are required for preparation of reference TEM specimens of the primary asbestos minerals. The NIST SRM 1866 contains well-characterized samples of chryostile, crocidolite, and amosite, although compositional data are not available for these standards. The UICC set of minerals may be used as additional reference materials.

8. REAGENTS

8.1. FRESHLY-DISTILLED WATER

A supply of freshly-distilled water must be available. The water must be produced using an all-glass still. Any water used for re-dispersal of ash, or for final rinsing of glassware must have been distilled no longer than 24 hours prior to use.

8.2. DIMETHYL FORMAMIDE, ANALYTICAL GRADE

8.3. GLACIAL ACETIC ACID, ANALYTICAL GRADE

8.4. ACETONE, ANALYTICAL GRADE

8.5. HYDROCHLORIC ACID, ANALYTICAL GRADE

WARNING - Use the reagents in accordance with the appropriate health and safety regulations.

9. AIR SAMPLE COLLECTION

Sampling procedures for this method were developed in consideration of and designed to be compatible with the analytical requirements of this method including, primarily, the need to achieve the desired analytical sensitivity.

Analytical sensitivity is a function of the volume of air sampled, the active area of the collection filter, the dilution or concentration factor introduced during specimen preparation, and the area of the TEM specimen over which asbestos structures are counted. If total airborne dust levels are high, it may be necessary to terminate sampling before the required volume has been sampled. If this happens, the analytical sensitivity required can be achieved only by counting structures over a greater area of the TEM specimen, or by use of a specimen preparation technique that incorporates some selective concentration of asbestos structures relative to other particulate species. For example, depending on the nature of the suspended particulate, a concentration factor up to about 2 may be possible for 25 mm filters, if the entire filter is used in the analysis. Larger concentration factors are possible for larger sample filters. In either case, the number of grid openings to be examined will be proportionately reduced.

The number of grid openings that must be examined in order to achieve a particular analytical sensitivity, without any selective concentration of the asbestos structures, is shown in Table 9.1. An average grid opening size of 0.0081 mm^2 is assumed in this table. However, grid opening sizes vary depending on the manufacturer of the grid. When using this table to derive the number of grid openings to be counted to achieve a defined analytical sensitivity, the number of grid openings to be counted must be adjusted to preserve the total areas represented. For example, if a volume of 5000 liters of air is collected, the table indicates that 96 grid openings must be counted to achieve an analytical sensitivity of 0.1 s/L. This corresponds to an area of 0.78 mm^2 (96 x 0.0081). If the average grid opening size on another grid is 80 µm square (resulting in an average grid opening area of 0.0064 mm^2), then a total of 122 grid openings will have to be counted, to cover the same area and achieve the same analytical sensitivity. Under no circumstances should fewer than 4 grid openings be counted.

The volume in liters required to achieve a specified analytical sensitivity is calculated from the formula:

$$V = A_f/(N \times A_s \times S \times F)$$

where:

A_f = Active area of sample collection filter in mm^2

N = Number of grid openings to be examined. N shall be rounded upwards to the next highest integer

A_s = Mean area of grid openings in mm^2

S = Analytical sensitivity in structures/liter

F = Concentration factor.

Table 9.1 - Examples of the Minimum Number of Grid Openings Required to Achieve a Particular Analytical Sensitivity

Analytical sensitivity	Volume of air sampled (liters)					
s/L	2000	5000	7000	10000	15000	20000
0.01	2377	951	680	476	317	238
0.02	1189	476	340	238	159	119
0.03	793	317	227	159	106	80
0.04	595	238	170	119	80	60
0.05	476	191	136	96	64	48
0.07	340	136	98	68	46	34
0.1	238	96	68	48	32	24
0.2	119	48	34	24	16	12
0.3	80	32	23	16	11	8
0.4	60	24	17	12	8	6
0.5	48	20	14	10	7	5
0.7	34	14	10	7	5	4
1.0	24	10	7	5	4	4
2.0	12	5	4	4	4	4
3.0	8	4	4	4	4	4
4.0	6	4	4	4	4	4
5.0	5	4	4	4	4	4
7.0	4	4	4	4	4	4
10.0	4	4	4	4	4	4

NOTES

In this table, it is assumed that the collection filter area is 385 mm^2, there is no concentration or dilution introduced during specimen preparation, and the TEM grid openings are 90 μm square.

For grid openings with dimensions other than 90μm square, the minimum number of openings to be counted should be adjusted by the ratios of the squares of the grid opening dimensions, such that the area of the TEM specimen examined remains the same.

The concentration factor F arises from the fact that, when using an indirect specimen preparation technique, the fibers on a particular area of the TEM specimen have usually been transferred from a different area of the original sample collection filter. The concentration factor may correspond to a concentration or a dilution of the original fiber density on the collection filter. It is calculated from the ratio between the areas of the original and analytical filters, the volume of water used to re-disperse the ashed particulate, and the volume of the final dispersion used to prepare the analytical filter. For the purposes of calculating the required volume of air to be sampled in order to achieve the specified analytical sensitivity when a direct transfer preparation is to be used, the concentration factor (F) is assumed to be 1.

9.1. REQUIRED SENSITIVITY

The required analytical sensitivities for characterizing environmental samples is established in section 4.3 as 0.5 s/L and 0.02 s/L for asbestos structures of all sizes and asbestos structures longer than 5 μm, respectively. For other applications of this method, different analytical sensitivities should be established prior to sample collection. Air volumes to be collected in this method are selected to provide the desired analytical sensitivity when coupled with the counting of a manageable number of grid openings.

9.2. AIR VOLUME

The sampling rate and the period of sampling shall be selected to yield as high a sampled volume as possible, which will minimize the influence of filter contamination. Wherever possible, a volume of 15 cubic meters shall be sampled for those samples intended for analysis only by the indirect TEM preparation method (Phase 1 samples). For those samples to be prepared by both the indirect and the direct specimen preparation methods (Phase 2 samples), the volumes must be adjusted so as to provide a suitably-loaded filter for the direct TEM preparation method (See Section 4.2.1). It has been found that the volume cannot normally exceed 5 cubic meters in an urban or agricultural area, and 10 cubic meters in a rural area for samples collected on a 25 mm filter and prepared by a direct transfer technique.

Prior to planning a sampling program, air volumes to be collected should be adjusted based on available information characterizing typical local TSP concentrations (see Section 4.2.1). It has not proven possible to judge the loading of filters in real time during sampling so that filters may easily become overloaded to the point were analysis by the direct method becomes impossible. To minimize the potential that filters will have to be rejected for analysis due to overloading, conservative estimates of the loading rate based on typical TSP concentrations have been developed so that an "optimum" volume of air per unit area of filter may be collected. However, because such estimates tend to be conservative to avoid overloading, a fair percentage of the filters analyzed from such

a sampling effort tend to be underloaded forcing a corresponding increase in analysis costs.

NOTE

One option is to collect filters at several loadings to bracket the estimated optimum loading for a particular site. Such filters can be screened in the laboratory so that only those filters closest to optimal loading are analyzed.

9.3. FLOW RATE

To remain within known constraints on filter face velocities as reported in Section 2.2, an upper limit to the range of acceptable flow rates for this method is 15 L/min. In practice, available pumps that can be used for environmental sampling at remote locations operate under load at a maximum of approximately 12 L/min. Consequently, the recommended 15 m^3 of air for Phase 1 samples requires approximately 20 hours to collect. Such pumps typically draw 6 amps at full power so that 2 lead/acid batteries should provide sufficient power to collect a full sample. The lower volumes required for Phase 2 samples can be collected in shorter periods with a corresponding reduction in stored energy requirements. The use of line voltage, where available, eliminates the difficulties associated with transporting stored electrical energy.

At many locations, wind patterns exhibit strong diurnal variations. Therefore, intermittent sampling (sampling over a fixed time interval repeated over several days) may be necessary to accumulate 20 hours of sampling time over constant wind conditions. Other sampling objectives also may necessitate intermittent sampling. The objective is to design a sampling schedule so that samples are collected under uniform conditions throughout the sampling interval. This method provides for such options.

9.4. SAMPLING PROCEDURES

Before air samples are collected, unused filters shall be analyzed as described in Section 10.6 to determine the mean background asbestos structure count for the analytical procedure.

Air samples shall be collected using cassettes as described in 7.1.1. The cassettes used for sampling shall be new and unused. Sampling shall be conducted with the cassette open-face. During sampling, the filter cassette shall be supported on a stand so that it is isolated from the vibrations of the pump. The cassette shall be held facing vertically downwards at a height of approximately 1.5-2.0 m above ground/floor level and connected to the pump with a flexible tube. In many cases it will likely be sufficient to collect samples with a short cassette. If conditions dictate the need for additional protection, however, extension cowls may be affixed to the front of the cassette. Such cowls must be

constructed of electrically conducting material to minimize electrostatic effects.

The sampling flow-rate should be measured at the cassette at the beginning and at the end of the sampling period. Sampling equipment shall be selected such that either the sampling flow rate can be maintained to within ± 10%, using a flow controller or critical orifice; or flow rates shall be measured at least every 4 hours. If at any time the measurement indicates that the flow-rate has decreased by more than 30%, the sampling shall be terminated. The mean value of these flow-rate measurements shall be used to calculate the total air volume sampled. After sampling, a cap shall be placed over the open end of the cassette, and the cassette packed in a clean plastic bag for return to the laboratory. Field blank filters shall also be included, as described in Section 10.6, and processed through the remaining analytical procedures along with the samples.

Should intermittent sampling be required, sampling cassettes must be covered between active periods of sampling. To cover the sampler: turn the cassette to face upward, attach a rotameter to measure the flow rate, turn off the sampling pump, and place the cap. To resume sampling: remove the cap, turn on the sampling pump, attach a rotameter to measure the flow rate, and invert the cassette to face downward.

10. PROCEDURE FOR ANALYSIS

10.1. INTRODUCTION

Filters representing Phase 1 samples will be prepared by the indirect method. Filters representing Phase 2 samples will be split. Half of the Phase 2 filters will be prepared by the indirect method and a quarter sector of the filter will be prepared by the direct method.

10.1.1. Indirect TEM Specimen Preparation Method

The indirect method requires collection of particulate from a large volume of air on a 0.45 μm pore size MCE filter. In this procedure, half of the filter is ashed by treatment in a low temperature plasma asher, and the ashed residue is re-dispersed in distilled water. Immediately before filtration, the suspension of particles is acidified using hydrochloric acid to remove any acid-soluble species. The suspension is filtered through a 0.1 μm pore size MCE filter. The filter is collapsed using a mixture of dimethyl formamide, acetic acid and water. A sector of the collapsed filter is carbon coated, and TEM specimen grids are prepared from the carbon coated filter by the Jaffe washer technique, using dimethyl formamide as the solvent.

Use of the 0.1 μm pore size membrane filter eliminates the requirement for the plasma etching step incorporated into both NIOSH Method 7402 and the method of Burdett and Rood. In previous work by Chatfield, Dillon and Stott, no statistically-significant fiber losses could be detected when TEM grids were prepared from 0.1 μm pore size MCE filters without including the etching step.

Indirect preparation of TEM specimens for determination of asbestos is particularly sensitive to problems of contamination by extraneous asbestos. This method requires that very low detection limits be achieved so that control of extraneous asbestos contamination becomes even more important. The ability to meet the blank sample criteria is critically dependent on the cleanliness of equipment and supplies. All supplies such as microscope slides and glassware should be considered as potential sources of asbestos contamination. All solvents used should be filtered through a 0.2 μm pore size filter or they should be distilled in a glass still. It is necessary to wash all glassware before it is used and the final washing of glassware should be performed using freshly-distilled water. Any tools or glassware that come into contact with the air sampling filters or TEM specimen preparations should be washed both before use and between handling of individual samples. Where possible, disposable supplies should be used.

10.1.2. Direct TEM Specimen Preparation Method

The direct TEM specimen preparation method requires that the air volume sampled must not result in a filter that is over-loaded by particulate. Particulate is collected on a 0.45 μm pore size MCE filter. A quarter sector of the MCE filter is collapsed using a mixture of dimethyl formamide, acetic acid and water. The collapsed filter is etched for a short time in a low temperature plasma asher. The collapsed and etched filter is carbon coated, and TEM specimen grids are prepared from the carbon coated filter by the Jaffe washer technique, using dimethyl formamide as the solvent.

10.2. PREPARATION OF TEM SPECIMEN GRIDS BY THE INDIRECT METHOD

10.2.1. Cleaning of Sample Cassettes

Asbestos can adhere to the exterior surfaces of air sampling cassettes and this asbestos can be inadvertently transferred to the sample during handling. To prevent this possibility of contamination, and after ensuring that the cassette is tightly sealed, wipe the exterior surfaces of each sampling cassette with a clean, wet paper towel before it is taken into the clean facility or laminar flow hood.

10.2.2. Ashing of MCE Filter

Open the filter cassette and remove the MCE filter. Place the filter on a clean microscope slide and cut it in half, using a curved scalpel blade. Return one half of the filter to the cassette. Place the other half filter in the bottom of a 100 mL glass beaker and cover the top of the beaker with a piece of aluminum foil. Make approximately 8 1-2 mm perforations in the aluminum foil. Completely ash the half filter in the low temperature plasma asher, using operating conditions as defined in Appendix A.

10.2.3. Re-dispersal of Ashed Residues

After the filter has been completely ashed, remove the beaker from the asher, add 40 mL of distilled water, and place the beaker in the ultrasonic bath for a period of 15 minutes. Using a disposable plastic pipette, add 0.2 mL of concentrated hydrochloric acid and stir the suspension. After addition of the acid, place the beaker in the ultrasonic bath for a period of 5 minutes. Filtration of the suspension must be performed immediately after the ultrasonic treatment. Power in the ultrasonic bath should be maintained below 0.1 watt/mL.

10.2.4. Filtration of the Aqueous Suspension

The separation of suspended particulate by filtration of the aqueous suspension through a membrane filter is a critical step in the analytical procedure. The objective is to produce an analytical filter on which the suspended solids from the sample are distributed uniformly, and at an acceptable loading for the microscopical examination.

The volume to be filtered generally depends on either the total suspended solids content or the asbestos structure concentration of the sample, and the intention is to prepare a filter that has a loading not exceeding about 50 asbestos structures per grid opening and with a particulate coverage not exceeding 10% of the filter area. Some judgment is required to achieve the optimum loading and, if the asbestos concentration is very low, it will be found that the suspended solids concentration will limit the volume that can be filtered. The maximum particulate loading on the filter that can be tolerated is about 10 $\mu g/cm^2$.

In practice, the best procedure for optimizing the loading on the final filter is to prepare several filters using different volumes of the suspension. Any fraction of the 40 mL suspension (prepared as per Section 10.2.3) may be selected as a unit for filtration. For example, aliquots containing 1 mL, 4 mL, 8 mL, and 20 mL fractions from the original suspension may be used to prepare a series of increasingly loaded filters. However, if the concentration of suspended solids dictates that a very small volume of the suspension be filtered, small volume aliquots need to be further diluted prior to filtering. Do not attempt to filter a volume of less than 10 mL.

If aliquots smaller than 10 mL from the original suspension are to be filtered, it is difficult to ensure that a uniform deposit of particulate is obtained on the filter unless the aliquot is first diluted. Samples of high solids content, or of high asbestos concentration, may require filtration of volumes less than 10 mL. Such aliquots shall be diluted with freshly-distilled water so that the filtered volumes exceed the minimum of 10 mL. These dilutions shall be made by transferring a known volume of the sample (such as an aliquot from the original suspension) to a disposable plastic beaker and making the volume up to a known volume with freshly-distilled water. The mixture shall be stirred vigorously before sub-samples are taken for filtration.

The following instructions for filtration must be followed precisely:

(a) Assemble the filtration base and turn on the vacuum. The upper surface of the filtration base (both the glass

frit and the ground mating surface) must be dry before the membrane filters are installed. Place a 5 μm pore size MCE filter on the glass frit. If the filter appears to become wet by capillary action on residual water in the glass frit it must be discarded and replaced by another filter. Place a 0.1 μm pore size MCE filter, smooth side facing up, on top of the 5 μm filter. The mating surface of the reservoir component of the filtration apparatus should be dried by shaking off any surplus water and draining on paper towel or tissue. Position the reservoir on the filters and firmly clamp it, taking care not to disturb the filters. The vacuum should not be released until the filtration has been completed.

It is necessary to comment on the use of filtration equipment that is still wet after washing, since improper procedures at this point can seriously compromise the results. If the glass frit is wet when the first filter is applied to it, capillary action will result in some areas of the filter structure being filled by water. When the second, smaller pore size filter is added and the vacuum is applied, the differential pressure across the 5 μm pore size filter will be insufficient to overcome the surface tension of the water in the filled areas. Thus no filtration will take place through the corresponding areas of the 0.1 μm pore size filter, and a grossly non-uniform deposit of particulate will be obtained.

(b) Add the required volume of sample to the filtration funnel. Disposable plastic beakers and pipettes provide a means of measuring the sample volumes without introducing problems of sample cross-contamination. The reservoir may not be sufficiently large to accommodate the total volume of liquid to be filtered. In this case, more of the sample may be added during the filtration, but this should be done carefully and only when the reservoir is more than half full. In this way the addition of liquid will not disturb or affect the uniformity of particulate already deposited on the filter. Do not rinse the sides of the reservoir, and avoid other manipulations which may disturb the particulate deposit on the filter.

(c) Disassemble the filtration apparatus, and transfer the 0.1 μm pore size filter to a labelled, clean plastic petri dish. Place the cover loosely over the top of the dish to limit any deposition of material on the filter, and allow the filter to dry. Discard the 5 μm pore size filter.

10.2.5. Selection of Area of Filter for Preparation

Using clean tweezers, and a clean, curved scalpel blade, cut out a 90 degree sector of the filter while it is still in the plastic petri dish.

10.2.6. Preparation of Solution for Collapsing MCE Filters

Mix 35 mL of dimethyl formamide, 15 mL of glacial acetic acid with 50 mL of freshly-distilled water. Store this mixture in a clean bottle.

10.2.7. Filter Collapsing Procedure

Using a micropipette with a disposable tip, place approximately 30 µL of the collapsing solution on a cleaned microscope slide, and using the end of the pipette tip spread the liquid over the area to be occupied by the filter sector. Place the filter sector, active surface upwards, on top of the solution, lowering the edge of the filter at an angle of about 20 degrees so that air bubbles are not created. Remove any solution not absorbed by the filter by allowing a paper tissue to contact the liquid at the edge of the filter. More than one filter sector may be placed on one slide, and a laboratory blank filter sector shall also be prepared on each slide. Place the slide either on a thermostatically-controlled slide warmer at a temperature of approximately 65-70°C, or in an oven at the same temperature, for about 10 minutes. The filter collapses slowly to about 15% of its original thickness. The procedure leaves a thin, transparent plastic film adhering to the microscope slide, with particles and fibers embedded in the upper surface.

10.2.8. Carbon Coating of Filter Sectors

Place the glass slide holding the filter sectors on the rotation-tilting device, approximately 10-12 cm from the evaporation source, and evacuate the evaporator chamber to a vacuum better than 0.013 Pa. The evaporation of carbon shall be performed in very short bursts, separated by some seconds to allow the electrodes to cool. If evaporation of carbon is too rapid, the surface of the filter may be damaged. The thickness of carbon required is dependent on the size of particles on the filter, and approximately 30-50 nm has been found to be satisfactory. If the carbon film is too thin, large particles will break out of the film during the later stages of preparation, and there will be few complete and undamaged grid openings on the specimen. Too thick a carbon film will lead to a TEM image which is lacking in contrast, and the ability to obtain SAED patterns will be compromised. The carbon film thickness should be the minimum possible, while retaining most of the grid openings of the TEM specimen intact.

10.2.9. Preparation of the Jaffe Washer

Place several pieces of lens tissue on the stainless steel bridge, and fill the washer with dimethyl formamide to a level where the meniscus contacts the underside of the mesh, resulting in saturation of the lens tissue. If it is intended to complete the washing of the specimens in the condensation washer, the pieces of lens tissue should be of such a size that they will fit on to the stainless steel mesh of the condensation washer cold finger. Three TEM specimen grids shall be prepared from each of the coated analytical filters, and for each of the analytical filters, three new specimen grids should be placed on to one of the pieces of lens tissue.

10.2.10. Placing of Specimens Into the Jaffe Washer

Using a curved scalpel blade, cut along the radius of the coated filter sector, approximately 1 mm from the edge. Discard the thin strip of coated filter, which usually exhibits signs of edge effects introduced during the collapsing procedure. Starting from the freshly-cut edge, cut three approximately 3 mm square pieces of the coated filter. Select three squares to represent the center and the outer periphery of the active surface of the filter. Place each square of filter, carbon side up, on one of the TEM specimen grids already on the lens tissue in the Jaffe washer. Cover the Jaffe washer with the lid, and allow the washer to stand. Specimens are normally cleared after approximately 4 hours.

10.2.11. Rapid Preparation of TEM Specimens From MCE Filters

An alternative washing procedure may be used to prepare TEM specimens from MCE filters more rapidly than can be achieved by the Jaffe washing procedure. After the specimens have been washed in a Jaffe washer for approximately 1 hour, transfer the piece of lens tissue supporting the specimens to the cold finger of a condensation washer operating with acetone as the solvent. Operate the condensation washer for approximately 30 minutes. This treatment removes all remaining filter polymer.

10.3. PREPARATION OF TEM SPECIMEN GRIDS BY THE DIRECT METHOD

10.3.1. Cleaning of Sample Cassettes

After ensuring that the cassette is tightly sealed, wipe the exterior surfaces of each sampling cassette with a clean, wet paper towel as described in 10.2.1 before it is taken into the clean facility or laminar flow hood.

10.3.2. Selection of Area of Filter for Preparation

Using clean tweezers, remove the MCE filter from the filter cassette, and cut a sector as described in 10.2.5.

10.3.3. Filter Collapsing Procedure

Collapse the filter sector as described in 10.2.7.

10.3.4. Plasma Etching of the Filter Surface

The conditions required in a particular plasma asher shall first be established using the procedure defined in Appendix A. Place the microscope slide holding the collapsed filter sectors in the plasma asher, and etch for the time and under the conditions determined. Care should be taken to ensure that the correct conditions are used. After etching, admit air slowly to the chamber and remove the microscope slide.

10.3.5. Carbon Coating of Filters

Carbon coat the microscope slide holding the collapsed filter portions as described in 10.2.8.

10.3.6. Preparation of the Jaffe Washer

Prepare the Jaffe washer as described in 10.2.9.

10.3.7. Placing of Specimens into the Jaffe Washer

Place specimens in the Jaffe washer as described in 10.2.10. Specimens are normally cleared after approximately 4 hours.

10.3.8. Rapid Preparation of TEM Specimens from MCE Filters

The alternative rapid washing procedure, described in 10.2.11, may be used to prepare TEM specimens from MCE filters more rapidly than can be achieved by the Jaffe washing procedure.

10.4. CRITERIA FOR ACCEPTABLE TEM SPECIMEN GRIDS

Examine the TEM specimen grid in the TEM at a magnification sufficiently low (300-1000) so that complete grid openings can be inspected. Reject the grid if:

(a) the TEM specimen has not been cleared of filter medium by the filter dissolution step. If the TEM specimen exhibits areas of undissolved filter medium and, if at least two of the three specimen grids are not cleared, either additional solvent washing shall be carried out or new specimens shall be prepared from the filter;

(b) the sample is over-loaded with particulate. If the specimen grid exhibits more than approximately 10% obscuration on the majority of the grid openings, the specimen will be designated as over-loaded. This filter cannot be analyzed satisfactorily because the grid is too heavily loaded with debris to allow separate examination of individual particles by ED and EDXA and obscuration of structures by other particulate would lead to under-estimation of the structure count;

(c) the particulate deposits on the specimen are not uniformly distributed from one grid opening to the next. If the particulate deposits on the specimen are obviously not uniform from one grid opening to the next, the specimen will be designated as non-uniform. This condition is caused either by use of an unsatisfactory procedure for the water filtration or it is a consequence of the fundamental nature of the airborne particulate. Such a filter cannot be analyzed satisfactorily;

(d) the TEM grid is too heavily loaded with asbestos structures to make an accurate count. Accurate counts cannot be made if the grid has more than approximately 7000 asbestos structures/mm^2; or,

(e) less than approximately 75% of the grid openings have unbroken carbon film over the whole grid opening. Since the breakage of carbon film is usually more frequent in areas of heavy deposit, counting of the intact openings can lead to an underestimate of the structure count. An additional carbon coating may be applied to the carbon coated filter and new specimen grids prepared. The larger particles can often be supported by using a thicker carbon film. If this action does not produce acceptable specimen grids, this filter cannot be analyzed and a filter prepared with a lighter loading of particulate should be selected.

If any one or more of the conditions described in (b), (c), (d) or (e) exists, the specimen grids cannot be analyzed.

10.5. PROCEDURE FOR STRUCTURE COUNTING BY TEM

10.5.1. Introduction

The TEM examination consists of a count of asbestos structures, which are present on a specified number of grid openings. Asbestos structures are classified into groups on the basis of morphological observations, ED patterns and EDXA spectra. The total number of asbestos structures to be counted depends on the statistical precision desired. In the absence of asbestos structures, the area of the TEM specimen grids which must be examined depends on the analytical sensitivity required. The

precision of the asbestos structure count depends not only on the total number of asbestos structures counted, but also on their uniformity from one grid opening to the next. Additional asbestos structure counting will be necessary if greater precision is required.

In order that the estimate of the asbestos structure density on the sampling filter shall not be based on the asbestos structure deposits found within the small area represented by one specimen grid, grid openings shall be examined on at least two of the three specimen grids prepared. The results shall then be combined in the calculation of the asbestos structure density. Structure counts shall be made at a magnification of approximately 20000, and will be terminated at the number of asbestos structures as defined below, except that the count shall be continued until a minimum of 4 grid openings have been examined. Otherwise, the asbestos structure count shall continue to that number of grid openings at which the specified analytical sensitivity has been achieved.

10.5.2. Measurement of Mean Grid Opening Area

The grid opening area shall be measured for the type of TEM specimen grids in use. This may be performed either on an optical microscope at a calibrated magnification of about 400, or at a calibrated magnification in the TEM. A mean value for the grid opening dimensions may be used if this value can be shown to be sufficiently precise. If a mean value is used, the standard deviation of the mean of 10 openings selected randomly from each of 10 grids shall be less than 5%. Alternately, the dimensions of one grid opening on each of the grids examined shall be measured and the mean of these used in the calculations for the particular analysis.

Values for the number of grid openings to be counted to achieve a desired level of sensitivity (Table 9.1) will be adjusted based on the average grid opening size derived in this section. The procedure for performing the conversion is presented in a note at the bottom of Table 9.1.

10.5.3. TEM Alignment and Calibration Procedures

Before structure counting is performed, align the TEM according to instrumental specifications. Calibrate the TEM and EDXA system according to the procedures described in Appendix B.

10.5.4. Determination of Stopping Point

Before structure counting is commenced, the area of specimen to be examined in order to achieve the required analytical

sensitivity shall be calculated. The maximum number of grid openings to be examined shall be calculated from the formula:

$$N = A_f/(A_s \times V \times S \times F)$$

where:

N = Number of grid openings to be examined. N shall be rounded upwards to the next highest integer

A_f = Area of collection filter in mm^2
A_s = Area of TEM specimen grid opening in mm^2

V = Volume of air sampled in liters

S = Required analytical sensitivity in asbestos structures/liter

F = Concentration factor.

The concentration factor F arises from the fact that the asbestos structures on a particular area of the TEM specimen have usually been transferred from a different area of the original sample collection filter. The concentration factor may correspond to a concentration or a dilution of the original asbestos structure density on the collection filter. It is calculated from the ratio between the areas of the original and analytical filters, the volume of water used to re-disperse the ash, and the volume of the final dispersion used to prepare the analytical filter. F is calculated from the following formula:

$$F = A_a \times V_f/(A_f \times V_a)$$

where:

A_a = Area of filter ashed
A_f = Area of analytical filter
V_a = Volume of water used to re-disperse ashed particulate
V_f = Volume of dispersion filtered through analytical filter

10.5.5. General Procedure for Structure Counting and Size Analysis

Use at least two specimen grids prepared from the filter in the structure count. Select at random several grid openings from each grid, and combine the data in the calculation of the results.

Use a form similar to that shown in Figure 10.1 to record the structure counting data. Insert the first specimen grid into the TEM. Select a typical grid opening and set the screen magnification to the calibrated value (approximately 20,000). Adjust the sample

TEM ASBESTOS COUNT

Page ___ of ___

Preparation Date: ___/___/___ By:____
Analysis Date: ___/___/___ By:____
y m d

Report Number: _____
Filename: _____
Sample Description: _____
_____
_____
Sample Number: _____

Sample Filter Area: _____sq.mm.
Area of Filter Taken: _____sq.mm.
Analytical Filter Area: _____sq.mm.
Redispersal Volume: _____mL
Liquid Volume Filtered: _____mL
Magnification: _____
Air Volume: _____Liters
Grid Opening Dimensions: _____um

Grid Opening	Structure Number	Class	Structure Type	Length (mm)	Width (mm)	Comments

Figure 10.1: Counting Form

height until the features in the center of the TEM viewing screen are at the eucentric point. Set the goniometer tilt angle to zero.

In column 1 of the structure counting form, record the sequential number of the grid opening. Position the specimen so that the grid opening is positioned with one corner visible on the screen. Move the image by adjustment of only one translation control, carefully examining the sample for asbestos structures, until the opposite side of the grid opening is encountered. Move the image by a pre-determined distance less than one screen diameter, using the other translation control, and scan the image in the reverse direction. Continue the procedure in this manner until the entire grid opening has been inspected in a pattern similar to that shown in Figure 10.2.

When a fibrous structure is detected, assign a sequential number in column 2, perform the identification procedures required as detailed in Appendix D, and enter the appropriate compositional classification on the fiber counting form in column 3. Assign a morphological classification to the structure according to the procedures in Appendix C, and record this in column 4. Measure on the TEM viewing screen the length and width of the image of each of the components of the fibrous structure in mm and record these measurements in columns 5 and 6 of the structure counting form.

If fibrous structures are present that are of obvious biological origin or that are determined to be non-asbestos, record data for a minimum of the first 10 such structures; further recording of data from non-asbestos structures is optional. After a fibrous structure has been examined and measured, re-locate the original field of view accurately before continuing scanning of the specimen. Failure to do this may cause asbestos structures to be overlooked or counted twice. The point at which the examination is to be terminated depends on the specimen, and is defined by either (a), (b) or (c) below:

(a) for Phase 1 samples, which have been prepared by the indirect TEM specimen preparation method only, complete the examination at the end of the grid opening on which the 50th asbestos structure is counted;

(b) for Phase 2 samples, which have been prepared both by the direct and indirect TEM specimen preparation methods, complete the examination at the end of the grid opening on which the 100th asbestos structure is counted; or

(c) for either of the sample types, until the number of grid openings required to achieve the specified analytical sensitivity for asbestos structures of all lengths, calculated according to 10.5.4, have been inspected, whichever occurs

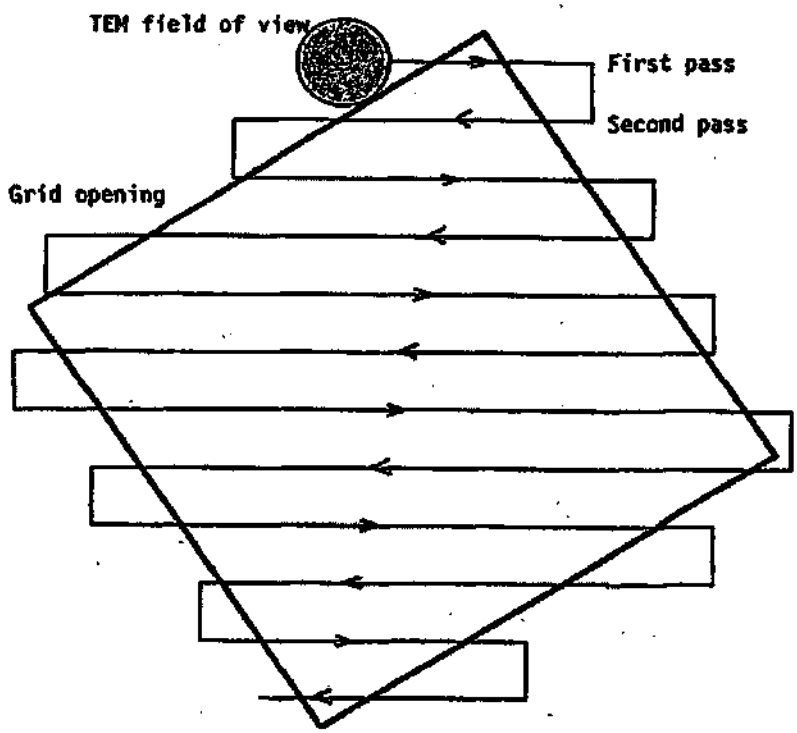

Figure 10.2: Scanning Procedure for TEM Specimen Examination

first. The data shall be drawn approximately equally from the two grids. Regardless of the value calculated according to 10.5.4, asbestos structures shall be counted on a minimum of 4 grid openings. Table 10.1 presents a summary of the counting requirements for Phase 1 and Phase 2 samples.

(d) Only those structures that are identified as, or are suspected to be, either chrysotile or one of the amphibole minerals, will be included in either the original or the extended structure count. Other materials, such as gypsum, cellulose fibers, and filter artifacts such as undissolved filter strands, will not be included in the structure count. This restriction is intended to ensure that the best statistical validity is obtained for the materials of interest.

10.5.6. Measurement of Concentration for Asbestos Structures Longer than 5 μm

To increase the statistical validity of the measurement, this method requires that an extended count be made of those asbestos structures with aspect ratios equal to or greater than 5:1 and longer than 5 μm. This separate structure count takes account only of the asbestos structures longer than 5 μm, and scanning of the TEM specimen shall be performed using a magnification of approximately 10,000.

Measurement of the asbestos structure lengths shall be performed at a magnification of 10,000, but asbestos structure diameters shall be measured at a magnification of a minimum of 20,000. For Phase 1 samples, which have been prepared by indirect transfer only, the count is continued until either 50 asbestos structures have been recorded or an area of specimen has been examined that achieves the specified analytical sensitivity for asbestos structures longer than 5 μm. For Phase 2 samples, which have been prepared both by indirect and direct transfer, the count is continued until either 100 asbestos structures have been counted or an area of specimen has been examined that achieves the specified analytical sensitivity for asbestos structures longer than 5 μm. Table 10.1 presents a summary of the counting requirements for Phase 1 and Phase 2 samples.

Only those structures that are identified as, or are suspected to be, either chrysotile or one of the amphibole minerals, will be reported in either the original or the extended structure count. Other materials, such as gypsum, cellulose fibers, and filter artifacts such as undissolved filter strands, will not be included in the structure count. This restriction is intended to ensure that the best statistical validity is obtained for the materials of interest.

TABLE 10.1: SPECIFICATIONS FOR PHASE 1 AND 2 SAMPLING AND ANALYSIS

Setting	Definitions	Phase 1 Samples: High Magnification Scan	Phase 1 Samples: Low Magnification Scan	Phase 2 Samples: High Magnification Scan	Phase 2 Samples: Low Magnification Scan
	Structure sizes to be counted	total	length > 5 μm	total	length > 5 μm
	Number of structures to be counted	50	50	100	100
Ambient:	Volume of air to be collected is 15 m^3				
	Maximum area to be scanned	0.05 mm^2	1.3 mm^2	0.11 mm^2	25 mm^2
	Maximum number of grid openings to be counted	7	160	13	307
	Minimum number of grid openings to be counted	4	4	4	4
Rural:	Volume of air to be collected is 10 m^3				
	Maximum area to be scanned	0.08 mm^2	2.0 mm^2	0.16 mm^2	3.7 mm^2
	Maximum number of grid openings to be counted	10	240	20	460
	Minimum number of grid openings to be counted	4	4	4	4
Urban and Agricultural:	Volume of air to be collected is 5 m^3				
	Maximum area to be scanned	0.15mm^2	3.9 mm^2	0.32 mm^2	7.5 mm^2
	Maximum number of grid openings to be counted	20	480	40	920
	Minimum number of grid openings to be counted	4	4	4	4
Activity Specific:	Volume of air to be determined in the field				
	Maximum area to be scanned	TBD	TBD	TBD	TBD
	Maximum number of grid openings to be counted	TBD	TBD	TBD	TBD
	Minimum number of grid openings to be counted	4	4	4	4

TABLE 10.1
(continued)

NOTES:

Phase 1 samples will be prepared by the indirect technique. Filters from Phase 2 samples will be split. Half will be prepared by the indirect technique and half will be prepared by the direct technique. Note that the required sensitivity for the Phase 2 samples is higher than that necessary for the Phase 1 samples. Consequently, it is assumed that 20 structures need to be counted for phase 2 samples at a minimum.

The information presented in this table is derived from the equation presented in the Section 10.5.4 assuming that A_f = 385 mm^2, A_s = 0.0081 mm^2, F = 1, and that 10 structures will have to be counted for statistical significance. Based on a review of reported background concentrations and typical concentrations encountered in previous studies, it is also assumed that the critical concentration for total structures is 5.0 structures/liter and the critical concentration for structures longer than 5 micrometers is 0.2 structures/liter.

During any site investigation, it will likely be necessary to characterize both ambient air concentrations and concentrations of asbestos associated with specific human activities. Although the method is designed for ambient conditions, it will be possible to adapt it to activity specific sampling by accounting with some adjustment for expected differences in conditions between the two types of samples. The principle difference likely is that a much higher concentration of non-asbestos particulates will likely be contained in air sampled in association with specific, dust-generating activities. As a consequence, the estimated values for maximum tolerable air volumes presented in section 4.2.1 of the text can not be applied to activity specific sampling and some measure of TSP will have to be provided to derive alternate sampling volumes. Thus, "TBD" means: to be determined.

It must be emphasized that the volume of air recommended for collection in each of the environmental settings listed represents a conservative estimate (based on published data) of the maximum that can be collected and still allow analysis without overloading the filter. If additional information regarding the local average concentration of ambient TSP, air volumes to be collected in any of these settings should be optimized accordingly.

To achieve the desired analytical sensitivity, the numbers of grid openings required for counting (as presented in this Table) may either be distributed over a set of specimen grids prepared from a single sample or a larger set of specimen grids prepared from several samples collected in a manner assuring that the samples are appropriately homogeneous (see Section 4.3 of the Technical Background Document, Part 2 of this report).

10.6. BLANK AND QUALITY CONTROL DETERMINATIONS

Before air samples are collected, a minimum of 2 unused 0.45 μm pore size MCE filters from each lot of 100 filters shall be sectioned and analyzed both by the indirect and the direct transfer procedure to determine the mean background asbestos structure concentration. If the mean concentration for all types of asbestos structures, expressed as the concentration per unit area of the sample collection filter, is found to be more than 10 structures/mm^2, the reasons for the high blank values shall be determined and the situation corrected before air samples are collected.

To ensure that contamination by extraneous asbestos structures during specimen preparation is insignificant compared with the results reported on samples, it is necessary to establish a continuous program of blank measurements. The number of field blanks incorporated into the program shall be at least 10% of the total number of samples, and all of these shall be analyzed. In addition, one unused 0.1 μm pore size filter shall be included with every group of samples prepared on one microscope slide.

It is further recommended that laboratory blanks be collected intermittently at all critical phases of the laboratory program. For example, in addition to the filter blanks and field blanks, suspension blanks should be prepared by subjecting a clean ashing tube to the asher, rinsing the tube and completing the standard indirect preparation on the "clean" rinse. Other blanks may be constructed to test for the potential introduction of contamination at other phases of the process. Such blanks need not be analyzed but may be stored to provide a chain for trouble-shooting should a problem with contamination occur.

Initially, and also included at random points in the sampling program, it is necessary to ensure that samples of known asbestos structure concentrations can be analyzed satisfactorily by this procedure. There are many opportunities in the procedure for a low recovery to be obtained, and the recovery must be measured frequently, particularly if more than one laboratory performs the analysis. Reference filters of known concentration will be incorporated, and shall constitute a minimum of 5% of the total analyses. These reference filters shall not be identified to the analytical laboratory prior to the analysis of any group of samples. The results of these reference analyses shall not differ at the 5% significance level from the mean value obtained by a selected group of experienced laboratories.

Since there is a subjective component in the structure counting procedure, it is necessary that re-counts of some specimens be made by different microscopists, in order to minimize the subjective effects. Such recounts provide a means of maintaining comparability between counts made by different microscopists. Variability between and within microscopists and between laboratories shall be characterized. These quality assurance

measurements shall constitute a minimum of approximately 10% of the analyses. Repeat results should not differ at the 5% significance level.

10.7. CALCULATION OF RESULTS

Calculate the results using the procedures detailed in Appendix E. Prior to the TEM examination of the specimens, the level of analysis was specified. Before the results are calculated, the compositional and morphological classifications to be included in the result shall be specified. The chi-squared uniformity test shall be conducted using the number of isolated asbestos structures (individual entities) found on each grid opening, prior to the application of the cluster and matrix counting criteria. The concentration result shall be calculated using the numbers of asbestos structures reported after the application of the cluster and matrix counting criteria.

11. PERFORMANCE CHARACTERISTICS

11.1. INTERFERENCES AND LIMITATIONS OF STRUCTURE IDENTIFICATION

Unequivocal identification of every chrysotile structure is not possible, due to both instrumental limitations and the nature of some of the component fibers. The requirement for a calibrated ED pattern eliminates the possibility of an incorrect identification of the structure selected. However, there is a possibility of misidentification of other chrysotile structures for which both a morphology and ED pattern are reported on the basis of visual inspection only. 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 ED pattern.

As in the case of chrysotile structures, complete identification of every amphibole structure is not possible due to instrumental limitations and the nature of some of the component fibers. Moreover, complete identification of every amphibole structure is not practical due to the limitations of both time and cost. Particles of a number of other minerals having compositions similar to those of some amphiboles could be erroneously classified as amphibole when the classification criteria do not include zone-axis ED techniques. However, the requirement for quantitative EDXA measurements on all structures as support for the random orientation ED technique makes misidentification very unlikely, particularly when other similar structures in the same sample have been identified as amphibole by zone-axis methods. 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.

11.2. PRECISION AND ACCURACY

11.2.1. Precision

The analytical precision that can be obtained is dependent upon the number of structures counted, and also on the uniformity of the particulate deposit on the original filter. Assuming that the structures are randomly deposited on the filter, if 100 structures are counted and the loading is at least 3.5 fibers/grid opening, computer modeling of the counting procedure shows that a coefficient of variation of about 10% can be expected. As the number of structures counted decreases, the precision will also decrease approximately as $\sqrt{N}$, where N is the number of structures counted. In practice, particulate deposits obtained by filtration are rarely ideally distributed, and it is found that the precision is correspondingly reduced from that predicted by the Poisson distribution. This degradation is a consequence of:

(a) non-uniformity of the filtered particulate deposit;

(b) distortion of the structure distribution by application of the structure counting criteria;

(c) variation between microscopists in their interpretation of the fibrous structures; and,

(d) variation between microscopists and instruments in their ability to detect and identify structures.

The 95% confidence interval about the mean for a single structure concentration measurement using this analytical method should be about ±25% when about 100 structures are counted.

11.2.2. Accuracy

There is no independent method available to determine accuracy.

11.3. ANALYTICAL SENSITIVITY

Analytical sensitivity is the concentration corresponding to observation of one asbestos structure in an analysis. The analytical sensitivity of the method can be varied by choice of the area of the collection filter, the volume of air sampled, the dilution or concentration factor used in the specimen preparation and the area of the specimen examined in the TEM. The analytical sensitivity shall be quoted for each sample analysis.

NOTE

In practice, the lowest achievable value of analytical sensitivity is frequently determined by the total suspended particulate concentration in the air sample, since each particle on the TEM specimen must be separated from adjacent ones by a sufficient distance that the particle can be identified without interference. Particulate loadings on filters greater than about 25 $\mu g/cm^2$ usually preclude preparation of TEM specimens.

If the analysis is to be performed with an acceptable expenditure of time, the area of the specimen examined in the TEM must also be limited. The analytical sensitivity that can be achieved for any particular sample depends on the air volume, the area of TEM specimen examined, and the dilution or concentration factor used in the analysis, which in turn is controlled by the amount of particulate that is not removed by the selective concentration procedures.

11.4. LIMIT OF DETECTION

Should asbestos be observed during analysis, it is generally important to distinguish whether such asbestos originated in the sampled medium or if it was introduced as contamination during analysis. Asbestos that can be attributed to the sampled medium is generally considered to have been "detected". Thus, a detection limit is defined as the smallest measurement that is unlikely (probability less than a specified value) to be due entirely to contamination from sources other than the air being sampled. Detection limits are generally quantified by considering the magnitude and frequency of occurrence of the analytical background associated with a particular method. However, detection limits for asbestos methods are difficult to quantify because the distribution of analytical background associated with asbestos analysis tends to be poorly behaved.

To avoid the problems typically associated with defining a detection limit for asbestos analysis, an alternate procedure for distinguishing sampled asbestos from contamination due to analytical background has been incorporated into this method[3]. A statistical test is performed to compare structure concentrations observed on sample filters with structure concentrations observed on appropriate blanks to determine whether differences between the concentrations observed on the two sets of filters are statistically significant.

NOTE

Due to the difficulty in characterizing contributions to observed asbestos on individual samples from contamination introduced as analytical background, tests to distinguish "detected" asbestos from such contamination are better applied to groups of samples. Adjustments to account for analytical background are not recommended for individual samples.

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[3] A detection limit is more appropriate for analytical methods that employ wet-chemical or spectroscopic techniques rather than structure counts (as in the case of asbestos) because the former are invariably associated with a lower limit to the amount (number of molecules) that can be detected. For asbestos, however, analysis by TEM is sufficiently sensitive to detect the smallest, single asbestos structure. Thus the issues involved in setting detection limits for TEM analyses of asbestos structures are quite different than from those involved with detecting populations of molecules such as in gas chromatograph-mass spectrometry (GC-MS) analyses, for example.

12. REPORTING REQUIREMENTS

Reporting requirements for the laboratory include the individual documentation for each sample (sample analysis report) and the cumulative report for any batch of samples from a particular study. The structure counting form to be used by the analyst was presented in Figure 10.1. Required data reduction by the site data reviewer is presented at the end of this chapter, Section 12.3. It is critical that the following documentation requirements be followed exactly to preserve the ability to interpret results without the need to re-examine samples.

12.1. SAMPLE ANALYSIS REPORT

The sample analysis report for each sample shall include at least the following information:

(a) reference to this analytical method;

(b) identification of the air sample and the batch number of the collection filter used;

(c) the dates and times of the air sample collection period;

(d) the volume of air passed through the sample collection filter, the area of filter used for the sample preparation, the volume of water used for re-dispersal, the batch number of the filters used for filtration of the aqueous suspension, and, for the set of TEM specimen grids examined, the volume of the aliquot used in the aqueous suspension filtered;

(e) a complete listing of the structure counting data. The dimensions of the TEM grid openings shall be specified, and for each asbestos structure (or structure component) the following data shall be included: grid opening number, structure number, identification category (morphological classification), structure type (compositional classification), length and width of the structure in μm, and any comments concerning the structure;

(f) a statement of the minimum acceptable identification category and the maximum identification category attempted;

(g) a statement specifying which identification and structure categories have been used to calculate the concentration values;

(h) separate concentration values for chrysotile and amphibole structures, expressed in asbestos structures/liter[4];

(i) separate concentration values for chrysotile and amphibole structure components, expressed in asbestos structures/liter[4];

(j) the 95% confidence limits for the concentration values, expressed in asbestos structures/liter;

(k) the analytical sensitivity, expressed in asbestos structures/liter;

(l) compositional data for the principal varieties of amphibole, if present;

(m) items d - l for the separate count of asbestos structures longer than 5 μm;

An example of a suitable format for the structure counting data is shown in figure 12.1.

NOTE

As improved definitions of the dimensions of asbestos structures critical to the determination of risk become available, the reporting requirements for this method may be modified accordingly. At the same time, sufficient information is preserved in these reporting requirements to allow later re-evaluation of existing analysis results without the need to re-examine the original sample.

12.2. SAMPLE BATCH REPORT

In addition to the sample analysis report for each sample, a summary page should be provided for each batch of samples representing an entire project. The summary sheet should include the following information:

(a) project title;

(b) date samples received and data results reported;

(c) a summary listing of sample results with chrysotile and amphibole reported separately including:

- the sample number;

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[4] If asbestos is not detected during a particular analysis, the resulting mean concentration for that sample should be reported as "NF" for "not found".

- the analytical sensitivity for each size range characterized (structures of all sizes and structures longer than 5 mm)

- the total number of structures counted;

- the concentration of asbestos structures of all sizes in structures per liter with appropriate 95% confidence limits;

- the concentration of asbestos structure components in structures per liter with appropriate 95% confidence limits;

- the concentration of asbestos structures longer than 5 μm in structures per liter with appropriate 95% confidence limits;

- the concentration of asbestos structure components longer than 5 μm in structures per liter with 95% confidence limits;

A sample summary sheet is presented in figure 12.2.

12.3. DATA REVIEW REPORT

Once the Sample Batch Report is received from the laboratory, the following reporting procedure will be performed by the project data reviewer. The data reviewer shall provide a summary sheet reflecting specific steps in data reduction. The summary data sheet shall include the following information:

(a) the sample number;

(b) the type of sample (lab blank, field blank, sampling station identifier);

(c) the analytical sensitivity for each size fraction reported (structures of all sizes and structures longer than 5 μm);

(d) the number of total structures counted;

(e) the concentration of asbestos structures of all sizes in structures per liter with 95% confidence limits;

(f) the concentration of asbestos structure components in structures per liter with 95% confidence limits;

(g) the concentration of asbestos structures longer than 5 μm in structures per liter with 95% confidence limits;

(h) the concentration of asbestos structure components longer than 5 μm in structures per liter with 95% confidence limits;

(i) the ratio of free asbestos fibers to total asbestos structures;

SAMPLE ANALYSIS INFORMATION (Page 1)

Laboratory name | Report number | Date

SAMPLE: 456 Queen Street
San Diego
Downwind sample 1988-09-25
Collected: 8 a.m. to 4 p.m.

ANALYSIS: Name of method
Reference to method

Air volume:	12500.0 liters
Area of collection filter:	385.0 mm^2
Area of filter ashed:	96.3 mm^2
Volume of water used for re-dispersal:	40.0 mL
Volume of suspension filtered:	35.0 mL
Area of analytical filter:	199.0 mm^2
Magnification used for fiber counting:	20500 (high magnification) 10200 (low magnification)
Mean dimension of grid openings:	90.0 μm

<u>High Magnification Scan</u>

Number of grid openings examined:	38
Analytical Sensitivity:	0.5 s/L
Number of chrysotile structures counted:	63 (including F, M, C and B)
Number of chrysotile structure components counted:	91
Number of amphibole structures counted:	12 (F only)
Number of amphibole structure components counted:	12

<u>Low Magnification Scan</u>

Number of grid openings examined:	357
Analytical sensitivity:	0.02 s/L
Number of long chrysotile structures counted:	3 (B and C)
Number of long chrysotile structure components counted:	14
Number of long amphibole structures counted:	0
Number of long amphibole structure components counted:	0

NOTES: Only structures with components longer than 5μm counted during the low magnification scan.

Figure 12.1A: Format for Reporting of Counting Data, Page 1

SAMPLE ANALYSIS INFORMATION (Page 2)

Laboratory name | Report number | Date

SAMPLE: 456 Queen Street
San Diego
Downwind sample 1988-09-25
Collected: 8 a.m. to 4 p.m.

ANALYSIS: Name of method
Reference to method

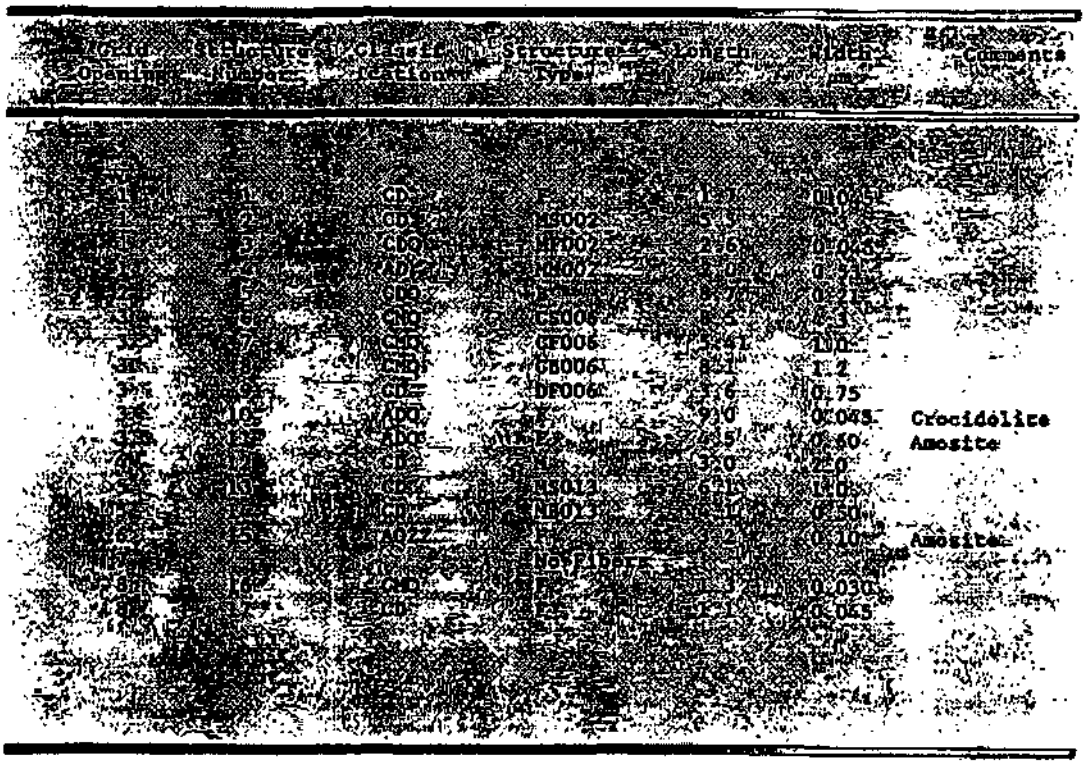
	Mean (s/L)	95% Confidence Limits Range (s/L)
Concentration of chrysotile structures:	31.5	24 - 38
Concentration of chrysotile structure components:	45.5	37 - 54
Concentration of long chrysotile structures:	0.06	0 - 0.12
Concentration of long chrysotile structure components:	0.28	0.16 - 0.4
Concentration of amphibole structures:	6	3.1 - 8.9
Concentration of amphibole structure components:	6	3.1 - 8.9
Concentration of long amphibole structures:	NF	0 - 0.07
Concentration of long amphibole structure components:	NF	0 - 0.07

NOTES: NF means Not Found

Long structures and structure components are those greater than 5μm in length.

Figure 12.1B: Format for Reporting of Counting Data, Page 2

TEM ASBESTOS COUNT – RAW DATA (Page 3 and higher)



* Classification codes listed in Tables D1 and D2

Figure 12.1C: Format for Reporting Counting Data, Page 3 and Subsequent Pages

Batch #: Sample Medium:

Project: Analysis Type:

Lab: Preparation Technique:

Date:

Sample I.D. Date Received:								
Structures counted: Structure concentration (s/L): Mean Range | Low / High Analytical sensitivity:								
Components counted: Component concentration (s/L): Mean Range | Low / High Analytical sensitivity:								
Long structures counted: Long structure concentration (s/L): Mean Range | Low / High Analytical sensitivity:								
Long components counted: Long component concentration (s/L): Mean Range | Low / High Analytical sensitivity:								

NOTES:

NF = not found

Long structures or components are those longer than 5 μm.

The range presented represents the 95% confidence limits constructed as discussed in Section E.4.

Provide separate sheets for chrysotile and amphiboles or total asbestos, as necessary.

Figure 12.2: Format for the Summary Batch Report

(j) the ratio of free asbestos fibers to total asbestos structures and components;

(k) the ratio of matrices to total asbestos structures;

(l) the ratio of bundles to total asbestos structures;

(m) the ratio of clusters to total asbestos structures;

(n) items i-m for asbestos structures longer than 5 μm;

(o) a complete listing of the structure counting data for each sample (item "e" of section 12.1).

An example of the summary form for data review is provided in Figure 12.3.

ANALYTICAL RESULTS

Case No.:
Site:
Lab:
Reviewer:
Date:

Analysis Type: Air for TEM asbestos
Indirect Preparation

Concentration of Asbestos (Chrysotile unless otherwise noted)

Sample I.D. (number and type)																
Analysis/Preparation																
Parameter	Mean	95% CL Low	95% CL High	Mean	95% CL Low	95% CL High	Mean	95% CL Low	95% CL High	Mean	95% CL Low	95% CL High	Mean	95% CL Low	95% CL High
Number of liters sampled																
HIGH MAGNIFICATION																
Number of grids examined																
Structures/area viewed																
Fibers/area viewed																
Bundles/area viewed																
Clusters/area viewed																
Matrices/area viewed																
Structures/L																
Components/L																
Long structures/L																
Long components/L																
Analytical sensitivity																
LOW MAGNIFICATION																
Number of grids examined																
Structures/area viewed																
Fibers/area viewed																
Bundles/area viewed																
Clusters/area viewed																
Matrices/area viewed																
Long structures/L																
Long components/L																
Analytical sensitivity																

NOTES:
NF = not found
95% CL = 95% confidence limit
The range presented represents the 95% upper or lower confidence limit constructed as discussed in Section B.4.

Figure 12.3: Format for Data Review Summary Report

APPENDIX A - DETERMINATION OF OPERATING CONDITIONS FOR PLASMA ASHER

A.1 INTRODUCTION

It is important to establish standard conditions for operation of the plasma asher, so that:

(a) ashing conditions for MCE filters and particulate are sufficient and remain constant; and,

(b) so that a known amount of etching of MCE filters can be achieved on the surface of filters being prepared by the direct-transfer method.

Therefore, the plasma asher shall be calibrated so that a known material is completely ashed in a constant time. This is carried out by adjusting the radio-frequency power output and the oxygen flow rate, and measuring the time taken to completely oxidize an unused 25 mm diameter 0.45 μm pore size MCE filter.

A.2 PROCEDURE

Place an unused, 25 mm diameter, 0.45 μm pore size MCE filter in the center of a glass microscope slide. Position the slide approximately in the center of the asher chamber. Close the chamber and evacuate to a pressure of approximately 40 Pa, while admitting oxygen to the chamber at a rate of 8-20 cc/min. Adjust the tuning of the system so that the intensity of the plasma is maximized. Measure the time required for complete oxidation of the filter. Determine the power setting that result in complete oxidation of the filter in a period of approximately 15 minutes.

NOTE

Plasma oxidation at high radio-frequency powers will cause the filter to shrink and curl, followed by sudden violent ignition. At lower powers the filter will remain in position, and will become slowly thinner until it becomes nearly transparent. It is recommended that the radio-frequency power be used is selected such that violent ignition does not occur. If powers are used such that the particulate ignites violently, fibers will possibly be disturbed and lost from the analysis.

APPENDIX B - CALIBRATION PROCEDURES

B.1 CALIBRATION OF THE TEM

B.1.1 Calibration of TEM Screen Magnification

The electron microscope should be aligned according to the specifications of the manufacturer. Initially, and at regular intervals, calibrate the magnifications used for the analysis using a diffraction grating replica. Adjust the specimen height to the eucentric position before carrying out the calibration. Measure the distance on the fluorescent viewing screen occupied by a convenient number of repeat distances of the grating image, and calculate the magnification. Always repeat the calibration after any instrumental maintenance or change of operating conditions. The magnification of the image on the viewing screen is not the same as that obtained on photographic plates or film. The ratio between these is a constant value for the particular model of TEM.

B.1.2 Calibration of ED Camera Length

Calibrate the camera length of the TEM when used in ED mode. Use a specimen grid supporting a carbon film on which a thin film of gold has been evaporated or sputtered. Form an image of the gold film and select ED conditions. Adjust the objective lens current to optimize the pattern obtained, and measure on the fluorescent viewing screen the diameters of the innermost two rings. Calculate the camera constant, $\lambda \times L$, from the relationship:

$$\lambda \times L = \frac{a \times D}{\sqrt{h^2 + k^2 + l^2}}$$

where:

λ = the wavelength of the incident electrons
$\lambda \times L$ = the effective camera length in mm
a = the unit cell dimensions of gold in Ångström units
D = the diameter of the (hkl) diffraction ring in mm

Using gold as the calibration material, the camera constant is given by:

$\lambda \times L = 2.3548 \times D$ (smallest ring)
$\lambda \times L = 2.0393 \times D$ (second ring)

B.2 CALIBRATION OF THE EDXA SYSTEM

Energy calibration of the EDXA system for a low energy and high energy peak shall be performed regularly. Calibration of the intensity scale of the EDXA system permits quantitative composition data, at an accuracy of about 10% of the elemental concentration, to be obtained from EDXA spectra of silicate minerals involving the elements Na, Mg, Al, Si, K, Ca, Mn, and Fe. SRM 2063 should also be used to calibrate the EDXA system for Mg, Ca, and Fe relative to Si. If quantitative determinations are required for minerals containing other elements, reference standards other than those referred to below will be required. Well-characterized mineral standards permit calibration of any TEM-EDXA combination that meets the instrumental specifications of 6.3.1 and 6.3.2, so that EDXA data from different instruments can be compared. Reference minerals are required for the calibration; the criteria for selection being that they should be silicate minerals with matrices as close as possible to those of the amphiboles or serpentine, and that individual small fragments of the minerals are homogeneous in composition within a few percent.

Determine the compositions of these standards by electron microprobe analysis or chemical methods, and prepare TEM grids from aqueous dispersions of crushed fragments of the same selected mineral specimens. These TEM specimens can then be used to calibrate any TEM-EDXA system so that comparable compositional results can be obtained from different instruments.

NOTE

The microprobe analyses of the mineral standards are made by conventional techniques, which can be found in the bibliography. The mineral is first embedded in a mount of polymethyl methacrylate or epoxy resin. The mount is then ground and polished to achieve a flat, polished surface of the mineral fragment. This surface is then analyzed, using suitable reference standards, preferably oxide standards of the individual elements wherever these are available. It is necessary to take account of the water concentration in the minerals, which in the case of chrysotile amounts to 13% by weight.

Express the results of the electron microprobe analyses as atomic ratios relative to silicon. X-ray peak ratios of the same elements relative to silicon, obtained from the EDXA system, can then be used to calculate the relationship between peak area ratio and atomic ratio. The technique was described by Cliff and Lorimer (See Bibliography).

The X-rays generated in a thin specimen by an incident electron beam have a low probability of interacting with the specimen. Thus mass absorption and fluorescence effects are negligible. In a specimen composed of elements i and j, the following relationship can be used to perform quantitative analyses in the TEM.

$$\frac{A_i}{A_j} = \frac{k\ C_i}{C_j}$$

where:

A_i = the elemental integrated peak areas for element i
A_j = the elemental integrated peak area for element j
C_i = the concentration or atomic proportion of element i
C_j = the concentration or atomic proportion of element j
k = a constant

To incorporate correction for the particle size effect on peak area ratios (See Small et al. in Bibliography), extend the Cliff and Lorimer technique by obtaining separate values of the constant k for different ranges of fiber diameter. It is recommended that about 20 EDXA measurements be made for each range of fiber diameter. Suitable ranges of fiber diameter are <0.25 μm, 0.25-0.5 μm, 0.5-1.0 μm and >1.0 μm.

Insert the TEM grid into the TEM, obtain an image at the calibrated higher magnification of about 20,000, and adjust the specimen height to the eucentric point. If the x-ray detector is a side-entry variety, tilt the specimen towards the x-ray detector. Select an isolated fiber or particle less than 0.5 μm in width, and accumulate an EDXA spectrum using an electron probe of suitable diameter. When a well-defined spectrum has been obtained, perform a background subtraction and calculate the background-corrected peak areas for each element listed, using energy windows centered on the peaks. Calculate the ratio of the peak area for each specified element relative to the peak area for silicon. All background-subtracted peak areas used for calibration shall exceed 400 counts.

Repeat this procedure for 20 particles of each mineral standard. Reject analyses of any obviously foreign particles. Calculate the arithmetic mean peak area to concentration ratio, k, for each specified element of each mineral standard and for each of the fiber diameter ranges. Routine checks to ensure that there has been no degradation of the detector performance shall be carried out. These k-values are used to calculate the elemental concentrations of unknown fibers, using the Cliff and Lorimer relationship.

APPENDIX C - STRUCTURE COUNTING CRITERIA

C.1 INTRODUCTION

In addition to isolated fibers, other assemblages of particles and fibers frequently occur in air samples. Groupings of asbestos fibers and particles, referred to as "asbestos structures", are defined as fiber bundles, clusters and matrices. The numerical result of a structure count depends strongly on whether the analyst assigns each such assemblage of fibers as a single entity, or as the estimated number of individual fibers which form the assemblage. It is therefore important that a logical system of counting criteria be defined, so that the interpretation of specific structures is the same for all analysts, and so that the numerical result is meaningful. Imposition of specific structure counting criteria generally requires that some interpretation, partially based on uncertain health effects information, be made of each asbestos structure found. It is not the intention of this method to make interpretations based on health effects. Rather, it is intended that a clear separation shall be made between recording of structure counting data and later interpretation of those data. The system of coding specified in this method permits a clear morphological description of the structures to be recorded in a concise manner suitable for later interpretation, if necessary, by a range of different criteria, without the necessity for re-examination of the specimens. The system requires each asbestos structure to be assigned a predominant classification as fiber, bundle, cluster or matrix and in some cases individual components of a structure are separately enumerated. Examples of the various types of morphological structures and the manner in which these shall be recorded are shown in Figure C1.

C.2 STRUCTURE DEFINITIONS AND TREATMENT

C.2.1 Fiber

Any particle with parallel or stepped sides, with an aspect ratio of 5:1 or greater, shall be defined as a fiber. For chrysotile asbestos, the single fibril shall be defined as a fiber. A fiber with stepped sides shall be assigned a width equal to the average of the minimum and maximum widths. This average shall be used as the width in determination of the aspect ratio.

C.2.2 Bundle

A grouping composed of apparently attached parallel fibers shall be counted as a bundle of a width equal to an estimate of the mean bundle width, and a length equal to the maximum length of the structure. The overall aspect ratio of the bundle may be any value, provided that the individual constituent fibers have aspect ratios equal to or greater than 5:1.

C.2.3 Cluster

An aggregate of randomly oriented fibers, with or without bundles, shall be defined as a cluster. Clusters occur as two varieties:

Cluster type A: a disperse and open network, in which both ends of individual fibers and bundles can be separately identified and their dimensions measured. If the cluster consists of up to 5 such fibers or bundles, the individual fibers and bundles comprising the cluster shall be separately counted, measured, and recorded as individual fibers and bundles that are components of the overall cluster. If the cluster consists of more than 5 fibers or bundles it shall be noted as such on the count sheet. In addition to characterizing and measuring individual components, the overall outer dimensions of the cluster will be recorded as prescribed for type B clusters.

Cluster type B: a complex and tightly bound network, in which one or both ends of each individual fiber or bundle are obscured, such that the dimensions of individual fibers cannot be measured. In this case the cluster shall be recorded as a single cluster with no components, and the overall dimensions in the two perpendicular directions defining the maximum and minimum dimensions shall be recorded.

The recording of clusters shall be based on the predominant characteristics of the structures. For example, a cluster consisting predominantly of 4 fibers, but with smaller regions of attached material containing fibers, shall be considered as a type A cluster of 4 fibers to be recorded separately as fibers that are components of the overall cluster.

The procedure for treatment of clusters is illustrated by examples in Figure C2.

C.2.4 Matrix

A fiber, fibers, or bundles, may be attached to, or partially concealed by, a single particle or group of overlapping non-fibrous particles. This structure shall be defined as a matrix. The TEM image does not discriminate between particles that are attached to fibers, and those that, by chance, overlap in the TEM image. It is not known, therefore, whether such a structure is actually a complex particle, or whether it has arisen by a simple overlapping of particles and fibers on the filter.

Since a matrix structure may involve more than one fiber, it is important to define in detail how matrices shall be counted. Matrices exhibit different characteristics, and three types can be defined:

Matrix type A: a disperse grouping of overlapping fibers and/or bundles and associated equant particles or groups of particles in which some

fibers have less than one third of their lengths obscured. If the matrix consists of up to 5 such fibers or bundles, the fibers or bundles shall be recorded as separate fibers or bundles that are components of the overall matrix, and their lengths and widths shall also be recorded. If the matrix consists of more than 5 fibers or bundles, it shall denoted as such on the count sheet. In addition to characterizing and measuring individual components, the overall outer dimensions of the matrix will be recorded as prescribed for type B matrices.

Matrix type B: a structure consisting of an equant particle or linked group of particles, in which the ends of fibers or bundles project from the particles, but the other ends of the fibers or bundles are obscured. Fibers and bundles shall be treated differently, depending on whether the obscured length could not possibly be more than one third of the total length. If the matrix exhibits up to 5 fibers or bundles, those fibers for which the obscured length could not be more than one third of the total length shall be counted as separate fibers or bundles that are components of the overall matrix. The assigned length for each partially-obscured fiber or bundle shall be equal to the visible length plus the maximum possible contribution from the obscured portion. Fibers or bundles which appear to cross the matrix, and for which both ends can be located approximately, shall be included in the maximum of 5 and counted as separate fibers or bundles that are components of the overall matrix. If more than 5 such fibers or bundles can be individually identified, this will be denoted on the count sheet. The residual matrix, if it exhibits additional fiber terminations that cannot be separately counted because the unobscured lengths are too short, shall be recorded as one matrix. All other matrices of type B shall each be recorded as one matrix with no components. The overall dimensions of each matrix in the two perpendicular directions defining the maximum and minimum dimensions shall be recorded.

Matrix type C: a structure in which fibers can be seen and identified in the interior, but which incorporates no fibers which project from the outside edges. This type of matrix can originate as a result of partial dissolution of binders during specimen preparation, when the original particle was a composite material containing asbestos. This type of matrix shall be recorded as a single matrix with no components. The overall dimensions of the matrix in the two perpendicular directions defining the maximum and minimum dimensions shall be recorded.

In practice, structures can occur in which different areas exhibit features of the three types of matrix, and more than one variety of asbestos may be incorporated in the same structure. In this case, the predominant characteristic of the structure should be determined, and then a logical procedure should be followed, in which predominant fibers and bundles are enumerated first up to a maximum of 5 followed by assignment of the remaining asbestos structures according to the counting rules. Complex structures shall be assigned no more than 6 separate components. Examples of the procedure which shall be followed are shown in Figure C3.

C.3 OTHER STRUCTURE COUNTING CRITERIA

C.3.1 Structures that Intersect Grid Bars

Structures that intersect grid bar shall be counted only for two adjacent sides of the grid opening, as illustrated in Figure C4. The length of the structure shall be recorded as twice the unobscured length. Structures intersecting either of the other two sides shall not be included in the count. This procedure ensures that the numerical count will be accurate, and that the best average estimate of length has been made.

C.3.2 Structures That Extend Outside the Field of View

During scanning of a grid opening, systematically count structures that extend outside of the field of view, so as to avoid double-counting. In general, a rule should be established so that structures extending outside the field of view in only two quadrants are counted. Structures without terminations within the field of view shall not be counted. The procedure is illustrated by Figure C5. Measure the length of each such structure by moving the specimen to locate the other end of the structure, and then return to the original field of view before continuing to scan the specimen.

C.4 PROCEDURE FOR DATA RECORDING

When a fibrous structure is detected during the structure count, the components of the structure are first identified according to the procedures in Appendix D. The procedure for recording the morphological description starts by classifying the predominant characteristic of the structure as a fiber, bundle, cluster, or matrix. The code F, B, C or M shall be the first component of the morphological descriptor. Depending on the nature of the individual structure, additional coded descriptions will be added to classify separate components of the structure.

C.4.1 Fibers

On the counting form, isolated fibers as defined in C.2.1 shall be recorded by the simple designation "F". If the fiber is a separately-counted part of a cluster or matrix structure, the sequential structure number shall be attached as a suffix, to identify all such constituent components of the particular cluster or matrix structure. For example, CF004 shall be used to denote a fiber forming part of structure number 004, the overall and predominant characteristic of which is a cluster.

C.4.2 Bundles

On the counting form, isolated bundles as defined in C.2.2 shall be recorded by the designation "B". If the bundle is a

separately-counted part of a cluster or matrix structure, the sequential structure number shall be attached as a suffix, to identify all such constituent components of the cluster or matrix structure. For example, MB004 shall be used to denote a bundle forming part of structure number 004, the overall and predominant characteristic of which is a matrix.

C.4.3 Clusters

On the fiber counting form, isolated clusters, as defined in C.1.4, shall be recorded by the designation "C". The dimensions of the overall structure are recorded. If some components of the cluster have been separately counted, the cluster shall be recorded by the designation CS, followed by the sequential structure number "n". For example, the code CS004 indicates that the primary structure number 004 was a cluster, for which the overall dimensions are specified. Thus if a localized cluster is attached to a group of fibers or bundles, and the fibers and bundles have been counted separately because this procedure was defined by the predominant characteristics of the structure, the sequential structure number shall be attached as a suffix, to identify all constituent components of the structure. For example, CF004 shall be used to denote a fiber forming part of structure number 004, the overall and predominant characteristic of which is a cluster. When more than 5 components can be individually identified, the cluster is still recorded by the designation CS and the overall dimensions of the structure are recorded, but the sequential structure number is excluded from the code and components are not recorded separately.

C.4.4 Matrices

On the fiber counting form, isolated matrices of type B or C, as defined in C.1.5, shall be recorded by the designation "M". The dimensions of the overall structure are recorded. If the type B or C matrix is attached to a group of fibers or bundles, and the fibers and bundles have been counted separately because this procedure was defined by the predominant characteristics of the structure, the sequential structure number shall be attached as a suffix, to identify all constituent components of the structure. For example, the primary matrix structure shall be designated as MS004, and the overall structure dimensions recorded. All separately-counted components of the structure shall carry the suffix 004. Thus the code MB004 shall be used to denote a bundle forming part of structure number 004, the overall and predominant characteristic of which is a matrix. When more than 5 components can be individually identified, the matrix is still recorded by the designation MS and the overall dimensions of the structure are recorded, but the sequential counting number is excluded from the code and components are not recorded separately.

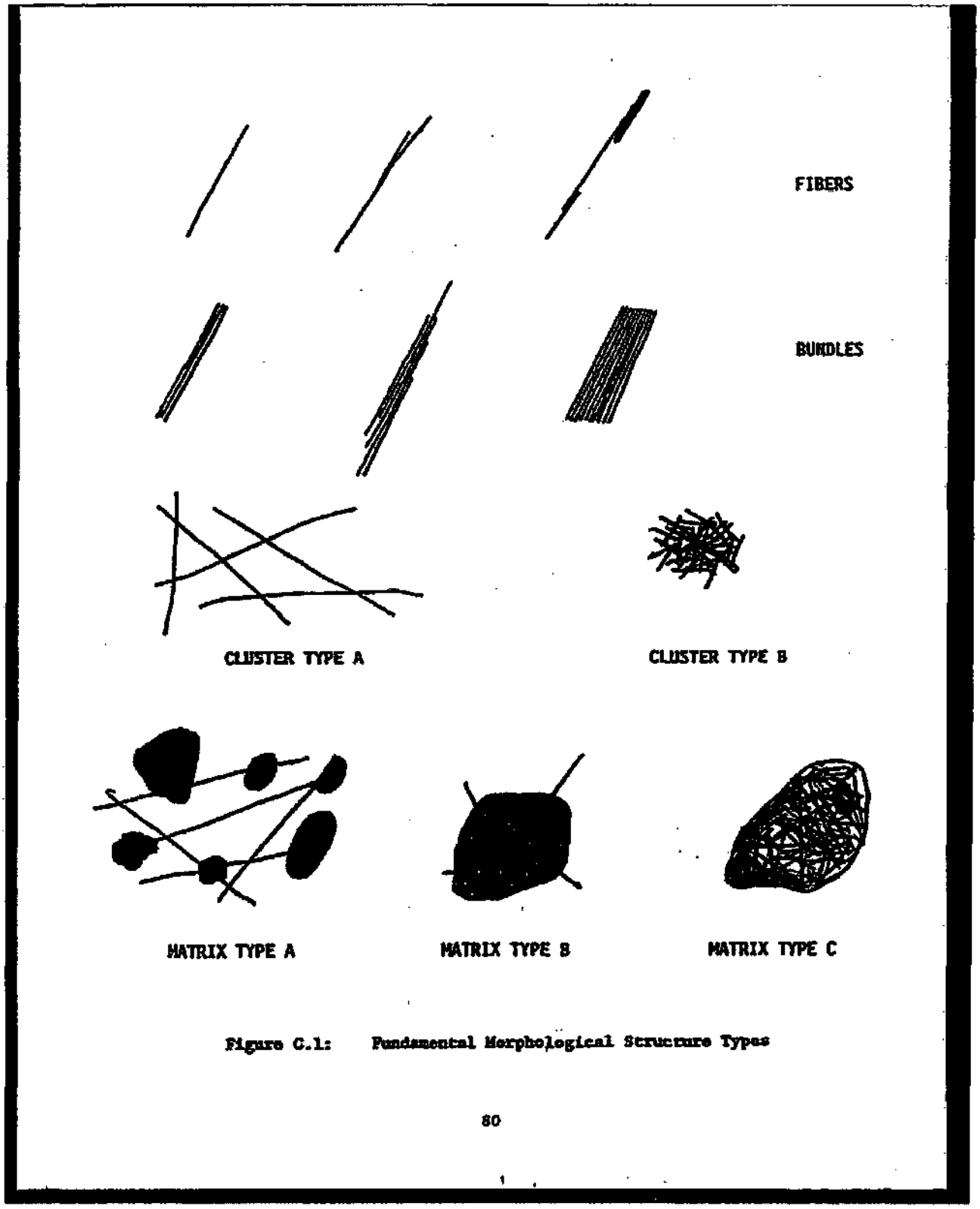
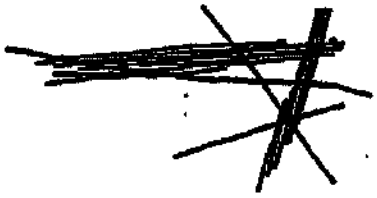


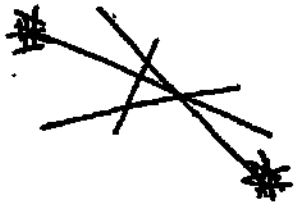
Figure C.1: Fundamental Morphological Structure Types

Record primary structure as one cluster designated C.

Record primary structure as 1 cluster designated as CSn. Record 5 fibers as components. Use code CFn for the component fibers.



Record primary structure as 1 cluster designated CSn. Record 3 fibers and 2 bundles as components. Use code CFn for component fibers and code CBn for component bundles.



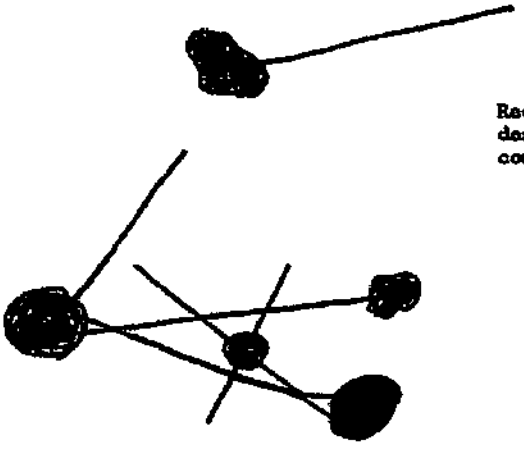
Record primary structure as 1 cluster designated as CSn. Record 4 fibers and 2 sub-clusters as components. Use code CFn for component fibers and code CCn for component clusters.

Notes:

"n" is a three digit number identifying the structure number of the primary structure that each component belongs to.

Dimensions assigned to each component represent the best estimate of the maximum length and mean width of that particular component. Dimensions recorded for a primary structure represent the best estimate of the overall outside dimensions of that structure.

Figure C.2: Examples of Recording of Complex Asbestos Clusters



Record primary structure as 1 matrix designated as MSn. Record 1 fiber as a component. Use code MFn for component fiber.

Record primary structure as 1 matrix designated as MSn. Record 5 fibers as components. Use code MFn for component fibers.

Record primary structure as 1 matrix designated as MSn. Record 3 fibers and 1 sub-matrix as components. Use code MFn for component fibers and code MMn for component matrix.

Record as 1 matrix designated as M.

Notes:

"n" is a three digit number identifying the structure number of the primary structure that each component belongs to.

Dimensions assigned to each component represent the best estimate of the maximum length and mean width of that particular component. Dimensions recorded for a primary structure represent the best estimate of the overall outside dimensions of that structure.

Submatrices are only recorded when they incorporate asbestos fibers not accounted for after separation of fiber, bundle, and cluster components from the primary structure.

Figure C.3: Examples of Recording of Complex Asbestos Matrices

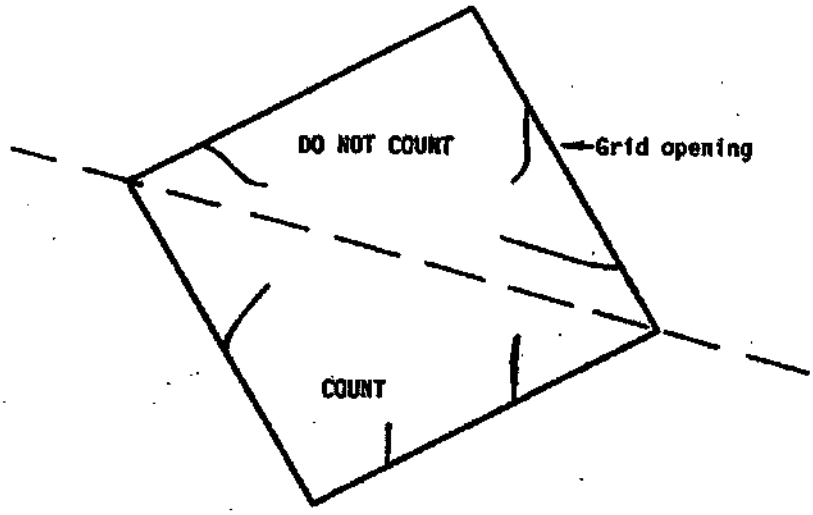


Figure C.4: Counting of Structures That Intersect Grid Bars

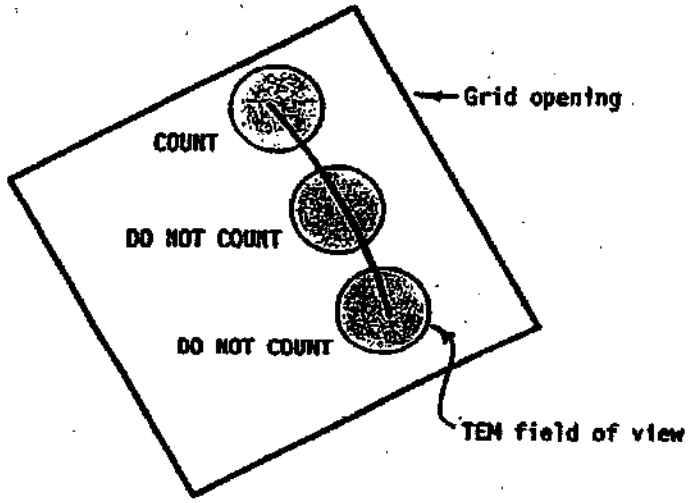


Figure C.5: Counting of Structures That Extend Outside the Field of View

APPENDIX D - FIBER IDENTIFICATION PROCEDURE

D.1 INTRODUCTION

Fibrous structures are classified as asbestos structures by identifying one or more of the constituent fibers or bundles as asbestos. Accordingly, this section refers to identification of fibers rather than structures.

The criteria which shall be used for identification of asbestos fibers are selected, depending on the intended use of the fiber counting results. In some circumstances, there can be a requirement that fibers shall be unequivocally identified as a specific mineral species. In other circumstances there can be sufficient knowledge about the sample that rigorous identification of each fiber need not be carried out. The time required to perform the analysis, and therefore the cost of analysis, can vary widely depending on the identification criteria which are considered to be sufficiently definitive. The combination of criteria considered definitive for identification of fibers in a particular analysis shall be specified before the analysis is made, and this combination of criteria shall be referred to as the "Level" of analysis. Various factors related to instrumental limitations and the character of the sample may prevent satisfaction of all of the specified fiber identification criteria for a particular fiber. Therefore, a record shall be made of the identification criteria which were satisfied for each suspected asbestos fiber included in the analysis. For example, if both ED and EDXA were specified to be attempted for definitive identification of each fiber, fibers with chrysotile morphology which, for some reason, do not give an ED pattern but which do yield an EDXA spectrum corresponding to chrysotile, are categorized in a way that conveys the level of confidence to be placed in the identification.

D.2 ED AND EDXA TECHNIQUES

D.2.1 General

Initially, classify fibers into two categories on the basis of morphology: those fibers with tubular morphology, and those fibers without tubular morphology. Conduct further analysis of each fiber using ED and EDXA methods. The following procedures should be used when fibers are examined by ED and EDXA.

The crystal structures of some mineral fibers, such as chrysotile, are easily damaged by the high current densities required for EDXA examination. Therefore, investigation of these sensitive fibers by ED shall be completed before attempts are made to obtain EDXA spectra from the fibers. When more stable fibers, such as the amphiboles, are examined, EDXA and ED may be used in either order.

D.2.2 ED Techniques

The ED technique can be either qualitative or quantitative. Qualitative ED consists of visual examination, without detailed measurement, of the general characteristics of the ED pattern obtained on the TEM viewing screen from a randomly orientated fiber. ED patterns obtained from fibers with cylindrical symmetry, such as chrysotile, do not change when the fibers are tilted about their axes, and patterns from randomly oriented fibers of these minerals can be interpreted quantitatively. For fibers that do not have cylindrical symmetry, only those ED patterns obtained when the fiber is oriented with a principal crystallographic axis closely parallel with the incident electron beam direction can be interpreted quantitatively. This type of ED pattern shall be referred to as a zone-axis ED pattern. In order to interpret a zone-axis ED pattern quantitatively, it shall be photographed and its consistency with known mineral structures shall be checked. A computer program may be used to compare measurements of the zone-axis ED pattern with corresponding data calculated from known mineral structures. The zone-axis ED pattern obtained by examination of a fiber in a particular orientation can be insufficiently specific to permit unequivocal identification of the mineral fiber, but it is often possible to tilt the fiber to another angle and to photograph a different ED pattern corresponding to another zone-axis. The angle between the two zone-axes can also be checked for consistency with the structure of a suspected mineral.

For visual examination of the ED pattern, the camera length of the TEM should be set to a low value of approximately 250 mm and the ED pattern then should be viewed through the binoculars. This procedure minimizes the possible degradation of the fiber by the electron irradiation. However, the pattern is distorted by the tilt angle of the viewing screen. A camera length of at least 2 m should be used when the ED pattern is photographed, if accurate measurement of the pattern is to be possible. It is necessary that, when obtaining an ED pattern to be evaluated visually or to be photographed, the sample height shall be properly adjusted to the eucentric point and the image shall be focussed in the plane of the selected area aperture. If this is not done there may be some components of the ED pattern which do not originate from the selected area. In general, it will be necessary to use the smallest available ED aperture.

For routine sample analysis, calibration films of evaporated gold or other materials shall not be applied to the TEM grids. Such films seriously degrade the visibility of fine chrysotile fibers so that they may not be detected by the EM operator. Moreover, the visibility of ED patterns from chrysotile fibers are also seriously degraded resulting in failure to identify them positively. Both effects lead to significant reduction of the asbestos structure concentrations reported.

To form an ED pattern, move the image of the fiber to the center of the viewing screen and insert a suitable selected area aperture into the electron beam so that the fiber, or a portion of it, occupies a large proportion of the illuminated area. The size of the aperture and the portion of the fiber shall be such that particles other than the one to be examined are excluded from the selected area. Observe the ED pattern through the binoculars. During the observation, the objective lens current should be adjusted to the point where the most complete ED pattern is obtained. If an incomplete ED pattern is still obtained, move the particle around within the selected area to attempt to optimize the ED pattern, or to eliminate possible interferences from neighboring particles.

If a zone-axis ED analysis is to be attempted on the fiber, the sample shall be mounted in the appropriate holder. The most convenient holder allows complete rotation of the specimen grid and tilting of the grid about a single axis. Rotate the sample until the fiber image indicates that the fiber is oriented with its length coincident with the tilt axis of the goniometer, and adjust the sample height until the fiber is at the eucentric position. Tilt the fiber until an ED pattern appears, which is a symmetrical, two dimensional array of spots. The recognition of zone-axis alignment conditions requires some experience on the part of the operator. During tilting of the fiber to obtain zone-axis conditions, the manner in which the intensities of the spots vary should be observed. If weak reflections occur at some points on a matrix of strong reflections, the possibility of multiple diffraction exists, and some caution should be exercised in the selection of diffraction spots for measurement and interpretation. A full discussion of electron diffraction and multiple diffraction can be found in the references by J. A. Gard, P.B. Hirsch et al, and H. R. Wenk, included in the Bibliography. Not all zone-axis patterns that can be obtained are definitive. Only those which have closely-spaced reflections corresponding to low indices in at least one direction should be recorded. Patterns in which all d-spacings are less than about 0.3 nm are not definitive. A useful guideline is that the lowest angle reflections should be within the radius of the first gold diffraction ring (111), and that patterns with smaller distances between reflections are usually the most definitive.

Five spots, closest to the center spot, along two intersecting lines of the zone-axis pattern shall be selected for measurement, as illustrated in Figure D1. The distances of these spots from the center spot and the four angles shown provide the required data for analysis. Since the center spot is usually very over-exposed, it does not provide a well-defined origin for these measurements. The required distances shall therefore be obtained by measuring between pairs of spots symmetrically disposed about the center spot, preferably separated by several repeat distances. The distances must be measured with a precision of better than 0.3 mm, and the angles to a precision of better than 2.5°. The diameter of the first or second ring of the calibration pattern (111 and 200) must also be measured with a precision of better than 0.3 mm.

Using gold as the calibration material, the camera constant is given by:

$$\lambda \times L = 2.3548 \times D \text{ (first ring)}$$
$$\lambda \times L = 2.0393 \times D \text{ (second ring)}$$

D.2.3 EDXA Measurements

Interpretation of the EDXA spectrum may be either qualitative or quantitative. For qualitative interpretation of a spectrum, the elements originating from the fiber are recorded. For quantitative interpretation, the net peak areas, after background subtraction, are obtained for the elements originating from the fiber. This method provides for quantitative interpretation for those minerals which contain silicon. To obtain an EDXA spectrum, move the image of the fiber to the center of the screen and remove the objective aperture. Select an appropriate electron beam diameter and deflect the beam so that it impinges on the fiber. Depending on the instrumentation, it may be necessary to tilt the specimen towards the X-ray detector, and in some instruments, to use Scanning Transmission Electron Microscopy (STEM) mode of operation.

The time for acquisition of a suitable spectrum varies with the fiber diameter, and also with instrumental factors. For quantitative interpretation, spectra should have statistically valid number of counts in each peak. Analyses of small diameter fibers which contain sodium are the most critical, since it is in the low energy range that the X-ray detector is least sensitive. Accordingly, it is necessary to acquire a spectrum for a sufficiently long period that the presence of sodium can be detected in such fibers. It has been found that satisfactory quantitative analyses can be obtained if acquisition is continued until the background subtracted silicon Kα peak integral exceeds 10,000 counts. The spectrum should then be manipulated to subtract the background and to obtain the net areas of the elemental peaks.

After quantitative EDXA classification of some fibers by computer analysis of the net peak areas, it may be possible to classify further fibers in the same sample on the basis of comparison of spectra at the instrument. Frequently, visual comparisons can be made after somewhat shorter acquisition times.

D.3 INTERPRETATION OF FIBER ANALYSIS DATA

D.3.1 Chrysotile

The morphological structure of chrysotile is characteristic, and with experience, can be recognized readily. However, a few other minerals have similar appearance, and morphological observation by itself is inadequate for most samples. The ED pattern obtained from chrysotile is quite specific for this mineral if the specified characteristics of the

pattern correspond to those from reference chrysotile. However, depending on the past history of the fiber, and on a number of other factors, the crystal structure of a particular fiber may be damaged, and it may not yield an ED pattern. In this case, the EDXA spectrum may be the only data available to supplement the morphological observations.

D.3.2 Amphiboles

Since the fiber identification procedure for asbestos fibers other than chrysotile can be involved and time-consuming, computer program such as developed by Rhoades (see Bibliography) can be used for interpretation of zone-axis ED patterns. The published literature contains composition and crystallographic data for all of the fibrous minerals likely to be encountered in TEM analysis of air samples, and the compositional and structural data from the unknown fiber should be compared with the published data. Demonstration that the measurements are consistent with the data for a particular test mineral does not uniquely identify the unknown, since the possibility exists that data from other minerals may also be consistent. It is, however, unlikely that a mineral of another structural class could yield data consistent with that from an amphibole fiber identified by quantitative EDXA and two zone axis ED patterns.

Suspected amphibole fibers should be classified initially on the basis of chemical composition. Either qualitative or quantitative EDXA information may be used as the basis for this classification. From the published data on mineral compositions, a list of minerals which are consistent in composition with that measured for the unknown fiber should be compiled. To proceed further, it is necessary to obtain the first zone axis ED pattern, according to the instructions in D.2.1.

It is possible to specify a particular zone-axis pattern for identification of amphibole, since a few patterns are often considered to be characteristic. Unfortunately, for a fiber with random orientation on a TEM grid, no specimen holder and goniometer currently available will permit convenient and rapid location of two pre-selected zone-axes. The most practical approach has been adopted, which is to accept those low index patterns which are easily obtained, and then to test their consistency with the structures of the minerals already pre-selected on the basis of the EDXA data. Even the structures of non-amphibole minerals in this pre-selected list must be tested against the zone-axis data obtained for the unknown fiber, since non-amphibole minerals in some orientations may yield similar patterns consistent with amphibole structures.

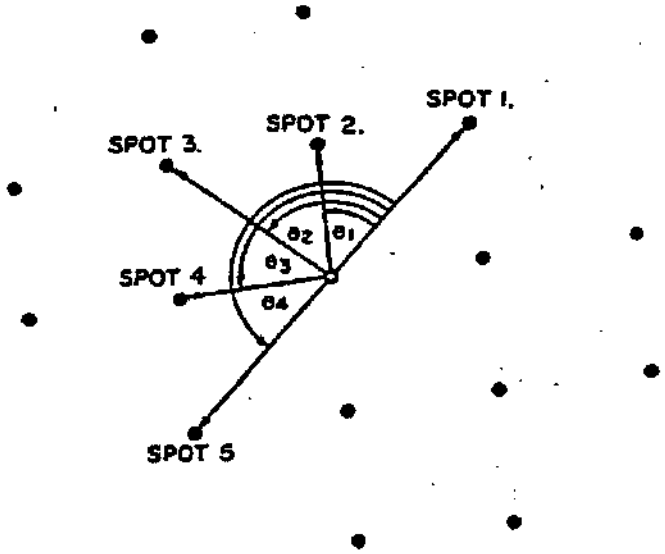


Figure D.1: Measurement of Zone Axis SAED Patterns

The zone-axis ED interpretation must include all minerals previously selected from the mineral data file as being chemically compatible with the EDXA data. This procedure will usually shorten the list of minerals for which solutions have been found. A second set of zone-axis data from another pattern obtained on the same fiber can then be processed, either as further confirmation of the identification, or to attempt elimination of an ambiguity. In addition, the angle measured between the orientations of the two zone-axes can be checked for consistency with the structures of the minerals. Caution should be exercised in rationalizing the inter-zone axis angle, since if the fiber contains c-axis twinning the two zone-axis ED patterns may originate from the separate twin crystals. In practice, the full identification procedure will normally be applied to very few fibers, unless for a particular reason precise identification of all fibers is required.

D.4 FIBER CLASSIFICATION CATEGORIES

It is not always possible to proceed to a definitive identification of a fiber; this may be due to instrumental limitations or to the actual nature of the fiber. In many analyses a definitive identification of each fiber may not actually be necessary if there is other knowledge available about the sample, or if the concentration is below a level of interest. The analytical procedure must therefore take account of both instrumental limitations and varied analytical requirements. Accordingly, a system for fiber classification is used to permit accurate recording of data. The classifications are shown in Tables D1 and D2, and are directed towards identification of chrysotile and amphibole respectively. Fibers shall be reported in these categories.

The general principle to be followed in this analytical procedure is first to define the most specific fiber classification which is to be attempted, or the "level" of analysis to be conducted. Then, for each fiber examined, record the classification which is actually achieved. Depending on the intended use of the results, criteria for acceptance of fibers as "identified" can then be established at any time after completion of the analysis.

In an unknown sample, chrysotile will be regarded as confirmed only if a recorded, calibrated ED pattern from one fiber in the CD categories is obtained, or if measurements of the ED pattern are recorded at the instrument. Amphibole will be regarded as confirmed only by obtaining recorded data which yields exclusively amphibole solutions for fibers classified in the AQZ, AZZ or AQZZ categories.

D.4.1 Procedure for Classification of Fibers with Tubular Morphology, Suspected to be Chrysotile

Occasionally, fibers are encountered which have tubular morphology similar to that of chrysotile, but which cannot be characterized further either by ED or EDXA. They may be non-crystalline, in which case ED

techniques are not useful, or they may be in a position on the grid which does not permit an EDXA spectrum to be obtained. Alternatively, the fiber may be of organic origin, but not sufficiently definitive that it can be disregarded. Accordingly, there is a requirement to record the fiber, and to specify how confidently each fiber can be identified. Classification of fibers will meet with various degrees of success. Figure D2 shows the classification procedure to be used for fibers which display any tubular morphology. The chart is self explanatory, and every fiber is either rejected as a non-asbestos mineral (NAM), or classified in some way which by some later criterion could still contribute to the chrysotile fiber count.

Morphology is the first consideration, and if this is not similar to that usually seen in chrysotile standard samples, designate the initial classification as TM. Regardless of the doubtful morphology, examine the fiber by ED and EDXA methods according to Figure D2. Where the morphology is more definitive, it may be possible to classify the fiber as having chrysotile morphology (CM).

For classification as CM, the morphological characteristics required are:

(a) the individual fibrils should have high aspect ratios exceeding 10:1, and be about 20 to 40 nm in diameter;

(b) the electron scattering power of the fiber at 60 to 100 kV accelerating potential should be sufficiently low for internal structure to be visible; and,

(c) there should be some evidence of internal structure suggesting a tubular appearance similar to that shown by reference UICC chrysotile, which may degrade in the electron beam.

Examine every fiber having these morphological characteristics by the ED technique, and classify as chrysotile by ED (CD) only those which give diffraction patterns with the precise characteristics shown in Figure D3. The relevant features in this pattern for identification of chrysotile are as follows:

(a) the (002) reflections should be examined to determine that they correspond approximately to a spacing of 0.73 nm;

(b) the layer line repeat distance should correspond to 0.53 nm; and,

(c) there should be "streaking" of the (110) and (130) reflections.

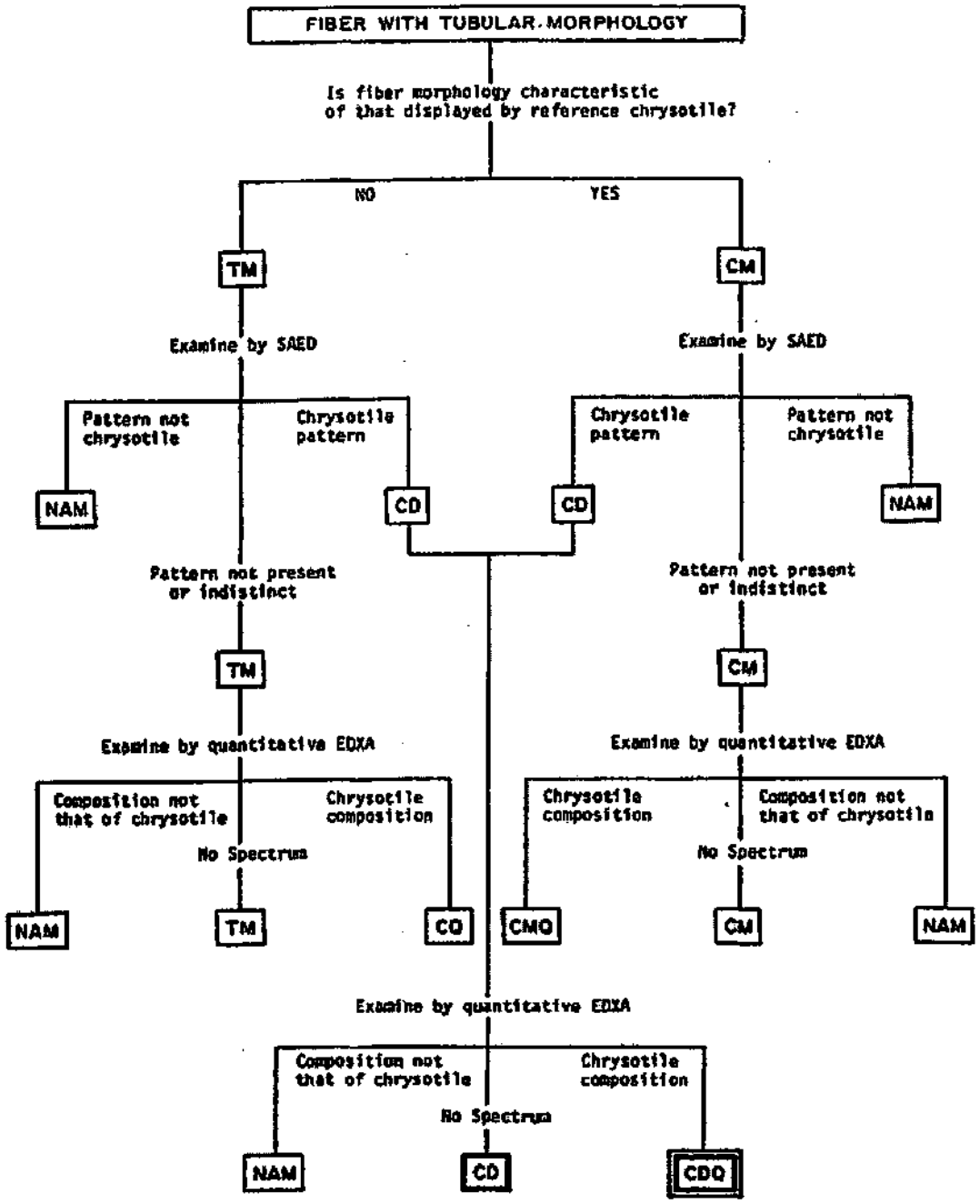


Figure D.2: Classification Chart for Fiber With Tubular Morphology

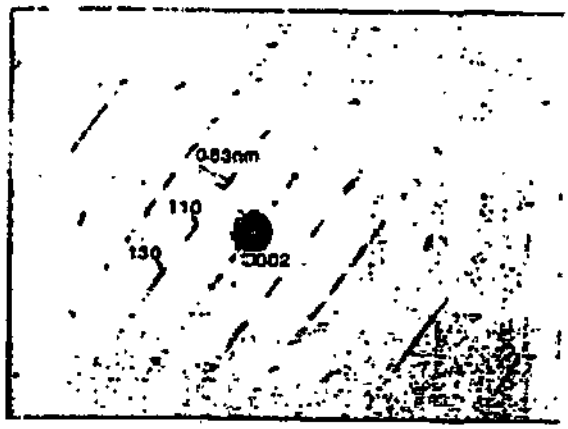


Figure D.3: Chrysotile SAED Pattern

Using the millimeter calibrations on the TEM viewing screen, these observations can readily be made at the instrument. If documentary proof of fiber identification is required, record a TEM micrograph of at least one representative fiber, and record its ED pattern on a separate film or plate. This film or plate shall also carry calibration rings from a known polycrystalline substance such as gold. This calibrated pattern is the only documentary proof that the particular fiber is chrysotile, and not some other tubular or scrolled species such as halloysite, palygorskite, talc or vermiculite. The proportion of fibers which can be successfully identified as chrysotile by ED is variable, and to some extent dependent on both the instrument and the procedures of the operator. The fibers that fail to yield an identifiable ED pattern will remain in the TM or CM categories unless they are examined by EDXA.

In the EDXA analysis of chrysotile there are only two elements which are relevant. For fiber classification, the EDXA analysis must be quantitative. If the spectrum displays prominent peaks from magnesium and silicon, with their area in the appropriate ratio, and with only minor peaks from other elements, classify the fiber as chrysotile by quantitative EDXA, in the categories CQ, CMQ, or CDQ, as appropriate.

D.4.2 Procedure for classification of Fibers Without Tubular Morphology, Suspected to be Amphibole

Every particle without tubular morphology and which is not obviously of biological origin, with an aspect ratio of 5:1 or greater, and having parallel or stepped sides, shall be considered as a suspected amphibole fiber. Further examination of the fiber by ED and EDXA techniques will meet with a variable degree of success, depending on the nature of the fiber and on a number of instrumental limitations. It will not be possible to identify every fiber completely, even if time and cost were of no concern. Moreover, confirmation of the presence of amphibole can be achieved only by quantitative interpretation of zone-axis ED patterns, a very time-consuming procedure. Accordingly, for routine samples from unknown sources, this analytical procedure limits the requirement for zone-axis ED work to a minimum of one fiber representative of each compositional class reported. In some samples, it may be necessary to identify more fibers by the zone-axis technique. When analyzing samples from well-characterized sources, the cost of identification by zone-axis methods may not be justified.

The 0.53 nm layer spacing of the random orientation ED pattern is not by itself diagnostic for amphibole. However, the presence of c-axis twinning in many fibers leads to contributions to the layers in the patterns by several individual parallel crystals of different axial orientations. This apparently random positioning of the spots along the layer lines, if also associated with a high fiber aspect ratio, is a characteristic of amphibole asbestos, and thus has some limited diagnostic value. If a pattern of this type is not obtained, the identity of the

fiber is still ambiguous, since the absence of a recognizable pattern may be a consequence of an unsuitable orientation relative to the electron beam, or the fiber may be some other mineral species.

Figure D4 shows the fiber classification chart to be used for suspected amphibole fibers. This chart shows all the classification paths possible in analysis of a suspected amphibole fiber, when examined systematically by ED and EDXA. Two routes are possible, depending on whether an attempt to obtain an EDXA spectrum or a random orientation ED pattern is made first. The normal procedure for analysis of a sample of unknown origin will be to examine the fiber by random orientation ED, qualitative EDXA, quantitative EDXA, and zone-axis ED, in this sequence. The final fiber classification assigned will be defined either by successful analysis at the maximum required level, or by the instrumental limitations. Record the maximum classification achieved for each fiber on the counting sheet in the appropriate column. The various classification categories can then be combined later in any desired way for calculation of the fiber concentration, and a complete record of the results obtained when attempting to identify each fiber is maintained for reassessment of the data if necessary.

In the unknown sample, zone-axis analysis will be required if the presence of amphibole is to be unequivocally confirmed. For this level of analysis, attempt to raise the classification of every suspected amphibole fiber to the ADQ category by inspection of the random orientation ED pattern and the EDXA spectrum. In addition, examine at least one fiber from each type of suspected amphibole found by zone-axis methods to confirm their identification. In most cases, some ambiguity of identification can be accepted, because information exists about possible sources of asbestos in close proximity to the air sampling location. Lower levels of analysis can therefore be accepted for these situations.

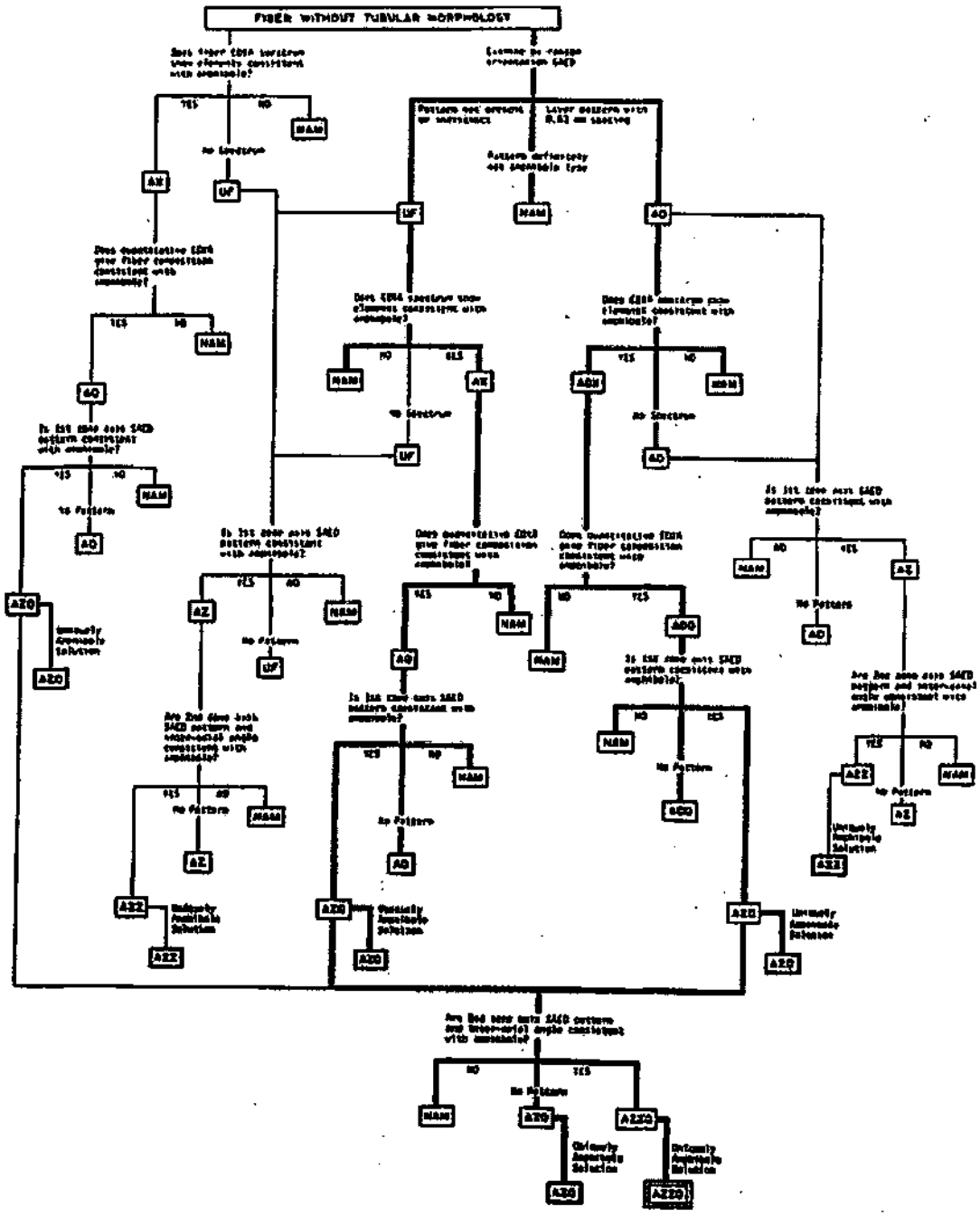


Figure D.4: Classification Chart for Fibers Without Tubular Morphology

Table D1 - Classification of fibers with tubular morphology

TM	-	Tubular Morphology, not sufficiently characteristic for classification as chrysotile
CM	-	Characteristic Chrysotile Morphology
CD	-	Chrysotile SAED pattern
CQ	-	Chrysotile composition by Quantitative EDXA
CMQ	-	Chrysotile Morphology and composition by Quantitative EDXA
CDQ	-	Chrysotile SAED pattern and composition by Quantitative EDXA
NAM	-	Non-asbestos Mineral

Table D2 - Classification of fibers without tubular morphology

UF	-	Unidentified Fiber
AD	-	Amphibole by random orientation SAED (shows layer pattern of 0.53 nm spacing)
AX	-	Amphibole by qualitative EDXA. Spectrum has elemental components consistent with amphibole
ADX	-	Amphibole by random orientation SAED and qualitative EDXA
AQ	-	Amphibole by quantitative EDXA
AZ	-	Amphibole by one Zone-Axis SAED pattern
ADQ	-	Amphibole by random orientation SAED and Quantitative EDXA
AQZ	-	Amphibole by Quantitative EDXA and one Zone-Axis SAED pattern
AZZ	-	Amphibole by two Zone-Axis SAED patterns with consistent inter-axial angle
AQZZ	-	Amphibole by Quantitative EDXA, two Zone-Axis SAED patterns, and consistent inter-axial angle
NAM	-	Non-asbestos Mineral

APPENDIX E - CALCULATION OF RESULTS

E.1 INTRODUCTION

Calculate the results using the procedures described below. The results can be conveniently calculated using a computer program.

E.2 TEST FOR UNIFORMITY OF FIBER DEPOSITS ON TEM GRIDS

A check shall be made using the chi-square test, to determine whether the asbestos structures found on individual grid openings are randomly and uniformly distributed among the grid openings. If the total number found in k grid openings is n, and the areas of the k individual grid openings are designated A_1 to A_k, then the total area of TEM specimen examined is:

$$A = \sum_{i=1}^{i=k} A_i$$

The fraction of the total area examined which is represented by the individual grid opening area, p_i, is given by A_i/A. If the structures are randomly and uniformly dispersed over the k grid openings examined, the expected number of structures falling in one grid opening with area A_i is np_i. If the observed number of structures found on that grid opening is n_i, then:

$$\chi^2 = \sum_{i=1}^{i=k} \frac{(n_i - np_i)^2}{np_i}$$

This value shall be compared with significance points of the χ^2 distribution, having (k - 1) degrees of freedom. Significance levels lower than 0.1% may be cause for the sample analysis to be rejected, since this corresponds to a very inhomogeneous deposit. If the structure count fails this test, the precision of the result will be uncertain, and new analytical filters should be prepared from the original collection filter.

E.3 CALCULATION OF THE ANALYTICAL SENSITIVITY

Calculate the analytical sensitivity S, in structures/liter, using the formula:

$$S = A_f/(N \times A_s \times V \times F)$$

where:

A_f = Active area of sample collection filter in mm^2
N = Number of grid openings examined
A_s = Mean area of grid openings
V = Volume of air sampled in liters
F = Concentration factor

E.4 CALCULATION OF THE MEAN AND CONFIDENCE LIMITS FOR A REPORTED ASBESTOS STRUCTURE CONCENTRATION

In the asbestos structure count made according to this method, a number of grid openings have been sampled from a population of grid openings and it is required to determine the mean grid opening asbestos structure count for the population on the basis of this small sample. The upper and lower 95% confidence limits are also to be reported with every mean. When such limits are constructed as indicated in Section E.4.2, the interval between the two limits should contain the true mean 90% of the time (95% of the time if the lower confidence limit is zero).

E.4.1 Calculation of the Mean Asbestos Structure Concentration

Calculate the mean concentration from a particular measurement, C, in structures/liter:

$$C = S \times N \times n$$

where:

S = Analytical sensitivity in structures/liter
N = Number of grid openings examined
n = mean number of asbestos structures found per grid opening

E.4.2 Calculation of Upper and Lower 95% Confidence Limits

If the total structures counted from a filter is 30 or less, the Poisson distribution is recommended to be used for constructing confidence limits. Let P(x;m) represent the cumulative probability function from a Poisson distribution with mean m. That is, P(x;m) is the probability of observing x or fewer counts given by

$$P(x;m) = e^{-m}(1 + m + m^2/2! + \ldots + m^x/x!).$$

The upper 95% confidence limit, x_u, on the mean count, based on an observed count of x, is the value that satisfies:

$$P(x,x_u) = 0.05,$$

and the lower 95% confidence limit, x_l, on the mean count, based on an observed count of x, is the value that satisfies

$$1 - P(x-1,x_l) = 0.05$$

(e.g., Miller and Freund 1965)[3]. Tables of P(x;m) for values of m less than 26 may be found in Miller and Freund. A computer program can be easily written for calculating P(x;m) for larger values of m. If such a program is not available, confidence limits may alternately be based on the normal distribution approximation to the Poisson distribution (see Miller and Freund 1965). Corresponding approximations to the upper and lower 95% confidence limits are

$$x_u = x + 1.65*x^{1/2}$$

and

$$x_l = x - 1.65*x^{1/2}.$$

The Poisson distribution is correct whenever structures are randomly distributed in the sampled air and on the filter. However, as a hedge

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[3] Confidence limits derived as described in this section are one-tailed limits meaning that 95% of the distribution lies below the upper 95% confidence limit and, similarly, 95% of the distribution lies above the lower 95% confidence limit. If the two limits are combined to create a confidence interval, the interval between the two limits represents 90% of the distribution (which is different than a 95% confidence interval).

Construction of confidence limits in this manner, rather than the more traditional approach of constructing confidence intervals (where, for example, the 95% confidence interval corresponds to upper and lower 97.5% confidence limits), is recommended to avoid confusion when referring to an asymmetric distribution or when the lower confidence limit falls to zero. When the lower confidence limit falls to zero, for example, the upper 95% confidence limit now corresponds to the upper 95% confidence interval (rather than the 90% confidence interval, as indicated in the last paragraph) because the lower confidence limit (zero) removes nothing from the distribution. Thus, we have chosen to refer to one-tailed confidence limits throughout and avoid emphasis on confidence intervals.

against possible clumping of structures, it is recommended that, whenever the total structure count on a filter is greater than 30, confidence limits also be calculated using the following procedure and the more extreme limits (i.e., larger x_u and smaller x_l) be reported.

Suppose k grid openings are examined and let x_i be the structure count for the ith grid opening. Note that a minimum of four grid openings (k>4) should be examined and they should be of equal size. Then 95% upper and lower confidence limits are calculated according to the formulas

$$x_u = 1.65s$$

$$x_l = 1.65s$$

Where

$$s^2 = [k/(k-1)] \sum_{i=1}^{k} (x_i - x/k)^2,$$

and 1.65 is the cumulative 95th percentage point of the standard normal distribution.

Upper or lower confidence limits for airborne concentrations derived from a particular measurement are computed by multiplying the corresponding upper or lower confidence limits derived as described above for the structure count by the analytical sensitivity of the measurement.

E.4.3 Reporting the Mean and Confidence Limits for a Measured Concentration

Where a result is to be interpreted as a single isolated measurement, the following procedures shall be used for reporting:

No structures detected - the concentration shall be reported as "NF" meaning "not found" and it should be accompanied by the upper and lower 95% confidence limits derived as described in Section E.4.2. Note that the lower 95% confidence limit is zero in this case.

At least 1 structure detected - report the mean concentration derived as described in Section E.4.1 along with the upper and lower 95% confidence limits derived as described in Section E.4.2.

Rules for combining the results of individual measurements as part of a site evaluation are presented in the Technical Background Document, Part 2 of this report.

E.5 DISTINGUISHING DETECTED ASBESTOS FROM ANALYTICAL BACKGROUND

The non-parametric Wilcoxon Test (Hollander and Wolfe 1973) is employed in this method to test for significant differences between asbestos concentrations (in units of s/cm^2) observed on sample filters and concentrations observed on blanks. It must be emphasized, however, that the most useful application of this test is to groups of sample results rather than individual results. This should not be a severe limitation because conclusions based on the totality of data from an entire study tend to be more important than the results from individual analyses.

The Wilcoxon test performs better on multiple samples. A test comparing the concentration from an individual filter to those from a group of blanks can be conducted, but such a test is likely to be of low power (insensitive to small differences). In fact, data from at least 20 blanks are likely required before the test becomes capable of exhibiting a statistically significant difference ($p < -0.05$) between blank concentrations and the concentration observed on a single sample filter. The power of the test will similarly be limited when comparing multiple sample filter analyses to a limited number of blanks. For example, at least seven sample filters likely would have to be combined to find a significant difference from two blanks.

Results of blank analyses will generally be available from two sources: study specific and historical. Within a particular study, two filter blanks are generally analyzed from each lot of filters. Typically, there will also be field blanks. Depending on the length of time that a particular laboratory has been using this method, the laboratory should also be accumulating a historical database of blank analyses. Either set of blank data may be combined with care to provide the needed data to perform the Wilcoxin Test and distinguish sampled asbestos from analytical contamination. However, if different lots of filters exhibit different levels of background contamination or if results from a laboratory change over time (due possibly to changes in methodology or analysts) then it may not be appropriate to include historical blank data in an analysis. On the other hand, if the historical data are representative, use of this data may greatly increase the power of the analysis for detecting airborne concentrations. If historical blank data are used in a statistical test in this method, this is to be noted and results of corresponding tests based only on concurrent blanks shall also be reported.

The following procedure can be used for applying the Wilcoxon test to asbestos analytical results. More detailed discussions of the procedure are available from numerous sources (including Hollander and Wolfe 1973). Suppose M blank and N non-blank samples are available. Let $X_1, \ldots, X_M$ be the measured filter concentrations from the blank samples and $Y_1, \ldots, Y_N$ the measured concentrations from the non-blank samples (in units of structures per mm^2). Combine the M+N concentrations and rank them from smallest to largest; assign sequential integers (beginning with 1) to each concentration arranged from smallest to largest. If there are ties (two or more samples

exhibiting the same concentration) among the M+N observations, assign the average rank to each group of tied observations. (E.g., if the observations are 0, 0, 0, 0, 0.001, 0.002, then the corresponding ranks would be 2.5, 2.5, 2.5, 2.5, 5, 6.) Let W be the sum of the ranks assigned to the concentrations from the non-blank samples. Large values of W are evidence that concentrations from non-blank samples are larger than those from blank samples, and the hypothesis that the blank and non-blank samples have equal concentrations of fibers is rejected if W is large enough.

Tables are available for determining when W is large enough to be statistically significant in cases in which there are no ties and M and N are small (see e.g., Table A.5 in Hollander and Wolfe 1973). As an example of the application of these tables, if N = 4 and M = 5, then W, the sum of the ranks of the concentrations from the non-blank samples, would have to be 28 or larger before it could be concluded at the 0.05 level of significance that the concentrations from the non-blanks are higher than those from the blanks (see Table A.8 in Hollander and Wolfe 1973, page 276).

If ties are present in the data or if the number of samples is so large that tables are not available, a normal approximation to the distribution of W may be used. Define

$$W^* = \frac{W - [N(M+N+1)/2]}{[\mathrm{Var}(W)]^{1/2}}$$

where

$$\mathrm{Var}(W) = \frac{MN}{12}\left[M+N+1 - \frac{\sum_{j=1}^{g} t_j(t_j^2-1)}{(M+N)(M+N-1)} \right]$$

where g is the number of groups of tied concentrations (included among the combined set of blank and nonblank samples) and t_j is the size of tied group j. The hypothesis test is based on an assumed normal distribution for W^*. Thus one would reject the hypothesis of equal concentrations on blanks and non-blanks at the 0.05 level of significance if $W^* \geq 1.65$. (Note: This normal approximation may not work well if the number of samples is small. Consequently, if there are ties in the data and N or M is less than about 4, consideration should be given to consulting with a statistician regarding a more accurate approach to computing the statistical significance of W.)

E.6 CALCULATION OF ASBESTOS STRUCTURE LENGTH, WIDTH, AND ASPECT RATIO DISTRIBUTIONS

The distributions all approximate to logarithmic-normal, and so the size range intervals for calculation of the distribution shall be spaced logarithmically. The other characteristics required for the choice of size intervals are that they should allow for a sufficient number of size classes, while still retaining a statistically-valid number of asbestos

structures in each class. Interpretation is also facilitated if each size class repeats at decade intervals. A ratio from one class to the next of 1.47 satisfies all of these requirements and this value shall be used. The distributions, being approximately logarithmic-normal, when presented graphically, shall be plotted using a logarithmic ordinate scale and a Gaussian abscissa.

E.6.1 Calculation of Asbestos Structure Length Cumulative Number Distribution

This distribution allows the fraction of the total number of asbestos structures either shorter or longer than a given length to be determined. It is calculated using the relationship:

$$C(N)_k = \frac{\sum_{i=1}^{i=k} n_i}{\sum_{i=1}^{i=N} n_i} \times 100$$

where:

$C(N)_k$ - Cumulative fraction asbestos structures (expressed in percent) which have lengths less than the upper bound of the k'th class

n_i - Number of asbestos structures in the i'th length class

N - Total number of length classes.

E.6.2 Calculation of Asbestos Structure Width Cumulative Number Distribution

This distribution allows the fraction of the total number of asbestos structures either narrower or wider than a given width to be determined. It is calculated in a similar way to that used in E.6.1, but using the asbestos structure widths.

E.6.3 Calculation of Asbestos Structure Aspect Ratio Cumulative Number Distribution

This distribution allows the fraction of the total number of asbestos structures which have aspect ratios either smaller or larger than a given aspect ratio to be determined. It is calculated in a similar way to that used in E.6.1, but using the asbestos structure aspect ratios.

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Superfund Method for the Determination of Asbestos in Ambient Air

Part 2: Technical Background Document

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Contents

Figures v
Tables vi
Acknowledgements vii

1. Introduction 1
2. Overview 2
3. Background 4
   3.1. Biological Activity 4
   3.2. Risk Factors 5
   3.3. Morphology of Asbestos Dusts 7
   3.4. Published Size Distributions 8
   3.5. Representative Asbestos Concentrations 30
   3.6. Representative Total Dust Concentrations 39
   3.7. Analytical Techniques Used to Monitor Asbestos 40
   3.8. Existing TEM Analytical Methods 42

4. Risk Assessment Objectives and Method Requirements 49
   4.1. Consideration of Biological Activity 49
   4.2. Precision Requirements 50
   4.3. Sensitivity Requirements 52
   4.4. Reporting Requirements 53
   4.5. Method Specifications 54

5. Overview of Method 55
   5.1. Sample Collection 55
   5.2. Sample Preparation 55
      5.2.1. Indirect TEM Specimen Preparation 55
      5.2.2. Direct TEM Specimen Preparation 55
   5.3. Analysis 56

6. Method Components 58
   6.1. Factors Affecting Characterization of Asbestos Structures 58
      6.1.1. Analytical Technique 58
      6.1.2. Magnification/Resolution 61
      6.1.3. Rules for Counting, Characterization and Recording 62
   6.2. Factors Affecting Sensitivity 62
      6.2.1. Filter Loading 64
      6.2.2. Sampled Air Volumes 65
      6.2.3. Area to be Scanned for Analysis 66
      6.2.4. Filter Preparation 67
      6.2.5. Existing Analytical Methods 73
      6.2.6. Analytical Technique 74
      6.2.7. Magnification/Resolution 74
   6.3. Factors Affecting the Limit of Detection 76
      6.3.1. Filter Blank Contamination 76
         6.3.1.1 Contamination on MCE Filters 77
         6.3.1.2. Asbestos Contamination on Polycarbonate Filters 78
         6.3.1.3. Estimating Filter Blank Contamination Levels 79
      6.3.2. Conclusions 81
   6.4 Factors Affecting Precision 81
      6.4.1. Analytical Technique 81
      6.4.2. Magnification/Resolution 82
      6.4.3. Uniformity of Filter Deposit 83
      6.4.4. Rules for Counting, Characterization, and Recording 83

7. Recommended Procedures for Manipulating Analytical Data 86
7.1. Computing Average Concentrations from Multiple Samples 86
7.2. Computing Confidence Limits for Averages of Homogeneous Samples 87
7.3. ComputingConfidence Limits for Averages of Non-Homogeneous Samples 87
7.4. Testing for Differences Between Concentrations Derived from Different Sets of Samples 88
7.5. Adjusting Measured Concentrations for Contributions from Analytical Background Contamination 89

References 90

Figures

3.1: Components of Respirable Particles

6.1: The Relationship Between Analytical Sensitivity, Sampled Air Volume, and the Number of Grid Openings Counted

6.2: The Relative Analytical Sensitivities of Published TEM Methods

Tables

3.1	The Fraction of Asbestos Fibers in Total Fibers and Total Dust
3.2	Fiber Size Distributions Measured in Various Environments For Several Asbestos Minerals
3.3	Fiber Size Distributions Measured in Various Environments For Chrysotile
3.4	Published Asbestos Concentrations Representing Background
3.5	Published Asbestos Concentrations Representative of Environmental Concentrations
3.6	Comparison of Available Asbestos Analytical Techniques

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1. INTRODUCTION

A sampling and analysis method for the determination of asbestos in air is presented in Part 1 of this report, under separate cover. This method is designed specifically to provide results suitable for supporting risk assessments at Superfund sites, although it is applicable to a wide range of ambient air situations. Considerations addressed during the development of the method are presented in this companion technical background document. Also, in the interest of facilitating the use and interpretation of analytical results derived from the method presented in Part 1, recommended procedures for manipulating such data as part of a site evaluation are provided in Section 7 of this document.

Asbestos presents a complex challenge to investigators evaluating risks at Superfund sites. Unlike the majority of other chemicals frequently monitored at hazardous wastes sites, asbestos exposures can not be adequately characterized by a single concentration parameter. This is because the different size ranges of airborne asbestos particles, even when they are of the same mineral variety, exhibit different dose/response relationships. Thus a more accurate characterization of asbestos exposure is that arising from a family of substances independently contributing to toxicity rather than that of exposure to a single chemical. Therefore, proper characterization of asbestos exposure requires that the relative contributions from each of the many components of exposure be defined.

Existing equipment and methods used to measure asbestos are limited in their ability to fully characterize asbestos exposures. In addition, the toxicity of asbestos is currently a subject of scientific debate. Consequently, monitoring asbestos in a manner that satisfies the needs of a risk assessment requires innovations that tax the limits of available technology. Several variations were considered during development and the method presented in Part 1 of this report represents a workable compromise among several technical constraints.

The purpose for documenting the data and assumptions used to develop the method proposed in Part 1 is to facilitate critical evaluation while highlighting the needs for additional research and for better documentation of existing analytical results. Considerations addressed in this report that have been documented in the literature are cited accordingly. Considerations that remain largely a subject of conjecture are also noted. Due to the current level of interest and activity provoked by asbestos, further improvements in asbestos sampling, analysis, and evaluation are anticipated.

2. OVERVIEW

Development of the method presented in Part 1 of this report began with consideration of the kinds of data required to perform a risk assessment. Because the primary objective is to provide analysis results that reflect potential health risks, factors contributing to the biological activity of asbestos were considered to identify specific asbestos exposure parameters that relate to risk assessment objectives.

Published risk factors are expressed in terms of airborne concentrations as determined by phase contrast microscope (PCM) counts. However, a range of dimensional parameters have been shown to relate to biological activity in addition to PCM counts. Therefore, to address persisting controversies in the interpretation of asbestos biological activity, several parameters were selected for characterization by the method presented in this report. To maximize flexibility, sampling and analysis results will be recorded in this method so as to allow for re-interpretation of results without the need to re-evaluate the original sample specimens.

The characteristics of the kinds of environmental samples likely to be collected and analyzed by the proposed method were considered in order to identify the sampling and analysis criteria, which are required to satisfy the objectives of a risk assessment. The morphologies of asbestos structures and total dusts typically found in these samples were considered in order to determine requirements for distinguishing and characterizing components that potentially relate to biological activity.

To determine the required level of analytical sensitivity for the method, concentrations typical of environmental samples were estimated from the limited published data. A range of typical concentrations was defined from measured background and concentrations expected to be observed in the immediate vicinity of asbestos sources. Precision criteria defined for the method were developed by considering the requirements for delineating spatial and temporal trends in environmental asbestos concentrations as they apply to risk assessment.

After the dimensional parameters to be characterized had been established and the criteria for the sensitivity and precision of the method had been defined, the available sampling and analysis technologies were evaluated to determine what combinations would be capable of providing the required information. Because published risk factors are expressed in terms of PCM counts, phase contrast microscopy was considered for use as the analysis technique but this was rejected for a variety of severe, inherent limitations.

Despite the relationship between PCM measurements and existing risk factors, measurement of the PCM equivalent fraction of asbestos in environmental samples has been shown to be less important for assessing risks

than characterizing fractions of asbestos that are more directly associated with biological activity (Chesson et al 1989a). Therefore, transmission electron microscopy (TEM) was selected as the appropriate tool for analysis of environmental samples.

Because sensitivity and precision are functions both of sample characteristics and of rules for analysis, alternate combinations of sampling procedures, sample preparation techniques, and analysis methods were evaluated to identify the most cost-effective combination capable of providing the kinds of analytical results necessary to support a risk assessment. Published TEM analytical methods were evaluated for their applicability to this problem. Combined with several specific modifications, many of the features of the published analytical methods were incorporated into the method developed in this report. For sampling, alternate types of collection filters and the degree of filter loading were addressed. For TEM specimen preparation, both a direct and an indirect preparation technique were considered. For analysis, the area of the filter to be scanned was defined as a function of sample loading and the required analytical sensitivity. In addition, counting rules and recording rules were modified to assure that data would be preserved in a manner allowing detailed re-interpretation after analysis was completed. Finally, a two-phased approach for sample collection and analysis was adopted for cost-effectiveness.

3. BACKGROUND

Available data from published studies of asbestos exposure and biological activity were reviewed to define method requirements as they relate to risk assessment objectives. Existing sampling and analysis technologies were considered to identify an approach that best satisfies the method requirements.

3.1. BIOLOGICAL ACTIVITY

It is generally recognized that health risks posed by exposure to asbestos dusts depend predominantly on the concentrations and physical dimensions of the individual fibrous structures in the dust. Fibrous structures may include fibers, bundles, clusters or matrices because airborne asbestos is often found as a mix of such complex structures in addition to single fibers. Asbestos structures may also be found aggregated with equant (non-asbestos) particles. Somewhat arbitrarily, fibrous structures have been defined as those exhibiting aspect ratios greater than 3:1 to distinguish them from isometric particles (Walton, 1982). Isometric particles have not been shown to exhibit the same types of biological activity as fibrous structures (Elmes, 1982). However, a cutoff in aspect ratio below which biological activity can be considered insignificant has not been formally established.

Although it has been shown qualitatively that a relationship exists between the physical dimensions of fibrous structures and biological activity, the form of the relationship appears to be complex (see for example: USEPA 1986a). The relationship between certain types of structures and biological activity has been investigated in a series of animal studies. Based on these studies, asbestos toxicity varies directly with the length and inversely with the width (thickness) of asbestos fibers. Thus, the longest and thinnest fibers tend to be the most potent. However, there has not been general agreement on a minimum length below which the biological activity of asbestos can be considered insignificant (USEPA 1986a). Therefore, the exact shape of the relationship between length, diameter, and potency is still a subject of controversy.

Unlike fibers, the biological activities of bundles, clusters, and matrices have not been investigated directly. Some investigators (see for example: Nicholson, 1988) believe that biological activity correlates best with the mass of the structure. However, other investigators do not share this view (see for example: Bertrund and Pezerat, 1980). Although, various individuals have proposed theories concerning the relationship between aggregates (bundles, clusters, and matrices) and biological activity, due to the lack of data, such theories currently remain in the realm of conjecture.

Several studies (see for example: Bonneau et al, 1986) indicate that the mineralogy of a structure also plays a role in determining the biological activity of the structure. The majority of studies relating biological activity to physical dimensions indicate that structures bearing appropriate dimensions all tend to exhibit similar biological activity provided that the

structures are durable in vivo (See for example: USEPA 1986a). In contrast, the Bonneau paper is one of a small number of papers that reports that structures with identical dimensions that are composed of different minerals exhibit different levels of biological activity.

Based on a review of the published studies, it is apparent that the characteristics of an asbestos dust that best relate to biological activity are still a subject of scientific debate. The principle outstanding issues are:

(a) whether the total structure count adequately tracks the biological activity of asbestos or whether a specified, minimum length can be defined below which contributions to biological activity may be considered unimportant;

(b) whether asbestos aggregates (bundles, clusters, and matrices) should be counted as single entities or weighted in proportion to the number of individual fibers present in the aggregate to properly represent their contributions to risks;

(c) whether biological activity correlates with the overall mass of an asbestos structure;

(d) whether durable, non-asbestos fibers with similar morphologies to asbestos are biologically active;

(e) whether to employ different criteria for different asbestos mineral types or for different exposure circumstances (eg. exposure to mine tailings verses exposure to textile wastes, etc.).

Ideally, the results of an analytical method should be sufficiently flexible to allow for interpretation based on any of the prevailing theories concerning biological activity so that such results will retain validity even as the understanding of asbestos dose/response relationships matures.

3.2. RISK FACTORS

Although animal studies provide an indication of the qualitative relationship between physical characteristics and potency, they are not useful for quantifying dose/response relationships for humans. Such risk factors have been derived for asbestos from existing epidemiological studies in which the exposures of the cohorts evaluated are based on a combination of analytical methods including, primarily, midget impinger and phase contrast microscopy (USEPA, 1986a). However, phase contrast microscopy (PCM) and midget impinger measurements provide only crude indices of exposure and do not necessarily track characteristics representing the biological activity of asbestos.

The traditional methods of characterizing exposure used in existing epidemiology studies have not proven adequate to develop a dose/response relationship that uniformly applies under all exposure circumstances. Published risk factors vary by a factor of 50 depending on the specific exposure setting studied (USEPA, 1986a). This may be due to one or a combination of several factors that include errors and uncertainty in the quantification of mortality, errors and uncertainty in the quantification of exposure, differences in the size distributions of structures, and differences in the way that the various analytical techniques respond to dusts containing varied structure size distributions. Due to these limitations, such risk factors should be viewed as order-of-magnitude estimates at best. Despite such limitations, however, published risk factors (properly modified by considering other factors that have been shown to define biological activity) represent the only reasonable tools currently available for estimating risks from measured asbestos exposures.

Published risk factors are generally expressed in terms of PCM counts. When data from different types of analytical techniques were combined in the existing epidemiology studies, results were generally normalized and converted to PCM equivalent counts before completing the evaluation. However, despite the fact that published risk factors are generally expressed in terms of PCM equivalent counts, quantification of the PCM visible fraction of asbestos in current measurements does not provide the best index for comparing current asbestos measurements to existing risk factors.

It has been shown that the use of identical methods for measuring asbestos exposure in two different settings (such as two different factories) is not sufficient to assure the direct comparability of the two measurements, at least in terms of comparing risks (Chesson et al 1989a). Measurements from two different exposure settings, where the characteristics of asbestos dusts may vary, only indicate relative risk when the characteristics of asbestos that determine biological activity are measured directly. Other measures of asbestos exposure that do not relate directly to biological activity may not remain proportional to the characteristics that determine biological activity in different exposure settings. Therefore, PCM counts and other measures of exposure that do not relate directly to biological activity do not provide results from different exposure settings that are proportional to risk.

The best approach for monitoring current asbestos exposures to estimate risks is to measure the characteristics that relate directly to biological activity and to compare the results to existing risk factors using adjustment factors that relate PCM counts from the historical studies to proper exposure characteristics representative of the historical exposures. Such adjustment factors may be estimated from published asbestos characterizations determined using transmission electron microscopy (TEM) by pairing characterizations from specific exposure settings with existing risk factors derived from similar exposure settings. Alternately, exposure settings (selected for their similarity to settings originally studied to derive the existing risk factors) could be re-characterized using the method presented in this report to provide improved adjustment factors.

The approach recommended in the last paragraph for estimating risks presupposes a concise definition of asbestos characteristics that relate to biological activity. Because the definition of such characteristics remains a subject of controversy, the method presented in this report is designed to retain sufficient information from each analysis to span the range of characteristics currently considered likely to impact biological activity. Thus, results may be easily re-evaluated in the future.

3.3. MORPHOLOGY OF ASBESTOS DUSTS

The size distributions of asbestos structures are not the only characteristics of asbestos exposure that are potentially critical to biological activity. This is because asbestos structures will always be accompanied by other materials as components of dust, even in the workplace.

The major components of dust in all environments (occupational and environmental) are nonfibrous, isometric particles. Fibrous structures consistently represent a minor fraction of total dust. In addition, even among fibrous structures, asbestos represents a variable fraction of the total present. The relationship between these fractions is depicted in Figure 3.1.

Figure 3.1 is a Venn diagram depicting the universe of particles present in dust. Fibrous structures represent a minor fraction of total dust. Long fibrous structures represent a subset of total fibrous structures. Optically visible fibrous structures, those that are visible using a phase contrast microscope (optical microscope), represent a subset of long fibrous structures. The importance of the optical fraction of asbestos dusts is addressed in Section 3.2. Although not depicted, each of the subsets of fibrous structures could potentially be further subdivided into individual fibers, bundles, clusters, and/or matrices.

Particles composed of asbestiform minerals represent an independent subset of total particles that is distinct from fibrous materials. The overlap between asbestiform minerals and fibrous structures represents the fraction of fibrous structures that are asbestos. Correspondingly, subsets of long, fibrous structures and optically visible, fibrous structures (the PCM equivalent fraction) are also composed of asbestos.

The large square in Figure 3.1 represents the fraction of respirable particles. Subsets of asbestiform particles, and the three fractions of fibrous structures are also respirable. The large circle at the bottom of the diagram represents the fraction of particles traditionally counted by midget impinger. Note the limited overlap with fibrous structures. The shaded area of Figure 3.1 represents the fraction of respirable structures that are fibrous. The cross-hatched area represents the fraction of asbestos dusts (the long structures) currently believed to be the most biologically active.

Figure 3.1 is a qualitative representation. The relative size and orientation of the various fractions depicted will change as a function of the occupational or environmental setting considered. For example, the overlap

between asbestiform structures and fibrous structures is expected to be much greater in asbestos occupational settings than in environmental settings. In this manner, Figure 3.1 is probably more representative of an environmental setting.

Only a very limited number of available studies address the issue of the fraction of dust particles composed of asbestos. This is because in general, either the analytical technique used in a study has been incapable of distinguishing asbestos from non-asbestos or, if the analytical technique was capable of distinguishing asbestos from non-asbestos, only asbestos particles are traditionally characterized. Table 3.1 presents data from the few studies where the fraction of asbestos particles in dust was considered.

Unfortunately, much of the data in Table 3.1 provide an indication of the fraction of total particles composed of asbestos, while it is the fraction of fibers composed of asbestos that is of principle interest. In some exposure settings, a measurement of the fraction of asbestos in total particles may simply reflect the fraction of total fibers in total particles with no manner of distinguishing asbestos from non-asbestos fibers.

The data presented in the table from Cherrie et al (1987) and from Altree-Williams and Preston (1985) indicate that, while the vast majority of fibers in an asbestos (textile) factory may be composed of asbestos (which may be representative of occupational settings), the fraction of fibers composed of asbestos in other exposure settings (including environmental settings) may vary over a wide range.

3.4. PUBLISHED SIZE DISTRIBUTIONS

In any exposure setting, airborne asbestos exists as a series of simple fibers and complex structures of varying length, width, and breadth. Historically, available analytical techniques were capable of detecting only a fraction of the population of asbestos structures existing in an exposure setting. Thus, only an index of exposure could be measured.

In the last 15 or 20 years, measurements with the superior resolving power and magnification of the transmission electron microscope (TEM) have allowed complete characterization of the distribution of sizes and morphologies of asbestos structures that exist within an exposure population. Comprehensive characterization of asbestos exposures by scanning electron microscope (SEM) have also been reported, although the visibility of SEM (resulting from the combined constraints on resolution and contrast) is not generally sufficient to detect the smallest and finest asbestos structures (Walton 1982).

Table 3.2 presents published asbestos size distributions that were characterized by electron microscopy. The first column of the table lists the exposure setting in which the distribution was measured. The second column provides the type of asbestos monitored. The next several columns provide

FIGURE 3.1: COMPONENTS OF RESPIRABLE PARTICLES

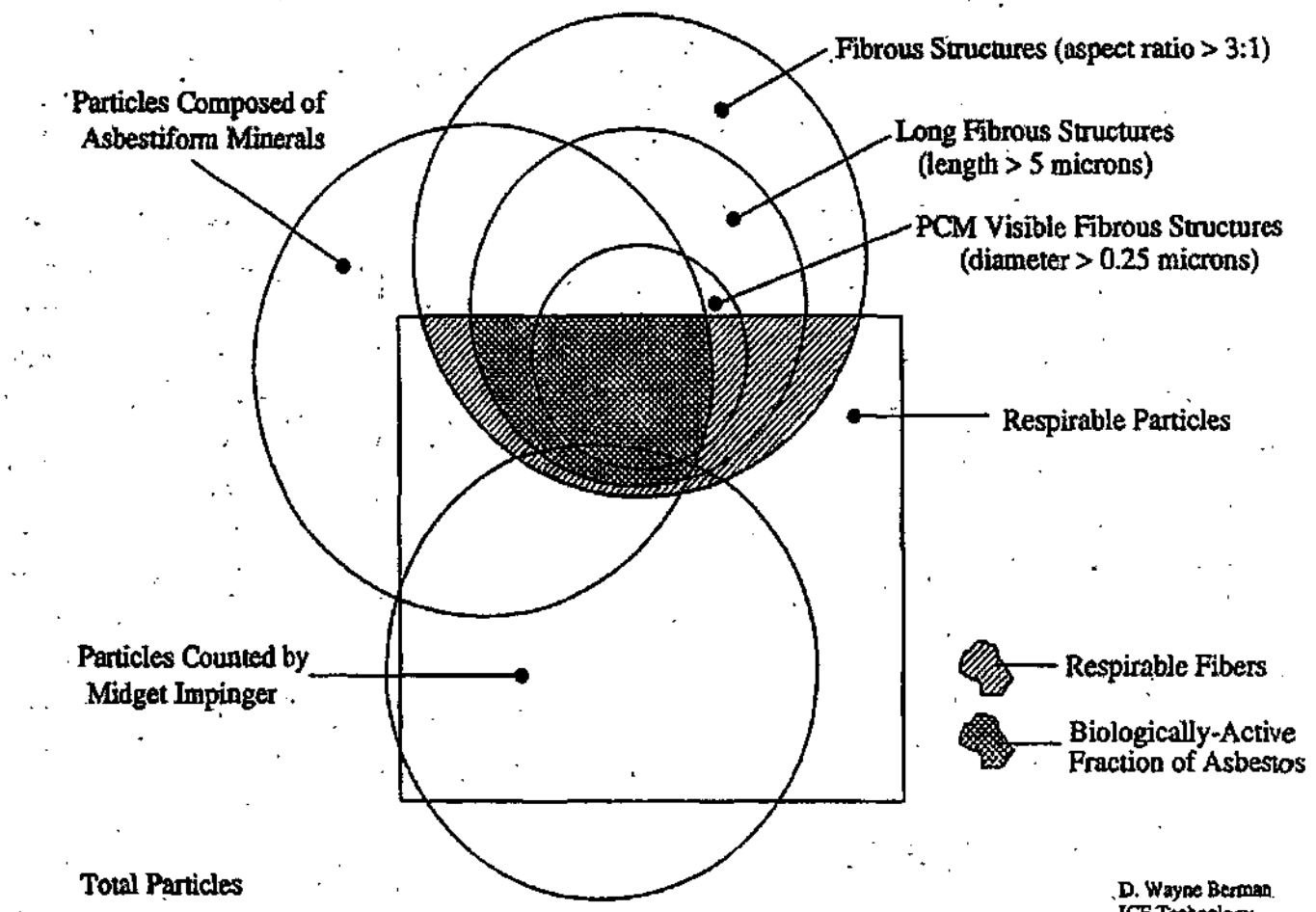

TABLE 3.1: PUBLISHED MEASUREMENTS OF THE FRACTION OF ASBESTOS IN DUST

Exposure Setting	Fiber Type	Analytical Method	Preparation Technique	Fraction of Asbestos in Shape Categories (% by count) of total particles	of total fibers	of optical fibers	Reference
Cape/Mine	Cro	PCM		41			(1)
Cape/Mill	Cro	&		51			
Tran/Mine	Cro	K		28			
Tran/Mill	Cro	&		59			
Mine	Ams	TP		33			
Mill	Ams	"		55			
Mine	Chr	"		45			
Mill	Chr	"		52			
Txt	Chr&Cro	TEM	D/M		82	94	(2)
Txt/Out	PChr	"	"		21	41	
Clearance	PAms	"	"		48	59	
Bldg w/Inst	PAms	"	"		15	36	
Urban	No re	"	"		0	0	
Bldg	Chr					20	(3)

Table 3.1 (continued)

Key

Exposure Settings:

AC	Dust from asbestos/cement sanding
AP	Asbestos production plant
ATB	Dust from asbestos tape and board
BC	Dust from a brake and clutch plant
Bag	Dust from bagging
Cape	Capetown, South Africa
Csr	Dust from construction
CP	Asbestos/cement pipe manufacture
Card	Dust from carding
Crsh	Dust from crushing
Cut	Dust from cutting
Dump	Dust from raw material dumping
Dry	Dust from drying
Fin	Dust from fiber finishing
FP	Friction products plant
Fprp	Dust from fiber preparation
Form	Dust from forming
Grnd	Dust from grinding
Insl	Site where insulation is applied
Mill	Dust from asbestos milling
Mine	Dust from asbestos mining
Mix	Dust from fiber mixing
Orest	Dust from ore storage
Out	Outside Plant
Pinsl	Pipe insulation manufacturers
Que	Quebec, Canada
RR	Railroad factory
Spec	Nonstandard clearance, etc.
Spin	Dust from fiber spinning
Tran	Transvaal, South Africa
Twst	Dust from fiber twisting
Txt	Dust from textile plant
UICC	Sample of UICC standards
Weav	Dust from weaving

Fiber Types:

Acin	Actinolite
Amph	Non-amosite amphibole
Ams	Amosite
Chr	Chrysotile
Crc	Crocidolite
Pr	Predominantly

Preparation Techniques:

D	Direct
I	Indirect
M	Mixed cellulose ester filter
N	Polycarbonate filter
Spec	Nonstandard preparation

Analytical Methods:

PCM	Phase contrast microscopy
K	Konimeter
MI	Midget impinger
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
TP	Thermal precipitator

Miscellaneous:

( )	Extrapolated from data
(T)	Total fibers

TABLE 3.1 (continued)

REFERENCES

1. Gibbs and du Toit, 1979
2. Cherrie et al., 1987
3. Altree-Williams and Preston, 1985

information on the techniques used in the study to generate the size data. These columns list, respectively, the method used to prepare the samples, the type of microscope used to determine the size distribution, and the magnification employed. The next two columns present reported median lengths and median diameters of the fibers in the distribution characterized.

Size distributions are presented in Table 3.2 as the number percent of total asbestos structures for structures in each of a series of size ranges that are listed in the headings of the next several columns. The column headed "percent with lengths less than 5 μm" represents the fraction of short structures detected within the particular distribution listed. The next two columns list structures with lengths greater than 5 μm. The second of these columns presents the fraction of structures with diameters (widths) greater than 0.25 μm, which represent the fraction of PCM equivalent structures detected. Since 0.25 μm is the limit of resolution generally quoted for phase contrast microscopy (PCM), this column represents the fraction of structures that would be detected by optical microscopy[1]. The first of the two columns representing structures longer than 5 μm lists structures that are too thin to be resolved by optical microscopy: the long, thin structures. The sum of PCM equivalent structures and long, thin structures represent the fraction of structures defined as long structures.

The four columns to the right of long, thick structures on Table 3.2, provide number percent fractions for two other ranges of length within the component represented by long structures. These are shown because it is possible that the most biologically active asbestos structures may be more restricted than indicated in Section 3.1 and 3.2 and Figure 3.1. Each length range is also subdivided into those resolvable and those unresolvable by optical microscopy (PCM).

Because chrysotile dusts have been characterized most frequently among the published fiber size distributions, the data for chrysotile (grouped by exposure setting) are presented in Table 3.3. Table 3.3 includes size distributions for dusts characterized both as "chrysotile" and as "predominantly chrysotile", that includes approximately half of all of the distributions presented in Table 3.2. The format of Table 3.3 is identical to that used in Table 3.2.

Although data are compressed and extrapolated in Table 3.3 (and Table 3.2) so that results from each study could be presented in a common format, the gross features of chrysotile size distributions can be summarized based on the detailed results of the studies listed. Where such studies provide sufficient detail, similar features are found for all chrysotile dusts

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[1] Note that the limit of resolution of PCM depends on a variety factors including the type and the condition of the instrument. The typical ranges of resolution quoted for PCM is between 0.2 and 0.4 μm. The impact of such variation on the interpretation of results is considered in later sections.

TABLE 3.2: STRUCTURE SIZE DISTRIBUTIONS MEASURED IN VARIOUS ENVIRONMENTS FOR SEVERAL ASBESTOS MINERALS

Exposure Setting	Fiber Type	Analytical Methods	Preparation Techniques	Magnification	Median Length	Median Diameter	Fraction of Asbestos Fibers in Size Categories (% by Count)							Reference
							L<5 µm	L > 5 µm D < 0.25 µm	L > 5 µm D > 0.25 µm	L 5 - 10 µm D < 0.25 µm	L 5 - 10 µm D > 0.25 µm	L > 10 µm D < 0.25 µm	L > 10 µm D > 0.25 µm	
Cape/Mine	Cro	PCM & TEM	D/N	-	0.95	0.08	96	3.72	0.7	3.02	0.57	0.7	0.13	(1)
Cape/Bag	Cro				1.16	0.08	93	5.88	1.2	5.1	1.09	0.78	0.11	
Tran/Mine	Amo				1.83	0.26	88	3.85	8.53	3.42	7.03	0.43	1.30	
Tran/Bag	Amo				2.53	0.26	77	5.56	17.8	4.42	12.6	1.14	5.2	
Que/Mine	Chr				0.34	0.06	99	0.40	0.2	0.36	0.7	0.04	0.13	
Que/Bag	Chr				0.55	0.06	96	2.06	1.72	1.56	1.31	0.5	0.41	
AP/Dump	Amo	SEM	D/N	2500	3.9	0.41	54	(3)	(43)	(3)	(27)	-	(16)	(2)
Ins/App	Amo			2500	2.5	0.37	69	(2)	(29)	(1.5)	(23)	-	(6)	
AP/Dump	Cro			6000	2.5	0.25	79	3	(18)	(3)	(14)	-	(4)	
Mill/Dry	Chr			6000	1.25	0.17	88.9	1.5	10	(1.5)	(7)	-	(3)	
Mill/Bag	Chr			6000	1.35	0.16	95.2	1	(4)	-	(3)	(1)	(1)	
Txt/Card	Chr			6000	1.0	0.15	97.7	0.40	(2.1)	(0.4)	(1)	-	(1)	
Cape/Mine	Cro	TEM & SEM & PCM	D/M,N	400 to 15,000	0.95	0.07	95.9	3.33	0.71	2.57	0.56	0.76	0.15	(3)
Cape/Crest					1.07	0.10	93.6	-	-	-	-	-	-	
Mill/Crsh					1.00	0.08	96.4	-	-	-	-	-	-	
Mill/Bag					1.16	0.09	92.9	5.89	1.21	5.11	1.1	0.78	0.11	
AP/Dump					0.91	0.08	98.1	1.41	0.44	1.27	0.38	0.14	0.06	
AP/Mix					1.25	0.10	93.9	-	-	-	-	-	-	
AP/Cut					0.50	0.04	99	-	-	-	-	-	-	

TABLE 3.2 (CONTINUED)

Exposure Setting	Fiber Type	Analytical Methods	Preparation Techniques	Magnification	Median Length	Median Diameter	Fraction of Asbestos Fibers in Size Categories (% by Count)						Reference
							L < 5 µm	L > 5 µm D < 0.25 µm	L > 5 µm D > 0.25 µm	L 5 - 10 µm D < 0.25 µm	L 5 - 10 µm D > 0.25 µm	L > 10 µm D < 0.25 µm	L > 10 µm D > 0.25 µm	
Special	Ams	PCM & SEM	D/Spec	400 to 10,000			21-40	52	81	26	37	26	44	(4)
Special							57	(43)		26		17		
Special							50	50		26		24		
UICC							77	23		21		2		
Cape/Mine	Crc	PCM & KON	–	–			88							(5)
Cape/Mill	Crc						84							
Tran/Mine	Crc						82							
Tran/Mill	Crc						73							
Mine	Ams						91							
Mill	Ams						65							
Mine	Chr						93							
Mill	Chr						87							
Txt/Fprp	Chr	PCM & TEM	–	–	1.4		96							(6)
Txt/Spin					1.0		98							
FP/Mix					0.9		98							
FP/Grnd					0.8		98							
CP/Mix					0.9		98							
CP/Fin					0.7		99							

TABLE 3.2 (CONTINUED)

Exposure Settings	Fiber Type	Analytical Methods	Preparation Techniques	Magnification	Median Length	Median Diameter	Fraction of Asbestos Fibers in Size Categories (% by Count)							Reference
							L < 5 µm	L > 5 µm D < 0.25 µm	L > 5 µm D > 0.25 µm	L 5 - 10 µm D < 0.25 µm	L 5 - 10 µm D > 0.25 µm	L > 10 µm D < 0.25 µm	L > 10 µm D > 0.25 µm	
Txt/Fprep	Chr	PCM	D/M	900			79	7.9	12.8	4.5	5.7	3.4	7.1	(7)
Txt/Twst		&		to			83.4	8.0	8.6	3.6	2.9	4.3	5.7	
Txt/Weav		TEM		17,000			81.2	9.0	9.7	4.9	3.1	4.2	6.6	
FP/Mix							90.4	6.3	3.4	2.9	1.2	3.4	2.2	
FP/Form							90.7	5.9	3.4	3.2	1.5	2.8	2.0	
FP/Fin							83.8	6.3	9.9	4.2	3.4	2.0	6.5	
CP/Mix							87.6	6.0	6.4	3.3	3.1	2.7	3.3	
CP/Form							90.7	6.4	2.9	3.8	1.4	2.5	1.6	
CP/Fin							95.0	3.0	2.1	2.0	0.8	0.9	1.2	
Pinsl/Mix	Ams						59.2	8.9	32.0	5.9	15.0	2.9	16.9	
Pinsl/Form	Ams						63.2	9.4	27.4	6.1	11.9	3.2	15.5	
Pinsl/Fin	Ams						65.1	6.6	28.4	6.1	14.4	0.4	14.0	
Que/ Oui/Mine	Chr	TEM	I				92.86	7.14	0.00					(8)
	Chr	TEM	D				84.54	3.54	11.92					
BC	Chr	PCM	D/M	500			44	(<6.1)	(49.9)					(9)
RR/ATB		&		to			75	(<2.5)	(22.5)					
RR/AC		TEM		10,000			78	(<2.4)	(19.6)					

TABLE 3.2 (CONTINUED)

Exposure Settings	Fiber Type	Analytical Methods	Preparation Techniques	Magnification	Median Length	Median Diameter	Fraction of Asbestos Fibers in Size Categories (% by Count)							Reference
							L < 5 µm	L > 5 µm D < 0.25 µm	L > 5 µm D > 0.25 µm	L 5 - 10 µm D < 0.25 µm	L 5 - 10 µm D > 0.25 µm	L > 10 µm D < 0.25 µm	L > 10 µm D > 0.25 µm	
Cstr	Chr	PCM	D/M				86.2	8.7	5.1					(10)
Bag		&					94.3	1.9	3.8					
Cut		STEM					53.4	13.3	33.3					
Cut							87.5	10.2	2.4					
Mill/Crsh							91.2	2.0	6.8					
Mill/Crsh							97.9	0.2	1.9					
Mill/Crsh							88.9	4.6	6.5					
Crsh							83.1	4.8	12.1					
CP/Mix							97.8	0.2	2.0					
Clearance	PAm						68.9	11.7	19.5					
Clearance	PAm						65.2	11.6	23.2					
Insl/Rmvl	PChr						64.2	10.2	25.6					
Insl/Rmvl	PAm						66.1	12.7	21.2					
Mine	Amph						96.9	0	3.1					
Mine	Amph						85.3	0	14.7					
Crsh	Actn						87.4	0	12.6					
Crsh	Actn						87.6	0	12.4					

TABLE 3.2 (CONTINUED)

Exposure Settings	Fiber Type	Analytical Methods	Preparation Techniques	Magnification	Median Length	Median Diameter	Fraction of Asbestos Fibers in Size Categories (% by Count)							Reference
							L<5μm	L>5μm D<0.25μm	L>5μm D>0.25μm	L 5-10μm D<0.25μm	L 5-10μm D>0.25μm	L>10μm D<0.25μm	L>10μm D>0.25μm	
Laboratory	ChrB	SEM	I/M				69							(11)
	ChrB	TEM					89							
	Ams	SEM					74							
	Ams	TEM					84							
	Crc	SEM					88							
	Crc	TEM					93							
	Tmi	SEM					50							
	Tmi	TEM					59							
Txt	Chr & Crc	SEM	D/M				78 (T)							
Txt		TEM					60 (T)							
Txt/Out	PChr	SEM					81 (T)							
Txt/Out	PChr	TEM					-							
Clearance	PAms	SEM					66 (T)							
Clearance	PAms	TEM					82 (T)							
Bldg w/Insl	PAms	SEM					63 (T)							
Bldg w/Insl	PAms	TEM					(91)(T)							
Urban	None	SEM					69 (T)							
Urban	None	TEM					-							

TABLE 3.2 (CONTINUED)

Exposure Settings	Fiber Type	Analytical Methods	Preparation Techniques	Magnification	Median Length	Median Diameter	Fraction of Asbestos Fibers in Size Categories (% by Count)							Reference
							L < 5 µm	L > 5 µm D < 0.25 µm	L > 5 µm D > 0.25 µm	L 5 - 10 µm D < 0.25 µm	L 5 - 10 µm D > 0.25 µm	L > 10 µm D < 0.25 µm	L > 10 µm D > 0.25 µm	
Mine	Amo	PCM		10,000			35.5							(12)
Crsh		&					46.4							
Bag		EM					55.8							
Assay							66.1							
Mine	Chr						56.6							
BC							45.5							
Mill							61.4							
Mine	Cro						77.1							
Mill	Cro						63.7							
Mill	Chr	PCM					93.7	(6.2)	(0.11)					(13)
Mill		&					96.1	(3.9)	(0.02)					
Mill		TEM					98.5	(1.5)	(0.01)					
Mill							98.6	(1.4)	(0.06)					
BC/Card	PChr						98.7	(1.3)	(0.04)					
BC							97.1	(2.9)	(0.05)					
CP							98.6	(1.4)	(0.03)					
CP							97.5	(2.5)	(0.06)					
Txt							87.5	(12.4)	(0.1)					
Txt							94.4	(5.5)	(0.08)					
Txt							93.7	(6.2)	(0.1)					

Table 3.2 (continued)

Key

Exposure Settings:

AC	Dust from asbestos/cement sanding
AP	Asbestos production plant
ATB	Dust from asbestos tape and board
BC	Dust from a brake and clutch plant
Bag	Dust from bagging
Cape	Capetown, South Africa
Catr	Dust from construction
CP	Asbestos/cement pipe manufacture
Card	Dust from carding
Crsh	Dust from crushing
Cut	Dust from cutting
Dump	Dust from raw material dumping
Dry	Dust from drying
Fin	Dust from fiber finishing
FP	Friction products plant
Fprp	Dust from fiber preparation
Form	Dust from forming
Grnd	Dust from grinding
Insl	Site where insulation is applied
Mill	Dust from asbestos milling
Mine	Dust from asbestos mining
Mix	Dust from fiber mixing
Orest	Dust from ore storage
Out	Outside Plant
Pinsl	Pipe insulation manufacturers
Que	Quebec, Canada
RR	Railroad factory
Spec	Nonstandard clearance, etc.
Spin	Dust from fiber spinning
Tran	Transvaal, South Africa
Twst	Dust from fiber twisting
Txt	Dust from textile plant
UICC	Sample of UICC standards
Weav	Dust from weaving

Fiber Types:

Actn	Actinolite
Amph	Non-amosite amphibole
Ams	Amosite
Chr	Chrysotile
Crc	Crocidolite
Pr	Predominantly

Preparation Techniques:

D	Direct
I	Indirect
M	Mixed cellulose ester filter
N	Polycarbonate filter
Spec	Nonstandard preparation

Analytical Methods:

PCM	Phase contrast microscopy
K	Konimeter
MI	Midget impinger
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
TP	Thermal precipitator

Miscellaneous:

( )	Extrapolated from data
(T)	Total fibers

TABLE 3.2 (continued)

REFERENCES

1. Gibbs and Hwang, 1980.
2. Gibbs and Hwang, 1975.
3. Hwang and Gibbs, 1981.
4. Beckett and Jarvis, 1979.
5. Gibbs and du Toit, 1979.
6. Lynch et al., 1970.
7. Demant and Harris, 1979.
8. Sebastien et al., 1984.
9. Marconi et al., 1984.
10. Snyder et al., 1987.
11. Cherrie et al., 1987.
12. Rendall and Skilne, 1980.
13. Winer and Cossette, 1979.

analyzed. Briefly, the distribution of structure lengths is unimodal and skewed with the mode occurring between 0.8 and 1.2 μm. The tail of the distribution extends out so that longer structures are present, but at decreasing frequencies. Structures longer than 5 μm constitute no more than 25% of the total and frequently constitute less than 5%. Structures longer than 10 μm constitute no more than half of the concentration of structures longer than 5 μm and frequently represent less than 2% of the total structures counted. Thus, representative counts of longer structures can only be guaranteed if counting procedures direct that long structures be counted independently from short structures and that 5 to 10 times as much area is scanned for the count of long structures. Such a procedure is termed statistically-balanced counting (Sebastien et al, 1982).

The reported magnitude of the fraction of short structures needs to be addressed with caution. Because the distribution of structures peaks in the vicinity of 1 μm and on the order of one half of the short structures are shorter than 1 μm, the relative number of short structures in a total distribution is extremely sensitive to the minimum size of the structures characterized. For example, counting all structures longer than 0.2 μm in length is likely to yield a structure size distribution where structures longer than 5 μm constitute less than 5% of the total while the same distribution truncated at 1 μm minimum length will yield contributions from long structures of 20%. The lower length limit counted is not reported in the majority of these studies. However, those where the limit is known all show short structure contributions greater than 90% (with corresponding contributions by long structures of less than 10%).

Diameters for chrysotile structures also vary. The median diameter of a typical structure less than 5 μm in length lies between 0.02 and 0.03 μm and virtually all of the structures less than 5 μm long have diameters less than 0.05 μm. Mean diameters increase with increasing length, but the increase is not proportional, so that the aspect ratios of long chrysotile structures are much larger than the aspect ratios of short structures. The thinnest chrysotile structures exhibit diameters on the order of 0.02 μm.

The data in Tables 3.2 and 3.3 must be interpreted carefully. Although the majority of the listed studies employed TEM to derive structure size distributions, lack of standardization of sample preparation techniques, of rules for counting and characterizing structures, and of the criteria used for establishing the mineralogy of counted structures potentially contribute to analytical variation between the studies (see Sections 6.1 and 6.4). For this reason, comparisons of distributions within a study are more reliable than comparisons between studies. In fact, the data in Table 3.2 clearly indicate that at least 2 studies are quantitatively different than the other studies. For the same fiber type in similar exposure settings, Rendall and Skikne (1980) and Marconi et al (1984) consistently report distributions containing a greater fraction of long structures than the other studies. Unfortunately, none of the studies contain sufficient documentation of the methods used to derive size distributions to determine the cause of the apparent discrepancies.

TABLE 3.3: STRUCTURE SIZE DISTRIBUTIONS MEASURED IN VARIOUS ENVIRONMENTS FOR CHRYSOTILE ASBESTOS

Exposure Setting	Fiber Type	Analytical Methods	Preparation Techniques	Median Length	Median Diameter	Magnification	Fraction of Asbestos Fibers in Size Categories (% by Count)							Reference
							L < 5 µm	L > 5 µm D < 0.25 µm	L > 5 µm D > 0.25 µm	L 5 - 10 µm D < 0.25 µm	L 5 - 10 µm D > 0.25 µm	L > 10 µm D < 0.25 µm	L > 10 µm D > 0.25 µm	
Que/Mine	Chr	PCM/TEM	D/	0.34	0.06		99	0.40	0.2	0.36	0.7	0.04	0.13	1
Mine		PCM/K, TP	D/				93							5
Que		TEM	I				92.86	7.14	0.00					8
Out/Mine		TEM	D				84.54	3.54	11.92					8
Mine		PCM/EM				10,000	56.6							12
Mill/Dry		SEM	D/N	1.25	0.17	6000	88.9	1.5	10	(1.5)	(7)		(3)	2
Mill/Crsh		PCM/STEM	D/M			10-70,000	91.2	2.0	6.8					10
Mill/Crsh		PCM/STEM	D/M				97.9	0.2	1.9					10
Mill/Crsh		PCM/STEM	D/M				88.9	4.6	6.5					10
Crsh		PCM/STEM	D/M				83.1	4.8	12.1					10
Mill		PCM/K,TP	D/				87							5
Mill		PCM/TEM					93.7	(6.2)	(0.11)					13
Mill							96.1	(3.9)	(0.02)					13
Mill							98.5	(1.5)	(0.01)					13
Mill							98.6	(1.4)	(0.06)					13
Mill		PCM & EM				10,000	61.4							12
Lab	ChrB	TEM	I/M				89 (T)							11
Lab	ChrB	SEM	I/M				69 (T)							11

TABLE 3.3 (CONTINUED)

Exposure Settings	Fiber Type	Analytical Methods	Preparation Techniques	Median Length	Median Diameter	Magnification	Fraction of Asbestos Fibers in Size Categories (% by Count)							Reference
							L < 5 µm	L > 5 µm D < 0.25 µm	L > 5 µm D > 0.25 µm	L 5 - 10 µm D < 0.25 µm	L 5 - 10 µm D > 0.25 µm	L > 10 µm D < 0.25 µm	L > 10 µm D > 0.25 µm	
FP/Mix	Chr	PCM/TEM	D/M			900	90.4	6.3	3.4	2.9	1.2	3.4	2.2	7
FP/Form			D/M			to	90.7	5.9	3.4	3.2	1.5	2.8	2.0	7
FP/Fin			D/M			17,000	83.8	6.3	9.9	4.2	3.4	2.0	6.5	7
(FP)/BC			D/M			10,000	44	(<6.1)	(49.9)					9
FP/Mix				0.9			98							6
FP/Grnd				0.8			98							6
BC/Card							98.7	(1.3)	(0.04)					13
BC							97.1	(2.9)	(0.05)					13
BC		PCM & EM				10,000	45.5							12
RR/ATB		PCM/TEM	D/M			10,000	75	(<2.5)	(22.5)					9
RR/AC			D/M			10,000	78	(<2.4)	(19.6)					9
CP/Mix			D/M			900	87.6	6.0	6.4	3.3	3.1	2.7	3.3	7
CP/Form			D/M			to	90.7	6.4	2.9	3.8	1.4	2.5	1.6	7
CP/Fin			D/M			17,000	95.0	3.0	2.1	2.0	0.8	0.9	1.2	7
CP/Mix				0.9			98							6
CP/Fin				0.7			99							6
CP/Mix		PCM/STEM	D/M				97.8	0.2	2.0					10
CP		PCM/TEM					98.6	(1.4)	(0.03)					13
CP		PCM/TEM					97.5	(2.5)	(0.06)					13

TABLE 3.3 (Continued)

Exposure Settings	Fiber Type	Analytical Methods	Preparation Techniques	Median Length	Median Diameter	Magnification	Fraction of Asbestos Fibers in Size Categories (% by Count)							Reference
							L < 5 μm	L > 5 μm D < 0.25 μm	L > 5 μm D > 0.25 μm	L 5 - 10 μm D < 0.25 μm	L 5 - 10 μm D > 0.25 μm	L > 10 μm D < 0.25 μm	L > 10 μm D > 0.25 μm	
Bag	Chr	PCM/STEM	D/M			10-70,000	94.3	1.9	3.8					10
Que/Bag		TEM/PCM	D/	0.55	0.06		96	2.06	1.72	1.36	1.31	0.50	0.41	1
Mill/Bag		SEM	D/N	1.35	0.16	6000	95.2	1	(4)		(3)	(1)	(1)	2
Cut		STEM/PCM	D/M			10-70,000	53.4	13.3	33.3					10
Cut		STEM/PCM	D/M			10-70,000	87.5	10.2	2.4					10
Cut		STEM/PCM	D/M			10-70,000	86.2	8.7	5.1					10
Insl/Rmvl	PChr	STEM/PCM	D/M				64.2	10.2	25.6					10
Txt/Card	Chr	SEM	D/N	1.0	0.15	6000	97.7	0.4	(2.1)	(0.4)	(1)		(1)	2
Txt/Fprp		TEM/PCM	D/M			900	79	7.9	12.8	4.5	5.7	3.4	7.1	7
Txt/Twst						to	83.4	8.0	8.6	3.6	2.9	4.3	5.7	7
Txt/Weav						17,000	81.2	9.0	9.7	4.9	3.1	4.2	6.6	7
Txt/Fprp				1.4			96							6
Txt/Spin				1.0			98							6
Txt/Out	PChr	SEM					81(T)							11
Txt/Out	PChr	TEM					—							11
Txt	Chr	TEM/PCM					87.5	(12.4)	(0.1)					13
Txt							94.4	(5.5)	(0.08)					13
Txt							93.7	(6.2)	(0.1)					13

Table 3.3 (continued)

Key

Exposure Settings:

AC	Dust from asbestos/cement sanding
AP	Asbestos production plant
ATB	Dust from asbestos tape and board
BC	Dust from a brake and clutch plant
Bag	Dust from bagging
Cape	Capetown, South Africa
Cstr	Dust from construction
CP	Asbestos/cement pipe manufacture
Card	Dust from carding
Crsh	Dust from crushing
Cut	Dust from cutting
Dump	Dust from raw material dumping
Dry	Dust from drying
Fin	Dust from fiber finishing
FP	Friction products plant
Fprp	Dust from fiber preparation
Form	Dust from forming
Grnd	Dust from grinding
Insl	Site where insulation is applied
Mill	Dust from asbestos milling
Mine	Dust from asbestos mining
Mix	Dust from fiber mixing
Orest	Dust from ore storage
Out	Outside Plant
Pinsl	Pipe insulation manufacturers
Que	Quebec, Canada
RR	Railroad factory
Spec	Nonstandard clearance, etc.
Spin	Dust from fiber spinning
Tran	Transvaal, South Africa
Twst	Dust from fiber twisting
Txt	Dust from textile plant
UICC	Sample of UICC standards
Weav	Dust from weaving

Fiber Types:

Actn	Actinolite
Amph	Non-amosite amphibole
Amo	Amosite
Chr	Chrysotile
Cro	Crocidolite
Pr	Predominantly

Preparation Techniques:

D	Direct
I	Indirect
M	Mixed cellulose ester filter
N	Polycarbonate filter
Spec	Nonstandard preparation

Analytical Methods:

PCM	Phase contrast microscopy
K	Konimeter
MI	Midget impinger
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
TP	Thermal precipitator

Miscellaneous:

()	Extrapolated from data
(T)	Toml fibers

TABLE 3.3 (continued)

REFERENCES

1. Gibbs and Hwang, 1980.
2. Gibbs and Hwang, 1975.
3. Hwang and Gibbs, 1981.
4. Beckett and Jarvis, 1979.
5. Gibbs and du Toit, 1979.
6. Lynch et al., 1970.
7. Dament and Harris, 1979.
8. Sebastian et al., 1984.
9. Marconi et al., 1964.
10. Snyder et al., 1987.
11. Cherrie et al., 1987.
12. Randall and Skilton, 1980.
13. Winer and Cossette, 1979.

The extent that the published size distributions presented in Tables 3.2 and 3.3 are representative of the exposure settings examined also needs to be considered when comparing results between studies. In most cases, each of the distributions presented is derived from a single grab sample. Since it is not known to what extent the size characteristics of an asbestos dust in a particular exposure setting vary over time, it is unclear how to relate single grab samples to the general features of an asbestos dust in a particular exposure setting. Nevertheless, this is currently the only data available for providing an indication of the distribution of particle size in (environmental and occupational) asbestos dusts.

Despite the above caveats, the data in Table 3.2 clearly indicate that different asbestos mineral types tend to produce dusts with distinctive size characteristics. Amosite dusts appear to contain the greatest fraction of long and thick structures. Averaged over the 26 distributions for amosite and predominantly amosite dusts, 34% of total amosite structures are longer than 5 μm. This compares with 10% of the total structures in the 53 chrysotile and predominantly chrysotile dusts and 12% of the 18 crocidolite dusts. The difference between amosite and the other two mineral types is further exaggerated if Marconi et al (1984) and Rendall and Skikne (1980) are removed from the data set.

NOTE

The absolute magnitudes of the fraction of long structures in a distribution all represent upper limits and may be revised downward depending on the minimum lengths counted in each of the studies. However, the relative values (between mineral type) are less likely to be affected because the observed differences are also apparent within individual studies, including the Rendall and Skikne study.

Although chrysotile and crocidolite dusts appear to contain comparable length distributions, the data in Table 3.2 indicate that chrysotile dusts tend to contain thicker structures than crocidolite. For example, the fraction of dusts visible by optical microscopy (those thicker than 0.25 μm) represents an average of 9% of chrysotile dusts with a range of 1 to 50%. Only an average 4% of crocidolite dusts are visible by optical microscopy (range 1 to 18%). The range of diameters across chrysotile dusts may actually be narrower, if inter-study variation is considered. Removing the Marconi et al (1984) data and one apparent outlier from Snyder et al (1987), the range of the fraction of structures visible by optical microscopy in chrysotile dusts is reduced to between 1 and 18% with an average of 8%. Note that the Winer and Cossette (1979) data were not included in the above averages because thick structures were counted by optical microscopy in this study and such results cannot be compared directly with the TEM data presented for counts of total long structures.

As indicated previously, amosite dusts appear to contain the greatest fraction of thick structures. Between 8 and 43% of total amosite structures are thick enough to be visible by optical microscopy with an average 25% among

amosite dusts. Interestingly, the median diameters reported for dusts do not appear to track the fractions of dusts represented by specific ranges of thicknesses. This is not surprising and simply indicates that size ranges are heavily skewed so that the mean and the median of the distributions are widely separated.

Overall, the fraction of long structures in crysotile and crocidolite typically represent less than 25% of total structures. Approximately 20 to 70% of long chrysotile structures or 1 to 18% of total chrysotile structures are visible by optical microscopy. Although the data is sparse for crocidolite, approximately 10 to 50% of long crocidolite structures are visible by optical microscopy. In contrast, the majority of long amosite structures are visible optically and long structures typically represent up to 50% of the total number of amosite structures present. In general, therefore, the fraction of amosite dust visible by optical techniques is up to 5 times higher than for chrysotile or crocidolite, although all three show a wide range.

As indicated earlier, the magnitude of the range and average fractions of long structures and structures visible by optical microscopy reported for the various size distribution may be revised downward subject to the minimum size counted in the distribution. For example, assuming published size distributions could be adjusted so that structures longer than 0.2 μm were included uniformly, long structures likely represent less than 10% of total structures in chrysotile size distributions and 4% may represent a better average fraction of structures in chrysotile that are visible by optical microscopy. This is in contrast to the numerical averages for these size fractions (25% and 8%, respectively), which were calculated directly from the data and presented above. Similarly, only 2% of crocidolite structures may fall within the range visible by optical microscopy.

It must be emphasized that the published size distributions presented in Tables 3.2 and 3.3 do not generally indicate whether aggregates are included or excluded from the distribution presented. However, aggregates likely represent major components of all asbestos dusts found in the environment (see for example: Chatfield, 1985b). In other examples, data generated at the Atlas and Coalinga Superfund sites in California indicate that 50% of the structures collected in the vicinity of the Atlas and Coalinga mines are matrices or other aggregates. Data from the South Bay Superfund site in California indicate that aggregates represent between 10 and 30% of the structures found in fibrous dust at this site. In two studies, Sebastien et al (1984 and 1986) report that aggregates constitute 40% of the fibrous structures in asbestos dusts found in the vicinity of the asbestos mines in Quebec.

One observation concerning size distributions that relates to biological activity address the spread in apparent risk factors derived from different exposure settings (see Section 3.2). Variations in fiber size distributions in dusts from different exposure settings have been identified as one of several possible causes of the observed spread in reported risk factors from

published epidemiology studies (see for example: USEPA 1986a). However, such variations are not readily apparent in Table 3.3. Overall, a slight trend toward increasing length for structures in dusts from mining and milling (except for bagging), through bagging, to textiles appears when distributions for these exposure settings are averaged. Structures in dusts from friction products and asbestos/cement pipe both appear shorter than textiles, although they cannot be distinguished from either mining, milling, or bagging.

Within individual studies, trends relating asbestos structure dimensions to exposure setting should be easier to distinguish since inter-study effects do not interfere. The clearest trend appears in the data reported by Dement and Harris (1979) with structures in dusts increasing in length from asbestos/cement pipe, through friction products, to textiles. Such trends are not apparent in the data of Winer and Cossette (1979) or Snyder (1987). However, the representativeness of the samples in terms of the types of dusts generated in the exposure settings examined has not been addressed in any of the studies except for Dement and Harris. None of the other studies listed in Tables 3.2 and 3.3 contain sufficient data for a single mineral type over a broad enough range of exposure settings to address this question. To resolve issues concerning the quantification of the risk associated with asbestos exposure, additional structure size characterizations may need to be developed using a standardized analytical method.

3.5. REPRESENTATIVE ASBESTOS CONCENTRATIONS

A limited number of published studies contain measurements of ambient concentrations of asbestos. Unfortunately, the utility of the data from these studies is restricted by the use of different analytical techniques and methods of reporting so that the results from different studies generally are not directly comparable (Chatfield, 1983b). Another problem with the available database is that several of the studies report results in mass equivalent concentrations and it has been shown that mass concentrations do not generally correlate with structure counts at ambient concentrations (Chatfield, 1985c). The high mass measurements that sporadically occur within any set of ambient samples are frequently due to the presence of a single, large aggregate on the sample filter, while the overall structure count remains low.

Available ambient asbestos measurements are presented in Tables 3.4 and 3.5. Table 3.4 presents representative background concentrations in various environmental settings and Table 3.5 presents several representative concentrations near sources of contamination. To distinguish ranges of concentrations that likely represent natural background from anthropogenic contamination, both the range of concentrations and the median concentrations reported in each study are presented in the tables. The median concentrations probably are better to base judgments upon since they tend to smooth the effects of outliers that potentially skew the limits of the reported ranges.

To the extent that the data are available in each study, concentrations are expressed in the tables in three formats: total asbestos structures per

unit air volume, asbestos structures visible by optical microscopy (PCM equivalent) per unit air volume, and asbestos mass per unit air volume. As addressed in later sections of this report, each of these formats has particular advantages and disadvantages. As indicated in the first paragraph of this section, however, the concentration formats are virtually independent variables; correlations between these formats that have been reported in the literature have been weak at best.

Comparisons between results presented in each of these tables should be made only with extreme care. Although the data presented is entirely from studies employing transmission electron microscopy, comparisons between studies must address the type of preparation (direct or indirect) employed and the specific size fraction of asbestos counted. Unfortunately, documentation for several of the studies is insufficient to determine precisely what size fraction was counted and whether aggregates are included or excluded from the count.

A few of the studies referenced in Tables 3.4 and 3.5 address the question of aggregates and provide a qualitative indication of size distributions. Sebastien et al (1986) indicate that in and around the mines of Quebec, 14% of structures longer than 5 μm are aggregates. This corresponds closely to the fraction of the long structures that represents the PCM equivalent fraction. Several authors report that asbestos structures encountered as background at remote locations are all short (less than 5 μm) chrysotile fibers (see, for example, Chatfield, 1985c). It is unclear, however, whether the detection of only short structures is solely due to statistical limitations in the number of structures encountered (longer structures are one to two orders of magnitude less abundant than short structures in most chrysotile distributions) or whether such observations represent a valid phenomenon indicating that the detection of contamination representative of a anthropogenic source can be limited to searching for long structures.

Due to the severe limitations of this database, it is difficult to identify reasonable target concentrations that represent the boundary between natural background and anthropogenic contamination. However, certain generalizations are apparent. For example, the data in Tables 3.4 and 3.5 suggest that analysis of samples prepared by an indirect-transfer technique generally yield results that are higher than analyses of samples prepared by a direct-transfer technique. However, because the concentration of indirectly prepared samples can be optimized to facilitate analysis, it is the limiting concentrations observed on directly prepared samples that is of principle concern for development of the method.

Of the results identified as derived from directly prepared samples in Table 3.4, the range of median concentrations reported for total structures spans from less than 0.4 s/L to 6 s/L. Note that this range does not include the median of less than 0.01 reported in one study (Tuckfield et al 1988), which appears to lie outside the range of the majority of other studies. Results reported in studies where the preparation technique was not specified

TABLE 3.4: PUBLISHED BACKGROUND CONCENTRATIONS OF ASBESTOS

Environmental Setting	Median of Reported Concentrations [a] s/l	PCME/l	ng/m^3	Range of Reported Concentrations [b] s/l	PCME/l	ng/m^3	Conversion (PCME/l to ng/m^3)	Analytical Sensitivity [c]	Preparation Technique	Reference
Outside Schools	25	(0.05) [d]	0.12	0-2000	—	0-8	—	—	indirect	①
Outside Schools	10	0.3 [d]	0.08	0-100	0-8 [d]	0-0.9	0.1-100, (4) [e,f]	—	indirect	②
Outside Schools	4	—	0.02	0-10	—	0-0.07	—	—	indirect	③
Outside Schools	—	—	0.5	—	—	0-100	—	—	—	④
Outside Public Buildings	<0.01	—	—	—	—	—	—	—	direct	⑤
Urban	2	<2	0.03	0-8	0-4	0-20	0.4 [g]	2	direct	⑥
Urban Toronto	6	<2 [h]	0.07	0-45	0-4 [h]	0-0.3	—	2	direct	⑥
Urban Paris	—	—	0.4	—	—	0.1-9	—	—	Indirect	⑦
Urban	—	—	1	—	—	1-10	—	—	—	⑧
Urban	—	—	—	0-10 [i]	—	—	—	—	—	⑨
New York	—	—	10	—	—	0-100	—	—	—	⑩
US Cities	—	—	1	—	—	0-50	—	—	—	⑪
US Cities	—	—	3	—	—	0-15	—	—	—	⑫
Canadian Cities	—	0.7 [h,j]	—	—	0.6-0.9 [h,j]	—	—	—	Indirect	⑬
Canadian Cities	—	0.1 [h,j]	1 [j]	—	0-3 [h]	0-6	0.1	—	—	⑭
English Cities	—	—	—	—	—	0.1-1	—	—	—	⑮
German Cities	3 [g]	—	—	0-10	—	—	—	—	—	⑯
Urban Switzerland	—	0.4 [h,j]	0.75 [h,j]	—	—	—	0.5	—	—	⑰
Upwind of Asbestos Plants	0.2	—	0.03	0-11	—	0-170	—	0.3	—	⑱

TABLE 3.4 (CONTINUED)

Environmental Setting	Median of Reported Concentrations[a]			Range of Reported Concentrations[b]			Conversion	Analytical Sensitivity[c]	Preparation Technique	Reference
	s/l	PCME/l	ng/m^3	s/l	PCME/l	ng/m^3	(PCME/l to ng/m^3)			
Suburban	0.6	<0.6[d]	0.003	0-6	0-2	0-9	0.2[g]	0.6	direct	⑥
Remote	<0.4	<0.4	—	0-0.4	<0.4	0-0.008	—	0.4	direct	⑥
Remote	—	—	0.3	—	—	0.1-2	—	—	—	⑲
Remote (FRG)	—	—	—	0.03-0.9	—	—	—	—	direct	⑳
Rural Ontario	2	<2[h]	0.002	0-30	<2[h]	0-0.2	—	2	direct	⑥
Rural	—	—	—	0-0.3[i]	—	—	—	—	—	⑨
Rural Austria	—	<0.1[h,j]	—	—	—	—	—	—	—	㉑

TABLE 3.4 (CONTINUED)

FOOTNOTES:

a. Values in these columns represent estimated median values for the range of concentrations reported in the study. In some cases, due to the form of data presentation, values presented in this table represent the median of a range of averages. In other cases, which are marked accordingly, only mean values could be derived from the study.

b. The lowest and highest values reported in each study are presented here as the limits of the range of reported concentrations. In some cases, due to the form of data presentation, the values presented in this table represent a range of average values from multiple locations.

c. Values in this column represent an estimated average of the analytical sensitivities reported for each measurement in the study.

d. These values are based on PCM analyses rather than TEM analyses.

e. This is a range derived from the manipulation of paired PCM, TEM analyses.

f. This value is simply the quotient of the median values for the parameters indicated.

g. These values are derived from a single measurement.

h. These values represent total structures longer than 5 um rather than PCM equivalent structures.

i. These values are estimated values.

j. These values are the mean of a range of concentrations rather than the median.

k. This is a range of means of multiple samples from several locations.

TABLE 3.4 (continued)

REFERENCES

1. Tuckfield, et al., 1988.
2. Chesson, et al., 1985.
3. Chesson, et al., 1986.
4. Constant, et al., 1983.
5. Hatfield, et al., 1988.
6. Chatfield, 1983b.
7. Sebastien et al., 1979.
8. Nicholson, et al., 1979.
9. Steen, et al., 1983.
10. Nicholson, et al., 1971.
11. Nicholson, et al., 1975.
12. U.S. EPA, 1974.
13. Sebastien, et al., 1986.
14. Nicholson, 1988.
15. Rickards, 1972.
16. Friedrichs, et al., 1983.
17. Litistorf, et al., 1985.
18. John, et al., 1976.
19. Sebastien, 1985.
20. Spurny and Stober, 1978.
21. Felbermayer, 1983.

TABLE 3.5: PUBLISHED ASBESTOS CONCENTRATIONS PROXIMAL TO ASBESTOS SOURCES

Environmental Setting	Median of Reported Concentrations [a] s/l	PCME/l	ng/m^3	Range of Reported Concentrations [b] s/l	PCME/l	ng/m^3	Conversion (PCME/l to ng/m^3)	Analytical Sensitivity [c]	Preparation Technique	Reference
In Buildings:										
-with asbestos	<5	<1	<0.1	0-40	0-2	0-40 [d]			direct	①
-with damaged asbestos	0.6	—	—	—	—	—			—	②
-with asbestos	0.4	—	—	—	—	—			—	②
-with no asbestos	0.1	—	—	—	—	—			—	②
-with asbestos	—	—	12	—	—	0-750			—	③
Toronto subways	25	—	0.2	15-150	0-15 [e]	0.015-50	0.3 [e,f]		direct	④
Toronto subways	200	—	15	40-3000	0-55 [e]	0.2-200	0.9 [e,g]		indirect	④
Near mines	—	40 [e,h]	—	—	15-150 [e,i]	—			indirect	⑤
Near waste piles	60	7 [h]	—	13-340 [i]	0.5-65 [i]	—			direct	⑥
Paraoccupational	—	—	—	—	0-100 [e]	—			—	⑦

TABLE 3.5 (CONTINUED)

FOOTNOTES:

a. Values in these columns represent estimated median values for the range of concentrations reported in the study. In some cases, due to the form of data presentation, values presented in this table represent the median of a range of averages. In other cases, which are marked accordingly, only mean values could be derived from the study.

b. The lowest and highest values reported in each study are presented here as the limits of the range of reported concentrations. In some cases, due to the form of data presentation, the values presented in this table represent a range of average values from multiple locations.

c. Values in this column represent an estimated average of the analytical sensitivities reported for each measurement in the study.

d. The highest mass concentration in this range is reported for a sample composed largely of amphibole. The highest mass concentration reported for a chrysotile sample in this data set is 24 ng/m^3.

e. These values represent total structures longer than 5 um rather than PCM equivalent structures.

f. These values are derived from a single measurement.

g. This value is the mean of three measurements.

h. These values are the mean of a range of concentrations rather than the median.

i. This is a range of means of multiple samples from several locations.

TABLE 3.5 (continued)

REFERENCES

1. Burdett and Jaffray, 1986.
2. Hatfield, et al., 1988.
3. Sebastien, et al., 1979.
4. Chatfield, 1983b.
5. Sebastien, et al., 1986.
6. Ruch and Serper, 1977.
7. Steen, et al., 1983.

appear to be in general agreement with this range, as opposed to the range of medians reported for samples prepared by an indirect technique: 4 to 25 s/L. The range of background concentrations in urban environments appear higher than exhibited at rural locations. As indicated by the data presented in Table 3.5, the lower range of concentrations reported in the vicinity of asbestos sources appears similar to the range reported in Table 3.4 for background.

Deriving a range of background concentrations for PCM equivalent structures is more problematic. For directly prepared samples from urban settings, the reported medians range between 0.1 and less than 2 s/L. However, for rural locations, PCM equivalent structures were largely undetected on directly prepared samples. Interestingly, median concentrations for PCM equivalent structures reported for indirectly prepared samples appear to span a lower range than for directly prepared samples: 0.05 to less than 2 s/L. Thus, the true range of background concentrations for PCM equivalent structures may not have been identified in this dataset.

Rounded to the nearest half order of magnitude, a reasonable range for background asbestos concentrations appears to span 0.5 to 5 s/L for total asbestos structures. The lower end of the range appears to be closer to the median background for rural locations. Although a representative range for background concentrations of PCM equivalent structures (or long structures) is difficult to estimate from the available literature, such values may be estimated from the range reported for total structures given the data presented in Section 3.4. Conservatively, long structures (those longer than 5 μm) represent approximately 4% of total structures in most chrysotile size distributions encountered. Thus an estimate of the range of background concentrations for long structures spans 0.02 to 0.2 s/L. Presumably, PCM equivalent structures would be some major fraction of these concentrations.

3.6. REPRESENTATIVE TOTAL DUST CONCENTRATIONS

As addressed in Section 6.2.1, the concentration of total particulate in a dust affects the ability to characterize the asbestos fraction. Concentrations of total suspended particulate (TSP) vary over several orders of magnitude depending on location, time of day, and weather. Urban and agricultural sites tend to exhibit significantly higher concentrations of TSP than rural locations. In addition to variation in overall concentration, the composition of TSP also varies significantly as a function of location. At Urban sites and specific rural locations, the TSP tends to be composed principally of organic matter that can be ashed or inorganic substances that are soluble in acidified media. Agricultural locations and other rural locations frequently exhibit higher concentrations of refractory silicate particles. Due to the wide spatial and temporal variation in TSP concentrations, a general rule for estimating levels at a site can not be provided without data from a comprehensive survey.

3.7. ANALYTICAL TECHNIQUES USED TO MONITOR ASBESTOS

Analytical methods traditionally used to monitor asbestos include midget impinger (MI), phase contrast microscopy (PCM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Each method differs in its ability to detect the various size fractions typical of asbestos dusts including those most likely to relate to biological activity.

In the occupational studies used to develop dose/response relationships (addressed in section 3.2), asbestos dusts were analyzed by counting structures on a slide or filter using optical microscopes. Until the early 1970's the procedure involved collecting workplace dust with a midget impinger using a light microscope at a magnification of 100 to count the number of particles collected.

The midget impinger is a device in which a stream of contaminated air is forced through a restricted opening into a liquid (alcohol) where it emerges as a jet of bubbles that disperse the asbestos. The particles suspended in the liquid are then transferred to a 1 mm deep well and counted using a light microscope.

Because the counting rules employed during the examination of midget impinger samples require that all observed particles be counted, the resulting concentrations represent coarse total dust counts and have little direct relationship to the quantity of fibrous structures present. In Figure 3.1, the lack of significant overlap between the midget impinger circle and the circle representing fibrous particles illustrates the limited relationship between analytical results from this method and potentially important asbestos fibers. Thus, midget impingers provide only an indirect index of asbestos exposure.

In the 1970's the midget impinger was replaced by the membrane filter method, which has become the standard technique for monitoring asbestos in industry. This latter method involves collecting a sample of airborne dust on a membrane filter, rendering the filter transparent with an appropriate solvent, and counting fibers using a phase contrast microscope (PCM) at magnifications between 400 and 900.

Because of the increased magnification and because phase-contrast lighting increases the sensitivity to narrow objects in the field of view, it is possible to delineate the general morphology of the particles being counted so that measurements can be restricted to fibrous structures (defined as those longer than 5 μm, exhibiting aspect ratios greater than 3:1, and having largely parallel sides). However, the limitations imposed are somewhat arbitrary and not based on health effects (Chatfield, 1979). In addition, PCM techniques were standardized only recently so that counting procedures and attendant results changed over the period spanned by existing epidemiology studies (Chatfield, 1985d).

Due to the limited resolution of PCM, the internal components of complex structures (bundles, clusters, or matrices) are not generally distinguishable so that all such structures appear as single, solid particles. Consequently, all structures visible by PCM that satisfied the appropriate dimensional criteria were counted as individual structures (fibers). The term "structure" is used here for consistency, because frequently a specific "fiber" viewed by PCM is observed to be a complex structure when viewed with the increased resolution afforded by transmission electron microscopy. The purpose is to preserve the terminology used to describe a specific particle that may be viewed using any of several microscopic techniques.

Due to a combination of resolution and contrast, the minimum width of structures visible in the phase contrast microscope reportedly ranges between 0.2 and 0.4 μm, depending on the configuration and condition of the instrument. The most frequently quoted average limit to visibility is 0.25 μm. Partially by convention and partially due to the practical limitations associated with classifying structures with aspect ratios less than 10:1, structure counts were further limited to those longer than 5 μm (See for example: Chatfield, 1985d).

As indicated in Figure 3.1, structures visible by phase contrast microscopy correspond to a range that encompasses a significant portion of the structures believed to be biologically active. However, structures believed to be the most biologically active (the longest, thinnest structures) are not counted by this technique. Additionally, because the method is incapable of distinguishing asbestos from non-asbestos minerals, non-asbestos structures (to the extent that they are present in an exposure setting under study) are included in PCM counts. Although it is assumed that the vast majority of fibrous structures encountered in an occupational setting are asbestos, this is not the case for environmental samples. Consequently, these two stated limitations of PCM render it unsuitable for monitoring environmental asbestos.

More recently, techniques based on the electron microscope have been introduced. The scanning electron microscope (SEM) and the transmission electron microscope (TEM) count, respectively, asbestos structures on membrane filters or structures transferred from the filters to electron microscope grids.

Magnifications typically achieved with SEM range between 2,000 and 10,000, while TEM magnifications can easily reach 100,000. Thus, TEM is capable of resolving even the finest fibrous structure. Although the magnification implies that the resolution of SEM should be significantly better than PCM, in practice, the visibility of structures in the SEM is limited by a combination of contrast and electronic noise (Small et al 1983). Consequently, the minimum structure width visible in the SEM is only slightly better than PCM. Under optimum conditions, the minimum width of structures visible in the SEM is perhaps a factor of 5 better than PCM (Walton 1982). Given the increased expense and other instrumental constraints, however, there appears to be little advantage to SEM over PCM except for the ability to distinguish asbestos from non-asbestos structures. At the same time, SEM does

not appear to retain a significant cost advantage over TEM for the analysis of environmental samples. Therefore, SEM is not likely a useful technology for environmental analysis of asbestos.

Most electron microscopes (both TEMs a SEMs) are equipped to allow mineralogical and elemental analysis to confirm the composition of the structures counted. Thus, TEM is clearly capable of identifying populations currently believed to represent the most biologically active fraction of asbestos, the shaded area of Figure 3.1 (see Sections 3.1, 3.2, and 3.3). In fact, TEM is theoretically capable of distinguishing whatever appropriate size or mineralogical fraction that is likely to be linked to asbestos biological activity in the future.

3.8. EXISTING TEM ANALYTICAL METHODS

Several researchers have published analytical methods based on TEM for the analysis of asbestos samples collected on membrane filters. The principal features of the Yamate method (1984), NIOSH 7402 (NIOSH 1986), Hayward's method (Hayward, no date), and the method in the AHERA regulations (USEPA 1987), are summarized in Table 3.6. A PCM analysis method, NIOSH 7400 (NIOSH 1985) is also included in Table 3.6 for comparison. It should be emphasized, however, that procedures employed for PCM analysis have changed over time. Therefore, the NIOSH method for PCM presented in the table, which is a relatively recent method, differs in detail from the various procedures likely employed to collect the data used in published epidemiology studies. Nonetheless, it is included to allow comparison between TEM methods and the general features of a PCM method.

As indicated in Table 3.6 the methods incorporate procedures for preparation, counting rules, and rules for identifying and characterizing asbestos mineralogy. Generally, the methods also define a fixed area of the specimen grid to be counted. By comparing the requirements of a method suitable to support risk assessment to the features of the methods presented in Table 3.6, it is possible to determine which of the methods may be applicable to Superfund field investigations.

Except for differences in procedural details, the TEM methods presented in Table 3.6 share many common features. All rely on direct preparation, although the Yamate method contains a protocol for an optional indirect preparation should filters prove too loaded to analyze the filters when prepared by a direct technique. All of the TEM methods incorporate structure characterization at a magnification of 20,000. The principle differences between the methods involve counting rules and the inclusion in the Hayward method of a low magnification scan designed to provide a statistically balanced count of large asbestos structures (longer than 5 μm). Because long structures appear to play a major role in determining the biological activity of asbestos dusts (see Sections 3.1 and 3.2), the use of a low magnification scan to count long structures in a statistically balanced manner is incorporated in the method presented in Part 1 of this report.

TABLE 3.6: COMPARISON OF AVAILABLE ASBESTOS ANALYTICAL TECHNIQUES

METHOD:	NIOSH 7400	NIOSH 7402	YAMATE	HAYWARD	AHERA
Analytical Technique	PCM	TEM	TEM	TEM	TEM
Preparation Methodology	Direct	Direct	Direct (Optional Indirect)	Direct	Direct
Magnification	450x	10,000x	20,000x	High Magnification: 20,000x - 50,000x Low Magnification: 400x - 4000x	15,000x - 20,000x
Dimensions (µm)				High Magnification:	
Length (l):	l > 5	l > 1	l > 0.08 [a]	l > 0.08 [a]	l > 0.5
Width (w):	w > 0.25 [b]	3.0>w>0.04 [c]	w > 0.02 [c]	w > 0.02 [c]	w > 0.02 [c]
Aspect Ratio (ar):	ar > 3	ar > 3	ar > 3	ar > 3	ar > 5
				Low Magnification:	
				l > 5	
				w > 0.25	
				ar > 3	
Reported Sensitivity					
s/cm^2	---	---	---	---	0.005 s/cm^2
s/mm^2	---	---	---	---	70 s/mm^2

TABLE 3.6 (continued)

METHOD:	NIOSH 7400	NIOSH 7402	YAMATE	HAYWARD	AHERA
Counting Rules:					
Structures	Count all structures exhibiting l > 5 μm, w < 3.0 μm, and aspect ratios > 3.	Count all structures exhibiting l > 1 μm, w < 3.0 μm, and aspect ratio > 3. Note PCME fraction within count.	Count all structures exhibiting an aspect ratio > 3.	Count all structures exhibiting an aspect ratio > 3. Use low magnification scan to count PCME fraction.	Count all structures longer than 0.5 μm that exhibit an aspect ratio > 5. Record individual fibers within all groupings with fewer than 3 intersections. Count structures longer than 5 μm separately (PCME).
Bundles [a]	Bundles meeting overall dimensional criteria generally counted as single fibers unless up to 10 individual fiber ends can be distinguished within the bundle (representing 5 individual fibers).	Bundles meeting overall dimensional criteria generally counted as single fibers.	Bundles meeting overall dimensional criteria generally counted as single entities and noted as bundles on the count sheet.	Bundles meeting overall dimensional criteria generally counted as single entities and noted as bundles on the count sheet.	Bundles of 3 or more fibers that meet the overall dimensional criteria are counted as single entities and noted as bundles on the count sheet.

TABLE 3.6 (continued)

METHOD:	NIOSH 7400	NIOSH 7402	YAMATE	HAYWARD	AHERA
Clusters[e]	Within a cluster, count up to 10 individual fiber ends from (up to 5) fibers that meet the overall dimensional criteria. Otherwise, count a cluster as a single entity.	Within a cluster, count up to 3 individual fibers that meet the overall dimensional criteria. Otherwise, clusters that contain more than 3 fibers that meet the overall dimensional criteria are counted as single clusters.	Within a cluster, count up to 3 individual fibers that meet the overall dimensional criteria. Otherwise, clusters that contain more than 3 fibers that meet the overall dimensional criteria are counted as single clusters.	Within a cluster, count up to 3 individual fibers that meet the overall dimensional criteria. Otherwise, clusters that contain more than 3 fibers that meet the overall dimensional criteria are counted as single clusters. Clusters are defined in the low magnification scan by their overall dimensions.	A collection of fibers with more than 2 intersections where at least one individual projection meets the overall dimensional criteria is counted as a single cluster.
Matrices[f]	Count up to 5 fibers emanating from a clump (matrix). Each individual fiber must meet the dimensional criteria.	Count individually identifiable fibers within a matrix. Fibers must individually meet the dimensional criteria.	Count as a single matrix, all matrices with at least one protruding or embedded fiber that meets the dimensional criteria.	Count as a single matrix, all matrices with at least one protruding or embedded fiber that meets the dimensional criteria.	Count as a single matrix, all matrices with at least one protruding fiber such that the protruding section meets the dimensional criteria.
Maximum Number Counted	100 structures	100 structures	100 structures	100 structures at high magnification and 100 long structures at low magnification.	50 structures

TABLE 3.6 (continued)

METHOD:	NIOSH 7400	NIOSH 7402	YAMATE	HAYWARD	AHERA
Area Scanned	100 fields	100 openings [g]	10 openings	15 openings[h] at high magnification distributed over 4 grids from each sample and 100 openings at low magnification distributed over 4 grids from each sample.	Blanks: 10 openings Samples: 10 openings (given the defined sensitivity and the recommended air volumes).
Mineralogy Determined?	no	yes	yes, except matrix particles	yes, except matrix particles	yes, except matrix particles
Statistical Balanced Counting?	no	no	no	yes	no

a. The minimum fiber length to be counted has not been defined in this method. Presumably the minimum fiber length counted would correspond to 3 times the resolution limit of the width (due to the aspect ratio requirement). Since the presumed resolution limit is 0.001μm, the corresponding minimum length fiber that would be counted under this method is 0.003μm.

b. Width restrictions for PCM are due to limits in resolution. A width of 0.25 μm represents the average resolution reported for PCM.

c. The width restriction reported for TEM is based on an estimate of the resolution limit associated with typical magnification settings for the technique. The presumed limit of resolution at a typical magnification of 20,000x reported for this technique is 0.001μm.

d. Bundles are defined as a parallel arrangement of fibers separated by distances smaller than one fiber diameter.

e. Clusters are a collection of fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group.

f. Matrices (termed "mats" by Hayward) are one or more fibers embedded within or protruding from another particle.

g. The 100 grid opening limit for scanning is not stated directly in this method but can be inferred from the information provided.

h. This has been converted to a 200 mesh equivalent to be consistent with all of the other criteria presented in this row.

TABLE 3.6 (continued)

METHOD	REFERENCES
1. NIOSH 7400	Niosh, 1985.
2. NIOSH 7402	Niosh, 1986.
3. YAMATE	Yamate, et al., 1984.
4. HAYWARD	Hayward, no date.
5. AHERA	US EPA, 1987.

Changes in counting rules as the methods evolved (each one building on its predecessors) were designed to minimize subjective decisions by the analyst using the method. This serves to minimize observer-dependent variation, one of the major sources of variation associated with TEM analysis. Of the published methods, the counting rules presented in AHERA provide the best defined counting procedures for minimizing observer-dependant variation. However, the recording of structure dimensions is not required under AHERA, which potentially limits the ability to use such results in a risk assessment. A method designed to support a risk assessment must count structures in a manner that is consistent with the characteristics that relate to biological activity, which depend on structure size. Therefore, the counting rules defined in AHERA were modified for this method to better detect and record the range of characteristics that potentially relate to biological activity (see Sections 3.1 and 3.2).

One of the limitations common to most of the TEM methods presented in Table 3.6 is that the sensitivity of the analytical method cannot be defined. This is a consequence of defining a fixed area of the specimen to scan without simultaneously defining a fixed volume of air to be collected during sampling. One of the critical elements of this method is that the target sensitivity is defined and the combined effect of sample loading and the area to be scanned during analysis are addressed (see Section 6.2).

Another critical modification incorporated into this method that is only partially addressed by the TEM methods presented in Table 3.6, is a recording procedure that preserves sufficient information to allow extensive and flexible interpretation of the results without the necessity to re-analyze the specimen.

4. RISK ASSESSMENT OBJECTIVES AND METHOD REQUIREMENTS

To support a risk assessment, a method must address three objectives:

(a) to provide measurements that relate to the biological activity of asbestos;

(b) to provide sufficient sensitivity to measure asbestos at the low concentrations typically found in the environment near potential sources;

(c) to provide sufficient precision to elucidate spatial and temporal trends in asbestos concentrations.

An additional consideration to be addressed is the need to control sampling and analysis costs. Each of these objectives imposes a set of technical constraints on a method for asbestos sampling and analysis. Such constraints served as a basis for defining performance criteria for this method.

4.1. CONSIDERATION OF BIOLOGICAL ACTIVITY

Although published risk factors are expressed in terms of PCM measurements, other studies indicate that the biological activity of asbestos is a function of a broader range of the distribution of asbestos structure types and sizes, which PCM is not capable of distinguishing (see Sections 3.1, 3.2, and 3.7). Therefore, to properly address current concepts concerning the biological activity of asbestos, this method is designed to:

(a) track asbestos structures of all lengths (defined as all entities with components exhibiting an aspect ratio exceeding 5:1 that are longer than 0.5 μm);

(b) separately track asbestos structures longer than 5 μm;

(c) track such entities at sufficient magnification to resolve the thinnest asbestos structures (approximately 0.02 μm in diameter);

(d) distinguish among asbestos fibers, bundles, clusters, and matrices and independently track their concentrations;

(e) characterize the mineralogy of each structure counted;

(f) record the lengths and widths of each structure counted to allow for later selection and tracking of size fractions with particular emphasis on long structures (longer than 5 μm);

(g) provide a system for recording analytical results that preserves sufficient information concerning the mineralogical determination and morphological characteristics of each structure to allow for later re-interpretation without the need to re-evaluate the original specimen.

Regarding aggregates (bundles, clusters, and matrices), it is also important to estimate and record, if possible, the number of asbestos components present within each aggregate.

NOTE

Although it appears that the size fraction represented by "b" above can be derived from the count of total structures by selecting those exhibiting the appropriate length and width criteria, it is listed separately to emphasize the need to count long structures separately for statistical validity.

Recording the length and width of each structure preserves the ability to select size fractions out of the total structure count that may be of special interest. Examples of size fractions likely to be of special interest include the PCME fraction (defined as structures longer than 5 μm and thicker than 0.25 μm) or "Stanton" fibers (defined as structures longer than 8 μm and thinner than 0.25 μm).

4.2. PRECISION REQUIREMENTS

To support a risk assessment, the precision of this method should be sufficient to delineate spatial and temporal trends in the field data collected at a Superfund site. Primarily, this means distinguishing among environmental concentrations of asbestos attributable to local sources at a site from concentrations associated with general background. In the absence of site-specific information, it is assumed desirable to distinguish with high confidence a factor of five difference (half an order of magnitude) between concentrations. The purpose of this assumption is simply to define a target performance requirement from which the method could be developed. However, numerous sources of variation associated with asbestos measurement potentially contribute to the uncertainty of an analytical result so that a specific, desired level of precision may be difficult to achieve.

In practice, the precision of a method for the determination of asbestos is limited by several factors including the distribution of asbestos on sample filters, the characteristics of sampling and analysis tools, and the subjectivity of the analyst. In the absence of a database sufficient to establish levels of uncertainty (and, hence, the ability to quantify precision), a lower limit to the number of structures that must be counted in a measurement to achieve a desired level of precision may be estimated from consideration of the distribution of asbestos on an analytical filter, ignoring variation introduced by other factors. The variation potentially introduced by these other factors would tend to increase the minimum number of

structures that must be counted to achieve a desired level of precision. A more detailed discussion of the factors affecting precision can be found in the literature (see, for example, ISO 1981).

Assuming, as suggested above, that it may be important to distinguish concentrations that differ by a factor of five, the minimum number of structures that must be counted to achieve the defined level of precision can be estimated as follows. If it is assumed that structures deposited on a filter exhibit a Poisson distribution and that analytical contamination is zero so that observed asbestos represents sampled asbestos, the test statistic for evaluating whether two means ("m" and "n") can be distinguished is:

$$t = (m - n)/(m + n)^{1/2}.$$

The object is to find the lowest value of the two parameters (m and n) such that m = 5n and the difference between m and n is significant based on the standard normal distribution to the Poisson (Miller and Freund 1965). For n = 1 and m = 5, t = 1.6 which is not quite significant at the 5% level (where the cutoff is 1.65). For n = 2 and m = 10, t = 2.3, which is significant at the 5% level. Thus, since the variability in structure counts is probably larger than that predicted by the Poisson distribution due to contributions from other factors, it is reasonable to assume that a minimum of 10 structures need to be counted at the concentration of interest to distinguish concentrations that differ by a factor of five.

Determinations based on multiple samples can be based on fewer counts per sample as long as the precision of the aggregate array of samples is comparable to the precision indicated above for single sample determinations. This requirement applies both to the count of asbestos structures of all lengths and to the count of asbestos structures longer than 5 μm. However, these two size fractions must be counted separately to provide a statistically balanced representation (Sebastien et al, 1982).

NOTE

Although the foundation for the above assumptions may be a subject of some debate, the practical impact of the application of these assumptions to the development of the method were minimal (see Section 4.3).

As indicated above, method precision is also limited by instrument characteristics and analyst subjectivity, among other factors. To minimize the extent of variation contributed by these two factors, instrument settings and characteristics must be specified in the method, and counting rules must be specified sufficiently to minimize the opportunity for subjective decisions by the analyst.

4.3. SENSITIVITY REQUIREMENTS

Analytical sensitivity is defined as the estimated airborne concentration corresponding to the observation of one asbestos structure. The required analytical sensitivity for this method may be defined by considering the range of concentrations over which measurement is likely to be required.

Based on a qualitative evaluation of the data presented in Section 3.5, estimates of median background concentrations range between 0.5 and 5 s/L for total asbestos structures and 0.02 to 0.2 s/L for asbestos structures longer than 5 μm. Such median concentrations are reasonable targets for analysis because they likely represent the concentrations above which contributions from local sources may be distinguished.

To achieve the desired precision, as indicated in section 4.2, the assumed requirement for this method is the detection of 10 structures at the target concentrations specified. However, this requirement must be tempered against the probability that a particular asbestos concentration measured in the environment includes contributions from anthropogenic contamination over local background. At the low end of the range of median concentrations reported for background, it is highly unlikely that such a concentration may be attributed to anthropogenic contamination. At the high end of the range of background concentrations, however, the probability that the measurement of such a concentration represents contamination from anthropogenic sources is high. At the high end of the range for background, therefore, it may be important to distinguish among small changes in measured concentrations such as might occur between upwind and downwind samples in the vicinity of a potential source.

Setting the analytical sensitivity for this method at 0.5 s/L for total structures and 0.02 s/L for structures longer than 5 μm, achieves the dual purposes of providing sufficient sensitivity to measure concentrations down to levels at which anthropogenic contamination is unlikely while providing that at least 10 structures will be counted when measured concentrations fall into a range where it is important to distinguish among small differences in concentrations. However, it is also important to consider analytical background.

Should asbestos be observed during analysis, it is generally important to distinguish whether such asbestos originated in the sampled medium or if it was introduced as contamination during analysis. Asbestos that can be attributed to the sampled medium is generally considered to have been "detected". Thus, a detection limit is defined as the smallest measurement that is unlikely (probability less than a specified value) to be due entirely to contamination from sources other than the air being sampled. Detection limits are generally quantified by considering the magnitude and frequency of occurrence of the analytical background associated with a particular method.

However, unless the distribution of analytical background is known, detection limits are difficult to quantify. Consequently, an alternate method to account for analytical background is incorporated into this method: a statistical test to distinguish blank measurements from sample measurements. Factors affecting analytical background are addressed in Section 6.3.

As addressed in Section 4.2, it should be emphasized that the desired analytical sensitivity defined above does not have to be achieved for individual samples if such samples are part of a set of multiple samples that were collected so that they are representative of the same sampling environment. Under such conditions, it is sufficient that the desired analytical sensitivity be achieved by the aggregate of the set of samples as a whole. For example, assuming that 10 samples were collected in a manner assuring that they represent the same sampling environment, analyzing each individual sample so that the analytical sensitivity of the measurement is 5 s/L, yields an analytical sensitivity for the arithmetic mean of the 10 samples of 0.5 s/L.

4.4. REPORTING REQUIREMENTS

It is necessary to understand the potential sources of variation in a measurement before the results of an analysis can be properly interpreted. Sources of variation that potentially contribute to the result of an asbestos measurement include particularly the location of the subsection selected for analysis on the TEM specimen, the characteristics of the instrument used in the analysis, and the subjectivity of the analyst. Variation introduced by each of these factors means that there is a probability that the results of two or more consecutive measurements obtained from the same sample may differ within a finite range. The degree of variation introduced by such factors to the results of a measurement obtained using a specific method is usually represented by specifying a set of confidence limits in association with each reported measurement result. Consequently, rules for constructing appropriate confidence limits are incorporated as part of this method (see Appendix E of The Method, Part 1 of this report).

In addition to factors that potentially contribute to variation in the result of the measurement of a specific specimen, asbestos observed during the analysis may have originated either in the air sampled or may have been introduced from contamination during any of several phases of sample handling, preparation, and analysis (see Section 4.3). Therefore, it is important to distinguish contributions to a measurement that may be attributed to sampled asbestos from contributions that may be attributed to analytical background. Consequently, rules for conducting a statistical test to distinguish sampled asbestos from analytical background are incorporated as part of this method (see Appendix E of The Method, Part 1 of this report).

4.5. METHOD SPECIFICATIONS

The method presented in Part 1 of this report is a method where samples are collected on membrane filters and analyzed by TEM. None of the published TEM methods satisfy all of the above requirements; accordingly, several alternatives had to be evaluated to develop a procedure that is capable of satisfying the entire set of method requirements defined above. The following procedures for sample collection, preparation, handling, and analysis were combined.

5. OVERVIEW OF METHOD

In the method presented in Part 1 of this report, samples are collected and prepared for TEM examination by either one of two techniques. The majority of air samples (denoted Phase 1 samples) will be analyzed using an indirect procedure for preparation of TEM specimens, optimized to provide the required analytical sensitivity and precision. A small number of samples (denoted Phase 2 samples) will be collected in such a way that they can be analyzed using both indirect and direct procedures for preparation of TEM specimens, to allow comparisons to be made between the results from the two specimen preparation procedures. TEM examination procedures used for the two sets of samples also differ.

5.1. SAMPLE COLLECTION

A sample of airborne particle is collected by drawing a measured volume of air through a 25 mm diameter, 0.45 μm pore size MCE membrane filter by means of a pump. Air volumes collected on Phase 1 samples will be maximized. Air volumes collected on Phase 2 samples will be limited to provide optimum loadings for filters to be prepared by a direct-transfer procedure.

5.2. SAMPLE PREPARATION

TEM grids will be prepared according to either 5.2.1 or 5.2.2 below.

5.2.1. Indirect TEM Specimen Preparation

This preparation will be applied to all Phase 1 and Phase 2 samples. Half of the filter is ashed in a low-temperature plasma asher. The residual ash is ultrasonically dispersed in freshly-distilled water. The suspension is acidified using hydrochloric acid, and immediately filtered through a 25 mm diameter, 0.1 μm pore size MCE filter. The filter is dried and the filter structure is collapsed using a mixture of dimethyl formamide, acetic acid and water. A thin film of carbon is evaporated onto the collapsed filter surface and small areas are cut from the filter. These areas of filter are supported on TEM specimen grids and the filter medium is dissolved away by a solvent extraction procedure.

NOTE

An alternate procedure for indirect preparation, which incorporates washing the deposit off of MCE filters and ashing of the wash-suspended deposit, may be substituted into this method subject to the results of a pilot study.

5.2.2. Direct TEM Specimen Preparation

One quarter of the remaining filter sections from all Phase 2 samples will be prepared by this procedure. The quarter filter is collapsed using a mixture of dimethyl formamide, acetic acid and water. The collapsed filter is

etched for a short time in a low temperature plasma asher to remove the surface layer of filter polymer which may have encapsulated asbestos structures during the collapsing procedure. A thin film of carbon is evaporated onto the collapsed filter surface and small areas are cut from the filter. These areas of filter are supported on TEM specimen grids and the filter medium is dissolved away by a solvent extraction procedure.

5.3. ANALYSIS

The TEM specimen grids are examined at both low and high magnifications to check that they are suitable for analysis before carrying out a quantitative examination on randomly-selected grid openings. In addition to isolated fibers, ambient air samples often contain more complex aggregates of fibers, with or without equant particles. Some particles are composites of asbestos fibers with other materials. Individual fibers and more complex structures are collectively referred to as "asbestos structures". A coding system is used to record the type of fibrous structure, and to provide the optimum description of each of these complex structures.

The method requires that separate examinations be made for asbestos structures of all sizes (incorporating asbestos fibers with lengths greater than 0.5 μm) and for asbestos structures longer than 5 μm. In both cases, asbestos structures are defined as structures containing components exhibiting mean aspect ratios equal to or greater than 5:1. This TEM examination procedure allows for specification of a lower analytical sensitivity for the measurement of the concentration of asbestos structures longer than 5 μm.

In the TEM analysis, electron diffraction (ED) is used to examine the crystal structure of a fiber, or fibrous components of complex structures, and the elemental composition is determined by energy dispersive X-ray analysis (EDXA). For a number of reasons, it is not possible to identify (confirm the mineralogy of) each structure unequivocally and structures are classified according to the techniques that have been used to identify them. A simple code is used to record the manner in which each structure is classified.

The classification procedure is based on successive inspection of the morphology, the electron diffraction pattern, and the energy dispersive X-ray spectrum. Confirmation of the identification of chrysotile is only by quantitative ED, and confirmation of amphibole is by a combination of quantitative EDXA and quantitative zone-axis ED.

Several levels of analysis are specified, the higher levels providing a more rigorous approach to the identification of fibers. The procedure permits a minimum required asbestos identification procedure to be defined on the basis of previous knowledge, or lack of it, about the particular sample. Attempts are then made to achieve this defined minimum procedure for each asbestos structure, and the degree of success is recorded for each. The two codes remove from the microscopist the requirement to interpret observations made during the TEM examination, and allow this evaluation to be made later without the requirement for re-examination of the TEM specimens.

The lengths and widths of all classified asbestos structures are recorded. The number of asbestos structures found on a known area of the TEM specimen grids, together with the equivalent volume of air filtered through this area, are used to calculate the airborne concentration in asbestos structures/liter of air.

This method specifies minimum analytical sensitivities of 0.5 s/L and 0.02 s/L for the measurements of asbestos structures of all sizes (incorporating structures longer than 0.5 μm) and asbestos structures longer than 5 μm, respectively. In both cases, asbestos structures are defined as structures containing components exhibiting mean aspect ratios equal to or greater than 5:1.

It will not always be possible to achieve the defined analytical sensitivities, because the volume of air that can be sampled is dictated by the nature and concentration of the suspended particulate in the atmosphere being sampled. To some degree, this limitation can be overcome by selective concentration of asbestos structures during the specimen preparation procedures and by examination of a larger area of the TEM specimens. However, the ease and cost of achieving a specific value for the analytical sensitivity will vary from sample to sample.

6. METHOD COMPONENTS

The detailed protocols incorporated in this method were selected based on the state of knowledge of their presumed capabilities and limitations. In some cases, data are lacking so that additional laboratory work is needed to properly evaluate the efficacy of the proposed procedures.

6.1. FACTORS AFFECTING CHARACTERIZATION OF ASBESTOS STRUCTURES

The proper characterization of asbestos structures requires use of an analytical technique capable of resolving the thinnest asbestos structure and capable of distinguishing asbestos from non-asbestos minerals. Counting rules must be designed to facilitate distinguishing fibers, bundles, clusters, and matrices. Various fractions of potential interest must be easily extracted from analysis results and counts must be recorded in sufficient detail to allow later reinterpretation.

6.1.1. Analytical Technique

Because one of the goals of environmental sampling and analysis for the determination of asbestos is to provide a measurement that is comparable with published risk factors, which are expressed in terms of PCM counts, an obvious question that arises is whether to simply use PCM as the analytical technique for the analysis of environmental samples. However, environmental samples can not be properly characterized using PCM due to a combination of the limitations of PCM and the characteristics of environmental dusts. It is unfortunate that PCM can not be used to evaluate environmental samples because PCM is significantly less expensive than TEM.

PCM is inherently less sensitive than TEM at detecting asbestos structures. The sensitivity of PCM is limited both by increasing obscuration as filters become more loaded and by increasing observer-dependent variation as fibrous structures become less concentrated on the filter (Chatfield, 1985a). In one round-robin study of PCM laboratories (Crawford, 1985), observer-dependent variation approached a factor of 300.

Observer-dependent variation may be due to a combination of instrument limitations, differences in preparation techniques, and the subjective judgments of the analyst. In common with any technique in which measurements are made close to the lower limits of sensitivity, PCM results vary as a function of the condition of the instrument. Differences may be due to such factors as misalignment of the phase ring, failure to scan the full depth of focus, and differences in the interpretation of irregular fibers. Evidence for such variation is provided in several studies including the following:

(a) fiber counts made on identical samples were shown to increase by a factor of two if the count was made at twice the magnification (Lynch et al 1970);

(b) counting with a graticule verses full field counting increases counts by a factor of three (Beckett et al, 1976);

(c) for chrysotile, novice counters frequently count only 25% of the fibers observed by experienced counters (Beckett and Attfield 1974);

(d) there are large inter-laboratory differences in counts of the same sample (Beckett and Attfield 1974).

In contrast to the last observation, inter-laboratory agreement among TEM results has been achieved without the extensive inter-laboratory discussions that have been required to normalize the optical work.

The practical limit of sensitivity for PCM is approximately 10 s/L at the maximum total dust loading that can be tolerated in occupational samples (Chatfield, 1985d). PCM can be applied in occupational settings because contaminated work sites tend to exhibit elevated asbestos concentrations in relationship to total dust concentrations. This allows asbestos to be deposited on the filter at concentrations within the range of the sensitivity limits of PCM at the same time that dust deposited on the filter remains below the level at which obscuration precludes a proper PCM count.

In contrast, the ratio of asbestos structures to total dust particles is generally lower in environmental samples than in occupational samples. The absolute concentration of asbestos also tends to be lower in environmental samples. The lower ratio of asbestos to dust limits PCM sensitivity so that the typical asbestos concentrations found in environmental samples (ranging between 0.01 and 0.1 PCME s/L) can not be detected. The net result, in the absence of other factors, is the creation of large numbers of false negative analyses when PCM is used to analyze environmental samples.

PCM analysis of environmental samples can create false positive results due to the inability of PCM to distinguish asbestos structures from non-asbestos structures. Non-asbestos fibers (such as cellulose and gypsum) are ubiquitous in many environmental settings. In fact, asbestos generally constitutes a minority of the total fibrous structures typically present in environmental samples (Chatfield, 1985d). Because they can not be distinguished from asbestos, non-asbestos fibers that exhibit the appropriate dimensional criteria will be included as asbestos in a PCM count, creating falsely inflated asbestos counts.

The inability of PCM to track asbestos concentrations in environmental samples has been documented in numerous studies. In a comparison of four sampling and analysis methods (Gibbs et al, 1980), three EM methods generated data that showed trends in asbestos concentrations, which decreased with distance from known sources. The one PCM method included in the study was not capable of distinguishing between high environmental asbestos concentrations

near sources and low concentrations at background locations. This is presumably because the majority of structures observed by PCM were not asbestos.

Asbestos measurements by TEM and PCM were also compared in a series of studies of asbestos abatement projects (Tuckfield et al, 1988; Chesson et al, 1985; and Karaffa et al, 1986). Both indoor air and outdoor (ambient air) were monitored in these studies. In none of these studies was PCM capable of reliably determining indoor or ambient concentrations. In fact, it was reported in one study (Chesson et al, 1985) that PCM counts appeared to relate to human activity rather than total asbestos (as measured by TEM), suggesting that non-asbestos fibers were interfering with the analysis.

Perhaps the most important limitation associated with PCM is the inability to detect asbestos characteristics that best relate to biological activity and, therefore, determine risk (see Sections 3.1 and 3.2). Due to the limited resolution of PCM, only structures longer than 5 μm with diameters exceeding 0.25 μm can be counted. Therefore, the long, thin asbestos structures (which are believed to constitute the most biologically active structures) can not be resolved by PCM. In addition, PCM is incapable of resolving the internal detail of the structures counted so that it is frequently not possible to distinguish aggregates from simple fibers. Thus, PCM is not capable of characterizing many of the aspects of asbestos exposures generally believed to affect biological activity.

Over the range of conditions typical of environmental samples, PCM is not capable of providing measurements of asbestos in environmental settings that relate meaningfully to risk factors in occupational settings. PCM can not be used to properly evaluate environmental samples because:

(a) it is not sufficiently sensitive to detect asbestos at concentrations typical of environmental samples;

(b) it suffers from observer-dependent variation to a degree that is unacceptable for the level of precision required for environmental samples;

(c) it is not capable of distinguishing asbestos from non-asbestos structures, a requirement that is critical to the proper analysis of environmental samples; and

(d) it is not capable of distinguishing among the various types and sizes of asbestos structures that impact the biological activity of asbestos.

Therefore, TEM is the only analytical technique capable of characterizing asbestos exposures in a manner that is consistent with the method requirements defined in Section 4. TEM is the analytical technique to be used for the determination of asbestos in this method.

NOTE

SEM was also evaluated and eliminated from consideration for use in this method (see Section 3.7).

6.1.2. Magnification/Resolution

To properly characterize asbestos as dictated by the method requirements presented in Section 4, it is necessary to resolve all asbestos structures present in an airborne dust. The thinnest asbestos fibrils that retain the recognizable crystalline character of asbestos (chrysotile fibrils) generally range in diameter between 0.02 and 0.04 μm (see Section 3.4). Therefore, the resolution of the method must exceed 0.02 μm in order to detect all of the asbestos structures on a sample specimen. A magnification of 20,000 is more than sufficient to resolve asbestos structures over the total range of lengths and diameters common to asbestos. A magnification of 10,000 is sufficient to resolve TEM structures longer than 5 μm over the entire range of diameters common to asbestos. The advantage of conducting the scan for long structures at the lower magnification is the decreased time required and the corresponding cost savings. Thus, magnifications of 20,000 and 10,000 are employed in this method to scan for asbestos structures of all lengths and asbestos structures longer than 5 μm, respectively.

Method requirements presented in Section 4 also indicate that the width of detected asbestos structures must be properly delineated. At a magnification of 10,000, structures 0.02 μm in diameter appear as thick as 0.2 mm on the viewing screen of the TEM, which is easily seen, but which can not be easily distinguished from dimensions that vary by factors of less than 2. Thus, although structures of such dimensions may be detected at the magnification specified, structure dimensions must be characterized at higher magnifications to adequately distinguish among diameters. The objective is to discriminate among diameters that differ by 0.05 μm. Such precision can be obtained at magnifications of 20,000 and greater. The primary purpose for the clear delineation of diameters is to facilitate later classification of candidate size fractions of potential interest for assessing risks from among the structures recorded.

NOTE

The possibility of employing lower magnification (less than 10,000) to detect the long (greater than 5 μm) structure fraction was considered during method development. However, this tends to increase variation in the results due to a combination of instrument variation and differences in operator experience.

Asbestos counts derived at lower magnifications (500 - 2000) appear to vary as a function of magnification and other microscope characteristics. For example, at magnifications typically employed in a PCM analysis, small changes that affect the average visibility of asbestos structures potentially yield large changes in the number of structures

that can be detected. The visibility of small asbestos structures varies from instrument to instrument and with changes in operating conditions. For PCM, a visibility test slide is available for standardizing the visibility of each instrument despite differences in operating conditions. Use of the slide is specified in the method. However, no such test procedure is available for TEM. Consequently, if the TEM is to be used at these low magnifications, there is no mechanism for standardizing the visibility of structures to be counted.

Due to the increased variation in results that generally accompany the counting of asbestos structures at reduced magnification, such an approach for determining the longer structure fractions were not incorporated in this method. Rather, longer fractions of asbestos are characterized by counting structures longer than 5 μm at a magnification of 10,000, characterizing their diameters at increased magnifications, and distinguishing the various fractions of interest in the count by selecting the structures listed on the count sheet that exhibit appropriate lengths and diameters, with diameters determined to a precision of +/- 0.05 μm.

6.1.3. Rules for Counting, Characterization, and Recording

Counting, characterization, and recording rules for this method are designed to provide:

(a) a count of total asbestos structures (individual entities) whose distribution is best described by Poisson statistics;

(b) a count of structure components that is not skewed by an arbitrary cutoff for the number of individually recognizable components present in each structure; and

(c) counts of structures in the range of dimensional fractions that are believed to best relate to biological activity.

To achieve these objectives, several modifications to published procedures are incorporated in this method. First, the criterion for meeting specified counting limits is defined in terms of asbestos structures of all lengths, which represents a count of individual asbestos entities. Structure components are not counted individually. This is to assure appropriate statistical validity. Second, when structure components are counted (to establish an estimate of the total equivalent number of fibers) the count is not arbitrarily truncated at 5 components per structure. Rather, structures exhibiting more than 5 individually recognizable components are specially noted on the count sheet.

6.2. FACTORS AFFECTING SENSITIVITY

For TEM analysis, analytical sensitivity (the concentration corresponding to the observation of a single asbestos structure) is limited by the amount of asbestos that can be deposited on a given area of filter.

The amount (loading) of asbestos that can be deposited on a filter is limited by two factors: sampling constraints and the limit at which a filter becomes too loaded to allow for proper analysis. Although the second of these factors is generally more restrictive than the first, it is also influenced by the type of filter preparation employed. This latter limitation is due, primarily, to the concentration of total dust deposited on the filter in coincidence with the asbestos. Thus, the volume of air that can be usefully collected for asbestos analysis is restricted by the concentration of dust in the air and the quantity of dust that can be tolerated on the filter before analysis is prevented. This limit is higher for filters prepared by an indirect technique than for filters prepared by a direct technique.

The analytical sensitivity is a combined function of the volume of air sampled, the total area of the analytical filter, the dilution or concentration factor introduced during specimen preparation, and the area of the TEM specimen over which asbestos structures are counted. The interrelationship between each of these latter factors determines the relative cost of sampling and analysis.

The relationship between analytical sensitivity, the volume of air sampled, the total area of the analytical filter, the dilution or concentration factor introduced during specimen preparation, and the area of the TEM specimen over which asbestos structures are counted can be summarized by the following relationship:

$$C_s = A_f/(N \times A_g \times V \times F) \qquad (6\text{-}1)$$

where:

C_s = Analytical sensitivity in structures/liter
A_f = Total area of the analytical filter in mm^2
A_g = Area of a TEM specimen grid opening in mm^2
N = Number of grid openings examined
V = Volume of air sampled in liters
F = Concentration factor

The concentration factor, F, simply reflects the dilution or concentration of asbestos structures resulting from an indirect preparation. During indirect preparation, asbestos structures deposited on the original sample filter are dispersed in water and redeposited on the analytical filter. The density of structures on the final filter is a function of the relative area of the two filters and the fraction of the total dispersion that is refiltered:

$$F = A_a \times V_f/(A_f \times V_a) \qquad (6\text{-}2)$$

where:

A_a = Area of filter ashed in mm^2
A_f = Area of analytical filter in mm^2

V_s = Volume of water used to redisperse ashed particles in liters
V_f = Volume of dispersion filtered through analytical filter in liters

For samples prepared by direct preparation, the concentration factor is always unity.

Actually, equation 6-1 is a contraction of a more general equation relating the number of structures counted on a filter to the total airborne asbestos concentration:

$$C = A_f \times S/(N \times A_s \times V \times F) \quad (6\text{-}3)$$

where:

C = Airborne concentration of asbestos in structures/liter
S = Number of structures counted
A_f = Total area of the analytical filter in mm^2
A_s = Area of a TEM specimen grid opening in mm^2
N = Number of grid openings examined
V = Volume of air sampled in liters
F = Concentration factor

The original equation 6-1 can be obtained from equation 6-3 simply by setting the number of structures counted equal to one and substituting the analytical *sensitivity for the airborne concentration of asbestos.*

NOTE

The required analytical sensitivities for total asbestos structures and for structures longer than 5 μm were derived as indicated in Section 4.3 using equation 6-3 to relate target concentrations to analytical sensitivity. The range of target concentrations were derived as indicated in Section 3.5.

6.2.1. Filter Loading

TEM analysis can proceed as long as the majority of the particles on a filter are deposited directly on the filter and do not overlap other particles present. Both asbestos and non-asbestos structures may potentially obscure observation of other asbestos structures, but at concentrations typical of environmental samples, non-asbestos structures (total suspended particulate) generally determine the upper limit to filter loading.

Based on experience, analysis of a TEM grid becomes impossible at a relative coverage of 25%. Analysis is difficult when the fraction of the specimen grid covered by particles exceeds 10%. Based on private conversations with several microscopists, experience suggests that a loading of 10 $\mu g/cm^2$ on

a filter will result in 10% of the filter area being covered with particles. The data of Steen et al (1983) is in general agreement with this estimate but indicates a range of potentially appropriate values. Steen et al (1983) report that the maximum tolerated dusts on filters are in the range of 50 $\mu g/cm^2$ for filters prepared by direct preparation and 250 $\mu g/cm^2$ for filters prepared by indirect preparation. Higher loadings can be tolerated by indirect preparation because much of the interfering particle is removed during filter preparation.

The reported variation in tolerable loading is not surprising. The fraction of filter covered per unit weight of deposit varies as a function of the average size and density of the particles. For spherical particles, the fraction of coverage expected as a function of the size of the particles can be estimated by taking the quotient of the calculated mass of a particle (the product of volume and density) and the calculated circumference of the particle. The majority of the non-asbestos fraction of any dust found in the environment is expected to be composed primarily of particles that are approximately spherical. Based on such calculations, 10% of the filter will be covered at a loading of 10 $\mu g/cm^2$ when the average particle diameter on the filter is greater than approximately 1 μm at an average particle density of 2.3 g/cm^3 (the average density of many silicates). However, in different environments, this value may vary significantly. Generally, the smaller the particles, the less the mass that can be tolerated before the filter becomes overloaded. Much of the variation in loadings reported as tolerable is likely due to differences in the average size of the particles present in the dust.

6.2.2. Sampled Air Volumes

The volume of air that can be collected during asbestos analysis is limited both by the technical constraints of the sampling system and by the tolerable limit of total dust that may be deposited before asbestos analysis becomes impossible due to overloading (as described in Section 6.2.1). The volume of air that can be collected per unit area of filter is related to the tolerable loading by the airborne concentration of total suspended particulate (see Section 3.6). However, because of the extent of variation in the concentration of airborne particulate (TSP), it is difficult to derive usable general averages suitable for deriving the tolerable upper limit to the volume of air that can be sampled for an asbestos analysis. At the same time, some data are available from limited published studies that provide an indication of reasonable upper limits.

(a) In a series of studies of airborne asbestos both in the vicinity of the Quebec mines and at remote urban and rural locations, Sebastien et al (1984 and 1986) report that on the order of 1 m^3 of air may be collected per cm^2 of filter for filters that are prepared by a direct technique. The tolerable limit for indirectly prepared samples is approximately an order of magnitude higher.

(b) In a study of building atmospheres (Chatfield, ed. 1985) an optimum loading of 0.3 m^3 of air per cm^2 of filter was recommended when filters were to be prepared by a direct technique.

(c) In one of a series of abatement studies, Tuckfield et al (1988) report that 2 of 24 filters could not be analyzed due to overloading when 0.6 m^3 of air was collected per cm^2 of filter and the filters were prepared by a direct technique.

(d) In a study of rural, suburban, and urban air, Chatfield (1983b) reports that filters prepared by direct analysis were loaded to 1.2 m^3/cm^2.

(e) At the South Bay Superfund site in California, only a small number of filters out of a total of more than 60 filters could not be analyzed due to overloading when 1.1 m^3 of air was collected per cm^2 of filter and the filters were prepared using a direct technique (unpublished results).

(f) Burdett (1985a) reports from a study in England that directly prepared samples of amosite and crocidolite from abatement sites could be loaded for analysis to a level of 0.5 m^3/cm^2.

The above data indicate that slightly more than 1 m^3 of air can be collected per cm^2 of filter before environmental samples become overloaded so that analysis is prevented for filters prepared by a direct technique. The corresponding limit for abatement samples appears to be on the order of 0.5 m^3 per cm^2 of filter. Better estimates for optimum loading can be derived from data on local TSP concentrations, when such information is available.

6.2.3. Area to be Scanned for Analysis

Equation 6-1 may be graphed to depict the combination of sampling requirements and analytical requirements necessary to obtain a particular analytical sensitivity. The two curves presented in Figure 6.1 depict the relationship between the volume of air to be sampled and the number of grid openings to be counted to achieve an analytical sensitivity of 0.5 s/L and 0.02 s/L. These, in turn, represent the analytical sensitivities required to monitor, respectively, total asbestos structures and long asbestos structures in environmental samples (see Section 4.3). To generate the figure, it is assumed that an average grid opening size is 0.0081 mm^2, the area of the analytical filter is 25 mm^2, and the concentration factor is unity. The vertical axis for the curve representing an analytical sensitivity of 0.02 s/L is presented on the right vertical axis of the figure while the vertical axis for the 0.5 s/L curve is presented on the left axis.

Based on cost considerations, it is optimal to minimize the number of grid openings to be counted during analysis. Experience also indicates that analysts tire more rapidly when forced to count an excessive number of grid openings on a single sample, so that precision suffers. It is recommended

that no more than 20 grid openings be counted at the magnification of 20,000 recommended for characterizing total asbestos structures. Similarly, 100 grid openings is a recommended target for the 10,000 magnification employed to characterize long asbestos structures.

Based on the information presented in Figure 6.1, the recommended limit for counting grid openings can be satisfied by collecting a sample volume of 5,000 liters for characterizing total asbestos structures. For a 25 mm filter, this corresponds to 1.2 m^3/cm^2, which in most cases probably will not cause excessive overloading of filters prepared by direct analysis. However, a sample volume of 25,000 liters would be necessary to remain within the recommended grid opening count for long asbestos structures, corresponding to a loading of 6.5 m^3/cm^2. This clearly exceeds the recommended maximum loadings defined above for filters prepared by direct techniques. Therefore, indirect preparation is recommended to keep the number of grid openings that must be counted within reason (see Section 6.2.4).

NOTE

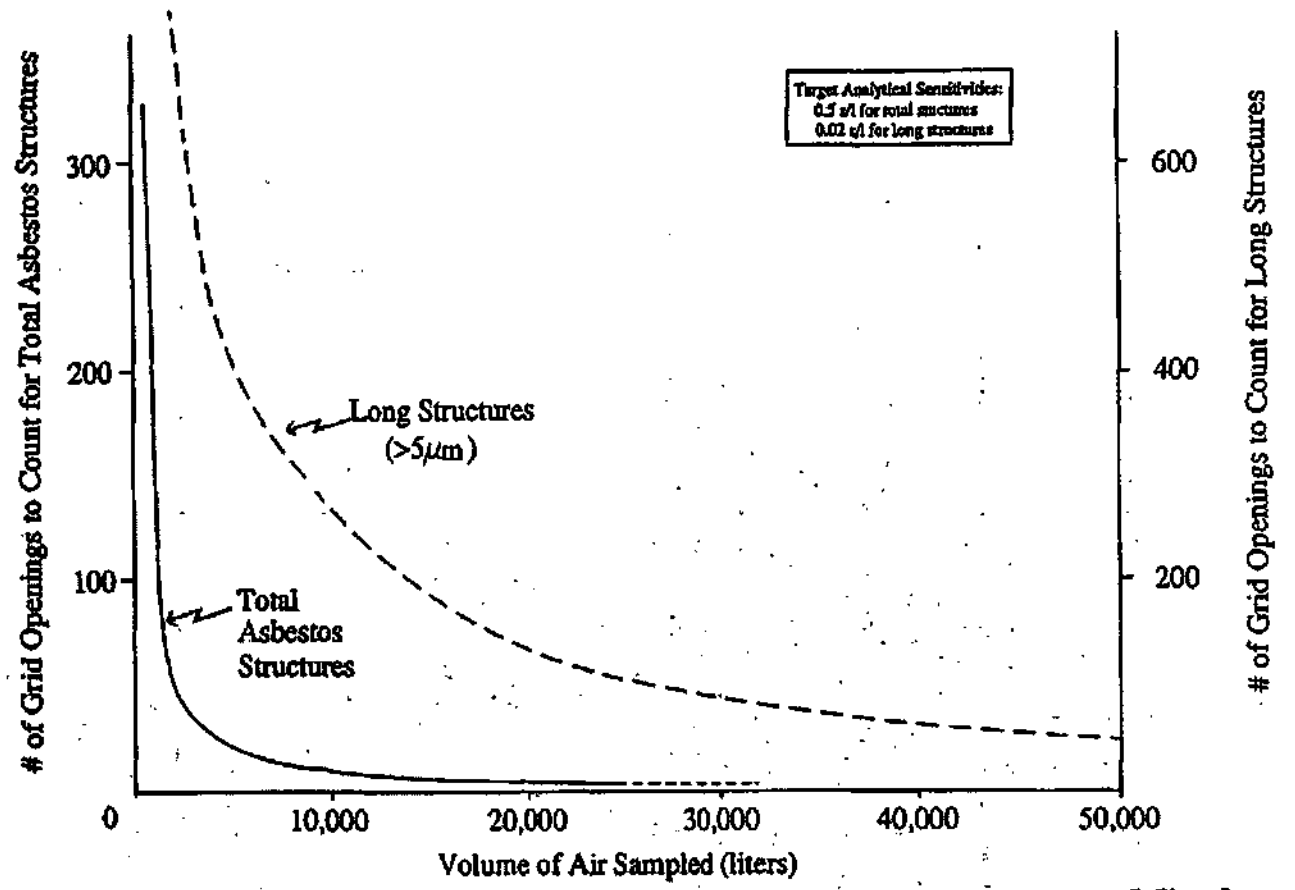
These calculations ignore the limitations imposed by filter blank contamination, which are addressed in Section 6.3.1.

6.2.4. Filter Preparation

To provide the required analytical sensitivity at asbestos levels typically found in the environment, it is necessary either to selectively concentrate asbestos, or to count structures over a much greater area of a TEM specimen grid than has traditionally been required. To the extent that it can be employed, selective concentration is the least costly of the alternatives for increasing sensitivity. The desired degree of concentration requires filter loadings in excess of what can generally be analyzed using direct-transfer methods for TEM specimen preparation. Consequently, an indirect technique for TEM specimen preparation is employed in this method.

In most ambient environments, a proportion of suspended particulate is organic, consisting of soot, spores, pollens and other debris from vegetation. Organic materials such as these can be removed from the analysis by low-temperature ashing. It is also common to find substantial numbers of calcium sulfate fibers (gypsum) in airborne particles collected in buildings and urban environments, and particularly in samples collected where demolition or construction work is in progress. The fibers are readily released when plasters and cement products are disturbed. Gypsum is also generated on air filters as a consequence of the chemical reaction between collected calcium carbonate particles and atmospheric sulfur dioxide. Gypsum can be removed by dissolution in water. Another major component of exterior atmospheres is limestone, either as calcite or dolomite. Such carbonates, along with some oxide species, can be removed by extraction with hydrochloric acid.

FIGURE 6.1: THE RELATIONSHIP BETWEEN ANALYTICAL SENSITIVITY, SAMPLED AIR VOLUME, AND THE NUMBER OF GRID OPENINGS



NOTE

Since the mineralogy of airborne dusts is related to local geological and meteorological conditions, the ability to concentrate asbestos from dusts by removal of soluble and ashable components varies as a function of location.

Removal of a large proportion of the suspended particulate by use of these techniques permits the asbestos present in the sample to be concentrated on to a smaller area of the TEM specimen. Consequently, the area of the TEM specimen that must be examined to achieve a particular analytical sensitivity is proportionately reduced. Also, many of the non-asbestos fibrous structures normally found in a directly-prepared TEM specimen, each of which must be identified and rejected from the asbestos structure count, do not appear on the indirectly-prepared TEM specimen.

A selective concentration of the asbestos structures, incorporating low temperature ashing, re-suspension in water, and acidification with HCl is therefore capable of removing substantial amounts of interfering particle from the analysis, and, for a particular analytical sensitivity, reduces the area of the TEM specimens that must be examined. If these procedures are carried out correctly, no chemical or crystallographic degradation of asbestos can be detected by routine methods of TEM analysis.

Indirect TEM specimen preparation techniques offer several advantages over direct preparation:

(a) air samples can be collected without regard to the amount of deposit on the filter surface. The filter loading can be adjusted in the laboratory to provide satisfactory TEM specimens;

(b) interfering particulate material can be completely or partially removed through a combination of dissolution and oxidation (ashing);

(c) the uniformity of distribution of asbestos structures on the filters to be analyzed is improved.

The middle of the above advantages has been addressed above. The first of the above advantages is addressed in greater detail in Section 6.2.1. The last of the advantages listed is addressed in Section 6.4.3.

Despite the advantages, use of indirect preparation techniques must be considered carefully because they also suffer from the following disadvantages:

(a) the size distribution of asbestos structures is modified;

(b) there is increased opportunity for fiber loss or introduction of extraneous contamination;

(c) when sample collection filters are ashed, any fiber contamination in the filter medium is concentrated.

The second of the above disadvantages can be minimized by maintaining a clean laboratory and following proper experimental procedures (see Section 6.3). The last of the above considerations is addressed in Section 6.3.1. The extent that structure size distributions are modified during the indirect preparation of a TEM specimen has been addressed in several studies.

(a) In a set of studies, Sebastien et al (1984 and 1986) found that samples prepared by an indirect technique exhibit 20 to 50 times as many short fibers as paired specimens prepared by a direct technique. Changes in the number of structures longer than 5 μm were not significant. It was noted in the study that the absolute concentration of aggregates encountered remained the same whether samples were prepared by the indirect or the direct technique. However, the average dimensions of the aggregates encountered on the indirectly prepared specimens appeared to be smaller than those observed on the directly prepared specimens. Because of the increase in the number of short structures, the fraction of total structures represented by long structures decreased. Possible sources of the increased number of short structures presented in the paper are losses during analysis of the directly prepared specimens due to obscuration, liberation of short structures from soluble matrices dissolved during indirect preparation, and disassociation of short structures loosely bound to the larger aggregates.

(b) Chatfield (1985d) in a comparison of specimens prepared by the direct and the indirect techniques found that structures shorter than 2.5 μm were 4 to 20 times more plentiful on specimens prepared by the indirect technique than on specimens prepared by the direct technique. The conversion factor for structures longer than 2.5 μm is less than a factor than 2. He also noted agreement in results between direct preparations of polycarbonate and MCE filters and indirect preparations of polycarbonate and MCE filters. For the indirect preparations, the deposits on polycarbonate filters were washed into suspension while deposits on MCE filters were ashed. Data were not reported for the process of washing MCE filters.

(c) In another study, Chatfield (1985b) reports that for single fibril suspensions of chrysotile, there is no difference in results obtained from specimens prepared by direct and indirect techniques either on polycarbonate or MCE filters. For suspensions that contain aggregates, which are more representative of the types of samples likely to be encountered in the environment, PCME counts were comparable for direct and indirect preparations on both polycarbonate and MCE filters but counts of structures shorter than 2.5 μm increased on indirectly prepared specimens. Chatfield

did not comment on the fate of long, thin structures in this study. It is unknown whether the source of the additional short structures observed following indirect preparation is from disaggregation or disintegration.

(d) In a study reported by Cook and Marklund (1980), the ratio of amphibole asbestos structure counts on paired specimens prepared, respectively, directly and indirectly varied between 0.3 and 5.1 with a mean of 2. Results from the direct and indirect preparations correlated to 95% significance. Cook and Marklund also report that results with chrysotile are much more problematic. Chrysotile samples that contained a high fraction of aggregates exhibited counts varying by four orders of magnitude from different laboratories (possibly due to lack of standardization). Unfortunately, there is insufficient documentation in this paper to determine the exact conditions under which specimens were prepared. However, they do report significant increases in the total mass of chrysotile observed on indirect preparations subjected to "harsh" preparation conditions.

NOTE

There may be a range of problems contributing to the observations presented in this study. At the time the study was conducted, little was known of the effects of organic materials on aqueous suspensions of asbestos so that four orders of magnitude variation could well have resulted from loss of asbestos from suspension onto container walls, etc.

(e) In a series of studies of abatement sites, Tuckfield et al (1988), Hatfield et al (1988), and Chesson et al (1985), directly prepared specimens consistently generated lower structure counts than indirectly prepared samples. Too few structures were counted on any of the preparations to derive size specific information. However, they did report that counts from paired direct and indirect preparations did not correlate.

(f) Chesson et al (1989b) performed a statistical evaluation of data from five independent studies where samples prepared, respectively, by direct-transfer and by indirect-transfer techniques could be paired for comparison. Results indicate that samples prepared by an indirect technique uniformly exhibit higher total structure counts and higher total fiber counts. Unfortunately, the data were not sufficient to determine the effect of indirect preparation on specific size fractions such as the long structures.

The Chesson et al study also indicates that, although counts on directly and indirectly prepared specimens are not strictly proportional across studies, they both track similar trends in

concentrations so that the choice of preparation method is unlikely to affect comparisons between samples from the same study. A statistically significant relationship was found in all five studies between the two sets of specimens (direct and indirect preparations). Quantitative relationships between samples prepared by the two techniques are expected to depend both on the characteristics of the dusts sampled and on the specifics of the sampling and analysis protocols employed in a particular study.

(g) In a study of amosite, Burdett (1985b) reported a high correlation between samples prepared by a direct technique and those prepared by an indirect technique. The observed ratio of indirect to direct varied between 1.7 to 1. The slight increase in counts on the indirectly prepared specimens was attributed to splitting of aggregates. Correspondingly, the average length and diameter measured for structures observed on indirect preparations were reported to decrease slightly. It is noted that surfactant was used during indirect preparation in this study.

Based on these findings, specimens prepared by an indirect technique are appropriate for comparing relative asbestos concentrations at a site. In addition, such specimens are likely to yield representative counts of long structures that correspond to counts on samples prepared by direct transfer, provided that gentle conditions are employed during the preparation. However, counts of asbestos structures of all sizes (which include short structures) or counts of individual components within complex asbestos structures of any size fraction likely vary when performed on specimens prepared by the indirect technique compared to specimens prepared by the direct technique. In either case, quantitative relationships between directly and indirectly prepared specimens are likely to be study specific due to a dependence on the sampling and analysis protocols employed.

The magnitude of differences between counts of long, thin structures (potentially the most biologically active) performed on directly prepared and indirectly prepared specimens has not been adequately addressed. Relationships between counts of asbestos structures, other than single, long fibers, obtained from specimens prepared by a direct technique or an indirect technique, respectively, appear to depend on the fraction of aggregates present in the asbestos dust. Therefore, they are likely to be site specific as well as study specific.

The question as to whether direct or indirect specimen preparation yields the "correct" result (in terms of representing biological activity) is currently unresolved. It can be argued that the direct methods yield an under-estimate of the asbestos structure concentration because many of the asbestos fibers present are concealed by other particulate material with which they are associated. Conversely, the indirect methods can be considered to

yield an over-estimate because some types of complex asbestos structures disintegrate during the preparation, resulting in an increase in the numbers of structures counted.

Because of critical limitations imposed by the sensitivity requirements for sampling environmental asbestos and because there is little reason to believe that direct preparations track relative risks better than indirect preparations, an indirect preparation technique is incorporated in the method presented in Part 1. However, assessing absolute risks requires that current measurements be compared with risk factors derived from historical measurements (PCM) that correspond to direct preparations. Therefore, the method also incorporates a procedure for evaluating the relationship between results from directly and indirectly prepared samples for any study employing this method.

A small subset of sample filters will be split so that one half of each filter may be prepared by a direct and one half by an indirect preparation technique to allow detailed evaluation of the effects of preparation. The total loading on this subset of filters will be restricted to assure that the level of dust can be tolerated by analysis of the directly prepared filter section. Consequently, a larger number of grid openings will be counted on these samples to achieve desired sensitivity and precision.

NOTE

The effect of an indirect preparation is heavily dependent on the specific procedures employed. A detailed protocol has been incorporated into this method.

As indicated by the Burdett study (1985b), variation in fiber counts between samples prepared by direct techniques versus indirect techniques appear to occur primarily for preparations of chrysotile. Preparations of amphiboles likely are relatively unaffected by the choice of preparation technique.

6.2.5. Existing Analytical Methods

Equation 6-3 is graphed in Figure 6.2 to illustrate the relationship between sampling and analysis constraints and the concentrations of airborne asbestos. In the log-log plot of Figure 6.2, the vertical axis represents the volume of air sampled, the horizontal axis represents the number of grid openings counted, and the diagonal lines represent the airborne concentrations (analytical sensitivities) of asbestos at which at least 1 structure can be expected to be encountered during analysis. Because it is a log-log plot, the hyperbolic curves depicted in Figure 6.1 are represented by straight lines in Figure 6.2. The upper curve corresponds to the required analytical sensitivity for structures longer than 5 μm (0.02 s/L) and the lower curve corresponds to the analytical sensitivity required to characterize asbestos

structures of all sizes in environmental samples (0.5 s/L). As Figure 6.1, it is assumed that an average grid opening is 0.0081 mm^2, that the analytical filter is 25 mm^2, and the concentration factor is unity.

The horizontal line representing 5 m^3 has been emphasized in Figure 6.2 because this volume of air corresponds to 1.2 m^3/cm^2 on a 25 mm filter, which is the maximum volume of air that can be tolerated for a direct preparation (see Section 6.2.1). Published TEM methods discussed in Section 3.8 are graphed along the 5 m^3 line at points corresponding to the number of grid openings specified to be counted in each method. Thus, each of these points represent the effective analytical sensitivities for the methods at the maximum reasonable volume of air that can be collected on a 25 mm filter. The Hayward method is depicted twice to represent the analytical sensitivities corresponding, respectively, to the high magnification scan and the low magnification scan defined in this method.

As is readily apparent from the figure, only NIOSH 7402 yields sufficient sensitivity to detect asbestos structures of all sizes over the range of concentrations expected to be found in environmental samples. The Yamate method, the method specified in AHERA, and the high magnification scan of the Hayward method would not provide sufficient sensitivity to detect the entire range of concentrations expected for environmental asbestos. None of the methods depicted in Figure 6.2 (including the low magnification scan of the Hayward method) are sufficiently sensitive to characterize the expected range of concentrations of long structures (longer than 5 μm) in environmental samples. In the method presented in Part 1 of this report, the number of grid openings to be counted is determined by specifying the analytical sensitivity, specifying the volume of air to be collected, and calculating the required number of grid openings to be counted using equation 6-1.

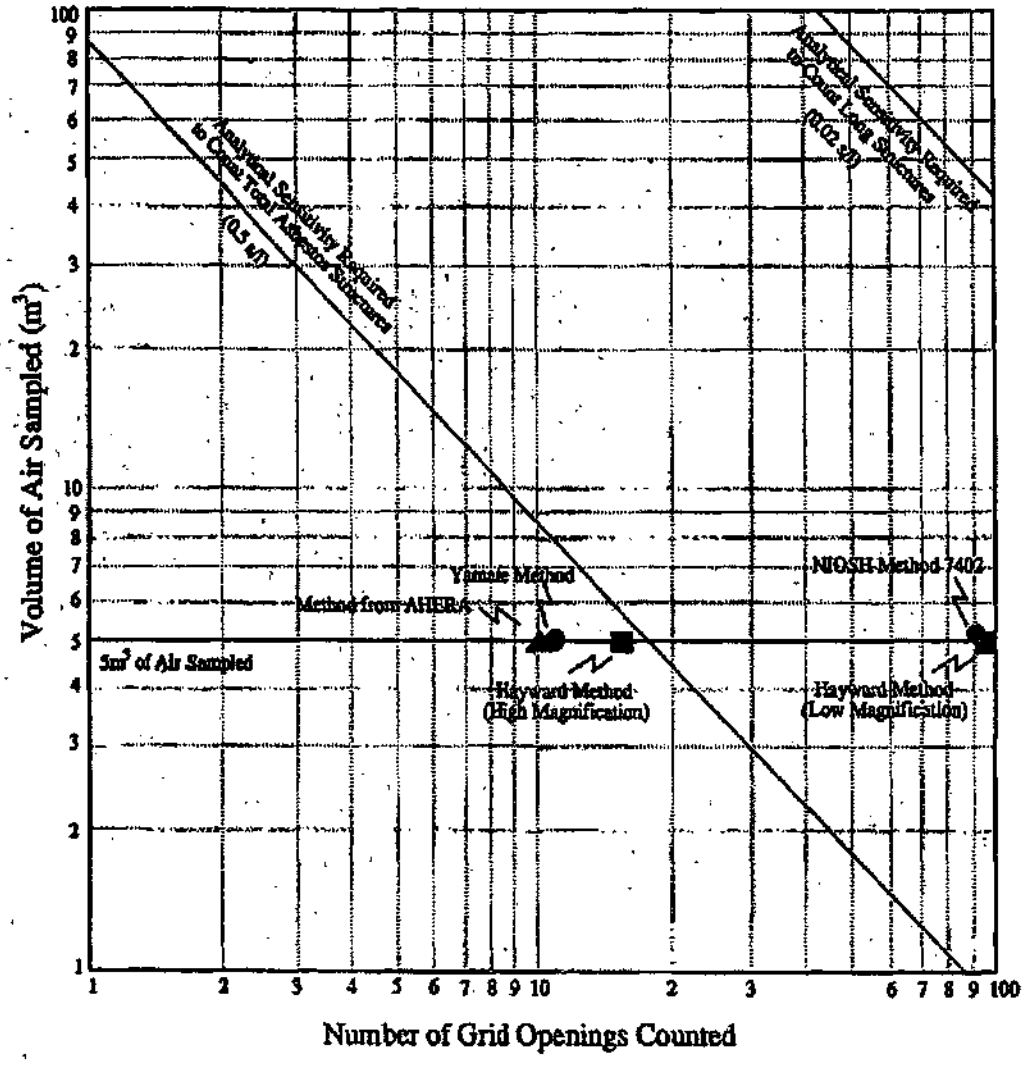
6.2.6. Analytical Technique

TEM is potentially capable of achieving the level of analytical sensitivity required for this method.

6.2.7. Magnification/Resolution

As noted in Section 3.4 and 4.2, total asbestos structures and asbestos structures longer than 5 μm are counted separately in this method because of the disparity in their abundance. Based on the size distributions discussed in Section 3.4, the probability of encountering an asbestos structure longer than 5 μm in an asbestos dust is only one tenth to one hundredth as likely as encountering an asbestos structure of any size. Given the disparity, the most cost-effective manner for analyzing the population of total structures and long structures representatively is to count them separately. If they were to be counted together and the limit for total structures was employed as the defined level of sensitivity, then the chance of encountering even 1 long structure while counting 10 total structures is very small. On the other hand, using the median for long structures as the defined limit of sensitivity

FIGURE 6.2: THE RELATIVE ANALYTICAL SENSITIVITIES OF PUBLISHED TEM METHODS



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ICF Technology
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means that likely 500 total asbestos structures would have to be counted at the same time that 10 long structures are identified. Characterizing such a large number of structures is extremely time consuming and expensive.

6.3. FACTORS AFFECTING THE LIMIT OF DETECTION

The limit of detection is defined as the smallest measurement that is unlikely to be due entirely to contamination from sources other than the air being sampled. More simply, it is the minimum environmental concentration that can be distinguished (with specified probability) from analytical background. To this point, the discussion of performance requirements (in terms of the analytical sensitivity) have been developed assuming zero analytical background. However, several published studies indicate that the analytical background for this method is potentially a critical factor affecting method performance.

Analytical background may derive both from filter blank contamination and from contamination contributed by the method of filter preparation. During an indirect preparation, for example, background contamination can arise from within the asher chamber, the containers in which the filters are ashed, the distilled water supply used for re-dispersing the ash, the pipettes used for transfer of the dispersion for filtration, and the filtration funnel[2]. However, while contamination introduced during filter preparation can be minimized by adherence to a rigid program of laboratory cleanliness, filter blank contamination is simply a function of the quality of commercially available filters.

6.3.1. Filter Blank Contamination

Asbestos contamination observed on membrane filters has varied historically. The ranges of filter blank contamination also differs for MCE and polycarbonate filters and may differ for different size filters of each type. In addition, the extent to which asbestos contamination on such membrane filters may contribute to background contamination during analysis depends on the procedure employed for preparing the sample. Unfortunately, studies reporting levels of asbestos filter blank contamination frequently omit critical information concerning either the filter type, size, date of purchase, or the preparation technique used in the analysis. The available

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[2] Some microscopists also suggest that asbestos losses may occur during sample collection and transport when asbestos structures are shaken off of the filter and redeposited on the cowl or cassette. Others contend that this is not a significant problem and that the data used to identify it is flawed by not controlling for the effects of the differences between direct and indirect preparation. The problem has yet to be confirmed in a published study.

data appear contradictory. Consequently, it is difficult to quantify a representative range of asbestos contamination levels for filter batches that are available today.

6.3.1.1. Contamination on MCE Filters

Prior to 1975, the level of contamination on MCE filters prepared by the direct method was reported in some batches to be sufficient to preclude use in standard asbestos analysis (Chatfield, 1985a). Prescreening of filter batches was therefore recommended. According to current consensus, levels of contamination detected on MCE filters prepared by a direct preparation now appear to be minimal and these filters are acceptable for most asbestos work. However, published data is limited and published data addressing 25 mm filters specifically is lacking.

(a) In a study of indoor asbestos contamination, Hatfield et al (1988) report 1 chrysotile asbestos fiber detected on 1 of 19 MCE filter blanks (37 mm, 0.45 μm pore size). This corresponds to an upper limit to background of less than 12 s/mm^2.

(b) The EPA study on filter blank contamination (USEPA, 1986b) concluded with the observation that contamination on MCE filters appeared to be composed predominantly of short chrysotile structures and that MCE filters are recommended for asbestos sampling and analysis.

(c) Chatfield (1985d) also reports that most of the contamination on filter blanks is chrysotile and that current levels of blank contamination are acceptable. This paper also suggests that short fibers predominate, although this observation may be due simply to lack of a statistically balanced count.

For MCE filters prepared by an indirect technique, the situation is less clear. General consensus indicates that levels of contamination are acceptable when MCE filters are ashed. But published data, especially for recent batches of 25 mm filters are limited. Published data that address background contamination resulting from the washing of MCE filters are lacking.

(a) In a study of abatement techniques, Chesson et al (1985) report detection of no asbestos contamination on laboratory blanks and field blank MCE filters (47 mm, 0.45 μm pore size) prepared by ashing the filter and resuspending the deposit in distilled water by sonication. This means that background contamination on the filters is less than 12 s/mm^2 based on the analysis performed. Note that the filtrate was redeposited on a polycarbonate filter (25 mm, 0.2 μm pore size) so that the background could also represent an upper limit to contamination on polycarbonate filters subjected to direct preparation.

(b) In a followup study of abatement techniques, Chesson et al (1986b) report levels ranging between 0 and 58 s/mm^2 with a mean of 30 s/mm^2 on laboratory blank MCE filters (47 mm, 0.45 μm pore size) prepared by ashing the filter and resuspending the deposit in distilled water by sonication. Field blanks appear to exhibit a slightly higher average asbestos contamination level than the laboratory blanks. Note that the filtrate was redeposited on a polycarbonate filter (25 mm, 0.2 μm pore size) so that the background could also represent contamination due to direct preparation of a polycarbonate filter.

(c) In another followup to the series of abatement papers, Tuckfield et al (1988) report levels ranging between 0 and 50 s/mm^2 with a mean of 20 s/mm^2 on 30 field and laboratory blank MCE filters (47 mm, 0.45 μm pore size) prepared by ashing the filter and resuspending the deposit in distilled water by sonication. Note that the filtrate was redeposited on a polycarbonate filter (25mm, 0.2 μm pore size) so that the background observed could also represent contamination due to direct preparation of polycarbonate filters.

(d) Individual microscopists (for example, G. Dunmeyer, 1988) have indicated in private conversations that indirect preparation by washing MCE filters results in efficient transfer of asbestos and that blank contamination is low (less than 15 s/mm^2). Filters considered were 25 mm, 0.8 μm pore size MCE filters. However, there are no published data to confirm such reports.

6.3.1.2. Asbestos Contamination on Polycarbonate Filters

The level of asbestos contamination on polycarbonate filters has historically been reported to be higher than MCE filters. Available data addressing current levels of contamination on polycarbonate filters is inconclusive for filters prepared by direct preparation and published data addressing background contamination on polycarbonate filters prepared by an indirect technique (either ashing or washing) appears to be lacking. General consensus indicates that background contamination on current batches of polycarbonate filters is generally higher than MCE filters when both are prepared by the direct technique. However, published data are limited.

(a) In an abatement study, Tuckfield et al (1988) prepared 47 mm polycarbonate filters (pore size not reported) by a direct technique and measured the background on a series of laboratory and field blanks. Levels reported range between 0 and 40 s/mm^2 with an arithmatic mean of 7 s/mm^2. Note that an amphibole fiber was detected on one of the blanks.

(b) In the workshop on blank contamination (USEPA, 1986b), it was reported that polycarbonate filters prepared by a direct technique exhibit variable levels of blank asbestos contamination. Levels

appear to average about 2-6 structures/10 grid openings (25-75 structures/mm^2) but particular lots may contain 10 times this amount. It was recommended that filter lots with more than 5 structures/10 grid openings (60 s/mm^2) be returned to the manufacturer. In the same study, use of MCE filters was recommended because polycarbonate blank contamination appears to include both chrysotile and amphibole and a significant fraction of structures longer than 5 μm were encountered.

(c) Chatfield (1985b) reports that although contamination of MCE filters appears to have been minimized, contamination of polycarbonate filters still appears to be a problem.

(d) The studies reported in Section 6.2.1.1 indicate that an upper limit to filter blank contamination on polycarbonate filters prepared by a direct technique is 30 s/mm^2 based on contamination observed following an indirect preparation of MCE filters in which polycarbonate filters were used to filter the final suspension.

(e) There appears to be a consensus among several microscopists that at least one group of microscopists consistently report lower values for polycarbonate filter contamination. However, there is no published data addressing this discrepancy and it has not been resolved.

6.3.1.3. Estimating Filter Blank Contamination Levels

Although qualitative conclusions may be drawn from the available data, additional research is recommended in this area. General consensus indicates that most current batches of MCE filters, when prepared by a direct technique, should yield filter blank contamination levels below 30 s/mm^2. MCE filters prepared by ashing should also yield background below 30 s/mm^2. Washing of polycarbonate filters yields comparably low levels of background contamination. Published data, however, is too sparse to support any of these numbers as typical. Therefore, at a minimum, duplicate filter blanks from every batch used in a study should be prepared and analyzed as per the technique to be used in the study to quantify the level of background.

If the range of filter blank contamination found during a preliminary evaluation is not clearly distinguishable from typical levels expected to be encountered during the study, either the filter batch should be returned for a new one (which must also be tested) or the study protocol should be redesigned. It is assumed for this method that filter blank contamination can be maintained below 30 s/mm^2. It is further assumed that the size distribution of asbestos appearing as filter blank contamination parallels typical chrysotile distributions as presented in Section 3.4. Therefore, if the count of structures of all sizes exceeds background, the count of any size fraction will also exceed the level of background corresponding to that

particular size fraction. To minimize interference with filter blank contamination, 60 s/mm^2 is set as a target minimum concentration for this method.

NOTE

The potential impact of asbestos contamination contributed by the membrane filter can be minimized by maximizing the volume of air collected per unit area of filter. However, collected air volumes must be limited to prevent overloading, especially for filters to be prepared by a direct-transfer technique (see Sections 6.2.1 and 6.2.4).

Within the range of the maximum air volume that can be tolerated on a filter due to constraints associated with filter loading, there is also the problem of the limit of the rate at which air can be pumped through a filter given the capabilities of available pumps (see Section 2.2 of The Method, Part 1 of this report). If the capabilities of the pump are limiting in a given situation, the total volume of air to be collected can theoretically be minimized without compromising analytical sensitivity by selecting the smallest filter (25 mm) that is practical for sample collection.

6.3.2. Conclusions

Although specification of a detection limit for this method requires knowledge of the range and variation of analytical background and is therefore study specific, general conclusions concerning the ability to achieve a reasonable limit of detection may be drawn from a comparison of the relative contributions to asbestos counts from environmental and analytical sources. For filters prepared for TEM analysis in a particular study, the absolute limit to detection is unlikely to be lower than the concentration at which the density of structures deposited on the filter from the air is just equal to the mean density of structures present on the blank filter (30 s/mm^2). Ideally, however, sampled asbestos should be deposited on the filter at several times the concentration of asbestos present due to analytical background so that distinguishing sampled asbestos from analytical contamination is trivial.

Given the upper range of the target concentrations derived in Section 3.5 (5 s/L for total asbestos structures and 0.2 s/L for long asbestos structures), an air volume of 1 m^3 of air per cm^2 of filter will deposit 50 asbestos structures of all sizes per mm^2 of filter and 2 long structures per mm^2 of filter respectively. The fact that the first of these numbers exceeds projected filter blank contamination levels by less than a factor of 2 indicates that preparing these filters by a direct technique, which is subject to the 1 m^3/cm^3 limitation, likely presents a marginally acceptable method for characterizing asbestos structures of all lengths, at best. Direct preparation is clearly unacceptable for characterizing the concentrations of long structures, because the major contribution to observed asbestos will be

from analytical background. Therefore, an indirect preparation protocol is incorporated in this method. The consequences of the selection of an indirect preparation procedure are addressed in Section 6.2.4.

NOTE

At a limit of 30 s/mm^2 on a 25 mm filter with 1 m^3/cm^2 air collected, 1 structure observed corresponds a concentration (analytical sensitivity) of 3 s/L for structures of all sizes and 0.1 s/L for structures longer than 5 μm. Clearly, limiting the quantity of air that can be collected to 1 m^3/cm^2 (as is the case for a direct preparation) is not sufficient to achieve the required analytical sensitivities defined for this method (see Sections 4.3 and 6.2).

6.4. FACTORS AFFECTING PRECISION

To maximize precision, potential sources of variation within the method must be controlled. To minimize systematic variation during sampling and analysis, the method specifies detailed procedures. Random variation is minimized by maximizing the number of structures to be identified and counted, which includes increasing the probability of encountering asbestos structures during analysis, as discussed in Section 6.2. In addition, detailed and unambiguous counting rules and rules for identification are specified in the method to minimize variation due to subjective interpretation. Specific factors that must be controlled to maximize precision are:

(a) generating a uniform deposit on the analytical filter;

(b) minimizing subjectivity in the interpretation of counting rules;

(c) minimizing variation introduced by small changes in instrument performance; and

(d) minimizing statistical variation from counting too small a number of structures.

6.4.1. Analytical Technique

TEM is the analytical tool selected for this method so that analysis can be performed at sufficient magnification to minimize variation due to small differences in the performance characteristics of the instrument or the experience of the analyst. However, several performance criteria must be specified to minimize the impact on precision.

In a comparison study, Steel and Small (1985) report that TEM instruments vary with age and type. Differences between instruments that contribute the most to analytical variation include image quality (resolution), contrast, and brightness. Electron beam dose and mechanical stage operation are also important. Resolution was judged by the ability to distinguish the hollow core of chrysotile fibrils. Contrast and brightness

are controlled by adjusting the voltage and aperture sizes of the instrument. In the study, not all instruments could be configured to perform equally well. Some of the oldest TEM instruments could not be adjusted to provide sufficient resolution to observe the central canals of chrysotile fibrils clearly.

The beam dose effects the counting process by causing damage. If too high a beam current is required by an instrument to achieve the required brightness or resolution, than the potential to damage the sample may become so great that the ability to scan properly may be lost. This is particularly true when attempting to generate SAED spectra.

The operation of the mechanical stage is crucial to achieve good precision in counts. Approximately 20-30 traverses are needed to complete a scan of a single grid opening. Thus, the extent that a stage wanders during a traverse may affect the time necessary and the ability to precisely count all fibers in a grid opening. Some of the stages tested by Steel and Small wandered by 30 μm during a single traverse. However, the TEM used for the operator tests in the study wandered by less than 1 μm in a 100 μm linear traverse.

Based on the work of Steel and Small, it is recommended that TEM instruments to be used with this method exhibit a resolution at 20,000 magnification that is sufficient to clearly resolve the central canal of the finest chrysotile fibril. In addition, the mechanical stage of the instrument must be shown to vander by less than 1 μm per 100 μm linear traverse. Finally, it is recommended that specific performance criteria addressing beam dose, brightness, and contrast be developed for instruments to be used with this method.

6.4.2. Magnification/Resolution

The precision of this method is affected by the magnification selected to the extent that the ability to detect asbestos structures lies close to the limit of the associated resolution. Microscopists vary in their ability to detect very small asbestos structures at magnifications used for scanning in this method, 10,000 and 20,000, (Steel and Small, 1985). It has been reported that only 50% of asbestos structures shorter than 0.5 μm were detected by all analysts in a round-robin study. Below this length, the fraction of analysts who regularly detect such structures decreases rapidly. At the same time, proper characterization of the size fractions present in an asbestos dust requires that the mode of the distribution be included in the count. The published size distributions presented in Section 3.4 indicate that the mode of asbestos length distributions in most dusts falls within a range from 0.8 μm to 1.2 μm. Therefore, as a reasonable compromise allowing the detection of the mode of asbestos distributions while addressing the limited ability of analysts to detect short structures, a minimum length of 0.5 μm has been defined as the shortest fiber to be incorporated in the reported results for this method.

The minimum detectable width for asbestos structures counted in this method is determined by the ability of an operator to detect them in a routine examination at the specified magnification of the method. The minimum magnification specified in this method (10,000) is more than sufficient to assure visibility of the thinnest chrysotile asbestos fibrils.

6.4.3. Uniformity of Filter Deposit

To meaningfully extrapolate structure counts performed over a limited area to the concentration of structures on an entire filter, the nature of the distribution of structures on the filter must be known and the distribution must be reasonably uniform. Chatfield (1985b) has shown that the distribution of structures on analytical filters prepared by a direct technique are not uniform. Using a chi-squared test, Chatfield found that 100% of the directly prepared filters that exhibited more than 30 structures/ 10 grid openings had to be rejected as non-uniform at a 1% level of significance. The fraction of filters that passed the chi-squared test for uniformity increased as the loadings on the filters decreased, but this was attributed to the presence of an insufficient number of structures to properly determine a distribution. In contrast, nearly all of the filters prepared by indirect preparation techniques exhibited uniform deposits at a 1% level of significance and 100% of the indirectly prepared samples could be shown to be uniform at reduced levels of significance.

Due to the apparent characteristics of air deposited asbestos, directly prepared filters do not exhibit uniform deposits. Consequently, indirect preparation has been incorporated into this method.

6.4.4. Rules for Counting, Characterization, and Recording

Based on a study by Steel and Small (1985), operator error is one of several potential sources of variation. The problem is worse if counting procedures are not standardized. It is also reported that this variation can be reduced when counting rules are standardized and specified in detail. Performance also varies as a function of observer experience and the degree of professional time pressure under which an analysis is performed. To account for these observations, detailed standardized counting rules have been incorporated in this method. It is also recommended that minimum requirements for experience be developed for the analysts. This may include, for example, mandatory participation by analysts in round-robin studies.

Once the concentration of structures collected on a filter is sufficient to be observed, the number of structures detected affects the precision of the measurement. Precision tends to increase as the square root of the number of structures counted. This implies that the incremental improvement in precision derived from each individual structure counted decreases as the total number of structures counted increases.

Burdett (1985a) got significant improvement in precision by increasing counts between 20 and 50 structures. He reports, however, that the incremental improvement in precision for structures counted beyond 50 are minimal. The characterization of structures to be counted in this method includes distinguishing among counts of several sub-types (bundles, clusters, matrices, and fibers). Based on Burdett's data, improvements in the precision of the count of each sub-type is expected until 50 structures of each sub type are counted. However, this would require counting more than of 400 total asbestos structures, an excessive requirement. Therefore, an upper limit to the count of total asbestos structures of 50 is defined for this method and a lower level of precision is considered acceptable for the count of each sub-type. A similar strategy is adopted for structures longer than 5 μm.

NOTE

A count of 100 total asbestos structures and 100 structures longer than 5 μm is defined in this method for Phase 2 samples because of a need for increased precision in the counts of sub-types in these samples (see Section 6.4.2.

7. RECOMMENDED PROCEDURES FOR MANIPULATING ANALYTICAL DATA

Because the principle objective of the sampling and analysis method presented in Part 1 of this report is to provide results suitable for supporting a risk assessment, this section addresses the kinds of data manipulation typically performed to complete a risk assessment. The procedures presented here are recommended for general application. It is recognized, however, that alternate valid approaches are also available so that the recommended procedures should not be viewed as strict requirements. In addition, specific circumstances may present special computational needs beyond the scope of what is addressed here. To preserve the integrity and the intent of data derived from this method, it is recommended that a statistician be consulted prior to the adoption of alternate computational procedures for manipulating data as part of a site evaluation.

In a risk assessment, field results are typically combined to provide average concentrations representative of a given location or a given period of time. Frequently, long-term (multiple-year) time averages are desired because lifetime rather than short-term risks are generally addressed. Depending on the particular purpose for the computation, average concentrations may be derived either from homogeneous samples (samples intended to represent the same sampling environment) or from non-homogeneous samples (samples collected in different sampling environments). Procedures for deriving averages from homogeneous and non-homogeneous sets of samples are provided in Section 7.1 along with instructions for constructing confidence limits around averages of homogeneous samples (Section 7.2) and non-homogeneous samples (Section 7.3).

Once average concentrations are computed, they are generally compared to distinguish anthropogenic contamination from background or to distinguish among levels of contamination potentially contributed from different sources. Such comparisons examine the relative differences of various measurements. However, long-term average exposure concentrations are also typically compared to existing risk factors to establish the magnitude of absolute risk. This last use of analytical data relies on the absolute concentration derived from a particular set of measurements. A procedure for conducting a statistical test to distinguish among the average concentrations derived from various sample sets is presented in Section 7.4.

The significance of considering the need for a relative verses an absolute measurement is to determine whether analytical results must be adjusted to account for analytical background contamination (see Section 11.4 of Part 1 of this report). While unnecessary and not recommended for the manipulation of relative values, the adjustment for analytical background is required to derive an absolute measurement. A procedure for adjusting measurements to account for analytical background is provided in Section 7.5.

NOTE

Statistical tests should all be performed on <u>unadjusted</u> analysis results.

7.1. COMPUTING AVERAGE CONCENTRATIONS FROM MULTIPLE SAMPLES

As indicated above, it is frequently helpful to summarize results from a set of samples by computing an average. For several reasons, it is recommended that arithmetic averages be used exclusively. First, averages are often used to judge risk to health and such risk is more likely to be a function of the arithmetic mean than, say, a geometric mean (e.g., the risk from breathing 0.2 f/ml 12 hours per day and 0.00002 f/ml 12 hours per day is probably closer to the risk from constantly breathing 0.1001 f/ml [the arithmetic mean] than breathing 0.002 f/ml [the geometric mean]). Second, the geometric mean is zero whenever one of the individual samples results is zero, and ad hoc adjustments to address this problem (such as replacing zero estimates by detection limits) can introduce serious biases.

If samples are considered to be homogeneous (designed to represent the same sampling environment), it is recommended that simple un-weighted arithmetic averages be calculated. For example, denote the calculated airborne concentrations from a group of K samples by C_1, ..., C_K. The estimated average concentration, $\bar{C}$, would then be

$$\bar{C} = (C_1 + \ldots + C_K)/K.$$

If the analytical sensitivities of the samples to be averaged differ considerably, it may be appropriate to compute a weighted average with weights set equal to the inverses of the analytic sensitivities. This is equivalent to computing the average by dividing the total number of structures found in all of the samples by the total volume of air passed through all of the sample filters.

Frequently, it will also be necessary to compute an average for a collection of non-homogeneous samples. For example, one might wish to calculate the average exposure of an individual who frequents several areas with varying estimated airborne concentrations of asbestos. It is recommended that such an average be obtained as follows. First, calculate an average concentration from each homogeneous area by averaging the set of samples collected in each such area as indicated above. Averages derived for the homogeneous areas should then be combined in a weighted average, with weights equal to the proportions of time the individual spends in each of the various areas. Thus, if $\bar{C}_1$, $\bar{C}_2$,...$\bar{C}_K$ are the average concentrations computed from K groups of homogeneous samples using the previous expression, then the overall average exposure concentration experienced by the individual under consideration, based on the respective weights W_1,...W_K, would be

$$C_W = (W_1\bar{C}_1 + \ldots + W_K\bar{C}_K)/(W_1 + \ldots + W_K).$$

If desired, one can test whether groups of samples are homogeneous using the Wilcoxon and Kruskal-Wallis tests (see Section 7.4).

7.2. COMPUTING CONFIDENCE LIMITS FOR AVERAGES OF HOMOGENEOUS SAMPLES

If there are no more than 30 structures in a combined group of K samples then the confidence limits for the average concentration is computed based on the Poisson distribution. Let x be the total number of structures on the combined filters (the number of structures in a single sample is the estimated concentration divided by the analytical sensitivity). Let x_U be the 95% upper confidence limit on the total count x, calculated from the Poisson distribution as described in detail in Section E.4.2 (Appendix E of The Method, Part 1 of this report). The upper 95% limit on the average concentration, C_U, is then x_U divided by the sum of the reciprocals of the analytical sensitivities, i.e.,

$$C_U = x_U/(1/S_1 + \ldots + 1/S_K).$$

The lower limit is determined from the same formula except that a suitably defined lower limit x_L replaces x_U.

If more than 30 structures are found in a combined group of K samples then confidence limits are also calculated by the following procedure and the most extreme values are reported. Let C_i be the calculated concentration for the ith sample, let $\bar{C}$ be the arithmetic average, and let s_c^2 be the corresponding sample variance,

$$s_c^2 = \frac{1}{k-1} \sum_{i=1}^{k} (C_i - \bar{C})^2.$$

Then the 95% confidence limits on the average concentration are computed as

$$C_U = \bar{C} + 1.65\ s_c$$

and

C_L equal to the larger of $\bar{C} - 1.65\ s_c$ or zero.

7.3. COMPUTING CONFIDENCE LIMITS FOR AVERAGES OF NON-HOMOGENEOUS SAMPLES

Suppose $\bar{C}_1, \ldots \bar{C}_K$ are average concentrations computed from K groups of homogenous samples. $s_{c1}^2 \ldots s_{cK}^2$ are the corresponding variance terms, to compute confidence limits for the weighted average concentration estimated by

$$C_W = (W_1\bar{C}_1 + \ldots + W_K\bar{C}_K)/(W_1 + \ldots + W_K).$$

Define

$$s_W^2 = (W_1^2 s_{\bar{c}1}^2 + \ldots + W_K^2 s_{\bar{c}K}^2)/(W_1 + \ldots + W_K)^2.$$

The 95% upper and lower limits, respectively, are then

$$C_{Wu} = C_W + 1.65\ s_W$$

and C_{Wl} equal to the larger of

$$C_W - 1.65\ s_W \text{ or zero.}$$

7.4. TESTING FOR DIFFERENCES BETWEEN CONCENTRATIONS DERIVED FROM DIFFERENT SETS OF SAMPLES

It is recommended that the Wilcoxon non-parametric test (Hollander and Wolfe 1973) be used to test whether airborne concentrations measured from one set of samples are significantly different from those measured by another set. Such a test can be used, for example, to determine whether concentrations are higher in one environmental area than another (e.g., near an asbestos disposal site verses a more distant control area). The test is also recommended to distinguish "sampled" asbestos from contamination attributed to analytical background.

The test should be applied directly to the unadjusted means reported for measurements of airborne concentrations. A mean of zero, "0", should be used in place of all measurements reported as "NF" for not found. A detailed description of the application of the Wilcoxon Test is provided in Section E.5 (Appendix E of The Method, Part 1 of this report).

Care should be taken to exclude possible sources of systematic bias when applying the Wilcoxon test or similar tests. Such bias can result, for example, if:

(1) different laboratories analyzed the groups of samples being compared;

(2) the groups of samples were analyzed by the same laboratory but by distinct analysts;

(3) the samples were analyzed by the same analysts at the same laboratory but before and after a modification to laboratory procedure was adopted;

(4) the samples from the two groups were collected on different types or batches of filters; or

(5) the groups of samples were collected or prepared using radically different procedures.

Such sources of bias can be minimized by keeping experimental conditions as similar as possible when collecting groups of samples intended for comparison, randomizing filters among laboratories or among analysts whenever multiple laboratories or analysts within laboratories are used to analyze filters, and withholding from analysts the identity of the source locations of the filters.

To test for non-homogeneities among airborne concentrations from several sites the non-parametric Kruskal-Wallis test can be used (Hollander and Wolfe 1973). This test is a generalization of the Wilcoxon test that can be applied to more than two groups of data. If analyses involve samples analyzed at separate laboratories, it is strongly suggested that laboratory be used as a blocking factor (Lehmann 1975), so that direct comparisons are made only among samples analyzed at the same laboratory.

7.5. ADJUSTING MEASURED CONCENTRATIONS FOR CONTRIBUTIONS FROM ANALYTICAL BACKGROUND CONTAMINATION

When it becomes necessary to determine the absolute magnitude of a measured asbestos concentration (as when measured concentrations are compared to existing risk factors, to establish the magnitude of risk), mean measurements of airborne concentrations must be adjusted to account for contributions from analytical background contamination. As addressed in Section E.5 (Appendix E to The Method, Part 1 of this report), it is strongly recommended that such adjustments be applied to averages of groups of samples rather than individual sample analysis results.

To adjust an average concentration computed for a group of samples weighted by the inverse of their individual analytical sensitivities, proceed as follows. Let x be the total number of structures detected in the used filters, A_T the total area examined from these filters, and BC the surface concentration of structures obtained from an appropriate set of blanks. The adjusted airborne concentration is calculated as

$$\frac{(x/A_T - BC)\,A_T}{1/S_1 + \ldots + 1/S_g}\ .$$

As indicated in Section E.5, blank analyses may be obtained either from filters analyzed from the same batch used for sample collection or from a cumulative database of historical blank analyses maintained by the laboratory performing the analyses. Depending on the given application, one or the other of these data sets may be considered most appropriate. When adjusting sample analysis results for contributions from analytical background, the source of the blank data must be specified.

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Test Method

Method for the Determination of Asbestos in Bulk Building Materials

EPA/600/R-93/116
July 1993

TEST METHOD

METHOD FOR THE DETERMINATION OF ASBESTOS IN BULK BUILDING MATERIALS

by

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TABLE OF CONTENTS

SECTION	PAGE
1.0 INTRODUCTION	1
1.1 References	3
2.0 METHODS	3
2.1 Stereomicroscopic Examination	3
2.1.1 Applicability	4
2.1.2 Range	4
2.1.3 Interferences	4
2.1.4 Precision and Accuracy	4
2.1.5 Procedures	5
2.1.5.1 Sample Preparation	5
2.1.5.2 Analysis	6
2.1.6 Calibration Materials	8
2.1.7 References	8
2.2 Polarized Light Microscopy	9
2.2.1 Principle and Applicability	9
2.2.2 Range	10
2.2.3 Interferences	10
2.2.4 Precision and Accuracy	10
2.2.5 Procedures	11
2.2.5.1 Sample Preparation	11
2.2.5.1.1 Qualitative Analysis Preparation	11
2.2.5.1.2 Quantitative Analysis Preparation	12
2.2.5.2 Analysis	13
2.2.5.2.1 Identification	13
2.2.5.2.2 Quantitation of Asbestos Content	16
2.2.5.2.3 Microscope Alignment	22
2.2.6 References	22
2.3 Gravimetry	23
2.3.1 Principle and Applicability	23
2.3.2 Interferences	24
2.3.3 Quantitation	25
2.3.4 Preliminary Examination and Evaluation	25
2.3.5 Sample Preparation	26
2.3.5.1 Drying	26
2.3.5.2 Homogenization/Grain Size Reduction	26
2.3.6 Procedure for Ashing	27
2.3.7 Use of Solvents for Removal of Organics	28
2.3.8 Procedure for Acid Dissolution	29
2.3.9 Determination of Optimal Precision and Accuracy	31
2.3.10 References	31
2.4 X-Ray Powder Diffraction	32
2.4.1 Principle and Applicability	32
2.4.2 Range and Sensitivity	35
2.4.3 Limitations	35

TABLE OF CONTENTS (cont'd)

SECTION PAGE

2.4.3.1 Interferences . . . 35
2.4.3.2 Matrix Effects . . . 38
2.4.3.3 Particle Size Dependence . . . 39
2.4.3.4 Preferred Orientation Effects . . . 39
2.4.3.5 Lack of Suitably Characterized Standard Materials . . . 39
2.4.4 Precision and Accuracy . . . 40
2.4.5 Procedure . . . 40
2.4.5.1 Sampling . . . 40
2.4.5.2 Analysis . . . 40
2.4.5.2.1 Sample Preparation . . . 41
2.4.5.2.2 Milling . . . 41
2.4.5.2.3 Ashing . . . 42
2.4.5.2.4 Acid Washing . . . 42
2.4.5.3 Qualitative Analysis . . . 42
2.4.5.3.1 Initial Screening of Bulk Material . . . 42
2.4.5.3.2 Detection of Minor or Trace Constituents . . . 43
2.4.5.4 Quantitative Analysis . . . 44
2.4.6 Calibration . . . 46
2.4.6.1 Preparation of Calibration Standards . . . 46
2.4.6.2 Analysis of Calibration Standards . . . 47
2.4.7 Calculations . . . 49
2.4.8 References . . . 51
2.5 Analytical Electron Microscopy . . . 51
2.5.1 Applicability . . . 51
2.5.2 Range . . . 52
2.5.3 Interferences . . . 52
2.5.4 Precision and Accuracy . . . 52
2.5.5 Procedures . . . 52
2.5.5.1 AEM Specimen Preparation for Semi-Quantitative Evaluation . . . 53
2.5.5.2 AEM Specimen Preparation for Quantitative Evaluation . . . 54
2.5.5.2.1 Identification . . . 54
2.5.6 References . . . 54
2.6 Other Methodologies . . . 53

3.0 QUALITY CONTROL/QUALITY ASSURANCE OPERATIONS- PLM . . . 55
3.1 General Considerations . . . 56
3.1.1 Training . . . 56
3.1.2 Instrument Calibration and Maintenance . . . 56
3.2 Quality Control of Asbestos Analysis . . . 57
3.2.1 Qualitative Analysis . . . 57
3.2.2 Quantitative Analysis . . . 58
3.3 Interlaboratory Quality Control . . . 59
3.4 Performance Audits . . . 60
3.5 Systems Audits . . . 60
3.6 References . . . 61

TABLE OF CONTENTS (cont'd)

APPENDIX A: GLOSSARY OF TERMS

APPENDIX B: APPARATUS FOR SAMPLE PREPARATION AND ANALYSIS

APPENDIX C: PREPARATION AND USE OF CALIBRATION STANDARDS FOR BULK ASBESTOS

APPENDIX D: SPECIAL-CASE BUILDING MATERIALS

TABLES

TABLE		PAGE
1-1	Simplified Flowchart for Analysis of Bulk Materials	2
2-1	Suggested Acceptable Errors For PLM Analysis	11
2-2	Optical Properties of Asbestos Fibers	19
2-3	Typical Central Stop Dispersion Staining Colors	20
2-4	Optical Properties of Man-Made Textile Fibers	20
2-5	Optical Properties of Selected Fibers	21
2-6	The Asbestos Minerals and Their Nonasbestiform Analogs	34
2-7	Principal Lattice Spacings of Asbestiform Minerals	34
2-8	Common Constituents in Building Materials	36
2-9	Interferences in XRD Analysis of Asbestiform Minerals	37

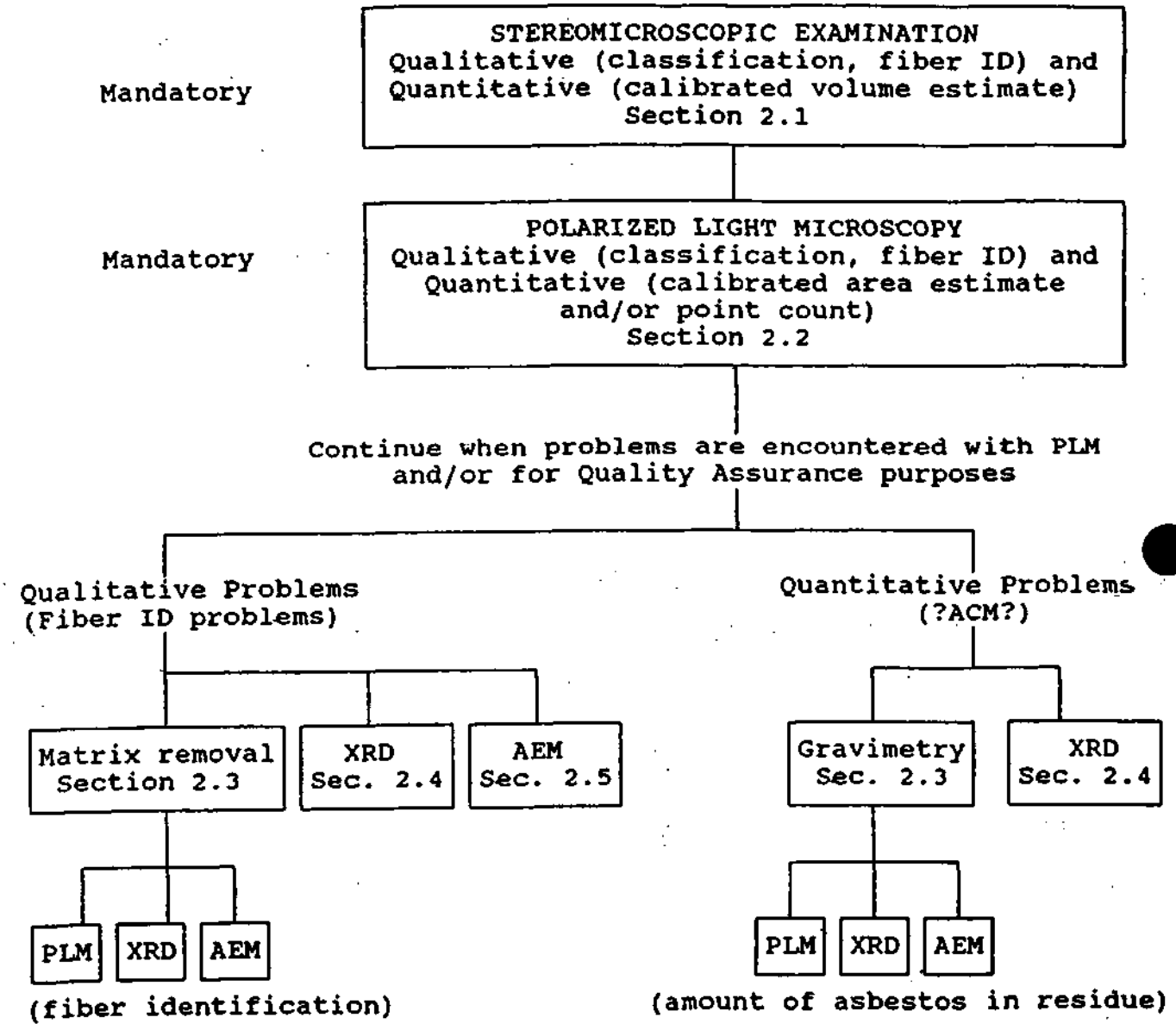
1.0 INTRODUCTION

Laboratories are now called upon to identify asbestos in a variety of bulk building materials, including loose-fill insulations, acoustic and thermal sprays, pipe and boiler wraps, plasters, paints, flooring products, roofing materials and cementitious products.

The diversity of bulk materials necessitates the use of several different methods of sample preparation and analysis. An analysis with a simple stereomicroscope is always followed by a polarized light microscopic (PLM) analysis. The results of these analyses are generally sufficient for identification and quantitation of major concentrations of asbestos. However, during these stereomicroscopic and PLM analyses, it may be found that additional techniques are needed to: 1) attain a positive identification of asbestos; 2) attain a reasonable accuracy for the quantity of asbestos in the sample; or 3) perform quality assurance activities to characterize a laboratory's performance. The additional techniques include x-ray diffraction (XRD), analytical electron microscopy (AEM), and gravimetry, for which there are sections included in the method. Other techniques will be considered by the Environmental Protection Agency (EPA) and may be added at some future time. Table 1-1 presents a simplified flowchart for analysis of bulk materials.

This Method for the Determination of Asbestos in Bulk Building Materials outlines the applicability of the various preparation and analysis methods to the broad spectrum of bulk building materials now being analyzed. This method has been evaluated by the EPA Atmospheric Research and Exposure Assessment Laboratory (EPA/AREAL) to determine if it offers improvements to current analytical techniques for building materials. This method demonstrated a capability for improving the precision and accuracy of analytical results. It contains significant revisions to procedures outlined in the Interim Method,[1] along with the addition of several new procedures. Each technique may reduce or introduce bias, or have some effect on the precision of the measurement, therefore results need to be interpreted judiciously. Data on each technique, especially those new to asbestos analysis, will be collected over time and carefully evaluated, with resulting recommendations for changes to the Method to be passed on to the appropriate program office within EPA.

TABLE 1-1. SIMPLIFIED FLOWCHART FOR ANALYSIS OF BULK MATERIALS



This is an analytical method. It is not intended to cover bulk material sampling, an area addressed previously[2,3,4,5] by the EPA. However, subsampling or sample splitting as it pertains to laboratory analysis procedures in this method, is discussed throughout.

1.1 References

1. **Interim Method for the Determination of Asbestos in Bulk Insulation Samples,** U.S. E.P.A. 600/M4-82-020, 1982.
2. **Asbestos-Containing Materials in School Buildings: A Guidance Document,** Part 1 and 2, U.S. E.P.A./O.T.S NO. C00090, 1979.
3. **Asbestos in Buildings: Simplified Sampling Scheme for Friable Surfacing Materials,** U.S. E.P.A. 560/5-85-030a, 1985.
4. **Guidance for Controlling Asbestos-Containing Materials in Buildings,** U.S. E.P.A. 560/5-85-024, 1985.
5. **Asbestos-Containing Materials in Schools: Final Rule and Notice,** 40 CFR Part 763, October, 1987.

2.0 METHODS

2.1 Stereomicroscopic Examination

A preliminary visual examination using a simple stereomicroscope is mandatory for all samples. A sample should be of sufficient size to provide for an adequate examination. For many samples, observations on homogeneity, preliminary fiber identification and semi-quantitation of constituents can be made at this point. Another method of identification and semi-quantitation of asbestos must be used in conjunction with the stereomicroscopic examination. A description of the suggested apparatus needed for stereomicroscopic examination is given in Appendix B.

The laboratory should note any samples of insufficient volume. A sufficient sample volume is sample-type dependent. For samples such as floor tiles, roofing felts, paper insulation, etc., three to four square inches of the layered material would be a preferred sample size. For materials such as ceiling tiles, loose-fill insulation, pipe insulation, etc., a sample size of approximately one cubic inch (~ 15cc) would be preferred. For samples of thin-coating materials such as paints, mastics, spray plasters, tapes, etc., a smaller sample

size may be suitable for analysis. Generally, samples of insufficient volume should be rejected, and further analysis curtailed until the client is contacted. The quantity of sample affects the sensitivity of the analysis and reliability of the quantitation steps. If there is a question whether the sample is representative due to inhomogeneity, the sample should be rejected, at least until contacting the client to see if: 1) the client can provide more material or 2) the client wishes the laboratory to go ahead with the analysis, but with the laboratory including a statement on the limited sensitivity and reliability of quantitation. If the latter is the case, the report of analysis should state that the client was contacted, that the client decided that the lab should use less material than recommended by the method, and that the client acknowledges that this may have limited the sensitivity and quantitation of the method. At the time the client is contacted about the material, he or she should be informed that a statement reflecting these facts will be placed in the report.

2.1.1 Applicability

Stereomicroscopic analysis is applicable to all samples, although its use with vinyl floor tile, asphaltic products, etc., may be limited because of small asbestos fiber size and/or the presence of interfering components. It does not provide positive identification of asbestos.

2.1.2 Range

Asbestos may be detected at concentrations less than one percent by volume, but this detection is highly material dependent.

2.1.3 Interferences

Detection of possible asbestos fibers may be made more difficult by the presence of other nonasbestos fibrous components such as cellulose, fiber glass, etc., by binder/matrix materials which may mask or obscure fibrous components, and/or by exposure to conditions (acid environment, high temperature, etc.) capable of altering or transforming asbestos.

2.1.4 Precision and Accuracy

The precision and accuracy of these estimations are material dependent and must be determined by the individual laboratory for the percent range involved. These values may be

determined for an individual analyst by the in-house preparation and analysis of standards and the use of error bars, control charts, etc.

The labs should also compare to National Voluntary Laboratory Accreditation Program (NVLAP) proficiency testing samples, if the lab participates in the Bulk Asbestos NVLAP, or to external quality assurance system consensus results such as from proficiency testing programs using characterized materials. However, at this time, consensus values for the quantity of asbestos have been shown to be unreliable. Only proficiency testing materials characterized by multiple techniques should be used to determine accuracy and precision.

2.1.5 Procedures

NOTE: Exposure to airborne asbestos fibers is a health hazard. Bulk samples submitted for analysis are oftentimes friable and may release fibers during handling or matrix reduction steps. All sample handling and examination must be carried out in a HEPA-filtered hood, a class 1 biohazard hood or a glove box with continuous airflow (negative pressure). Handling of samples without these precautions may result in exposure of the analyst to and contamination of samples by airborne fibers.

2.1.5.1 Sample Preparation

No sample preparation should be undertaken before initial stereomicroscopic examination. Distinct changes in texture or color on a stereomicroscopic scale that might denote an uneven distribution of components should be noted. When a sample consists of two or more distinct layers or building materials, each should be treated as a separate sample, when possible. Thin coatings of paint, rust, mastic, etc., that cannot be separated from the sample without compromising the layer are an exception to this case and may be included with the layer to which they are attached. Drying (by heat lamp, warm plate, etc.) of wet or damp samples is recommended before further stereomicroscopic examination and is mandatory before PLM examination. **Drying must be done in a safety hood.**

For nonlayered materials that are heterogeneous, homogenization by some means (mill, blender, mortar and pestle) may provide a more even distribution of sample components. It

may also facilitate disaggregation of clumps and removal of binder from fibers (rarely however, it may mask fibers that were originally discernable).

For materials such as cementitious products and floor tiles, breaking, pulverizing, or grinding may improve the likelihood of exposing fibrous components.

It may be appropriate to treat some materials by dissolution with hydrochloric acid to remove binder/matrix materials. Components such as calcite, gypsum, magnesite, etc., may be removed by this method. For materials found to possess a high organic content (cellulose, organic binders), ashing by means of a muffle furnace or plasma asher (for small, cellulosic samples), or dissolution by solvents may be used to remove interfering material. In either case, it is recommended that matrix removal be tracked gravimetrically.

Additional information concerning homogenization, ashing and acid dissolution may be found in Sections 2.2.5.1 and 2.3.

2.1.5.2 Analysis

Samples should be examined with a simple stereomicroscope by viewing multiple fields of view over the entire sample. The whole sample should be observed after placement in a suitable container (watchglass, weigh boat, etc.) substrate. Samples that are very large should be subsampled. The sample should be probed, by turning pieces over and breaking open large clumps. The purpose of the stereomicroscopic analysis is to determine homogeneity, texture, friability, color, and the extent of fibrous components of the sample. This information should then be used as a guide to the selection of further, more definitive qualitative and quantitative asbestos analysis methods. Homogeneity refers to whether each subsample made for other analytical techniques (e.g. the "pinch" mount used for the PLM analysis), is likely to be similar or dissimilar. Color can be used to help determine homogeneity, whether the sample has become wet (rust color), and to help identify or clarify sample labelling confusion between the building material sampler and the laboratory. Texture refers to size, shape and arrangement of sample components. Friability may be indicated by the ease with which the sample is disaggregated (see definitions in Appendix A) as received by the analyst. This does not necessarily represent the friability of the material as determined by the assessor at the collection site. The relative proportion of fibrous

components to binder/matrix material may be determined by comparison to similar materials of known fibrous content. For materials composed of distinct layers or two or more distinct building materials, each layer or distinct building material should be treated as a discrete sample. The relative proportion of each in the sample should be recorded. The layers or materials should then be separated and analyzed individually. Analysis results for each layer or distinct building material should be reported. If monitoring requirements call for one reported value, the results for the individual layers or materials should always be reported along with the combined value. Each layer or material should be checked for homogeneity during the stereomicroscopic analysis to determine the extent of sample preparation and homogenization necessary for successful PLM or other analysis. Fibers and other components should be removed for further qualitative PLM examination.

Using the information from the stereomicroscopic examination, selection of additional preparation and analytical procedures should be made. Stereomicroscopic examination should typically be performed again after any change or major preparation (ashing, acid dissolution, milling, etc.) to the sample. Stereomicroscopic examination for estimation of asbestos content may also be performed again after the qualitative techniques have clarified the identities of the various fibrous components to assist in resolving differences between the initial quantitative estimates made during the stereomicroscopic analysis and those of subsequent techniques. Calibration of analysts by use of materials of known asbestos content is essential.

The stereomicroscopic examination is often an iterative process. Initial examination and estimates of asbestos concentration should be made. The sample should then be analyzed by PLM and possibly other techniques. These results should be compared to the initial stereomicroscopic results. Where necessary, disagreements between results of the techniques should be resolved by reanalyzing the sample stereomicroscopically.

2.1.6 Calibration Materials

Calibration materials fall into several categories, including internal laboratory standards and other materials that have known asbestos weight percent content. These calibration materials could include:

- Actual bulk samples: asbestos-containing materials that have been characterized by other analytical methods such as XRD, AEM and/or gravimetry. (e.g. NVLAP test samples).

- Generated samples: in-house standards that can be prepared by mixing known quantities of asbestos and known quantities of asbestos-free matrix materials (by weight), and mixing (using blender, mill, etc.) thoroughly to achieve homogeneity; matrix materials such as vermiculite, perlite, sand, fiberglass, calcium carbonate, etc. may be used. A range of asbestos concentrations should be prepared (e.g. 1, 3, 5, 10, 20%, etc.). The relationship between specific gravities of the components used in standards should be considered so that weight/volume relationships may be determined.

- Photographs, drawings: photomicrographs of standards, computer-generated drawings, etc.

Suggested techniques for the preparation and use of in-house calibration standards are presented in Appendix C, and at greater length by Harvey et al.[1] The use of synthesized standards for analyst calibration and internal laboratory quality control is not new however, having been outlined by Webber et al.[2] in 1982.

2.1.7 References

1. Harvey, B. W., R. L. Perkins, J. G. Nickerson, A. J. Newland and M. E. Beard, "Formulating Bulk Asbestos Standards", Asbestos Issues, April 1991, pp. 22-29.

2. Webber, J. S., A. Pupons and J. M. Fleser, "Quality-Control Testing for Asbestos Analysis with Synthetic Bulk Materials". American Industrial Hygiene Associations Journal, 43, 1982, pp. 427-431.

2.2 Polarized Light Microscopy

2.2.1 Principle and Applicability

Samples of bulk building materials taken for asbestos identification should first be examined with the simple stereomicroscope to determine homogeneity and preliminary fiber identification. Subsamples should then be examined using PLM to determine optical properties of constituents and to provide positive identification of suspect fibers.

The principles of optical mineralogy are well-established.[1,2,3,4] A light microscope equipped with two polarizing filters is used to observe specific optical characteristics of a sample. The use of plane polarized light allows for the determination of refractive indices relative to specific crystallographic orientations. Morphology and color are also observed while viewing under plane polarized light. Observation of particles or fibers while oriented between polarizing filters whose privileged vibration directions are perpendicular (crossed polars) allows for determination of isotropism/anisotropism, extinction characteristics of anisotropic particles, and calculation of birefringence. A retardation plate may be placed in the polarized light path for verification of the sign of elongation. If subsamples are prepared in such a way as to represent all sample components and not just suspect fibers, semi-quantitative analysis may also be performed. Semi-quantitative analysis involves the use of calibrated visual area estimation and/or point counting. Visual area estimation is a semi-quantitative method that must relate back to calibration materials. Point counting, also semi-quantitative, is a standard technique used in petrography for determining the relative areas occupied by separate minerals in thin sections of rock. Background information on the use of point counting[3] and the interpretation of point count data[5] is available.

Although PLM analysis is the primary technique used for asbestos determination, it can show significant bias leading to false negatives and false positives for certain types of materials. PLM is limited by the visibility of the asbestos fibers. In some samples the fibers may be reduced to a diameter so small or masked by coatings to such an extent that they cannot be reliably observed or identified using PLM.

2.2.2 Range

The detection limit for visual estimation is a function of the quantity of sample analyzed, the nature of matrix interference, sample preparation, and fiber size and distribution. Asbestos may be detected in concentrations of less than one percent by area if sufficient material is analyzed. Since floor tiles may contain fibers too small to be resolved by PLM (< 0.25 μm in diameter), detection of those fibers by this method may not be possible. When point counting is used, the detection limit is directly proportional to the amount of sample analyzed, but is also limited by fiber visibility. Quantitation by area estimation, both visual and by point counting, should yield similar results if based on calibration standards.

2.2.3 Interferences

Fibrous and nonfibrous, organic and inorganic constituents of bulk samples may interfere with the identification and quantitation of the asbestos mineral content. Binder/matrix materials may coat fibers, affect color, or obscure optical characteristics to the extent of masking fiber identity. Many organic mastics are soluble in refractive index liquids and, unless removed prior to PLM examination, may affect the refractive index measurement of constituent materials. Fine particles of other materials may also adhere to fibers to an extent sufficient to cause confusion in identification. Gravimetric procedures for the removal of interfering materials are presented in Section 2.3.

2.2.4 Precision and Accuracy

Data obtained for samples containing a single asbestos type in a sample matrix have been reported previously by Brantley et al.[6] Data for establishing the accuracy and precision of the method for samples with various matrices have recently become available. Perkins,[7] Webber et al.[8] and Harvey et al.[9] have each documented the tendency for visual estimates to be high when compared to point-count data. Precision and accuracy must be determined by the individual laboratory for the percent range involved. If point counting and/or visual estimates are used, a table of reasonably expanded errors, such as those shown in Table 2-1, should be generated for different concentrations of asbestos.

If the laboratory cannot demonstrate adequate precision and accuracy (documented by control charts, etc), quantitation by additional methods, such as gravimetry, may be required. Refer to the Handbook for SRM Users[10] for additional information concerning the concepts of precision and accuracy.

TABLE 2-1. SUGGESTED ACCEPTABLE ERRORS FOR PLM ANALYSIS
(Based on 400 point counts of a reasonably homogeneous sample or 100 fields of view for visual estimate)

% Area Asbestos	Acceptable Mean Result	% Area Asbestos	Acceptable Mean Result
1	>0-3%	50	40-60%
5	>1-9%	60	50-70%
10	5-15%	70	60-80%
20	10-30%	80	70-90%
30	20-40%	90	80-100%
40	30-50%	100	90-100%

2.2.5 Procedures

NOTE: Exposure to airborne asbestos fibers is a health hazard. Bulk samples submitted for analysis are oftentimes friable and may release fibers during handling or matrix reduction steps. All sample and slide preparations must be carried out in a HEPA-filtered, a class 1 biohazard hood, or a glove box with continuous airflow (negative pressure). Handling of samples without these precautions may result in exposure of the analyst to and contamination of samples by airborne fibers.

2.2.5.1 Sample Preparation

Slide mounts are prepared for the identification and quantitation of asbestos in the sample.

2.2.5.1.1 Qualitative Analysis Preparation

The qualitative preparation must allow the PLM analysis to classify the fibrous components of the sample as asbestos or nonasbestos. The major goal of the qualitative

preparation is to mount easily visible fibers in appropriate refractive index liquids for complete optical characterization. Often this can be accomplished by making immersion grain mounts of random subsamples of the homogeneous material. Immersion liquids with refractive indices close to the suspected (see stereomicroscopic analysis) asbestos mineral should be used for the qualitative analysis so that n_D can be determined. Problem samples include those with inhomogeneities, coatings, small fibers, and interfering compounds. Additional qualitative preparations are often necessary for these types of samples. All samples, but especially those lacking homogeneity, may require picking of fibers from specific sample areas during the stereomicroscopic examination. Coatings on the fibers often need to be removed by mechanical or chemical means. Teasing the particles apart or use of a mortar and pestle or similar mechanical method often is sufficient to free fibers from coatings. Chemical means of removing some coatings and interfering compounds are discussed in Section 2.3, Gravimetry.

2.2.5.1.2 Quantitative Analysis Preparation

The major purpose of the quantitative preparation is to provide the analyst with a representative grain mount of the sample in which the asbestos can be observed and distinguished from the nonasbestos matrix. This is typically performed by using randomly selected subsamples from a homogeneous sample (see stereomicroscopic analysis). Particles should be mounted in a refractive index (RI) liquid that allows the asbestos to be visible and distinguished from nonasbestos components. Care should be taken to ensure proper loading and even distribution of particles. Both the qualitative and quantitative sample preparations are often iterative processes. Initial samples are prepared and analyzed. The PLM analysis may disclose problems or raise questions that can only be resolved by further preparations (e.g. through the use of different RI immersion liquids, elimination of interfering compounds, sample homogenization, etc.)

For layered materials, subsamples should be taken from each individual or discrete layer. Each of these subsamples should be treated as a discrete sample, but as stated in Section 2.1.5.2, the results for the individual layers or materials may be combined if called for by monitoring requirements.

Homogenization involves the use of any of a variety of devices, such as a mortar and pestle, mill, or blender to pulverize, disaggregate and mix heterogeneous, friable bulk materials. Selection of the appropriate device is dependent upon personal preference and the nature of the materials encountered. A blender or mortar and pestle may be adequate for homogenizing materials that lack appreciable amounts of tacky matrix/binder, and for separating interfering components from the fibers. For materials which are unusually sticky or tacky, or contain unusually long asbestos fibers, milling (especially freezer milling) may be more efficient. However, milling should be discontinued as soon as the material being milled appears homogeneous, in order to reduce the potential for mechanically reducing fiber size below the resolving power of the polarizing microscope. Hammer mills or cutting mills may also be used on these materials; however, the same precaution regarding reduction of fiber size should be taken. Blending /milling devices should be disassembled (to the extent possible) and thoroughly cleaned after each use to minimize contamination.

2.2.5.2 Analysis

Analysis of bulk building materials consists of the identification and semi-quantitation of the asbestos type(s) present, along with the identification, where possible, of fibrous nonasbestos materials, mineral components and matrix materials. If the sample is heterogeneous due to the presence of discrete layers or two or more distinct building materials, each layer or distinct material should be analyzed, and results reported. Total asbestos content may also be stated in terms of a relative percentage of the total sample.

2.2.5.2.1 Identification

Positive identification of asbestos requires the determination of the following optical properties:

- Morphology
- Color and, if present, pleochroism
- Refractive indices (± .005)
- Birefringence
- Extinction characteristics
- Sign of elongation

Descriptions of the optical properties listed above for asbestos fibers may be found in Appendix A, Glossary of Terms. Table 2-2 lists the above properties for the six types of asbestos and Table 2-3 presents the central stop dispersion staining colors for the asbestos minerals with selected high-dispersion index liquids. Tables 2-4 and 2-5 list selected optical properties of several mineral and man-made fibers. All fibrous materials in amounts greater than trace should be identified as asbestos or nonasbestos, with all optical properties measured for asbestos and at least one optical property measured for each nonasbestos fibrous component that will distinguish each from asbestos. Small fiber size and/or binder may necessitate viewing the sample at higher magnification (400-500x) than routinely used (100x).

Although it is not the purpose of this section to explain the principles of optical mineralogy, some discussion of the determination of refractive indices is warranted due to its importance to the proper identification of the asbestos minerals. Following is a brief discussion of refractive index determination for the asbestos minerals.

All asbestos minerals are anisotropic, meaning that they exhibit different optical properties (including indices of refraction) in different directions. All asbestos minerals are biaxial, meaning that they have one principal refractive index parallel (or nearly parallel) to the length of the fiber and two principal refractive indices (plus all intermediate indices between these two) in the plane perpendicular (or nearly so) to the length of the fiber. Although chrysotile (serpentine) is classified as a biaxial mineral, it behaves as a uniaxial mineral (two principal refractive indices) due to its scrolled structure. Amosite and crocidolite, although also biaxial, exhibit uniaxial properties due to twinning of the crystal structure and/or random orientation of fibrils in a bundle around the long axis of the bundle. For all of the asbestos minerals except crocidolite, the highest refractive index (γ) is aligned with the fiber length (positive sign of elongation). For crocidolite, the lowest refractive index (α) is aligned with the fiber length (negative sign of elongation). A more complete explanation of the relationship of refractive indices to the crystallographic directions of the asbestos minerals may be found in References 1, 2, 4, 11 and 12. It should be noted that for the measurement of refractive indices in an anisotropic particle (e.g. asbestos fibers), the orientation of the particle is quite critical. Orientation with respect to rotation about the axis

of the microscope (and thus with respect to the vibration directions of the polarizer and analyzer) and also to the horizontal plane (plane of the microscope stage) will affect the determination of the correct values for refractive indices. The refractive index that is measured will always correspond to a direction perpendicular to the axis of the microscope (i.e., lying in the plane of the stage) and is the direction in that horizontal plane parallel to the vibration direction of the polarizer, by convention E-W.

To determine $\gamma(n\parallel)$ for chrysotile, anthophyllite and amosite, the index is measured when the length of the fiber is aligned parallel to the vibration direction of the polarizer (E-W). Under crossed polars, the fiber should be at extinction in this orientation. To determine the lowest refractive index, α ($n\perp$), for chrysotile and amosite, the fiber should be oriented N-S (extinction position under crossed polars). The determination of $n\parallel$ and $n\perp$ with crocidolite is accomplished in the same manner as with amosite and chrysotile with the exception that the α and γ directions are reversed. For crocidolite, α is measured at the E-W position (parallel to the polarizer) and γ is measured at the N-S orientation (perpendicular to the polarizer). For anthophyllite, the fiber should be oriented N-S and the lowest and highest indices for this orientation should be measured. These correspond to α and β respectively.

The extinction behavior of tremolite-actinolite is anomalous compared to that of most monoclinic minerals due to the orientation of the optic axes relative to the crystallographic axes. This relationship is such that the refractive indices of the principal axes α and γ <u>are not measured</u> when the fiber is exhibiting the maximum extinction angle. The values measured at these positions are α' and γ'. The fiber exhibits an extinction angle within a few degrees of the maximum throughout most of its rotation. A wide range of refractive indices from α' to α, and from γ' to γ, are observed. For tremolite-actinolite, β is measured on those fibers displaying parallel extinction when oriented in the N-S position. The refractive index for α is also measured when the fiber is oriented generally in the N-S position and exhibits <u>the true extinction</u> angle; true α will be the <u>minimum</u> index. To determine the refractive index for γ, the fibers should be oriented E-W and exhibit the true extinction angle; true γ will be the <u>maximum</u> value for this orientation.

When viewing single fibers, the analyst may often be able to manipulate the microscope slide cover slip and "roll" the fibers to positions that facilitate measuring the true values of refractive indices. When viewing a large population of fibers with the microscope in the dispersion staining mode, the analyst can easily detect fibers that exhibit the highest and lowest indices (β and α) in the N-S position and the highest indices (γ) in the E-W position. Since individual asbestos fibrils cannot generally be resolved using polarized light microscopy, refractive indices are most commonly measured on fiber bundles. Such measurements would not result in true values for the indices and therefore by convention should be reported as α' and γ'.

Asbestos types chrysotile, amosite and crocidolite are currently available as SRM 1866 and actinolite, tremolite and anthophyllite as SRM 1867 from the Office of Standard Reference Materials, National Institute of Standards and Technology.

2.2.5.2.2 Quantitation of Asbestos Content

As described in Sections 2.1.5 and 2.1.6, a calibrated visual volume estimation of the relative concentrations of asbestos and nonasbestos components should be made during the stereomicroscopic examination. In addition, quantitation of asbestos content should be performed on subsample slide mounts using calibrated visual area estimates and/or a point counting procedure. Section 2.1.6 and Appendix C discuss the procedures for preparation and use of calibration standards. After thorough PLM analysis in which the asbestos and other components of the bulk material are identified, several slides should be carefully prepared from randomly selected subsamples. If the sample is not homogeneous, some homogenization procedure should be performed to ensure that slide preparations made from small pinch samples are representative of the total sample. Homogenization may range from gentle mixing using a mortar and pestle to a brief period of mixing using a blender equipped with a mini-sample container. The homogenization should be of short duration ($\sim$ 15 seconds) if using the blender technique so as to preclude a significant reduction in fiber size. The use of large cover slips (22x30mm) allows for large subsamples to be analyzed. Each slide should be checked to ensure that the subsample is representative, uniformly dispersed, and loaded in a way so as not to be dominated by superimposed (overlapping) particles.

During the qualitative analysis of the sample, the analyst should decide on the appropriate optical system (including magnification) to maximize the visibility of the asbestos in the sample while still allowing the asbestos to be uniquely distinguished from the matrix materials. The analyst may choose to alter the mounting medium or the optical system to enhance contrast. During the quantitative analysis, slides should be scanned using an optical setup that yields the best visibility of the asbestos. Upon finding asbestos, the parameters that were selected in the qualitative analysis for uniquely distinguishing it from the matrix should be used for identification. These properties will vary with the sample but include any or all of the parameters required for the qualitative analysis. For instance, low magnification allows for concurrent use of dispersion staining (focal screening), but compromises resolution of extremely small diameter fibers; use of a compensator plate and crossed polarizers frequently enhances the contrast between asbestos fibers and matrix material.

Visual area estimates should be made by comparison of the sample to calibration materials that have similar textures and fiber abundance (see Section 2.1.6 and Appendix C). A minimum of three slide mounts should be examined to determine the asbestos content by visual area estimation. Each slide should be scanned in its entirety and the relative proportions of asbestos and nonasbestos noted. It is suggested that the ratio of asbestos to nonasbestos material be recorded for several fields for each slide and the results be compared to data derived from the analysis of calibration materials having similar textures and asbestos content.

For point counting, an ocular reticle (cross-line or point array) should be used to visually superimpose a point or points on the microscope field of view. The cross-line reticle is preferred. Its use requires the scanning of most, if not all, of the slide area, thereby minimizing bias that might result from lack of homogeneity in the slide preparation. In conjunction with this reticle, a click-stop counting stage can be used to preclude introducing bias during slide advancement. Magnification used will be dictated by fiber visibility. The slide should be examined along multiple parallel traverses that adequately cover the sample area. The analyst should score (count) only points directly over occupied (nonempty) areas. Empty points should not be scored on the basis of the closest particle. If an asbestos fiber and a nonasbestos particle overlap so that a point is superimposed on their visual intersection,

a point should be scored for both categories. If the point(s) is/are superimposed on an area which has several overlapping particles, the slide should be moved to another field. While not including them in the total asbestos points counted, the analyst should record the presence of any asbestos detected but not lying under the reticle cross-line or array points. A minimum of 400 counts (maximum of eight slides with 50 counts each to minimum of two slides with 200 counts each) per sample is suggested, but it should be noted that accuracy and precision improve with number of counts. Point counting provides a determination of the projected area percent asbestos. Conversion of area percent to dry weight percent is not feasible unless the specific gravities and relative volumes of the different materials are known. It should be noted that the total amount of material to be analyzed is dependent on the asbestos concentration, i.e. the lower the concentration of asbestos, the larger the amount of sample that should be analyzed, in both the visual estimation and point counting methods. Quantitation by either method is made more difficult by low asbestos concentration, small fiber size, and presence of interfering materials.

It is suggested that asbestos concentration be reported as volume percent, weight percent or area percent depending on the method of quantitation used. A weight concentration cannot be determined without knowing the relative specific gravities and volumes of the sample components.

TABLE 2-2. OPTICAL PROPE[illegible] OF ASBESTOS FIBERS

Mineral	Morphology and Color[1]	Refractive Indices[2] α	Refractive Indices[2] γ[5]	Birefringence[6]	Extinction	Sign of Elongation
Chrysotile (asbestiform serpentine)	Wavy fibers. Fiber bundles have splayed ends and "kinks". Aspect ratio typically >10:1. Colorless[3]	1.493-1.546 1.532-1.549 1.529-1.559 1.544-1.553	1.517-1.557 1.545-1.556 1.537-1.567 1.552-1.561	0.004-0.017	Parallel	+ (length slow)
Amosite (asbestiform grunerite)	Straight to curved, rigid fibers. Aspect ratio typically >10:1. Colorless to brown, nonpleochroic or weakly so.[4] Opaque inclusions may be present	1.657-1.663 1.663-1.686 1.663-1.686 1.676-1.683	1.699-1.717 1.696-1.729 1.696-1.729 1.697-1.704	0.021-0.054	Usually parallel	+ (length slow)
Crocidolite (asbestiform riebeckite)	Straight to curved, rigid fibers. Aspect ratio typically > 10:1. Thick fibers and bundles common, blue to dark-blue in color. Pleochroic.	1.693 1.654-1.701 1.680-1.698	1.697 1.668-1.717 1.685-1.706	0.003-0.022	Usually parallel	- (length fast)
Anthophyllite-asbestos	Straight to curved fibers and bundles. Aspect ratio typically > 10:1. Anthophyllite cleavage fragments may be present with aspect ratios <10:1. Colorless to light brown.	1.598-1.652 1.596-1.694 1.598-1.674 1.6148[7]	1.623-1.676 1.615-1.722 1.615-1.697 1.6362[7]	0.013-0.028	Parallel	+ (length slow)
Tremolite-Actinolite-asbestos	Straight to curved fibers and bundles. Aspect ratio typically > 10:1. Cleavage fragments may be present with aspect ratios <10:1. Colorless to pale green	Tremolite 1.600-1.628 1.604-1.612 1.599-1.612 1.6063[7]	 1.625-1.655 1.627-1.635 1.625-1.637 1.6343[7]	0.017-0.028	Parallel and oblique (up to 21°); Composite fibers show parallel extinction.	+ (length slow)
		Actinolite 1.600-1.628 1.612-1.668 1.613-1.628 1.6126[7]	 1.625-1.655 1.635-1.688 1.638-1.655 1.6393[7]	0.017-0.028		

[1]Colors cited are seen by observation with plane polarized light.

[2]From references 2, 11, 12, and 18, respectively. Refractive indices for n_g at 589.3nm.

[3]Fibers subjected to heating may be brownish. (references 13, 14, and 15)

[4]Fibers subjected to heating may be dark brown and pleochroic. (references 13, 14, and 15)

[5]∥ to fiber length, except ⊥ to fiber length for crocidolite only.

[6]Maximum and minimum values from references 2, 11, 12, and 18 given.

[7]± 0.0007

TABLE 2-3. TYPICAL CENTRAL STOP DISPERSION STAINING COLORS[1]

Mineral	Cargille® RI Liquid	n∥	n⊥
Chrysotile	1.550HD	Magenta to light blue-green λ_0's ca. 520-620nm	Blue-green to pale blue λ_0's ca. 600-700nm
Amosite	1.680	Yellow to magenta λ_0's ca. 420-520nm	Blue magenta to light blue λ_0's ca. 560-660nm
Crocidolite	1.680	Yellow to magenta λ_0's ca. 420-520nm	Pale yellow to golden yellow λ_0's ca. 360-460nm
Anthophyllite-asbestos	1.605HD	Pale yellow to yellow λ_0's ca. 330-430nm	Golden yellow to light blue green λ_0's ca. 460-700nm
Tremolite-asbestos	1.605HD	Pale yellow to yellow λ_0's ca. 330-430nm	Golden yellow to light blue green λ_0's ca. 460-700nm
Actinolite-asbestos	1.605HD	Pale yellow λ_0's ca. 260-360nm	Pale yellow to golden yellow λ_0's ca. 360-460nm
	1.630HD	Yellow to magenta λ_0's ca. 420-520nm	Golden yellow to blue λ_0's ca. 450-600nm

[1]Modified from reference 16

TABLE 2-4. OPTICAL PROPERTIES OF MAN-MADE TEXTILE FIBERS[1,2]

Fiber Type	n∥	n⊥	n∥ - n⊥	Sign of Elongation
Polyester (Dacron®)	1.710	1.535	0.175	+
Polyamide (Nylon®)	1.582	1.514	0.063	+
Aramid (Kevlar®)	≈ 2.37	≈ 1.641	0.729	+
Olefin (Polyethylene)	1.556	1.512	0.044	+
Olefin (Polypropylene)	1.520	1.495	0.025	+
Viscose Rayon	1.535-1.555	1.515-1.535	0.020	+
Acetate	1.478-1.480	1.473-1.476	0.004-0.005	+
Acrylic (Orlon®)	1.505-1.515	1.507-1.517	0.004-0.002	-
Modacrylic (Dynel®)	1.535	1.532	0.002	+

[1]Modified from reference 17

[2]Refractive indices for specific fibers; other fibers may vary

TABLE 2-5. OPTICAL PROPERTIES OF SELECTED FIBERS[1]

FIBER TYPE	MORPHOLOGY	REFRACTIVE INDICES	BIREFRINGENCE ($n\parallel - n\perp$)	EXTINCTION ANGLE	SIGN OF ELONGATION	DISPERSION STAINING COLORS
Paper (Cellulose)	Tapered, flat ribbons	$n\parallel$ ~ 1.580 $n\perp$ ~ 1.530	High (0.05)	Parallel and incomplete	+	in 1.550HD $n\parallel$: yellow (λ_o's < 400nm) $n\perp$: pale blue (λ_o's > 700nm)
Olefin (polyethylene)	Filaments or shredded like chrysotile	$n\parallel$ ~ 1.556 $n\perp$ ~ 1.512	Moderate (0.044)	Parallel	+	in 1.550HD $n\parallel$: yellow to magenta (λ_o's = 440-540nm) $n\perp$: pale blue (λ_o's > 700nm)
Brucite (nemalite)	Straight fibers	$n\parallel$ ~ 1.560-1.590 $n\perp$ ~ 1.580-1.600	Moderate (0.012-0.020)	Usually parallel	- occasionally +	in 1.550HD $n\parallel$: golden yellow (λ_o's 440-460nm) $n\perp$: yellow (λ_o's 400-440nm)
Heated amosite	Similar to unheated, (brittle and shorter) pleochroic: $n\parallel$-dark brown $n\perp$ yellow	$n\parallel$ and $n\perp$ >1.700[2]	High (> 0.05)	Usually parallel	+	in 1.680HD $n\parallel$ & $n\perp$: both pale yellow to white (λ_o's < 400nm)
Glass fibers, Mineral wool	Exotic shapes, tear drips, single filaments	1.515-1.700	Isotropic	------	-	in 1.550HD usually pale blue to blue (λ_o's 580 to > 700nm)
Wollastonite	Straight needles and blades	$n\parallel$ ~ 1.630 $n\perp$ ~ 1.632 $n\perp$ also 1.610	Moderate to low (0.018 to 0.002)	Parallel and oblique	+ and -	in 1.605HD $n\parallel$ & $n\perp$: yellow to pale yellow (λ_o's < 460nm)
Fibrous talc	Thin cleavage ribbons and wavy fibers	$n\parallel$ ~ 1.60 $n\perp$ ~ 1.54	High (0.06)	Parallel and oblique	+	in 1.550HD $n\parallel$: pale yellow (λ_o's <400nm) $n\perp$: pale blue (λ_o's >660nm)

[1]From reference 19

[2]From references 13, 14, and 15

2.2.5.2.3 Microscope Alignment

In order to accurately measure the required optical properties, a properly aligned polarized light microscope must be utilized. The microscope is aligned when:

1) the privileged directions of the substage polarizer and the analyzer are at 90° to one another and are represented by the ocular cross-lines;

2) the compensator plate's privileged vibration directions are 45° to the privileged directions of the polarizer and analyzer;

3) the objectives are centered with respect to stage rotation; and,

4) the substage condenser and iris diaphragm are centered in the optic axis.

Additionally, the accurate measurement of the refractive index of a substance requires the use of calibrated refractive index liquids. These liquids should be calibrated regularly to an accuracy of 0.004, with a temperature accuracy of 2°C using a refractometer or R.I. glass beads.

2.2.6 References

1. Bloss, F. Donald, **An Introduction to the Methods of Optical Crystallography**, Philadelphia: Saunders College Publishing, 1989.

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2.3 Gravimetry

2.3.1 Principle and Applicability

Many components of bulk building materials, specifically binder components, can be selectively removed using appropriate solvents or, in the case of some organics, by ashing. The removal of these components serves the following purposes:

1) to isolate asbestos from the sample, allowing its weight to be determined;

2) to concentrate asbestos and therefore lower the detection limit in the total sample;

3) to aid in the detection and identification of fibrous components; and,

4) to remove organic (ashable) fibers which are optically similar to asbestos.

Common binder materials which are removed easily using the techniques described include: 1) calcite, gypsum, magnesite, brucite, bassanite, portlandite, and dolomite, using hydrochloric acid, and 2) vinyl, cellulose, and other organic components, by ashing. The removal of the binder components results in a residue containing asbestos, if initially present, and any other non-soluble or non-ashable components which were present in the original sample. Unless the procedures employed result in the loss of some asbestos, the weight percent of the residue is the upper limit for the weight percent of asbestos in the sample.

This section describes the procedure for removing acid-soluble and ashable components, and for determining the weight percent of the residue. However, the acid dissolution and ashing techniques can be used without the accompanying weight measurements to either liberate or clean fibers to aid in qualitative PLM or AEM analyses.

This technique is not an identification technique. Other methods, such as PLM, XRD, or AEM must be used to determine the identity of the components. A description of the suggested apparatus, reagents, etc. needed for the techniques described is included in Appendix B.

2.3.2 Interferences

Any components which cannot by removed from the sample by selective dissolution or ashing interfere with asbestos quantitation. These components include, but are not limited to, many silicates (micas, glass fibers, etc.) and oxides (TiO_2, magnetite, etc.). When interfering phases are present (the residue contains other phases in addition to asbestos), other techniques such as PLM, AEM, or XRD must be used to determine the percent of asbestos in the residue.

Care must be taken to prevent loss of or chemical/structural changes in the critical components (asbestos). Prolonged exposure to acids or excessive heating (above 500°C) can cause changes in the asbestos components in the sample and affect the optical properties.[1,2,3]

2.3.3 Quantitation

The weight of the residue remaining after solvent dissolution/ashing should be compared with the original weight of the material. Presuming no insoluble material is lost, the weight percent of the residue is the upper limit for the amount of asbestos in the sample. If the residue is comprised only of asbestos, then the weight percent of residue equals the weight percent of asbestos in the sample. If the residue contains other phases, then techniques such as PLM, XRD, or AEM must be employed to determine the relative abundance of asbestos in the residue.

The precision and accuracy of the technique are dependent upon the homogeneity of the material, the accuracy of the weight measurements, and the effectiveness of the sample reduction and filtering procedures. In practice, the precision can be equal to $\pm 1\%$, and the accuracy at 1 wt% asbestos can be less than or equal to $\pm 10\%$ relative.

The incomplete solution of components and the presence of other nonasbestos components in the residue contribute to producing a positive bias for the technique (falsely high percentages of asbestos).

2.3.4 Preliminary Examination and Evaluation

Stereomicroscopic and PLM examinations of the sample should already have been conducted prior to initiating this procedure. These examinations should have provided information about: 1) whether the sample contains components which can be removed by acid-washing, solvent dissolution, or ashing, and 2) whether the sample contains asbestos, or fibers that might be asbestos, or whether no asbestos was detected.

If the sample is friable and contains organic (ashable) components, the ashing procedure should be followed. If the sample is friable and contains HCl-soluble components, the acid dissolution procedure should be followed. If the sample is friable and contains both types of

components, the two procedures can be applied, preferably with acid dissolution following ashing.

If the sample is nonfriable (e.g. floor tiles), it is also recommended that the ashing procedure be used first, followed by the acid dissolution procedure. The ashing procedure reduces floor tiles to a material which is easily powdered, simplifying the sample preparation for acid dissolution.

2.3.5 Sample Preparation

2.3.5.1 Drying

Any moisture in the sample will affect the weight measurements, producing falsely low percentages of residue. If the sample is obviously wet, it should be dried at low temperature (using a heat lamp, or simply by exposure at ambient conditions, prior to starting the weighing procedure). If an oven is used, the drying temperature should not exceed 60°C. Drying by means of heat lamp or ambient air must be performed within a safety-filtered hood. Even if the sample appears dry, it can contain enough moisture to affect the precision and accuracy of the technique. The test for sample moisture involves placing the amount of sample to be used on the weighing pan; if the weight remains stable with time, then the sample is dry enough. If the weight decreases as the sample sits on the weighing pan, then the sample should be dried. Where conditions of moderate to high humidity are known to exist, all materials to be weighed should be allowed time to stabilize to these ambient conditions.

2.3.5.2 Homogenization/Grain Size Reduction

To increase the accuracy and precision of the acid dissolution technique, the sample should be homogenized prior to analysis. This reduces the grain size of the binder material and releases it from fiber bundles so that it may be dissolved in a shorter time period. Leaving the sample in the acid for a longer period of time to complete the dissolution process can adversely affect the asbestos components, and is not recommended. Homogenization of the sample also ensures that any material removed for analysis will more likely be representative of the entire sample.

Homogenization of friable samples prior to ashing may also accelerate the ashing process; however, the ashing time can simply be increased without affecting the asbestos in the sample. Nonfriable samples, such as vinyl floor tiles, can be broken or shaved into pieces to increase surface area and accelerate the ashing process.

Homogenization and grain size reduction can be accomplished in a variety of ways: 1) hand grinding in a mortar and pestle; 2) crushing with pliers or similar instrument; 3) mixing in a blender; 4) milling (i.e. Wylie mill, cryomill, etc.); or 5) any other technique which seems suitable. If the fibers are extremely long, a pair of scissors or similar implement can be used to reduce the fiber length.

2.3.6 Procedure for Ashing

1) **Weigh appropriate amount of material.**

   There is no restriction on the maximum weight of material used; however, a large amount of material may take longer to ash. Enough material should be used to avoid a significant contribution of weighing errors to the total accuracy and precision.

2) **Place material in crucible, weigh, and cover with lid.**

   Placing a lid on the crucible both minimizes the amount of oxygen available, slowing the rate of combustion of the sample, and prevents any foreign material from falling into the crucible during ashing.

3) **Place crucible into furnace, and ash for at least 6 hours.**

   The furnace temperature at the sample position should be at least 300°C but should not exceed 500°C. If the sample combusts (burns), the temperature of the sample may exceed 500°C. Chrysotile will decompose above approximately 500°C.

   The furnace area should be well-ventilated and the fumes produced by ashing should be exhausted outside the building.

   The ashing time is dependent on the furnace temperature, the amount of sample, and the surface area (grain size). Six hours at 450°C is usually sufficient.

4) **Remove crucible from furnace, allow contents to adjust to room temperature and humidity, and weigh.**

5) **Divide residue weight by starting weight and multiply by 100 to determine weight% residue.**

6) **Analyze residue and/or proceed to acid dissolution procedure.**

If the objective was to remove organic fibers that may be confused optically with asbestos, examine residue with PLM to determine whether any fibers remain.

If the sample is a floor tile, the acid dissolution procedure must now be performed. The residue does not have to be analyzed at this stage.

2.3.7 Use of Solvents for Removal of Organics

Solvent dissolution may be used as a substitute for low temperature ashing for the purpose of removing organic interferences from bulk building materials. However, solvent dissolution, because of the involvement of potentially hazardous reagents such as tetrahydrofuran, amyl acetate, 1-1-1, trichlorethane, etc., requires **that all work be performed with extreme caution inside a biohazard hood.** Material Safety Data Sheets should be reviewed before using any solvent. Solvent dissolution involves more apparatus than does ashing, and requires more time, mainly due to set-up and slow filtration resulting from viscous solvent/residue mixtures.

The following is a brief description of the solvent dissolution process.

1) **Weigh starting material.**

Place approximately 15-25ml of solvent in a 100ml beaker. Add 2.5-3.0 grams (carefully weighed for continued gravimetric tracking) of powdered sample.

2) **Untrasonicate sample.**

Place the beaker in an ultrasonic bath (or ultrasonic stirrer) for approximately 0.5 hours. The sample containers should be covered to preclude escape of an aerosol spray.

3) **Centrifuge sample.**

Weigh centrifuge vial before adding beaker ingredients. Wash beaker with an additional 10-15ml of solvent to remove any remaining concentrate. Then centrifuge

at approximately 2000-2500 rpm for 0.5 hour. Use solvent-resistant centrifuge tubes.

4) **Decant sample, reweigh.**

After separation by centrifuging, decant solvent by pipetting. Leave a small amount of solvent in the centrifuge vial to minimize the risk of decanting solid concentrate. Allow solid concentrate to dry in vial, then reweigh.

2.3.8 Procedure for Acid Dissolution

1) **Weigh starting material, transfer to acid resistant container.**

Small, dry sample weights between 0.1g and 0.5g are recommended (determined for 47mm filters - adjust amount if different diameter filters are used). If too much material is left after acid dissolution the filter can get clogged and prevent complete filtration. Very small samples are also to be avoided, as the weighing errors will have a large effect on the total accuracy and precision of the technique.

2) **Weigh filter.**

3) **Add HCl to sample in container, stir, allow to sit for 2-10 minutes.**

Either concentrated or dilute HCl can be used. If concentrated HCl is used, add enough acid to completely soak the material, allow the reaction to proceed to completion, and then dilute with distilled water. Alternatively, a dilute solution, made by adding concentrated HCl to distilled water, can be used in the place of concentrated HCl. A solution of 1 part concentrated HCl to 3 parts distilled water (approximately 3N solution) has been found to be quite effective in removing components within 5 minutes. For a sample size less than 0.5g, 20-30 ml of a 3N HCl solution is appropriate. In either case (using concentrated or dilute HCl), the reaction will be more effective if the sample has been homogenized first. All obvious signs of reaction (bubbling) should cease before the sample is filtered. Add fresh acid, a ml or two at a time, to ensure complete reaction. It should be noted that if dolomite is present, a 15-20 minute exposure to concentrated HCl may be required to completely dissolve the carbonate materials.

NOTE: Other solvents may be useful for selective dissolution of nonasbestos components. For example, acetic acid will dissolve calcite, and will not dissolve asbestos minerals. If any solvent other than hydrochloric acid is used for the dissolution of inorganic components, the laboratory must be able to demonstrate that the solvent does not remove asbestos from the sample.

4) **Filter solution.**

Use the pre-weighed filter. Pour the solution into the vacuum filter assembly, then rinse all material from container into filter assembly. Rinse down the inside walls of the glass filter basin and check for particles clinging to the basin after removal.

5) **Weigh dried filter + residue, subtract weight of filter from total.**

6) **Divide residue weight by starting weight and multiply by 100 to determine weight% residue.**

7) **Analyze residue.**

Perform stereomicroscopic examination of residue (can be performed without removing the residue from the filter). Note in particular whether any binder material is still present.

Perform PLM, AEM, or XRD analysis of residue to identify fibers and determine concentration as described in the appropriate sections of this method.

8) **Modify procedure if necessary.**

If removal of the acid soluble components was not complete, start with a new subsample of material and try any of the following:

a) Decrease grain size of material (by grinding, milling, etc.)
b) Put solutions on hot plate - warm slightly
c) Increase soak time (exercise caution)

9) **Calculate relative weight% asbestos in sample.**

wt% asbestos in sample = % asbestos in residue x wt% residue ÷ 100

For floor tiles, if the ashing procedure was used first, multiply the weight % of asbestos in the sample, as determined above, by the weight percent of the residue from the ashing procedure, then divide by 100.

Example:
A = wt% residue from ashing = 70%
B = wt% residue from HCl = 20%
C = wt% of asbestos in HCl residue = 50%

wt% asbestos after HCl dissolution = B x C ÷ 100 = 20 x 50 ÷ 100 = 10%

wt% asbestos in floor tile = (B x C ÷ 100) x A ÷ 100 = 10 x 70 ÷ 100 = 7%

If weights are expressed in decimal form, multiply the weight % of asbestos in the sample by the weight % of the residue from the ashing procedure, then multiply by 100.

wt% asbestos after HCl dissolution = B x C = 0.2 x 0.5 = 0.1 (x 100 = 10%)

wt% asbestos in floor tile = (B x C) x A = 0.1 x 0.7 = 0.07 (x 100 = 7%)

2.3.9 Determination of Optimal Precision and Accuracy

The precision of the technique can be determined by extracting multiple subsamples from the original sample and applying the same procedure to each. The optimal accuracy of the technique can be determined by applying gravimetric standards. Mixtures of calcite and asbestos (chrysotile, amosite, etc.) in the following proportions are recommended for testing the accuracy of the acid dissolution technique: 0.1 wt% asbestos/99.9 wt% calcite, 1.0 wt% asbestos/99.0 wt% calcite, and 10 wt% asbestos/90 wt% calcite. Mixtures of cellulose and asbestos are useful for testing the accuracy of the ashing technique.

Mixtures of only two components, as described above, are simplifications of "real-world" samples. The accuracy determined by analyzing these mixtures is considered optimal and may not apply directly to the measurement of each unknown sample. However, analyzing replicates and standards using the full laboratory procedure, including homogenization, ashing, acid dissolution, filtration, and weighing, may uncover steps that introduce significant bias or variation that the laboratory may then correct.

2.3.10 References

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2. Prentice, J. and M. Keech, "Alteration of Asbestos with Heat", **Microscopy and Analysis**, March 1989.

3. Laughlin, G. and W. C. McCrone, "The Effect of Heat on the Microscopical Properties of Asbestos", **The Microscope**, 37, 1989, pp. 8-15.

2.4 X-Ray Powder Diffraction

2.4.1 Principle and Applicability

The principle of x-ray powder diffraction (XRD) analysis is well established.[1,2] Any solid crystalline material will diffract an incident beam of parallel, monochromatic x-rays whenever Bragg's Law,

$$\lambda = 2d \sin \theta,$$

is satisfied for a particular set of planes in the crystal lattice, where

λ = the x-ray wavelength, Å;
d = the interplanar spacings of the set of reflecting lattice planes, Å and
θ = the angle of incidence between the x-ray beam and the reflecting lattice planes.

By appropriate orientation of a sample relative to the incident x-ray beam, a diffraction pattern can be generated that will be uniquely characteristic of the structure of the crystalline phases present.

Unlike optical methods of analysis, however, XRD cannot determine crystal morphology. Therefore, in asbestos analysis, XRD does not distinguish between fibrous and nonfibrous forms of the serpentine and amphibole minerals (Table 2-6). However, when used in conjunction with methods such as PLM or AEM, XRD techniques can provide a reliable analytical method for the identification and characterization of asbestiform minerals in bulk materials.

For qualitative analysis by XRD methods, samples should initially be scanned over limited diagnostic peak regions for the serpentine (~7.4 Å) and amphibole (8.2-8.5 Å) minerals (Table 2-7). Standard slow-scanning methods for bulk sample analysis may be used for materials shown by PLM to contain significant amounts of asbestos (>5 percent). Detection of minor or trace amounts of asbestos may require special sample preparation and step-scanning analysis. All samples that exhibit diffraction peaks in the diagnostic regions for asbestiform minerals should be submitted to a full (5°-60° 2θ; 1° 2θ/min) qualitative XRD scan, and their diffraction patterns should be compared with standard reference powder

diffraction patterns[3] to verify initial peak assignments and to identify possible matrix interferences when subsequent quantitative analysis will be performed.

Accurate **quantitative** analysis of asbestos in bulk samples by XRD is critically dependent on particle size distribution, crystallite size, preferred orientation and matrix absorption effects, and comparability of standard reference and sample materials. The most intense diffraction peak that has been shown to be free from interference by prior qualitative XRD analysis should be selected for quantitation of each asbestiform mineral. A "thin-layer" method of analysis[5,6] can be used in which, subsequent to comminution of the bulk material to $\sim 10\ \mu m$ by suitable cryogenic milling techniques, an accurately known amount of the sample is deposited on a silver membrane filter. The mass of asbestiform material is determined by measuring the integrated area of the selected diffraction peak using a step-scanning mode, correcting for matrix absorption effects, and comparing with suitable calibration standards. Alternative "thick-layer" or bulk methods[7,8] are commonly used for **semi-quantitative analysis.**

TABLE 2-6. THE ASBESTOS MINERALS AND THEIR NONASBESTIFORM ANALOGS

Asbestiform	Nonasbestiform	Chemical Abstract Service No.
Serpentine		
Chrysotile	Antigorite, lizardite	12001-29-5
Amphibole		
Anthophyllite asbestos	Anthophyllite	77536-67-5
Cummingtonite-grunerite asbestos (Amosite)	Cummingtonite-grunerite	12172-73-5
Crocidolite	Riebeckite	12001-28-4
Tremolite asbestos	Tremolite	77536-68-6
Actinolite asbestos	Actinolite	77536-66-4

TABLE 2-7. PRINCIPAL LATTICE SPACINGS OF ASBESTIFORM MINERALS[1]

Minerals	Principal d-spacings (Å) and relative intensities			JCPDS Powder diffraction file[2] number
Chrysotile (Serpentine)	7.31_{100} 7.36_{100} 7.10_{100}	3.65_{70} 3.66_{80} 2.33_{80}	4.57_{50} 2.45_{25} 3.55_{70}	21-543[3] 25-645 22-1162 (theoretical)
Amosite (Grunerite)	8.33_{100} 8.22_{100}	3.06_{70} 3.060_{85}	2.756_{70} 3.25_{70}	17-745 (nonfibrous) 27-1170 (UICC)
Anthophyllite	3.05_{100} 3.06_{100}	3.24_{60} 8.33_{70}	8.26_{55} 3.23_{50}	9-455 16-401 (synthetic)
Crocidolite (Riebeckite)	8.35_{100} 8.40_{100}	3.10_{55} 3.12_{55}	2.720_{35} 2.726_{40}	27-1415 (UICC) 19-1061
Actinolite	2.72_{100}	2.54_{100}	3.40_{80}	25-157
Tremolite	8.38_{100} 2.706_{100} 3.13_{100}	3.12_{100} 3.14_{95} 2.706_{60}	2.705_{80} 8.43_{40} 8.44_{40}	13-437[3] 20-1310[3] (synthetic) 23-666 (synthetic mixture w/richterite)

1. This information is intended as a guide only. Complete powder diffraction data, including mineral type and source, should be referred to ensure comparability of sample and reference materials where possible. Additional precision XRD data on amosite, crocidolite, tremolite and chrysotile are available from the U.S. Bureau of Mines, Reference 4.

2. From Reference 3

3. Fibrosity questionable

This XRD method is applicable as a confirmatory method for identification and quantitation of asbestos in bulk material samples that have undergone prior analysis by PLM or other optical methods.

2.4.2 Range and Sensitivity

The range and sensitivity of the method have not been determined. They will be variable and dependent upon many factors, including matrix effects (absorption and interferences), diagnostic reflections selected and their relative intensities, preferred orientation, and instrumental limitations. A detection limit of one percent is feasible given certain sample characteristics.

2.4.3 Limitations

2.4.3.1 Interferences

Since the asbestiform and nonasbestiform analogs of the serpentine and amphibole minerals (Table 2-7) are indistinguishable by XRD techniques unless special sample preparation techniques and instrumentation are used,[9] the presence of nonasbestiform serpentines and amphiboles in a sample will pose severe interference problems in the identification and quantitative analysis of their asbestiform analogs.

The use of XRD for identification and quantitation of asbestiform minerals in bulk samples may also be limited by the presence of other interfering materials in the sample. For naturally-occurring materials, the commonly associated asbestos-related mineral interferences can usually be anticipated. However, for fabricated materials, the nature of the interferences may vary greatly (Table 2-8) and present more serious problems in identification and quantitation.[10] Potential interferences are summarized in Table 2-9 and include the following:

- **Chlorite** has major peaks at 7.19 Å and 3.58 Å that interfere with both the primary (7.31 Å) and secondary (3.65 Å) peaks for serpentine (chrysotile). Resolution of the primary peak to give good quantitative results may be possible when a step-scanning mode of operation is employed.

- **Vermiculite** has secondary peaks at 7.14 Å and 3.56 Å that could interfere with the primary peak (7.31 Å) and a secondary peak (3.65 Å) of serpentine (chrysotile).

TABLE 2-8. COMMON CONSTITUENTS IN BUILDING MATERIAL
(From Ref. 10)

A. Insulation Materials

Chrysotile
Amosite
Crocidolite
*Rock wool
*Slag wool
*Fiber glass
Gypsum ($CaSO_4 \cdot 2H_2O$)
Vermiculite (micas)
*Perlite
Clays (kaolin)
*Wood pulp
*Paper fibers (talc, clay carbonate filters)
Calcium silicates (synthetic)
Opaques (chromite, magnetite inclusions in serpentine)
Hematite (inclusions in "amosite")
Magnesite
*Diatomaceous earth

B. Flooring Materials

Calcite
Dolomite
Titanium Oxide
Quartz
Antigorite
Chrysotile
Anthophyllite
Tremolite
*Organic binders
Talc
Wollastonite

C. Spray Finishes or Paints

Bassanite
Carbonate minerals (calcite, dolomite, vaterite)
Talc
Tremolite
Anthophyllite
Serpentine (including chrysotile)
Amosite
Crocidolite
*Mineral wool
*Rock wool
*Slag wool
*Fiber glass
Clays (kaolin)
Micas
Chlorite
Gypsum
Quartz
*Organic binders and thickeners
Hydromagnesite
Wollastonite
Opaques (chromite, magnetite inclusion in serpentine)
Hematite (inclusions in "amosite")

D. Cementitious Materials

Chrysotile
Amosite
Crocidolite
Micas
Fiber glass
Cellulose
Animal hair
Quartz
Gypsum
Calcite
Dolomite
Calcium silicates

E. Roofing Materials

Chrysotile
Cellulose
Fiber glass
Mineral Wool
Asphalt
Quartz
Talc
Micas

* Amorphous materials--contribute only to overall scattered radiation and increased background radiation.

TABLE 2-9 INTERFERENCES IN XRD ANALYSIS OF ASBESTIFORM MINERALS

Asbestiform Mineral	Primary diagnostic peaks (approximate d spacings in Å)	Interference
Serpentine Chrysotile	7.3	Nonasbestiform serpentines, (antigorite, lizardite), chlorite, vermiculite, sepiolite, kaolinite, gypsum
	3.7	Nonasbestiform serpentines (antigorite, lizardite), chlorite, vermiculite, halloysite, cellulose
Amphibole Amosite (Grunerite) Anthophyllite Crocidolite (Riebeckite)	3.1	Nonasbestiform amphiboles (grunerite-cummingtonite, anthophyllite, riebeckite, tremolite), mutual interferences, talc, carbonates
Tremolite Actinolite	8.3	Nonasbestiform amphiboles (grunerite-cummingtonite, anthophyllite, riebeckite, tremolite), mutual interferences

- **Sepiolite** produces a peak at 7.47 Å which could interfere with the primary peak (7.31 Å) of serpentine (chrysotile).
- **Halloysite** has a peak at 3.63 Å that interferes with the secondary (3.65 Å) peak for serpentine (chrysotile).
- **Kaolinite** has a major peak at 7.15 Å that may interfere with the primary peak of serpentine (chrysotile) at 7.31 Å when present at concentrations of > 10 percent. However, the secondary serpentine (chrysotile) peak at 3.65 Å may be used for quantitation.
- **Gypsum** has a major peak at 7.5 Å that overlaps the 7.31 Å peak of serpentine (chrysotile) when present as a major sample constituent. This may be removed by careful washing with distilled water, or by heating to 300°C to convert gypsum to plaster of paris (bassanite).
- **Cellulose** has a broad peak that partially overlaps the secondary (3.65 Å) serpentine (chrysotile) peak.[8]

- Overlap of major diagnostic peaks of the amphibole minerals, grunerite (amosite), anthophyllite, riebeckite (crocidolite), and tremolite, at approximately 8.3 Å and 3.1 Å causes mutual interference when these minerals occur in the presence of one another. In some instances adequate resolution may be attained by using step-scanning methods and/or by decreasing the collimator slit width at the x-ray port.

- Carbonates may also interfere with quantitative analysis of the amphibole minerals grunerite (amosite), anthophyllite, riebeckite (crocidolite), and tremolite-actinolite. Calcium carbonate ($CaCO_3$) has a peak at 3.035 Å that overlaps major amphibole peaks at approximately 3.1 Å when present in concentrations of >5 percent. Removal of carbonates with a dilute acid wash is possible; however, the time in acid should be no more than 20 minutes to preclude any loss of chrysotile.[11]

- A major talc peak at 3.12 Å interferes with the primary tremolite peak at this same position and with secondary peaks of actinolite (3.14 Å), riebeckite (crocidolite) (3.10 Å), grunerite (amosite) (3.06 Å), and anthophyllite (3.05 Å). In the presence of talc, the major diagnostic peak at approximately 8.3 Å should be used for quantitation of these asbestiform minerals.

The problem of intraspecies and matrix interference is further aggravated by the variability of the silicate mineral powder diffraction patterns themselves, which often makes definitive identification of the asbestos minerals by comparison with standard reference diffraction patterns difficult. This variability results from alterations in the crystal lattice associated with differences in isomorphous substitution and degree of crystallinity. This is especially true for the amphiboles. These minerals exhibit a wide variety of very similar chemical compositions, resulting in diffraction patterns characterized by having major (110) reflections of the monoclinic amphiboles and (210) reflections of orthorhombic anthophyllite separated by less than 0.2 Å.[12]

2.4.3.2 Matrix Effects

If a copper x-ray source is used, the presence of iron at high concentrations in a sample will result in significant x-ray fluorescence, leading to loss of peak intensity, increased background intensity, and an overall decrease in sensitivity. This situation may be corrected by use of an x-ray source other than copper; however, this is often accompanied both by loss of intensity and by decreased resolution of closely spaced reflections. Alternatively, use of a

diffracted beam monochromator will reduce background fluorescent radiation, enabling weaker diffraction peaks to be detected.

X-ray absorption by the sample matrix will result in overall attenuation of the diffracted beam and may seriously interfere with quantitative analysis. Absorption effects may be minimized by using sufficiently "thin" samples for analysis.[5,13,14] However, unless absorption effects are known to be the same for both samples and standards, appropriate corrections should be made by referencing diagnostic peak areas to an internal standard[7,8] or filter substrate (Ag) peak.[5,6]

2.4.3.3 Particle Size Dependence

Because the intensity of diffracted x-radiation is particle-size dependent, it is essential for accurate quantitative analysis that both sample and standard reference materials have similar particle size distributions. The optimum particle size (i.e., fiber length) range for quantitative analysis of asbestos by XRD has been reported to be 1 to 10 μm.[15] Comparability of sample and standard reference material particle size distributions should be verified by optical microscopy (or another suitable method) prior to analysis.

2.4.3.4 Preferred Orientation Effects

Preferred orientation of asbestiform minerals during sample preparation often poses a serious problem in quantitative analysis by XRD. A number of techniques have been developed for reducing preferred orientation effects in "thick layer" samples.[7,8,15] For "thin" samples on membrane filters, the preferred orientation effects seem to be both reproducible and favorable to enhancement of the principal diagnostic reflections of asbestos minerals, actually increasing the overall sensitivity of the method.[12,14] However, further investigation into preferred orientation effects in both thin layer and bulk samples is required.

2.4.3.5 Lack of Suitably Characterized Standard Materials

The problem of obtaining and characterizing suitable reference materials for asbestos analysis is clearly recognized. The National Institute of Standards and Technology can

provide standard reference materials for chrysotile, amosite and crocidolite (SRM 1866) and anthophyllite, tremolite and actinolite (SRM 1867).

In addition, the problem of ensuring the comparability of standard reference and sample materials, particularly regarding crystallite size, particle size distribution, and degree of crystallinity, has yet to be adequately addressed. For example, Langer et al.[18] have observed that in insulating matrices, chrysotile tends to break open into bundles more frequently than amphiboles. This results in a line-broadening effect with a resultant decrease in sensitivity. Unless this effect is the same for both standard and sample materials, the amount of chrysotile in the sample will be under-estimated by XRD analysis. To minimize this problem, it is recommended that standardized matrix reduction procedures be used for both sample and standard materials.

2.4.4 Precision and Accuracy

Neither the precision nor accuracy of this method has been determined. The individual laboratory should obtain or prepare a set of calibration materials containing a range of asbestos weight percent concentrations in combination with a variety of matrix/binder materials. Calibration curves may be constructed for use in semi-quantitative analysis of bulk materials.

2.4.5 Procedure

2.4.5.1 Sampling

Samples taken for analysis of asbestos content should be collected as specified by EPA[19]

2.4.5.2 Analysis

All samples must be analyzed initially for asbestos content by PLM. XRD may be used as an additional technique, both for identification and quantitation of sample components. **Note: Asbestos is a toxic substance. All handling of dry materials should be performed in a safety-hood.**

2.4.5.2.1 Sample Preparation

The method of sample preparation required for XRD analysis will depend on: (1) the condition of the sample received (sample size, homogeneity, particle size distribution, and overall composition as determined by PLM); and (2) the type of XRD analysis to be performed (qualitative or quantitative; thin-layer or bulk).

Bulk materials are usually received as heterogeneous mixtures of complex composition with very wide particle size distributions. Preparation of a homogeneous, representative sample from asbestos-containing materials is particularly difficult because the fibrous nature of the asbestos minerals inhibits mechanical mixing and stirring, and because milling procedures may cause adverse lattice alterations.

A discussion of specific matrix reduction procedures is given below. Complete methods of sample preparation are detailed in Sections 2.4.5.3 and 2.4.5.4. **Note: All samples should be examined microscopically before and after each matrix reduction step to monitor changes in sample particle size distribution, composition, and crystallinity, and to ensure sample representativeness and homogeneity for analysis.**

2.4.5.2.2 Milling

Mechanical milling of asbestos materials has been shown to decrease fiber crystallinity, with a resultant decrease in diffraction intensity of the specimen; the degree of lattice alteration is related to the duration and type of milling process.[20-23] Therefore, all milling times should be kept to a minimum.

For **qualitative analysis**, particle size is not usually of critical importance and initial characterization of the material with a minimum of matrix reduction is often desirable to document the composition of the sample as received. Bulk samples of very large particle size (>2-3 mm) should be comminuted to ~ 100 μm. A mortar and pestle can sometimes be used in size reduction of soft or loosely bound materials though this may cause matting of some samples. Such samples may be reduced by cutting with a razor blade in a mortar, or by grinding in a suitable mill (e.g., a microhammer mill or equivalent). When using a mortar for grinding or cutting, the sample should be moistened with ethanol, or some other

suitable wetting agent, to minimize exposure, and the procedure should be performed in a HEPA-filtered hood.

For accurate, reproducible quantitative analysis, the particle size of both sample and standard materials should be reduced to $\sim 10\ \mu m$. Dry ball milling at liquid nitrogen temperatures (e.g., Spex Freezer Mill®, or equivalent) for a maximum time of 10 minutes (some samples may require much shorter milling time) is recommended to obtain satisfactory particle size distributions while protecting the integrity of the crystal lattice.[5] Bulk samples of very large particle size may require grinding in two stages for full matrix reduction to $< 10\ \mu m$.[8,16]

Final particle size distributions should always be verified by optical microscopy or another suitable method.

2.4.5.2.3 Ashing

For materials shown by PLM to contain large amounts of cellulose or other organic materials, it may be desirable to ash prior to analysis to reduce background radiation or matrix interference. Since chrysotile undergoes dehydroxylation at temperatures between 550°C and 650°C, with subsequent transformation to forsterite,[24,25] ashing temperatures should be kept below 500°C. Use of a muffle furnace is recommended. In all cases, calibration of the furnace is essential to ensure that a maximum ashing temperature of 500°C is not exceeded (see Section 2.3).

2.4.5.2.4 Acid Washing

Because of the interference caused by gypsum and some carbonates in the detection of asbestiform minerals by XRD (see Section 2.4.3.1), it may be necessary to remove these interferences by a simple acid washing procedure prior to analysis (see Section 2.3).

2.4.5.3 Qualitative Analysis

2.4.5.3.1 Initial Screening of Bulk Material

Qualitative analysis should be performed on a representative, homogeneous portion of the sample, with a minimum of sample treatment, using the following procedure:

1. Grind and mix the sample with a mortar and pestle (or equivalent method, see Section 2.4.5.2.2) to a final particle size sufficiently small ($\sim 100\ \mu m$) to allow adequate packing into a sample holder.

2. Pack sample into a standard bulk sample holder. Care should be taken to ensure that a representative portion of the milled sample is selected for analysis. Particular care should be taken to avoid possible size segregation of the sample. **(Note: Use of back-packing method[26] for bulk sample preparation may reduce preferred orientation effects.)**

3. Mount the sample on the diffractometer and scan over the diagnostic peak regions for the serpentine (~ 7.4 Å) and amphibole (8.2-8.5 Å) minerals (see Table 2-7). The x-ray diffraction equipment should be optimized for intensity. A slow scanning speed of 1° 2θ/min is recommended for adequate resolution. Use of a sample spinner is recommended.

4. Submit all samples that exhibit diffraction peaks in the diagnostic regions for asbestiform minerals to a full qualitative XRD scan (5°-60° 2θ; 1° 2θ/min) to verify initial peak assignments and to identify potential matrix interferences when subsequent quantitative analysis is to be performed.

5. Compare the sample XRD pattern with standard reference powder diffraction patterns (i.e., JCPDS powder diffraction data[3] or those of other well-characterized reference materials). Principal lattice spacings of asbestiform minerals are given in Table 2-7; common constituents of bulk insulation and wall materials are listed in Table 2-8.

2.4.5.3.2 Detection of Minor or Trace Constituents

Routine screening of bulk materials by XRD may fail to detect small concentrations ($< 1\%$) of asbestos. The limits of detection will, in general, be improved if matrix absorption effects are minimized, and if the sample particle size is reduced to the optimal 1 to 10 μm range, provided that the crystal lattice is not degraded in the milling process. Therefore, in those instances when confirmation of the presence of an asbestiform mineral at very low levels is required, or where a negative result from initial screening of the bulk material by XRD (see Section 2.4.5.3.1) is in conflict with previous PLM results, it may be desirable to prepare the sample as described for quantitative analysis (see Section 2.4.5.4) and step-scan over appropriate 2θ ranges of selected diagnostic peaks (Table 2-7). Accurate

transfer of the sample to the silver membrane filter is not necessary unless subsequent quantitative analysis is to be performed.

2.4.5.4 Quantitative Analysis

The proposed method for quantitation of asbestos in bulk samples is a modification of the NIOSH-recommended thin-layer method for chrysotile in air.[6] A thick-layer bulk method involving pelletizing the sample may be used for semi-quantitative analysis;[7,8] however, this method requires the addition of an internal standard, use of a specially fabricated sample press, and relatively large amounts of standard reference materials. Additional research is required to evaluate the comparability of thin- and thick-layer methods for quantitative asbestos analysis.

For quantitative analysis by thin-layer methods, the following procedure is recommended:

1. Mill and size all or a substantial representative portion of the sample as outlined in Section 2.4.5.2.2.

2. Dry at 60°C for 2 hours; cool in a desiccator.

3. Weigh accurately to the nearest 0.01 mg.

4. Samples shown by PLM to contain large amounts of cellulosic or other organic materials, gypsum, or carbonates, should be submitted to appropriate matrix reduction procedures described in Sections 2.4.5.2.3 and 2.4.5.2.4. After ashing and/or acid treatment, repeat the drying and weighing procedures described above, and determine the percent weight loss, L.

5. Quantitatively transfer an accurately weighed amount (50-100 mg) of the sample to a 1-L volumetric flask containing approximately 200 mL isopropanol to which 3 to 4 drops of surfactant have been added.

6. Ultrasonicate for 10 minutes at a power density of approximately 0.1 W/mL, to disperse the sample material.

7. Dilute to volume with isopropanol.

8. Place flask on a magnetic-stirring plate. Stir.

9. Place silver membrane filter on the filtration apparatus, apply a vacuum, and attach the reservoir. Release the vacuum and add several milliliters of isopropanol to the reservoir. Vigorously hand shake the asbestos suspension and immediately withdraw

an aliquot from the center of the suspension so that total sample weight, W_T, on the filter will be approximately 1 mg. Do not adjust the volume in the pipet by expelling part of the suspension; if more than the desired aliquot is withdrawn, discard the aliquot and repeat the procedure with a clean pipet. Transfer the aliquot to the reservoir. Filter rapidly under vacuum. Do not wash the reservoir walls. Leave the filter apparatus under vacuum until dry. Remove the reservoir, release the vacuum, and remove the filter with forceps. (Note: Water-soluble matrix interferences such as gypsum may be removed at this time by careful washing of the filtrate with distilled water. Extreme care should be taken not to disturb the sample.)

10. Attach the filter to a flat holder with a suitable adhesive and place on the diffractometer. Use of a sample spinner is recommended.

11. For each asbestos mineral to be quantitated, select a reflection (or reflections) that has (have) been shown to be free from interferences by prior PLM or qualitative XRD analysis and that can be used unambiguously as an index of the amount of material present in the sample (see Table 2-7).

12. Analyze the selected diagnostic reflection(s) by step-scanning in increments of 0.02° 2θ for an appropriate fixed time and integrating the counts. (A fixed count scan may be used alternatively; however, the method chosen should be used consistently for all samples and standards.) An appropriate scanning interval should be selected for each peak, and background corrections made. For a fixed time scan, measure the background on each side of the peak for one-half the peak-scanning time. The net intensity, I_s, is the difference between the peak integrated count and the total background count.

13. Determine the net count, I_{Ag}, of the filter 2.36 Å silver peak following the procedure in step 12. Remove the filter from the holder, reverse it, and reattach it to the holder. Determine the net count for the unattenuated silver peak, I°_{Ag}. Scan times may be less for measurement of silver peaks than for sample peaks; however, they should be constant throughout the analysis.

14. Normalize all raw, net intensities (to correct for instrument instabilities) by referencing them to an external standard (e.g., the 3.34 Å peak of an α-quartz reference crystal). After each unknown is scanned, determine the net count, I°_r of the reference specimen following the procedure in step 12. Determine the normalized intensities by dividing the peak intensities by I°_r:

$$\hat{I}_a = \frac{I_a}{I_r^\circ}, \quad \hat{I}_{Ag} = \frac{I_{Ag}}{I_r^\circ}, \text{ and } \quad \hat{I}_{Ag}^\circ = \frac{I_{Ag}^\circ}{I_r^\circ}$$

2.4.6 Calibration

2.4.6.1 Preparation of Calibration Standards

1. Mill and size standard asbestos materials according to the procedure outlined in Section 2.4.5.2.2. **Equivalent standardized matrix reduction and sizing techniques should be used for both standard and sample materials.**

2. Dry at 100°C for 2 hours; cool in a desiccator.

3. Prepare two suspensions of each standard in isopropanol by weighing approximately 10 and 50 mg of the dry material to the nearest 0.01 mg. Transfer each to a 1-L volumetric flask containing approximately 200 mL isopropanol to which a few drops of surfactant have been added.

4. Ultrasonicate for 10 minutes at a power density of approximately 0.1 W/mL, to disperse the asbestos material.

5. Dilute to volume with isopropanol.

6. Place the flask on a magnetic stirring plate. Stir.

7. Prepare, in triplicate, a series of at least five standard filters to cover the desired analytical range, using appropriate aliquots of the 10 and 50 mg/L suspensions. For each standard, mount a silver membrane filter on the filtration apparatus. Place a few mL of isopropanol in the reservoir. Vigorously hand shake the asbestos suspension and immediately withdraw an aliquot from the center of the suspension. Do not adjust the volume in the pipet by expelling part of the suspension; if more than the desired aliquot is withdrawn, discard the aliquot and resume the procedure with a clean pipet. Transfer the aliquot to the reservoir. Keep the tip of the pipet near the surface of the isopropanol. Filter rapidly under vacuum. Do not wash the sides of the reservoir. Leave the vacuum on for a time sufficient to dry the filter. Release the vacuum and remove the filter with forceps.

2.4.6.2 Analysis of Calibration Standards

1. Mount each filter on a flat holder. Perform step scans on selected diagnostic reflections of the standards and reference specimen using the procedure outlined in Section 2.4.5.4, step 12, and the same conditions as those used for the samples.

2. Determine the normalized intensity for each peak measured, $\hat{I}^{\circ}_{std}$, as outlined in Section 2.4.5.4, step 14.

2.4.7 Calculations

For each asbestos reference material, calculate the exact weight deposited on each standard filter from the concentrations of the standard suspensions and aliquot volumes. Record the weight, w, of each standard. Prepare a calibration curve by regressing $\hat{I}^{\circ}_{std}$, on w. Poor reproducibility ($\pm$15 percent RSD) at any given level indicates problems in the sample preparation technique, and a need for new standards. The data should fit a straight-line equation.

Determine the slope, m, of the calibration curve in counts/microgram. The intercept, b, of the line with the $\hat{I}^{\circ}_{std}$ axis should be approximately zero. A large negative intercept indicates an error in determining the background. This may arise from incorrectly measuring the baseline or from interference by another phase at the angle of background measurement. A large positive intercept indicates an error in determining the baseline or that an impurity is included in the measured peak.

Using the normalized intensity, $\hat{I}_{Ag}$ for the attenuated silver peak of a sample, and the corresponding normalized intensity from the unattenuated silver peak $\hat{I}^{\circ}_{Ag}$, of the sample filter, calculate the transmittance, T, for each sample as follows:[27,28]

$$T = \frac{\hat{I}_{Ag}}{\hat{I}^{\circ}_{Ag}}$$

Determine the correction factor, f(T), for each sample according to the formula:

$$f(T) = \frac{-R(\ln T)}{1 - T^R}$$

where

$$R = \frac{\sin \theta_{Ag}}{\sin \theta_a}$$

θ_{Ag} = angular position of the measured silver peak (from Bragg's Law), and

θ_a = angular position of the diagnostic asbestos peak.

Calculate the weight, W_a, in micrograms, of the asbestos material analyzed for in each sample, using the absorption corrections:

$$W_a = \frac{\hat{I}_a f(t) - b}{m}$$

Calculate the percent composition, P_a, of each asbestos mineral analyzed for in the parent material, from the total sample weight, W_T, on the filter:

$$P_a = \frac{W_a(1 - .01L)}{W_T} \times 100$$

where

P_a = percent asbestos mineral in parent material;

W_a = mass of asbestos mineral on filter, in μg;

W_T = total sample weight on filter, in μg;

L = percent weight loss of parent material on ashing and/or acid treatment (see Section 2.4.5.4).

2.4.8 References

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2.5 Analytical Electron Microscopy

2.5.1 Applicability

Analytical electron microscopy (AEM) can often be a reliable method for the detection and positive identification of asbestos in some bulk building materials, both friable and nonfriable. The method is particularly applicable to bulk materials that contain a large amount of interfering materials that can be removed by ashing and/or dissolution and contain asbestos fibers that are not resolved by PLM techniques. Many floor tiles and plasters would be included in this type of sample. In combination with suitable specimen preparation techniques, the AEM method can also be used to quantify asbestos concentrations.

2.5.2 Range

The range is dependent on the type of bulk material being analyzed. The upper detection limit is 100%, and the lower detection limit can be as low as 0.0001% depending on the extent to which interfering materials can be separated during the preparation of AEM

specimens, the sophistication of the AEM preparation, and the amount of labor expended on AEM examination.

2.5.3 Interferences

The presence of a large amount of binder/matrix materials associated with fibers can make it difficult to positively identify fibers as asbestos. The portion of the fiber examined by either electron diffraction or energy dispersive x-ray analysis (EDXA) must be free of binder/matrix materials.

2.5.4 Precision and Accuracy

The precision and accuracy of the method have not been determined.

2.5.5 Procedures

The procedures for AEM specimen preparation depend on the data required. In analysis of floor tiles, the weighed residue after removal of the matrix components (see Section 2.3, Gravimetry) is often mostly asbestos, and the task is primarily to identify the fibers. In this situation the proportion of asbestos in the residue can be estimated by AEM and this estimate can be used to refine the gravimetric result. For many floor tiles, the final result is not very sensitive to errors in this estimation because the proportion of asbestos in the residue is very high. For samples in which this is not the case, precise measurements can be made using a quantitative AEM preparation, in which each grid opening of the specimen grid corresponds to a known weight of the original sample or of a concentrate derived from the original sample. Asbestos fibers on these grids are then identified and measured, using a fiber counting protocol which is directed towards a precise determination of mass concentration. This latter procedure is suitable for samples of low asbestos concentration, or for those in which it is not possible to remove a large proportion of the matrix material.

2.5.5.1 AEM Specimen Preparation for Semi-Quantitative Evaluation

The residual material from any ashing or dissolution procedures (see Section 2.3) used (usually trapped on a membrane filter) should be placed in a small volume of ethanol or another solvent such as acetone or isopropyl alcohol, in a disposable beaker, and dispersed

by treatment in an ultrasonic bath. A small volume of this suspension (approximately 3μl) should be pipetted onto the top of a carbon-coated TEM grid. The suspension should be allowed to dry under a heat lamp. The grid is then ready for examination.

Samples that are not conducive to ashing or dissolution may also be prepared in this way for AEM analysis. A few milligrams of the sample may be ground in a mortar and pestle or milled, dispersed in ethanol or another solvent using an ultrasonic bath, and pipetted onto a grid as described previously.

2.5.5.2 AEM Specimen Preparation for Quantitative Evaluation

The objective of this preparation is to obtain a TEM grid on which a known weight of the bulk sample is represented by a known area of the TEM grid. A known weight of the bulk sample, or of the residue after extraction, should be dispersed in a known volume of distilled water. Aliquots of this dispersion should then be filtered through 0.22 μm pore-size MCE or 0.2 μm pore-size PC filters, using filtration techniques as described for analysis of water samples.[1] In order to obtain filters of appropriate particulate loading for AEM analysis, it may be necessary to perform serial dilutions of the initial dispersion. TEM grids should then be prepared from appropriately-loaded filters, using the standard methods.[2]

Determination of the mass concentration of asbestos on the TEM grids requires a different fiber counting protocol than that usually used for determination of numerical fiber concentrations. Initially, the grids should be scanned to determine the dimensions of the largest asbestos fiber or fiber bundle on the specimens. The volume of this fiber or bundle should be calculated. The magnification of the AEM should be set at a value for which the length of this fiber or bundle just fills the fluorescent screen. Asbestos fiber counting should then be continued at this magnification. The count should be terminated when the volume of the initial large fiber or bundle represents less than about 5% of the integrated volume of all asbestos fibers detected. This counting strategy ensures that the fiber counting effort is directed toward those fibers which contribute most to the mass, and permits a precise mass concentration value to be obtained.

2.5.5.2.1 Identification

To document the positive identification of asbestos in a sample, the analyst should record the following physical properties: morphology data, electron diffraction data, EDXA data, and any other distinguishing characteristics observed. For fibrous structures identified as nonasbestos, the unique physical property or properties that differentiate the material from asbestos should be recorded.

The purpose of the identification data collected is to prevent or limit false negatives and false positives. This can be accomplished by having a system for measuring and recording the d-spacings and symmetry of the diffraction patterns, determining the relative abundance of the elements detected by EDXA, and comparing these results to reference data. The laboratory should have a set of reference asbestos materials from which a set of reference diffraction patterns and x-ray spectra have been developed. Also, the laboratory should have available reference data on the crystallography and chemical composition of minerals that might analytically interfere with asbestos.

2.5.6 References

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2.6 Other Methodologies

Additional analytical methods (e.g. Scanning Electron Microscopy) may be applicable for some bulk materials. However, the analyst should take care to recognize the limitations of any analytical method chosen. Conventional SEM, for example, cannot detect small diameter fibers ($\sim < 0.2\mu m$), and cannot determine crystal structure. It is, however, very useful for observing surface features in complex particle matrices, and for determining elemental compositions.

3.0 QUALITY CONTROL/QUALITY ASSURANCE OPERATIONS- PLM

A program to routinely assess the quality of the results produced by the PLM laboratory must be developed and implemented. **Quality Control** (QC) is a system of activities whose purpose is to control the quality of the product or service so that it meets the need of the users. This also includes **Quality Assessment**, whose purpose is to provide assurance that the overall quality control is being done effectively. While the essential elements of a quality control system are described in detail elsewhere,[1,2,3,4,5,6] only several of the elements will be discussed here. **Quality Assurance** (QA) is comprised of Quality Control and Quality Assessment and is a system of activities designed to provide assurance that a product or service meets defined standards of quality.

The purpose of the Quality Assurance program is to minimize failures in the analysis of materials prior to submitting the results to the client. Failures in the analysis of asbestos materials include false positives, false negatives, and misidentification of asbestos types. False positives result from identification or quantitation errors. False negatives result from identification, detection, or quantitation errors.

For the stereomicroscopic and PLM techniques, the quality control procedures should characterize the accuracy and precision of both individual analysts and the techniques. Analysts should demonstrate their abilities on calibration materials, and also be checked routinely on the analysis of unknowns by comparison with results of a second analyst. The limitations of the stereomicroscopic and PLM techniques can be determined by using a second analytical technique, such as gravimetry, XRD, or AEM. For example, stereomicroscopic and PLM techniques can fail in the analysis of floor tiles because the asbestos fibers in the sample may be too small to be resolved by light microscopy. An XRD or AEM analysis is not subject to the same limitations, and may indicate the presence of asbestos in the sample.

The accuracy, precision, and detection limits of all analytical techniques described in this method are dependent on the type of sample (matrix components, texture, etc.), on the preparation of the sample (homogeneity, grain size, etc.), and the specifics of the method (number of point counts for PLM, mass of sample for gravimetry, counting time for XRD,

etc.). These should be kept in mind when designing quality control procedures and characterizing performance, and are variables that must be tracked in the quality assurance system.

3.1 General Considerations

3.1.1 Training

Of paramount importance in the successful use of this or any other analytical method is the well-trained analyst. It is highly recommended that the analyst have completed course work in optical mineralogy on the collegiate level. That is not to say that others cannot successfully use this method, but the classification error rate[7] may, in some cases, be directly attributable to level of training. In addition to completed course work in optical mineralogy, specialized course work in PLM and asbestos identification by PLM is desirable. Experience is as important as education. A good laboratory training program can be used in place of course work. Analysts that are in training and not yet fully qualified should have all analyses checked by a qualified analyst before results are released. A QC Plan for asbestos identification would be considered incomplete without a detailed description of the analyst training program, together with detailed records of training for each analyst.

3.1.2 Instrument Calibration and Maintenance

Microscope alignment checks (alignment of the polarizer at 90° with respect to the analyzer, and coincident with the cross-lines, proper orientation of the slow vibration direction of the Red I compensator plate, image of the field diaphragm focussed in the plane of the specimen, centering of the central dispersion staining stop, etc.) should be performed with sufficient frequency to ensure proper operations. Liquids used for refractive index determination and those optionally used for dispersion staining should have periodic refractive index checks using a refractometer or known refractive index solids. These calibrations must be documented.

Microscopes and ancillary equipment should be maintained daily. It is recommended that at least once per year each microscope be thoroughly cleaned and re-aligned by a professional microscope service technician. Adequate inventories of replaceable parts

(illumination lamps, etc.) should be established and maintained. All maintenance must be documented.

3.2 Quality Control of Asbestos Analysis

3.2.1 Qualitative Analysis

All analysts must be able to correctly identify the six regulated asbestos types (chrysotile, amosite, crocidolite, anthophyllite, actinolite, and tremolite) using combined stereomicroscopic and PLM techniques. Standards for the six asbestos types listed are available from NIST, and should be used to train analysts in the measurement of optical properties and identification of asbestos. These materials can also be used as identification standards for XRD and AEM.

Identification errors between asbestos types (e.g. reporting amosite when tremolite is present) implies that the analyst cannot properly determine optical properties and is relying on morphology as the identification criteria. This is not acceptable. Each analyst in the lab should prove his or her proficiency in identifying the asbestos types; this can be checked through use of calibration materials (NVLAP proficiency testing materials, materials characterized by an independent technique, and synthesized materials) and by comparing results with another analyst. The identification of all parameters (e.g. refractive indices, birefringence, sign of elongation, etc.) leading to the identification should fall within control limits determined by the laboratory. In addition, a subset of materials should be analyzed using another technique to confirm the analysis.

As discussed earlier, the qualitative analysis is dependent upon matrix and asbestos type and texture. Therefore, the quality assurance system should monitor for samples that are difficult to analyze and develop additional or special steps to ensure accurate characterization of these materials. When an analyst is found to be out of the control limits defined by the laboratory, he or she should undergo additional training and have confirmatory analyses performed on all samples until the problem has been corrected.

3.2.2 Quantitative Analysis

The determination of the amount of asbestos in a sample can be accomplished using the various techniques outlined in this method. The mandatory stereomicroscopic and PLM examinations provide concentrations in terms of volume, area, or weight, depending upon the calibration procedure. Gravimetric and quantitative XRD techniques result in concentrations in units of weight percent. Specific guidelines for determining accuracy and precision using these techniques are provided in the appropriate sections of this method. In general, however, the accuracy of any technique is determined through analysis of calibration materials which are characterized by multiple independent techniques in order to provide an unbiased value for the analyte (asbestos) in question. The precision of any technique is determined by multiple analyses of the sample. The analyst is the detector for stereomicroscopic and PLM techniques, as opposed to gravimetric and XRD techniques, and therefore must be calibrated as an integral part of the procedure.

As in the qualitative analysis, the laboratory should determine its accuracy and precision for quantitative asbestos analysis according to the type of material analyzed and the technique used for analysis. For example, the laboratory may determine that its analysts have a problem with calibrated area estimates of samples containing cellulose and chrysotile and therefore needs to make or find special calibration materials for this class of sample.

Calibration materials for quantitative analysis of asbestos are available through the Bulk Asbestos NVLAP as proficiency testing materials for those laboratories enrolled in NVLAP. In a report provided following a test round, the concentration of asbestos in each sample is given in weight percent with 95%/95% tolerance limits, along with a description of the major matrix components. Materials from other round robin and quality assurance programs for asbestos analysis may not have been analyzed by independent techniques; the concentrations may represent consensus PLM results that could be significantly biased. Therefore, values from these programs should <u>not</u> be used as calibration materials for quantitative analysis.

Calibration materials for quantitative analysis can also be synthesized by mixing asbestos and appropriate matrix materials, as described in Appendix C of this method. These

materials are usually simplifications of "real world" samples; therefore the accuracy and precision determined from analysis of these materials are probably ideal.

Limits on permissible analytical variability must be established by the laboratory prior to QC implementation. It is recommended that a laboratory initially be at 100% quality control (all samples reanalyzed.) The proportion of quality control samples can later be lowered gradually, as control indicates, to a minimum of 10%. Quantitative results for standards including the mean and error estimate (typically 95% confidence or tolerance intervals) should be recorded. Over time these data can be used to help determine control limits for quality control charts.

The establishment and use of control charts is extensively discussed elsewhere in the literature. [1,2,3,4,5] Several cautions are in order:

- Control charts are based on the assumption that the data are distributed normally. Using rational subgrouping, the means of the subgroups are approximately normally distributed, irrespective of the distribution of the individual values in the subgroups. Control charts for asbestos analysis are probably going to be based on individual measurements, not rational subgroups. Check the data for normality before proceeding with the use of control charts. Ryan[8] suggests a minimum of 50 analyses before an attempt is made to establish control limits. However, for this analysis, consider setting "temporary" limits after accumulating 20-30 analyses of the sample.

- Include <u>both</u> prepared slides as well as bulk samples in your reference inventory.

- Make certain that sample quantities are sufficient to last, and that the act of sampling will not alter the composition of the reference sample.

Data on analytical variability can be obtained by having analysts repeat their analyses of samples and also by having different analysts analyze the same samples.

3.3 Interlaboratory Quality Control

The establishment and maintenance of an interlaboratory QC program is fundamental to continued assurance that the data produced within the laboratory are of consistent high quality. Intralaboratory programs may not be as sensitive to accuracy and precision error, especially if the control charts (see Section 3.2.2) for all analysts in the laboratory indicate small percent differences. A routine interlaboratory testing program will assist in the detection of internal bias and analyses may be performed more frequently than proficiency

testing. Arrangements should be made with at least two (preferably more) other laboratories that conduct asbestos identification by PLM. Samples (the number of which is left to the participating laboratories, but at least 4-10) representing the types of samples and matrices routinely submitted to the lab for analysis should be exchanged with sufficient frequency to determine intralaboratory bias. Both reference slides and bulk samples should be used. Results of the interlaboratory testing program should be evaluated by each of the participating laboratories and corrective actions, if needed, identified and implemented. Since quantitation problems are more pronounced at low concentrations ($\leq$ 5%), it would be prudent to include approximately 30-50% from this concentration range in the sample selection process.

3.4 Performance Audits

Performance audits are independent quantitative assessments of laboratory performance. These audits are similar to the interlaboratory QC programs established between several laboratories, but with a much larger cohort (the EPA Asbestos Bulk Sample Analysis Quality Assurance Program had as many as 1100 participating laboratories). Participation in this type of program permitted assessment of performance through the use of "consensus" test materials, and served to assist in assessing the bias relative to individual interlaboratory, as well as intralaboratory programs. Caution should be exercised in the use of "consensus" quantitation results, as they are likely to be significantly responsible for the propagation of high bias in visual estimates. The current NIST/NVLAP[9] for bulk asbestos laboratories (PLM) does not use concensus quantitation results. Results are reported in weight percent with a 95% tolerance interval. The American Industrial Hygiene Association (AIHA)[10] also conducts a proficiency testing program for bulk asbestos laboratories. Quantitation results for this program are derived from analyses by two reference laboratories and PLM, XRD and gravimetric analysis performed by Research Triangle Institute.

3.5 Systems Audits

Where performance audits are quantitative in nature, systems audits are qualitative. Systems audits are assessments of the laboratory quality system as specified in the Laboratory

Quality Assurance Manual. Such an audit might consist of an evaluation of some facet of the QA Manual, or the audit may be larger in scope. For example, the auditor might request specific laboratory data sheets which will be evaluated against written procedures for data recording in the laboratory. Or, the auditor might request air monitoring or contamination control data to review for frequency of sampling, analysis methodology, and/or corrective actions taken when problems were discovered. The audit report should reflect the nature of the audit as well as the audit results. Any recommendations for improvement should also be reflected in such a report.

3.6 References

1. **Quality Assurance for Air Pollution Measurement Systems. Volume I, Principles.** EPA-600/9-76-005, March, 1976.

2. Juran, J. and F. Gryna, **Quality Planning Analysis**, 2nd edition, McGraw-Hill, Inc., 1980.

3. Taylor, J.R., **Quality Control Systems**, McGraw Hill, Inc., 1989.

4. Ratliff, T.A., **The Laboratory Quality Assurance System**, Van Nostrand Reinhold, 1990.

5. Taylor, J.K., **Quality Assurance of Chemical Measurements**, Lewis Publishers, 1987.

6. Bulk Asbestos Handbook, National Institute of Standards and Technology, National Voluntary Laboratory Accreditation Program, NISTIR 88-3879, October 1988.

7. Harvey, B.W., "Classification and Identification Error Tendencies in Bulk Insulation Proficiency Testing Materials," **American Environmental Laboratory**, 2(2), 4/90, pp. 8-14.

8. Ryan, T.P., **Statistical Techniques for Quality Improvement**, John Wiley & Sons, Inc., New York, 1989.

9. National Institute of Standards & Technology (NIST) National Voluntary Laboratory Accreditation Program (NVLAP), Building 411, Room A124, Gaithersburg, MD 20899, telephone (301) 975-4016.

10. American Industrial Hygiene Association (AIHA), 2700 Prosperity Avenue, Suite 250, Fairfax, VA 22031, (703) 849-8888.

APPENDIX A

Glossary Of Terms

APPENDIX A. GLOSSARY OF TERMS

Accuracy - The degree of agreement of a measured value with the true or expected value.

Anisotropic - Refers to substances that have more than one refractive index (e.g. are birefringent), such as nonisometric crystals, oriented polymers, or strained isotropic substances.

Asbestiform (morphology) - Said of a mineral that is like asbestos, i.e., crystallized with the habit of asbestos. Some asbestiform minerals may lack the properties which make asbestos commercially valuable, such as long fiber length and high tensile strength. With the light microscope, the asbestiform habit is generally recognized by the following characteristics:

- Mean aspect ratios ranging from 20:1 to 100:1 or higher for fibers longer than 5μm. Aspect ratios should be determined for fibers, not bundles.
- Very thin fibrils, usually less than 0.5 micrometers in width, and
- Two or more of the following:
  - Parallel fibers occurring in bundles,
  - Fiber bundles displaying splayed ends,
  - Matted masses of individual fibers, and/or
  - Fibers showing curvature

These characteristics refer to the population of fibers as observed in a bulk sample. It is not unusual to observe occasional particles having aspect ratios of 10:1 or less, but it is unlikely that the asbestos component(s) would be dominated by particles (individual fibers) having aspect ratios of $<$20:1 for fibers longer than 5μm. If a sample contains a fibrous component of which most of the fibers have aspect ratios of $<$20:1 and that do not display the additional asbestiform characteristics, by definition the component should not be considered asbestos.

Asbestos - A commercial term applied to the asbestiform varieties of six different minerals. The asbestos types are chrysotile (asbestiform serpentine), amosite (asbestiform grunerite), crocidolite (asbestiform riebeckite), and asbestiform anthophyllite, asbestiform tremolite, and asbestiform actinolite. The properties of asbestos that caused it to be widely used commercially are: 1) its ability to be separated into long, thin, flexible fibers; 2) high tensile strength; 3) low thermal and electrical conductivity; 4) high mechanical and chemical durability, and 5) high heat resistance.

Becke Line - A band of light seen at the periphery of a specimen when the refractive indices of the specimen and the mounting medium are different; it is used to determine refractive index.

Bias - A systematic error characterized by a consistent (non-random) measurement error.

Binder - With reference to a bulk sample, a component added for cohesiveness (e.g. plaster, cement, glue, etc.).

Birefringence - The numerical difference between the maximum and minimum refractive indices of an anisotropic substance. Birefringence may be estimated, using a Michel-Levy chart, from the interference colors observed under crossed polarizers. Interference colors are also dependent on the orientation and thickness of the grain, and therefore are used qualitatively to determine placement in one of the four categories listed below.

Qualitative	Quantitative(N-n)
none	0.00 or isotropic
low	≤0.010
moderate	0.011-0.050
high	>0.050

Bulk Sample - A sample of building material taken for identification and quantitation of asbestos. Bulk building materials may include a wide variety of friable and nonfriable materials.

Bundle - Asbestos structure consisting of several fibers having a common axis of elongation.

Calibration Materials - Materials, such as known weight % standards, that assist in the calibration of microscopists in terms of ability to quantitate the asbestos content of bulk materials.

Color - The color of a particle or fiber when observed in plane polarized light.

Compensator - A device with known, fixed or variable retardation and vibration direction used for determining the degree of retardation (hence the thickness or value of birefringence) in an anisotropic specimen. It is also used to determine the sign of elongation of elongated materials. The most common compensator is the first-order red plate (530-550nm retardation).

Control Chart - A graphical plot of test results with respect to time or sequence of measurement, together with limits within which they are expected to lie when the system is in a state of statistical control.

Detection Limit - The smallest concentration/amount of some component of interest that can be measured by a single measurement with a stated level of confidence.

Dispersion Staining (focal masking) - An optical means of imparting apparent or virtual color to transparent substances by the use of stops in the objective back focal plane; ir it is used to determine refractive indices.

Error - Difference between the true or expected value and the measured value of a quantity or parameter.

Extinction - The condition in which an anisotropic substance appears dark when observed between crossed polars. This occurs when the vibration directions in the specimen are parallel to the vibration directions in the polarizer and analyzer. Extinction may be complete or incomplete; common types include parallel, oblique, symmetrical and undulose.

Extinction Angle - For fibers, the angle between the extinction position and the position at which the fiber is parallel to the polarizer or analyzer privileged directions.

Fiber - With reference to asbestiform morphology, a structure consisting of one or more fibrils.

Fibril - The individual unit structure of fibers.

Friable - Refers to the cohesiveness of a bulk material, indicating that it may be crumbled or disaggregated by hand pressure.

Gravimetry - Any technique in which the concentration of a component is determined by weighing. As used in this document, it refers to measurement of asbestos-containing residues after sample treatment by ashing, dissolution, etc.

Homogeneous - Uniform in composition and distribution of all components of a material, such that multiple subsamples taken for analysis will contain the same components in approximately the same relative concentrations.

Heterogeneous - Lacking uniformity in composition and/or distribution of material; components not uniform. Does not satisfy the conditions stated for homogenous; e.g., layered or in clumps, very coarse grained, etc.

Isotropic - Refers to substances that have a single refractive index such as unstrained glass, un-oriented polymers and unstrained substances in the isometric crystal system.

Lamda Zero (λ_o) - The wavelength (λ_o) of the dispersion staining color shown by a specimen in a medium; both the specimen and medium have the same refractive inde at that wavelength.

Matrix - Nonasbestos, nonbinder components of a bulk material. Includes such components as cellulose, fiberglass, mineral wool, mica, etc.

Michel-Levy Scale of Retardation colors - A chart plotting the relationship between birefringence, retardation and thickness of anisotropic substances. Any one of the three variables can be determined if the other two are known.

Morphology - The structure and shape of a particle. Characterization may be descriptive (platy, rod-like, acicular, etc) or in terms of dimensions such as length and diameter (see asbestiform).

Pleochroism - The change in color or hue of colored anisotropic substance when rotated relative to the vibration direction of plane polarized light.

Point Counting - A technique used to determine the relative projected areas occupied by separate components in a microscope slide preparation of a sample. For asbestos analysis, this technique is used to determine the relative concentrations of asbestos minerals to nonasbestos sample components.

Polarization Colors - Interference colors displayed by anisotropic substances between two polarizers. Birefringence, thickness and orientation of the material affect the colors and their intensity.

Precision - The degree of mutual agreement characteristic of independent measurements as the result of repeated application of the process under specified conditions. It is concerned with the variability of results.

Reference Materials - Bulk materials, both asbestos-containing and nonasbestos-containing, for which the components are well-documented as to identification and quantitation.

Refractive Index (index of refraction) - The ratio of the velocity of light in a vacuum relative to the velocity of light in a medium. It is expressed as n and varies with wavelength and temperature.

Sign of Elongation - Referring to the location of the high and low refractive indices in an elongated anisotropic substance, a specimen is described as positive when the higher refractive index is lengthwise (length slow), and as negative when the lower refractive index is lengthwise (length fast).

Standard Reference Material (SRM) - A reference material certified and distributed by the National Institute of Standards and Technology.

Visual Estimate - An estimation of concentration of asbestos in a sample as compared to the other sample components. This may be a volume estimate made during stereomicroscopic examination and/or a projected area estimation made during microscopic (PLM) examination.

APPENDIX B

Apparatus For Sample Preparation And Analysis

B1.0 INTRODUCTION

The following lists the apparatus and materials required and suggested for the methods of sample preparation and analysis described in the test method.[1,2,3]

B2.0 STEREOMICROSCOPIC EXAMINATION

The following are suggested for routine stereomicroscopic examination.

- HEPA-filtered hood or class 1 biohazard hood, negative pressure
- Microscope: binocular microscope, preferably stereoscopic, 5-60X magnification (approximate)
- Light source: incandescent or fluorescent
- Tweezers, dissecting needles, scalpels, probes, etc. (for sample manipulation)
- Glassine paper, glass plates, weigh boats, petri dishes, watchglasses, etc. (sample containers)

The following are suggested for sample preparation.

- Mortar and pestle, silica or porcelain-glazed
- Analytical balance (readability less than or equal to one milligram) (optional)
- Mill or blender (optional)

B3.0 POLARIZED LIGHT MICROSCOPY

The laboratory should be equipped with a polarized light microscope (preferably capable of Köhler or Köhler-type illumination if possible) and accessories as described below.

- Ocular(s) binocular or monocular with cross hair reticle, or functional equivalent, and a magnification of at least 8X
- 10X, 20X, and 40X objectives, (or similar magnification)

- Light source (with optional blue "day-light" filter)
- 360-degree rotatable stage
- Substage condenser with iris diaphragm
- Polarizer and analyzer which can be placed at 90 degrees to one another, and can be calibrated relative to the cross-line reticle in the ocular.
- Accessory slot for wave plates and compensators (or demonstrated equivalent).
- Wave retardation plate (Red I compensator) with approximately 550 nanometer retardation, and with known slow and fast vibration directions.
- Dispersion staining objective or a demonstrated equivalent. (optional)
- Monochromatic filter (n_D), or functional equivalent. (optional)

In addition, the following equipment, materials and reagents are required or recommended.[1]

- NIST traceable standards for the major asbestos types (NIST SRM 1866 and 1867)
- Class I biohazard hood or better (see "Note", Section 2.2.5)
- Sampling utensils (razor knives, forceps, probe needles, etc.)
- Microscope slides and cover slips
- Mechanical Stage
- Point Counting Stage (optional)
- Refractive index liquids: 1.490-1.570, 1.590-1.720 in increments of less than or equal to 0.005; high dispersion, (HD) liquids are optional; however, if using dispersion staining, HD liquids are recommended.
- Mortar and pestle
- Distilled water
- HCl, ACS reagent grade concentrated

- Muffle furnace (optional)
- Mill or blender (optional)
- Beakers and assorted glassware (optional)
- Other reagents (tetrahydrofuran, amyl acetate, acetone, sodium hexametaphosphate, etc.) (optional)

B4.0 GRAVIMETRY

The following equipment, materials, and reagents are suggested.

- Scalpels
- Crucibles, silica or porcelain-glazed, with lids
- Muffle furnace - temperature range at least to 500°C, temperature stable to $\pm$ 10°C, temperature at sample position calibrated to $\pm$ 10°C
- Filters, 0.4 μm pore size polycarbonate
- Petri dishes
- Glass filtration assembly, including vacuum flask, water aspirator, and/or air pump
- Analytical balance, readable to 0.001 gram
- Mortar and pestle, silica or porcelain-glazed
- Heat lamp or slide warmer
- Beakers and assorted glassware
- Centrifuge, bench-top
- Class I biohazard hood or better
- Bulb pipettes
- Distilled water
- HCl, reagent-grade concentrated

- Organic solvents (tetrahydrofuran, amyl acetate,etc)
- Ultrasonic bath

B5.0 X-RAY DIFFRACTION

Sample Preparation

Sample preparation apparatus requirements will depend upon the sample type under consideration and the kind of XRD analysis to be performed.

- Mortar and pestle: agate or porcelain
- Razor blades
- Sample mill: SPEX, Inc., freezer mill or equivalent
- Bulk sample holders
- Silver membrane filters: 25-mm diameter, 0.45-μm pore size. Selas Corp. of America, Flotronics Div., 1957 Pioneer Road, Huntington Valley, PA 19006
- Microscope slides
- Vacuum filtration apparatus: Gelman No. 1107 or equivalent, the side-arm vacuum flask
- Microbalance
- Ultrasonic bath or probe: Model W140, Ultrasonics, Inc., operated at a power density of approximately 0.1 W/mL, or equivalent
- Volumetric flasks: 1-L volume
- Assorted pipets
- Pipet bulb
- Nonserrated forceps
- Polyethylene wash bottle
- Pyrex beakers: 50-mL volume

- Desiccator
- Filter storage cassettes
- Magnetic stirring plate and bars
- Porcelain crucibles
- Muffle furnace or low temperature asher
- Class 1 biohazard hood or better

Sample Analysis

Sample analysis requirements include an x-ray diffraction unit, equipped with:

- Constant potential generator; voltage and mA stabilizers
- Automated diffractometer with step-scanning mode
- Copper target x-ray tube: high intensity; fine focus, preferably
- X-ray pulse height selector
- X-ray detector (with high voltage power supply): scintillation or proportional counter
- Focusing graphite crystal monochromator; or nickel filter (if copper source is used, and iron fluorescence is not a serious problem)
- Data output accessories:
  - Strip chart recorder
  - Decade scaler/timer
  - Digital printer

  or

  - PC, appropriate software and Laser Jet Printer
- Sample spinner (optional)
- Instrument calibration reference specimen: α-quartz reference crystal (Arkansas quartz standard, #180-147-00, Philips Electronics Instruments, Inc., 85 McKee Drive, Mahwah, NJ 07430) or equivalent.

Reagents, etc.

Reference Materials - The list of reference materials below is intended to serve as a guide. Every attempt should be made to acquire pure reference materials that are comparable to sample materials being analyzed.

- Chrysotile: UICC Canadian, NIST SRM 1866 (UICC reference material available from: UICC, MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, Glamorgan, CF61XW, UK); (NIST Standard Reference Materials available from the National Institute of Standards and Technology, Office of Reference Standards, Gaithersburg, MD 20899)
- Crocidolite: UICC, NIST SRM 1866.
- "Amosite": UICC, NIST SRM 1866.
- Anthophyllite-Asbestos: UICC, NIST SRM 1867
- Tremolite Asbestos: Wards Natural Science Establishment, Rochester, NY; Cyprus Research Standard, Cyprus Research, 2435 Military Ave., Los Angeles, CA 900064 (washed with dilute HCl to remove small amount of calcite impurity); Indian tremolite, Rajasthan State, India; NIST SRM 1867.
- Actinolite Asbestos: NIST SRM 1867

Adhesive - Tape, petroleum jelly, etc. (for attaching silver membrane filters to sample holders).

Surfactant - 1 Percent aerosol OT aqueous solution or equivalent.

Isopropanol - ACS Reagent Grade.

B6.0 ANALYTICAL ELECTRON MICROSCOPY

AEM equipment requirements will not be discussed in this document; it is suggested that equipment requirements stated in the AHERA regulations be followed. Additional information may be found in the NVLAP Program Handbook for Airborne Asbestos Analysis.[3]

The following additional materials and equipment are suggested:

- Analytical balance, readable to 0.001 gram
- Ultrasonic bath
- Glass filtration assembly (25mm), including vacuum flask and water aspirator
- Mixed cellulose ester (MCE) filters (0.22μm pore size) or 0.2μm pore size polycarbonate filters
- MCE backing filters (5μm pore size)
- Silica mortar and pestle
- Beakers - glass and disposable
- Pipettes, disposable, 1,5, and 10 ml

B7.0 REFERENCES

1. National Institute of Standards and Technology (NIST) National Voluntary Laboratory Accreditation Program (NVLAP) Bulk Asbestos Handbook, NISTIR 88-3879, 1988.
2. **Interim Method for the Determination of Asbestos in Bulk Insulation Samples**, U.S. E.P.A. 600/M4-82-020, 1982.
3. National Institute of Standards and Technology (NIST) National Voluntary Laboratory Accreditation Program (NVLAP) Program Handbook for Airborne Asbestos Analysis, NISTIR 89-4137, 1989.

APPENDIX C

Preparation and Use of Bulk Asbestos
Calibration Standards

C1.0 INTRODUCTION

Evaluation of the results from national proficiency testing programs for laboratories analyzing for asbestos in bulk materials indicates that laboratories have had, and continue to have, problems with quantitation of asbestos content, especially with samples having a low asbestos concentration.[1] For such samples, the mean value of asbestos content reported by laboratories may be four to ten times the true weight percent value. It is assumed that the majority of the laboratories quantify asbestos content by visual estimation, either stereomicroscopically or microscopically; therefore, the problem of quantitation must be attributed to lack of or inadequate calibration of microscopists.

As calibration standards for asbestos-containing bulk materials are not currently commercially available, laboratories should consider generating their own calibration materials. This may be done rather easily and inexpensively.

C2.0 MATERIALS AND APPARATUS

Relatively pure samples of asbestos minerals should be obtained. Chrysotile, amosite and crocidolite (SRM 1866) and anthophyllite, tremolite and actinolite (SRM 1867) are available from NIST. A variety of matrix materials are commercially available; included are calcium carbonate, perlite, vermiculite, mineral wool/fiberglass, and cellulose. Equipment, and materials needed to prepare calibration bulk materials are listed below.

- Analytical balance, readable to 0.001 gram
- Blender/mixer; multi-speed, ~ one quart capacity
- Filtration assembly, including vacuum flask, water aspirator and/or air pump (optional)
- HEPA-filtered hood with negative pressure
- Filters, 0.4μm pore size polycarbonate (optional)
- Beakers and assorted glassware, weigh boats, petri dishes, etc.
- Hot/warm plate

- Asbestos minerals
- Matrix materials
- Distilled water.

C3.0 MATERIAL FORMULATION PROCEDURES

The formulation procedure involves first weighing appropriate quantities of asbestos and matrix material to give the desired asbestos weight percent. The following formula may be used to determine the weights of asbestos and matrix materials needed to give a desired weight percent asbestos.

$$\frac{WTa}{Wa} = \frac{WTm}{Wm}$$

Where:

WTa = weight of asbestos in grams (to 0.001 gram)
WTm = weight of matrix materials in grams (to 0.001 gram)
Wa = weight percent asbestos
Wm = weight percent matrix

Example: The desired total weight for the calibration sample is ~ 10 grams containing 5% asbestos by weight. If 0.532 grams of asbestos are first weighed out, what corresponding weight of matrix material is required?

WTa = 0.532 grams
Wa = 5%
Wm = 95%

$$\frac{0.532}{5} = \frac{WTm}{95}$$

Then: WTm = 10.108 grams

The matrix is then placed into the pitcher of a standard over-the-counter blender, the pitcher being previously filled to approximately one-fourth capacity (8-10 ounces) with distilled water. Blending is performed at the lowest speed setting for approximately ten seconds which serves to disaggregate the matrix material. The asbestos is then added, with additional blending of approximately 30 seconds, again at the lowest speed setting. Caution should be taken not to overblend the asbestos-matrix mixture. This could result in a significant reduction in the size of the asbestos fibers causing a problem with detection at normal magnification during stereomicroscopic and microscopic analyses. Ingredients of the

pitcher are then poured into a filtering apparatus, with thorough rinsing of the pitcher to ensure complete material removal. After filtering, the material is transferred to a foil dish which is placed on a hot plate. The material is covered and allowed to sit over low heat until drying is complete; intermittent stirring will speed the drying process. For fine-grained matrix materials such as gypsum, calcium carbonate, clays, etc., the sample is not filtered after the blending process. Instead, the ingredients in the pitcher are transferred into a series of shallow, glass (petri) dishes. The ingredients should be stirred well between each pouring to minimize the possible settling (and over-representation) of some components. The dishes are covered and placed on a hot plate until the contents are thoroughly dried. For small quantities of any matrix materials (15 grams or less), air-drying without prior filtering is generally very suitable for removing water from the prepared sample. For each material, the final step involves placing all formulated, dried subsamples into a plastic bag (or into one petri dish, for small quantities), where brief hand-mixing will provide additional blending and help to break up any clumps produced during drying. **All operations should be performed in a safety-hood with negative pressure.**

C4.0 ANALYSIS OF MATERIALS

All formulations should be examined with the stereomicroscope to determine homogeneity. Gravimetric analysis (ashing and/or acid dissolution) should be performed on those materials containing organic and/or acid-soluble components. Matrix materials to which no asbestos has been added should be analyzed by gravimetric analysis to determine the amount of nonashable or insoluble materials that are present. Several subsamples of each material should be analyzed by the gravimetric technique to provide information concerning the uniformity of the prepared materials. Experience has shown that the previously described formulation procedure results in relatively homogeneous materials.[2]

C4.1 Stereomicroscopic Analysis

Visual estimation of sample components using the stereomicroscope is in reality a comparison of the relative volumes of the components.[3] Therefore, differences in specific gravity between asbestos and matrix material must be considered and the relationship

between weight percent and volume percent must be determined.[4] Materials such as expanded vermiculite, perlite, and cellulose have specific gravities significantly lower than asbestos minerals. Table C1 lists the specific gravities for the three most commonly encountered asbestos varieties and several common matrix materials.

TABLE C1. SPECIFIC GRAVITIES OF ASBESTOS VARIETIES AND MATRIX MATERIALS

Asbestos Type	Specific Gravity	Matrix Type	Specific Gravity
Chrysotile	2.6	Calcium Carbonate	2.7
		Gypsum	2.3
Amosite	3.2	Perlite	~0.4
		Vermiculite (expanded)	~0.3
		Mineral Wool	~2.5
Crocidolite	3.3	Fiberglass	~2.5
		Cellulose	~0.9

The conversion of weight percent asbestos to equivalent volume percent asbestos is given by the following formula:

$$\frac{\frac{Wa}{Ga}}{\frac{Wa}{Ga} + \frac{Wm}{Gm}} \times 100 = Va$$

where:

Wa = weight percent asbestos
Ga = specific gravity of asbestos
Wm = weight percent matrix
Gm = specific gravity of matrix
Va = volume percent asbestos

Example: Chrysotile and perlite have been combined to form a 5% asbestos calibration standard, by weight. What is the equivalent volume percent asbestos?

$$Wa = 5\% \quad Ga = 2.6 \quad Wm = 95\% \quad Gm = 0.4$$

$$Va = \frac{\frac{5}{2.6}}{\frac{5}{2.6} + \frac{95}{0.4}} \times 100 = 0.8\%$$

Conversely, to convert volume percent asbestos to equivalent weight percent, the following formula may be used.

$$\frac{(Va)(Ga)}{(Va)(Ga)+(Vm)(Gm)} \times 100 = Wa$$

Vm = volume percent matrix

Example: A calibration standard consisting of amosite and cellulose is estimated to contain 2% asbestos, by volume. What is the equivalent weight percent asbestos?

$$Va = 2\% \quad Ga = 3.2 \quad Vm = 98\% \quad Gm = 0.9$$

$$Wa = \frac{(2)(3.2)}{(2)(3.2)+(98)(0.9)} \times 100 = 6.77\%$$

Volume percentages should be calculated for all calibration materials prepared so that visual estimates determined by examination with the stereomicroscope may be compared to true volume concentrations.

Figure C1 illustrates the relationship between volume percent and weight percent of chrysotile mixed with vermiculite and cellulose respectively. It should be noted that when asbestos in a low weight percentage is mixed with matrix materials having low specific gravities (vermiculite, perlite), the resulting volume concentration of asbestos is very low. For example, a mixture containing three percent chrysotile by weight in a cellulose matrix would result in a volume percent asbestos of approximately 1.1%; in a vermiculite matrix, the resulting volume percent asbestos would be approximately 0.4%. In the latter case especially, an analyst might possibly fail to detect the asbestos or consider it to be present in only trace amounts.

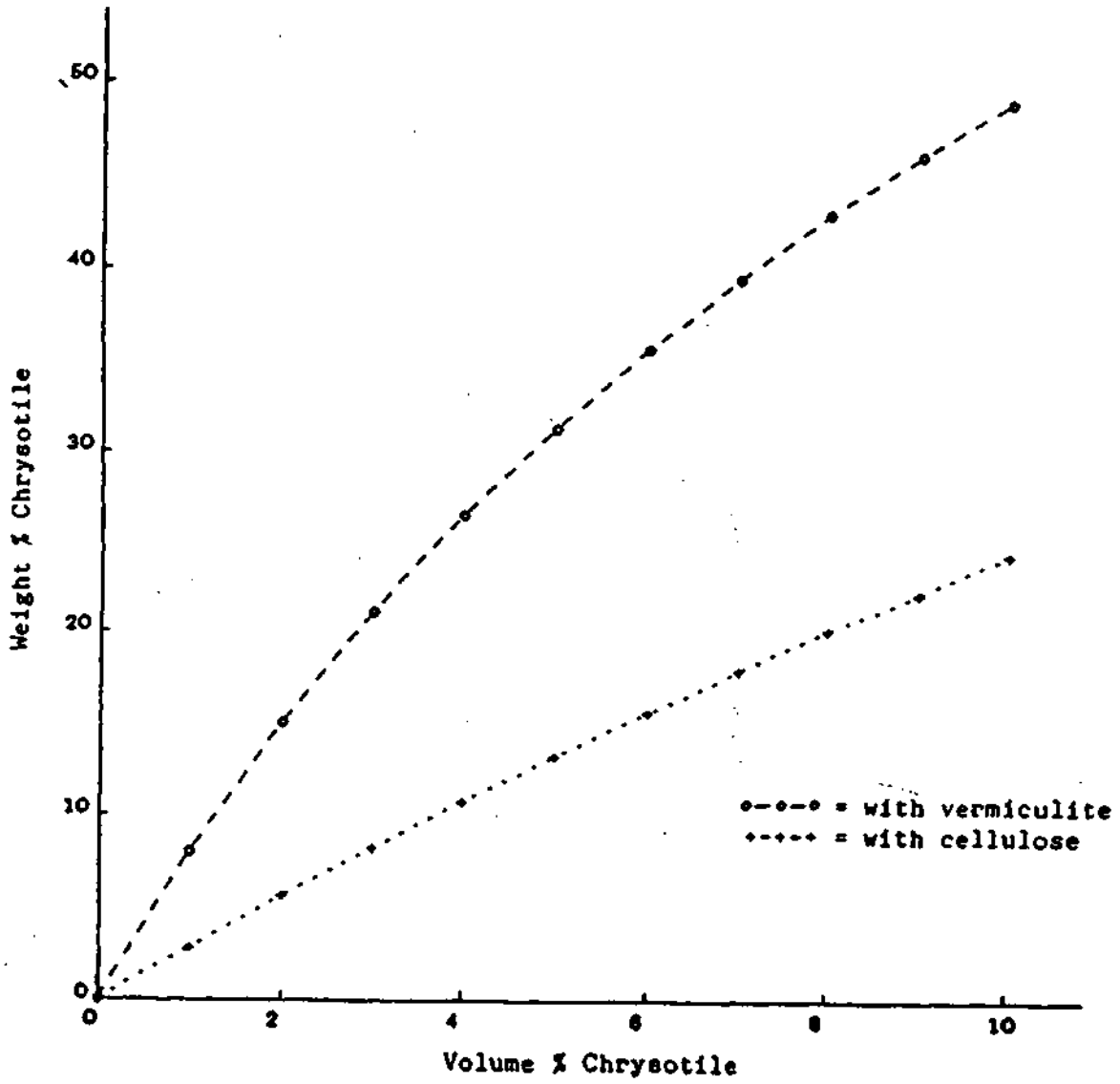


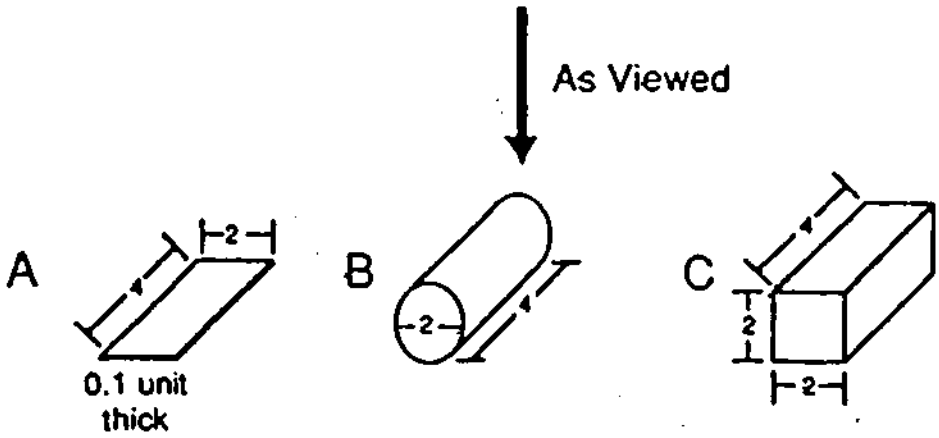
Figure C1. Relationship between volume % and weight % of chrysotile mixed with a)vermiculite and b) cellulose.

C4.2 Microscopical Analysis (PLM)

The polarized light microscope may be used to quantify asbestos and other components of a sample. Slide mounts are prepared from "pinch" samples of the calibration material and asbestos content is determined by visual area estimate and/or point counting. Both of these quantitation techniques are in fact estimates or measurements of the relative projected areas of particles as viewed in two dimensions on a microscope slide. For quantitation results to be meaningful, the following conditions should be met:

- The sample should be homogeneous for slide preparations, which are made from small pinches of the sample, to be representative of the total sample.
- Slide preparation should have an even distribution of particles and approach a one particle thickness (seldom achieved) to avoid particle overlap.
- All materials used should be identified and specific gravities determined in order to relate area percent to volume and/or weight percent.
- The size (thickness) relationship between matrix particles and asbestos fibers should be determined if the results based on projected area are to be related to volume and/or weight percent.

Particle characteristics can greatly affect the quantitation results obtained by visual area estimation or point counting. Figure C2 illustrates three hypothetical particle shapes of identical length and width (as viewed from above). Although the three-dimensional shape is different, the projected area is equal for all particles. The table accompanying Figure C2 presents data for each particle in terms of thickness, volume and projected area. It should be noted that although the projected areas may be equal, the volumes represented by the particles may vary by a factor of 20(0.8 vs 16 cubic units). It is obvious that quantitation of a sample consisting of a mixture of particles with widely ranging particle thicknesses could result in different results. For example, if a sample contained relatively thick bundles of asbestos and a fine-grained matrix such as clay or calcium carbonate, the true asbestos content (by volume) would likely be underestimated. Conversely, if a sample contained thick "books" of mica and thin bundles of asbestos, the asbestos content (by volume) would likely be overestimated.



Particle	Thickness	Volume	Projected Area
A	0.1 units	0.8 cubic units	8 sq. units
B	2 units	12.6 cubic units	8 sq. units
C	2 units	16 cubic units	8 sq. units

Note that although all particles have the same projected area, particle C volume is 20x that of particle A.

Figure C2. Relationship of projected area to volume and thickness for three different particles as viewed on a slide mount.

Table C2 illustrates several examples of expected results from area estimates or point counting of samples in which the asbestos fibers and matrix particles differ in thickness.

TABLE C2. RELATIONSHIP OF WEIGHT PERCENT, VOLUME PERCENT AND PARTICLE THICKNESS TO QUANTITATION RESULTS

Composition of Sample In Wt. %	Theoretical Vol. % Asbestos	Thickness Factor* (Matrix/Asbestos)	Expected Area %
1% Amosite 99% Calcium Carbonate	0.9	0.5	0.4
1% Amosite 99% Calcium Carbonate	0.9	1	0.9
1% Amosite 99% Calcium Carbonate	0.9	2	1.8
1% Amosite 99% Vermiculite	0.1	1	0.1
1% Amosite 99% Vermiculite	0.1	10	1.0
1% Amosite 99% Vermiculite	0.1	20	2.0
1% Amosite 99% Vermiculite	0.1	30	2.9

* Value represents the relationship between the mean thickness of the matrix particles compared to the mean thickness of the asbestos particles.

It should be noted that it is not uncommon for matrix particle thickness to differ greatly from asbestos fiber thickness, especially with matrix materials such as vermiculite and perlite; vermiculite and perlite particles may be 20 - 30 times as thick as the asbestos fibers.

The general size relationships between matrix particles and asbestos fibers may be determined by scanning slide mounts of a sample. A micrometer ocular enables the microscopist to actually measure particle sizes.

If a thickness factor can be determined for a calibration sample of known volume proportions of asbestos and matrix materials, an expected equivalent projected area asbestos can be calculated using the following formula:

$$\frac{Va}{\frac{Vm}{T} + Va} \times 100 = Aa$$

where:

Va = true volume percent asbestos
Vm = true volume percent matrix
T = thickness factor (mean size matrix particle/mean size asbestos fiber)
Aa = expected projected area percent asbestos

Example: A calibration standard of known weight percent asbestos is determined, by factoring in component specific gravities, to be 5.0% asbestos by volume. The matrix particles are estimated to be ten times thicker than the asbestos fibers. What would be the expected projected area percentage of asbestos?

Va = 5%
Vm = 95%
T = 10

$$Aa = \frac{5}{\frac{95}{10} + 5} \times 100 = 34.5\%$$

Conversely, to convert projected area percent asbestos to equivalent volume percent, the following formula may be used:

$$\frac{Aa}{T(Am) + Aa} \times 100 = Va$$

Where: Am = projected area matrix

Example: A slide containing a subsample of an amosite/mineral wool calibration standard is determined by point counting to have a projected area asbestos of 18.6%. If the mineral wool fibers are estimated to be six times the asbestos fibers, in diameter, what is the equivalent volume percent asbestos?

$Am = 81.4\%$
$Aa = 18.6\%$
$T = 6$

$$Va = \frac{(18.6)}{6(81.4) + 18.6} \times 100 = 3.67\%$$

Based on specific gravity values listed in Table 1C and on the above volume asbestos determination, what is the equivalent weight percent asbestos in the sample?

$Va = 3.67\%$
$Ga = 3.2$
$Vm = 96.33\%$
$Gm = 2.5$

$$Wa = \frac{(3.67)(3.2)}{(3.67)(3.2) + (96.33)(2.5)} \times 100 = 4.7\%$$

C5.0 USE OF CALIBRATION STANDARDS FOR QA/QC

Once the materials have been formulated and thoroughly characterized by all techniques to determine their suitability as calibration standards, a system for incorporating them into the QA/QC program should be established. Someone should be designated (QA officer, lab supervisor, etc.) to control the distribution of standards and to monitor the analysis results of the microscopists. Both precision and accuracy may be monitored with the use of suitable standard sets.

Records such as range charts, control charts, etc. may be maintained for volume (stereomicroscopic estimates), area (PLM) estimates and point counts. For point counts and area estimates, relatively permanent slides may be made using epoxy or Melt Mount®. Such slides may be very accurately quantified over time as to point count values, and due to their very long shelf life, may be used for QA/QC purposes almost indefinitely.

C6.0 REFERENCES

1. "Analysis Summaries for Samples used in NIST Proficiency Testing", National Institute of Standards and Technology (NIST) National Voluntary Laboratory Accreditation Program (NVLAP) for Bulk Asbestos, January 1989 to present.

2. Harvey, B. W., R. L. Perkins, J. G. Nickerson, A. J. Newland and M. E. Beard, "Formulating Bulk Asbestos Standards", **Asbestos Issues**, April 1991.

3. Perkins, R. L. and M. E. Beard, "Estimating Asbestos Content of Bulk Materials", **National Asbestos Council Journal**, Vol. 9, No. 1, 1991, pp. 27-31.

4. **Asbestos Content in Bulk Insulation Samples: Visual Estimates and Weight Composition**, U.S. Environmental Protection Agency 560/5-88-011, 1988.

APPENDIX D

Special-Case Building Materials

Asbestos laboratories are now called upon to analyze many types of bulk building materials that are very difficult to characterize by routine PLM analysis. These materials are dominantly nonfriable and can be grouped into the following categories:

- Cementitious Products (pipe, sheeting, etc.)
- Viscous Matrix Products (adhesives, cements, coatings, etc.)
- Vinyl Materials (vinyl floor tile, sheeting)
- Asphaltic Roofing Materials (shingles, roll roofing)
- Miscellaneous Products (paints, coatings, friction plates, gaskets, etc.)

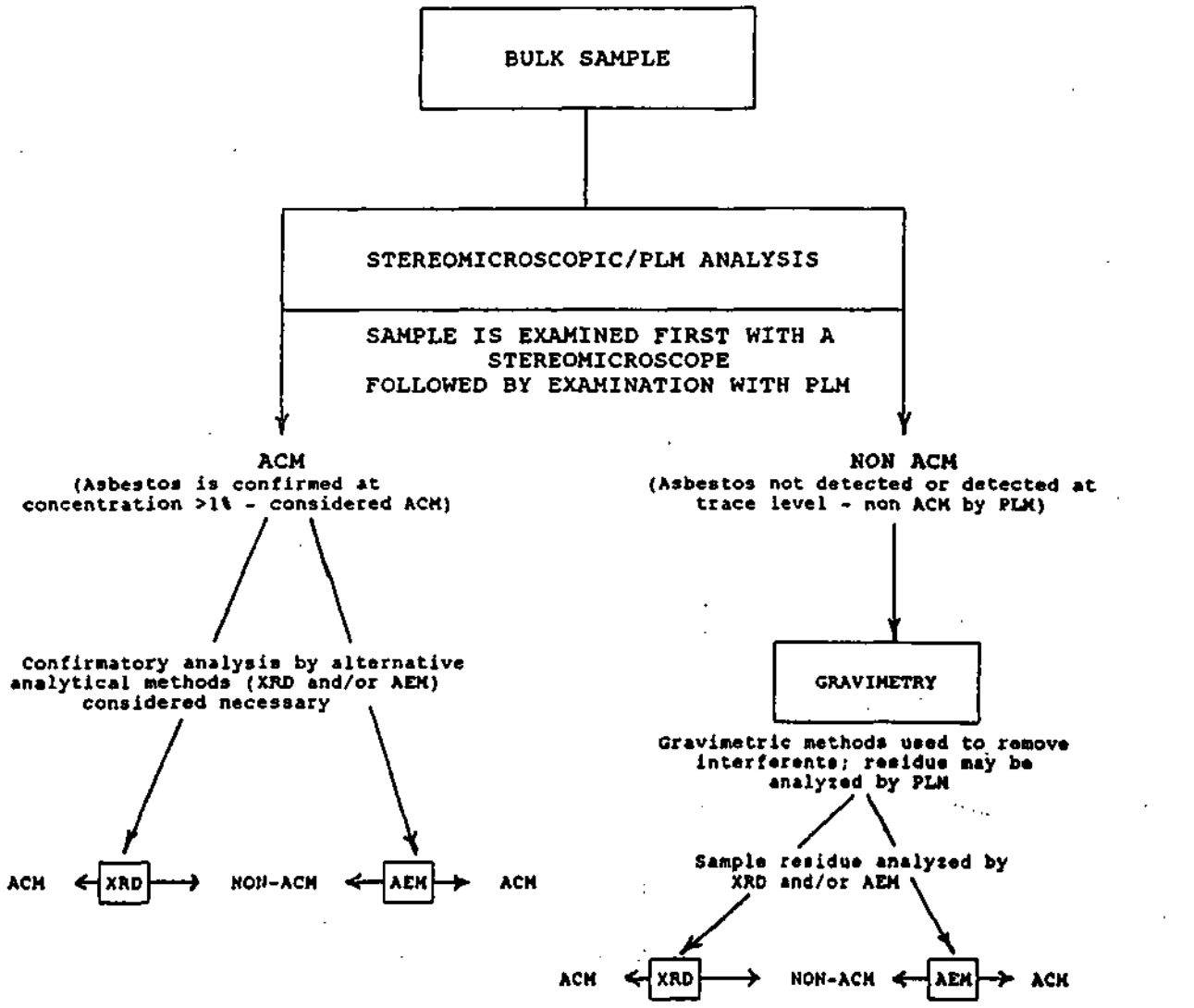
Materials characterized by interfering binder/matrix, low asbestos content, and/or small fiber size may require that additional sample treatment(s) and analysis be performed beyond routine PLM analysis. The sample treatment(s) required is(are) determined by the dominant nonasbestos sample components (see Section 2.3, Gravimetry). Materials containing an appreciable amount of calcareous material may be treated by dissolution with hydrochloric acid. Samples containing organic binders such as vinyl, plasticizers, esters, asphalts, etc. can be treated with organic solvents or ashed in a muffle furnace (preferred method) or low temperature plasma asher to remove unwanted components. Materials containing cellulose, synthetic organic fibers, textiles, etc. may also be ashed in a muffle furnace or low temperature plasma asher.

The method chosen for analysis of a sample after treatment is dependent on asbestos concentration and/or fiber size. An examination of the sample residue by PLM may disclose asbestos if the fibers are large enough to be resolved by the microscope, but additional analytical methods are required if the sample appears negative. Analysis by XRD is not fiber-size dependent, but may be limited by low concentration of asbestos and the presence of interfering mineral phases. In addition, the XRD method does not differentiate between fibrous and nonfibrous varieties of a mineral. Analysis by AEM is capable of providing positive identification of asbestos type(s) and semi-quantitation of asbestos content.

The following flowchart illustrates a possible scheme for the analysis of special-case building materials.

NOTE: Preliminary studies indicate that the XRD method is capable of detecting serpentine (chrysotile) in floor tile samples without extensive sample preparation prior to XRD analysis. XRD analysis of small, intact sections of floor tile yielded diffraction patterns that confirmed the presence of serpentine, even at concentrations of ~one percent by weight. TEM analysis of these same tiles confirmed the presence of chrysotile asbestos. With further investigation, this method may prove applicable to other types of nonfriable materials.

FLOWCHART FOR QUALITATIVE ANALYSIS OF SPECIAL CASE BUILDING MATERIALS SUCH AS FLOOR TILES, ASPHALTIC MATERIALS, VISCOUS MATRIX MATERIALS, ETC.*



*Although this flowchart is applicable to all bulk materials, it is primarily intended to be used with known problem materials that are difficult to analyze by PLM due to low asbestos concentration, and/or small fiber size, and/or interfering binder/matrix. In addition to being qualitative, the results may also be semi-quantitative. It should not be assumed that all samples need to be analyzed by AEM and XRD. The flowchart simply illustrates options for methods of analysis. Alternate methods such as SEM may be applicable to some bulk materials.

*U.S GOVERNMENT PRINTING OFFICE:1993 -750 -002/80237

Tabbed Page: 19

METHOD 100.2

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

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June 1994

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U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

METHOD 100.2

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

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 ≥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 (≤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 ≤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 ≤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 100th 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:

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

where--
no str = number of asbestos fibers counted

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

R_p = 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

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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.

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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.

Tabbed Page: 20

DRAFT 28 August 2002

Date: August 28, 2002 (Rev. #2) SOP No. EPA-LIBBY-01

Title: ASBESTOS ANALYSIS OF SOIL BY SCANNING MICROSCOPY AND ENERGY DISPERSIVE X-RAY SPECTROSCOPY

AUTHORS: Origination Date: 7-11-00

Authors: P. Frasca, R. De Malo, & J. Newton, EMSL Analytical, Inc.;
M. Goldade, USEPA, Region 8

SYNOPSIS: A standardized method for identifying and quantifying asbestos fibers in soil samples using Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy is provided. Equipment operating conditions, sample preparation and handling, and sample analysis are included. Prior to implementation of these procedures, the user must define which of three data collection methods is necessary to meet project requirements (Refer to Section 4.2).

APPROVALS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
USEPA Region 8, On-Scene Coordinator		
USEPA Region 8, Science Support Coordinator		
EMSL Analytical, Inc., Laboratory Director		

Revision Number	Revision Date	Reason for Revision
1	7/16/02	Added new prep procedure.
2	8/28/02	Sample quantification changes.

DRAFT 28 August 2002

1.0 PURPOSE

The purpose of this standard operating procedure (SOP) is to specify the proper methods and protocols to be used to identify and quantify the number and type of asbestos fibers found in soil samples using scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS). The SEM/EDS technique, instrument operation protocols, sample preparation, sample analysis, and quality assurance/quality control procedures developed for this method are discussed in the following sections.

2.0 SCOPE AND APPLICATION

2.1 This method is designed for the identification of asbestos fibers in talc-free (fibrous) soil by SEM/EDS. ***It is recognized that there is an inherent limitation in the use of SEM for mineral identification. This limitation is the inability of the SEM to perform selected area election diffraction (SAED.***

2.2 Table 1 lists the specific asbestos fibers (e.g., actinolite, chrysotile, etc.) for which instrument sensitivity, method precision and accuracy, and the optimum instrument conditions have been developed.

> *The appropriate table will be provided after results from the Performance Evaluation Study (EPA 2000) are available. Additionally, the table will be updated as new precision and accuracy data are generated.*

3.0 RESPONSIBILITIES

3.1 It is the responsibility of the laboratory supervisor and/or designates to ensure that all analyses and quality assurance procedures are performed in accord with this SOP. It is the responsibility of the laboratory supervisor to communicate anticipated situations that require any deviation from the SOP and must receive approval for the deviation before proceeding with sample preparation and analysis. Additionally, the laboratory supervisor must immediately communicate with oversight agencies or personnel any unforeseen deviations that occur during sample handling, preparation, or analysis.

3.2 Microscopists must be trained and experienced in identification and quantification of asbestos fibers. Further, SEM technicians must perform a proficiency test and/or

performance evaluation prior to analysis of field samples (Section 10.3).

4.0 METHOD SUMMARY

4.1 Prior to analysis, samples must be prepared using appropriate sample preparation methods (Section 6.0).

4.2 After sample preparation and mounting, soil samples are viewed using SEM and EDS. Any one of six data collection methods must be selected to identify and quantify asbestos in soil. These are:

Method A	Total Asbestos Content only (No Plates)
Method A_p	Total Asbestos Content only (With Plates)
Method B	Total Asbestos Content and Protocol Fiber Size Distribution (No Plates)
Method B_p	Total Asbestos Content and Protocol Fiber Size Distribution (With Plates)
Method C	Total Asbestos Content and Total Fiber Size Distribution (No Plates)
Method C_p	Total Asbestos Content and Total Fiber Size Distribution (With Plates)

The methods differ in how the detected asbestos fibers are measured and reported. The Total Asbestos Content technique provides an estimate of the mass percent of asbestos present in a sample, and is the least labor-intensive analytical technique. Total and Protocol Fiber Size Distribution techniques require fiber counting and sizing. The data collection method selected will depend on the technical and economic requirements of the project. Oversight agencies will provide a project plan or similar document that provides the rationale for the data collection method of choice. Each data collection technique is described below.

Total Asbestos Content. The amount of asbestos present in the soil sample is estimated and reported in units of mass percent.

Total Fiber Size Distribution. All asbestos fibers observed in the soil are identified, counted, and sized. Fiber counting rules are provided in the ISO 10312 Method (1995). Results are reported in two ways: 1) Raw data as individual fiber identity and size (Refer to the Data Collection Logsheet - Attachment 1); and 2) Number of asbestos fibers per gram of material.

Protocol Fiber Size Distribution. "Protocol" fibers are defined as asbestos fibers with aspect ratios of 5:1 or more, where the minimum fiber diameter is ≤0.5 µm

and fiber length is ≥5.0 µm. Counting rules for protocol fibers are provided in the ISO 10312 Method (1995). All protocol asbestos fibers observed in the soil are identified, counted, and sized to obtain the Protocol Fiber Size Distribution. Results are reported in two ways: 1) Raw data as the size and identity of individual protocol fibers; and 2) Number of protocol asbestos fibers per gram of material.

5.0 APPARATUS AND MATERIALS

Scanning Electron Microscope fitted with Energy Dispersive X-ray Spectrometer with the following attributes:

For SEM:

- Minimum Resolution: Having the ability to resolve a fiber 0.2 µm in diameter
- Accelerating Voltage: 1 to 25 kV
- Minimum Range of Magnification: x 10 to 180,000
- Imaging Mode: SEI (secondary electron image)
- Micron marker and magnification display on viewing screen
- Scan Rotation Accessory is recommended but optional

For EDS:

- Detector resolution having the ability to resolve Na, Mg, Al, Si, Ca, and Fe peaks in SRM 1866a and 1867 materials
- Analyzer capability of continuum background subtraction

Other Ancillary Equipment:

Analytical balance - accurate to 0.0001 g, range of 0.0001 g to 100 g.

Stainless steel or aluminum scoop, spoon or spatula - for transferring samples.

#10 mesh stainless steel sieve and catch pan - for coarse sieving sample to a particle size of less than 2 mm.

Gloves - for personal protection. May be plastic or latex. Disposable, powderless.

Laboratory logbook -used to record progress, any problems or observations.

Permanent marking pen - used to complete laboratory logbook notes.

Mortar and pestle - used for bulk sample homogenization.

General purpose laboratory oven – used for drying samples; capable of housing several samples at a time; must be capable of maintaining a constant temperature of approximately 103-105 °C.

Sample drying trays – capable of holding an even layer of the complete sample volume of each sample. To minimize the decontamination effort, disposable drying trays are recommended.

Porcelain crucible – used for sample ashing.

Negative Air Pressure, HEPA-filtered Hood – used to capture airborne dust, fibers, and other potential contaminants.

Muffle furnace – used for sample ashing.

Desiccator or glass petri dish – used for temporary storage of sample-mounted stubs.

Stub - used to mount samples for SEM analysis made of metal such as aluminum; covered with double stick carbon tape to secure the sample.

Sputter coater or carbon vacuum evaporator - The sputter coater is used to apply a layer of gold to the prepared SEM sample. Alternatively, a carbon vacuum evaporator may be used to apply a carbon or gold layer to the prepared SEM sample.

6.0 SAMPLE PREPARATION

6.1 General

Sample preparation must take place in a room that is free of asbestos fibers. It is recommended

that sample preparation and analysis be performed in separate rooms. Sample preparation should at minimum be performed under a negative air pressure, HEPA-filtered hood.

6.2 Bulk Soil Sample Preparation (Frasca, *et al.* 2000)

Field sample collection and preparation will be performed in accord with the Soil Sample Preparation SOP (SOP #ISSI-LIBBY-01). All samples submitted to the laboratory for SEM/EDS analysis will be representative of the sample as a whole. That is, the sample should be minimally prepared and handled prior to submittal to the analytical laboratory. Soil samples received in the laboratory, must be dried and sieved to particle size less than 2 mm. The steps for drying and sieving are provided below.

Bulk Soil Drying

Determine the dryness of the sample by performing a "squeeze test". Squeeze a portion of the sample between a freshly gloved thumb and forefinger. Sample dryness is indicated by a lack of cohesiveness in the soil. If the sample is already dry, no further drying is required. However, if the sample is moist, dry the sample as outlined below.

Set the oven temperature to 103-105 °C (not to exceed 115 °C). Prior to drying each sample, record the weight on the Sample Preparation Logbook Sheet (Attachment 2). Spread the sample on the drying tray in an even layer to promote even drying. Mark each tray with the sample ID number. Check the oven temperature to verify that the proper temperature has been reached. When the appropriate temperature is achieved, place the drying trays containing the samples in the oven. Leave the samples in the oven until they are completely dry (e.g., stable mass readings obtained 15 minutes apart). Record the weight after drying on the Sample Preparation Logbook Sheet.

Sieving

Soil Particulates larger than 1 mm in size are typically too large to be practically mounted for view using the SEM and therefore must be removed. Each sample is passed through a clean #10 stainless steel, or approximate equivalent, sieve. Non-platy particulates that are greater than 1 mm in size (particulates that are retained by the sieve) must be weighed (W_c) and reserved for analysis via Stereoscopic and Polarized Light Microscopy (PLM) (See Section 8.0). Remove any visible platy structures (i.e., vermiculite, biotite, etc.) present in the unsieved portion with tweezers and weigh them separately (W_p). Since asbestos fibers lying on the surface of the plates would go undetected by visual microscopy, these structures must be viewed with SEM. Any visible asbestos structures retained by the sieve are combined with the sieved material for later

homogenization. The fine soil fraction (particulates less than 1 mm in size) with the recombined visible asbestos structures from the sieved portion is also weighed (W_f). Record the sample masses for both soil fractions on the laboratory Logsheet (Attachment 2). Decontaminate the sieve between samples using decontamination procedures described in Section 11.0.

6.3 SEM Sample Preparation

The soil must be further prepared for SEM analysis. Prior to mounting onto a SEM stub, the soil must be homogenized, and if necessary ashed and acidified

Ashing

Soil samples containing visible (by eye or macroscopically) amounts of organic material (e.g., leaves, twigs, grass, etc.) must be ashed prior to mounting the sample. Accurately weigh approximately 2-5 grams of material and record the mass (Attachment 2). Place the material in a porcelain crucible with cover and place in a muffle furnace at 450 ±5 °C, for 3-6 hours, or until no further mass loss is observed. Cool, reweigh and calculate percent organic loss. Record the final mass and percent organic material lost.

Acid Treatment

Soils containing significant amounts of calcium carbonates to interfere with the analysis are further treated with concentrated hydrochloric acid (HCl). Place the sample into a small beaker and add enough HCl to completely cover the residue. Typically, 2 times the volume of the sample is an appropriate volume of acid. Allow the sample to react for about 15 minutes or until the sample is dry. Record the dry weight of the residue and calculate percent loss.
Note: Acid treatment may alter or remove asbestos fibers from the matrix.

Homogenization

The large asbestos structures retained by the sieve are added to the sieved soil and together they are ground down and homogenized with a mortar and pestle. The coarse portion of the soil retained by the sieve will contain relatively large rocks creating unnecessary background and is not included in the homogenization process.

Mounting the Sample

A strip of 8 mm wide double stick carbon tape is placed on the Al SEM stub and placed in an

upright, horizontal orientation. The mortar and pestle ground material is deposited with a clean micro-spatula. Upon careful distribution of the soil sample using a micro-spatula, the edge of a clean glass slide is used to gently scrape the material into an approximate monolayer. The flat surface of the glass slide is then used to press the sample onto the double-sided carbon tape used as a mounting medium. This serves to increase the surface area in contact with the conductive surface and to provide improved adhesion. The sample is then coated with a thin layer of gold using a sputter coater or with carbon or gold in a vacuum evaporator. This process provides a source of secondary electrons, which are responsible for generating the SEM image and also renders, the sample electrically conductive preventing charging of the sample. The procedures for each of these techniques are provided below.

Carbon Coating. Carbon may be evaporated onto the sample by running a current through sharpened carbon rods at pressures of $\leq 10^{-5}$ Torr. Likewise, gold wire secured within a tungsten basket can be evaporated onto the sample. Coating with carbon only will generally, provide a better signal to noise especially for the low energy peaks, but will however generally result in a noisier image.

Sputter Coating. Gold may be sputtered onto the sample at pressures of ≤0.1 Torr, preferably, but not necessarily, consisting of backfilled dry argon gas. Typical current settings are between 20-40 milliamps (mA) and duration of coating is approximately 30-60 seconds. Actual conditions and settings may vary among instruments. Too much gold may compromise the quality of the EDS spectrum, especially at the lower energy end, while too little may reduce the intensity of the image and result in undesirable "charging" effects, which not only perturb and distort the image, but potentially may also dislodge and eject asbestos fibers from the sample.

7.0 SEM/EDS ANALYTICAL REQUIREMENTS

Asbestos fibers will be characterized by two key parameters: identification and quantification. That is, the sample will be identified by classifying the asbestiform material (e.g., Libby amphibole chrysotile, etc.). The sample will be quantified using one of the six Methods A, A_p, B, B_p, C, or C_p (Section 4.2) by estimating asbestos content and/or classifying the length and diameter of the asbestos fibers. The steps necessary to characterize the asbestos in soil samples are provided in the subsequent sections.

7.1 General

Sample analysis must take place in a room that is free of asbestos fibers. It is recommended that

sample preparation and analysis be performed in separate rooms. To prevent cross contamination of samples, only one sample should be mounted onto a SEM stub at a time. Furthermore, SEM stubs containing the samples should be stored either in a large dessicator or in glass petri dishes. Plastic petri dishes must not be used for carrying or storing samples since loosely anchored asbestos fibers (and especially those classified AS Libby amphiboles, which exhibits particularly strong electrostatic effects) may be pulled off the sample stub onto the cover of the plastic petri dish.

7.2 Maintenance

Periodic instrument maintenance should be performed as necessary in accord with manufacturer's recommendations. All maintenance activities must be recorded in an instrument maintenance logbook (Attachment 3).

7.3 Standards

Standard Reference Materials

Standard reference materials (SRMs) are used to compare known fibrous materials with unknown field samples. The following SRMs should be retained at the analytical laboratory for comparison and verification including standards of the six asbestos minerals (Chrysotile, Anthophyllite, Actinolite, Tremolite, Amosite & Crocidolite) as provided by the National Institute of Standards and Technology (NIST SRM 1866a and SRM 1867). ***Additional Libby Amphibole reference material and a glass film reference will be supplied to all associated laboratories by the USGS.***

Calibration Standards

The following standards are recommended to be used for instrument calibration purposes. All procedures shall be carried out as per manufacturer's specifications for each individual instrument utilized for this method.

Magnification Grating. Magnification gratings (obtained from E.F. Fullam or appropriate vendor) are used to calibrate the true magnification of the microscope.

Manganese Standard. Manganese (obtained from the NBS or appropriate vendor) is used to calibrate the resolution of the EDS detector.

Intensity Calibration. Perform calibration in accordance with manufacturer's specification.

7.4 Initial Calibration Procedures

The SEM and EDS must be calibrated with four different calibration standards as outlined in this section. Instrument calibration must be performed at the following minimum frequency: 1) prior to initial receipt of field samples; 2) monthly after the first calibration; and 3) immediately after any maintenance activities are performed. In the event that any one of the four calibration standards do not meet the acceptance criteria provided below, further instrument maintenance or other corrective action(s) may be necessary. Analysis of investigative samples may not proceed until the instrument is properly calibrated.

Magnification Calibration. Insert the Magnification Grating into the SEM and obtain an image at 10X, 200X, 2,000X and/or 10,000X magnifications, as appropriate. The magnification calibration shall fall within ± 5% of the certified values as indicated in the calibration standard manufacturer's specifications. Record the results on the Data Collection Logsheet (Attachment 1).

Peak Centroid Calibration. Insert the Aluminum/Copper standard (or other appropriate standard) into the SEM and energize the filament to the appropriate energy (10-20 KeV). Acquire an EDS spectrum adjusting the SEM probe current setting to achieve a count per second rate of 1000 to 2000. Adjust the standard to enable acquisition of x-rays so that the aluminum and copper peaks are approximately the same height. Once the peak heights appear correct, acquire a fresh spectrum for a gross count of greater than 10,000. With the channel cursor, measure the peak centroids of both the aluminum and the copper Kα peaks. Record the results on the Data Collection Logsheet. The centroid of the aluminum peak should correspond to 1.487 (±0.01) KeV and the copper should correspond to 8.047 (±0.01) KeV. In the event that the peak centroids do not correspond to the appropriate energy reading (in KeV), then an alignment must be performed before proceeding.

Resolution Calibration. Insert the Manganese standard into the SEM and energize the filament to the appropriate energy (10-20 KeV). Acquire an EDS spectrum adjusting the SEM current setting to achieve a count per second rate of 1000 to 2000. Broaden the KeV scale to clearly view the region around manganese (5.2 to 6.5 KeV). Once the reading appears correct, acquire a fresh spectrum for a gross count of greater than 10,000. Perform a background correction and note the total counts for the peak centroid. Divide the peak centroid counts by 2 (one half maximum) and mark the low and high KeV channels that closely correspond the half maximum of the peak centroid. Count the number of channels between the low and high half-maximum

markers. Multiply the number of channels by the KeV per channel rating of the EDS unit for the resolution. The resolution must be no greater than 175 eV. Record the results on the Data Collection Logsheet.

Sodium (Na) Sensitivity. To assure low energy detection of the EDXA system, a resolvable Na kα peak must be measured using a standard crocidolite standard. Perform these measurements in accordance with manufacturer specifications.

7.5 Continuing Calibration Procedures

Laboratory Control Samples

The initial calibration for the SEM and EDS must be verified daily. An independent Laboratory Control Sample (LCS) must be analyzed with each analytical batch or once a day, whichever is more frequent. An analytical batch is comprised of 20 field samples. A LCS is a certified reference standard or performance evaluation sample that is independent from the calibration standards and consists of the asbestiform fibers of interest. The acceptance criteria for the LCS is presented in Table 3. In the event that any LCS does not meet the acceptance criteria, the analyses will be halted. The LCS and all field samples associated with the analytical batch must be reanalyzed once the source of problem is identified and resolved.

> *Currently, an asbestos in soil LCS is not available, but will be prepared and certified as part of the Performance Evaluation Study (EPA 2000) by USGS. In addition, the appropriate table will be provided after results from the Performance Evaluation Study (EPA 2000) are available. Additionally, the table will be updated as new precision and accuracy data*

7.6 SEM/EDS Sample Analysis Procedures (Frasca, *et al.* 2000)

Operate the SEM at a voltage of 10-25 kV, depending on the optimum operating specifications unique to the individual instrument. Load the sample onto the SEM. Care should be taken in loading the sample so as not to mechanically disturb the sample particulates. If charging is noted, add more gold or carbon coating to the sample (Section 6.3).

Asbestos fiber sizes in the sample can vary significantly. Generally, analysis at lower magnifications yields a larger area of material that can be counted and/or analyzed in fewer

magnification fields. However, only the larger the fibers will be seen at low magnification, and the smaller fibers will be missed. Therefore, more than one magnification must be used. Four (4) different magnifications will be applied during analysis: 10X, 200X, 2,000X and 10,000X. Analysis will progress from lowest to highest magnification. The SEM operator should, when necessary, proceed to higher magnifications to verify the nature of suspected asbestos fibers from its telltale asbestiform striations or fiber splitting features. Similarly, the SEM operator should refer to the EDS spectra for the characteristic elemental composition of the suspect fibers. Increasing the magnification for suspected fibrous structures may also eliminate a plate-like structure resting perpendicular to the viewer. Therefore, tilting of the stage is rarely necessary. After confirming the asbestos nature of the fiber or group of fibers in the field of view, an area percent estimate of asbestos to total material is obtained and recorded. ***To ensure uniformity of area estimates, all laboratories will use the area percent visual estimate aids produced by Dr. Shu-Chun Su (See attached).***

Fiber lengths and diameters are determined with the use of the micron marker and scan rotation accessory. The smallest diameter visible in the SEM is approximately 0.05 µm, while the fiber lower limit for identification by EDS is approximately 0.2 µm in diameter. These limits may need to be adjusted upward if matrix interferences are present. Example EDS ***spectra of several members of the designated Libby amphibole group*** (Richterite, Tremolite and Actinolite) are seen in Figures 1, 2, and 3, respectively.

For both SEM stubs, fiber distribution and asbestos content determination at each magnification are summarized below. Specific details for asbestos identification and counting rules are summarized in the ISO 10312 Method. Analysis may cease for each magnification if 100 fibers are counted or at least 15 acceptable fields of view have been examined (Refer to slide preparation evaluation under 200X magnification). Fibers, fiber bundles, matrices and clusters (F, B, M, C, respectively) are recorded on the worksheet where required.

10X. Set the magnification on the SEM to 10X. Scan the sample. Estimate and record the asbestos content by estimating the fraction (percent) of the total area that contains asbestos material present at this magnification. Identify, count, and record all asbestos fibers ≥50 µm in diameter (Methods A or B). Repeat this for 2-3 fields to view the full extent of the sample.

200X. Adjust the magnification on the SEM to 200X. Scan the sample. Evaluate the sample mounting technique by visually assessing whether the fields of view are loaded uniformly. This is done to avoid "particle stacking" or underloading the stub. Particle loading must cover at least 70% of the field of view. If less than 70% loading is observed, and only % asbestos content needs to be determined, the field should not be

assessed and another field of view selected. If calculating on a per mass basis is required, than all fibers in all fields must be counted and sized. If greater than 20% of the fields are rejected due to improper loading criteria, the analysis must cease and the entire sample must be re-mounted and reanalyzed. Track the sample mounting evaluation on the Data Collection Logsheet.

Estimate and record the fraction (percent) of the total area that contains asbestos material present at this magnification. Identify, count, and record all asbestos fibers ≥2 µm and <50 µm in diameter (Methods A or B). Repeat this for 100-200 fields of view ***or however many fields are needed to reach the desired sensitivity***. The lower the concentration of asbestos in a particular sample, the greater the number of fields required to achieve statistical confidence.

2,000X. Re-adjust the magnification on the SEM to 2000X. Scan the sample. Estimate and record the fraction (percent) of the total area that contains asbestos material present at this magnification. Identify, count, and record all asbestos fibers ≥0.5 and < 2 µm in diameter (Methods A or B). Repeat this for 100-200 fields of view ***or however many fields are needed to reach the desired sensitivity***.

10,000X. Finally, adjust the magnification on the SEM to 10, 000X. Scan the sample. The fields of view are selected randomly, in various locations and in such a pattern of motion, which prevents selection of the same area more than once. Estimate and record the fraction (percent) of the total area that contains asbestos material present at this magnification. Identify, count, and record all asbestos fibers ≥0.05 and <0.5 µm in diameter (Methods A or B). Repeat this for 100-200 fields of view ***or however many fields are needed to reach the desired sensitivity***.

For the platy structures, estimate the percent content of asbestos.

7.7 Imaging with SEM and EDS

Use the rapid rate-scanning mode from one field to the next to estimate asbestos content or to count and identify fibers. EDS analysis may be performed with the spot mode. ***Spectra will be collected and identified for all Libby amphiboles or other regulated minerals that would cause the analysis results to exceed 1 %. Thereafter, spectral analysis will continue at a reduced rate.***

Using the photo scanning mode, provide 1 SEM electron micrograph and accompanying EDS spectrograph for each type of asbestos present in a sample delivery group (SDG). A SDG is

defined as a single set of samples submitted to the laboratory in one day or every 25 samples, whichever is more frequent. Assign a unique laboratory identification number to each photograph and spectrograph and record in logbook. The microscopist may at times determine that unplanned or "opportunistic" micrographs and spectrographs are necessary. Any opportunistic photographs taken are also numbered, but must accompany a brief explanation of the reason for taking the picture. A logbook that tracks the photomicrographs and spectrographs must be maintained. An example logbook page is provided (Attachment 4).

8.0 STEREOMICROSCOPY AND PLM ANALYTICAL REQUIREMENTS

In order to determine the total asbestos content of a soil sample, the coarse fraction of the bulk soil sample (sample retained by the sieve) must also be analyzed. These particles may be too large to be analyzed with the SEM/EDS, but it is conceivable that the asbestos content in this fraction may significantly affect the reported total asbestos content in the sample. Therefore, these samples are analyzed for the presence of large asbestos structures via stereomicroscopy, coupled with PLM (Stereo/PLM) to confirm suspected asbestos fibers. Refer to NIOSH Method 9002 for a discussion of the analytical procedures (NIOSH 1994).

9.0 QUANTIFICATION OF ASBESTOS IN SOIL SAMPLES (Frasca, *et al.* 2000)

Procedures for quantifying the total asbestos content in soil are provided in the following subsections.

9.1 Total Asbestos Content

The percent of asbestos in the sample (defined as asbestos content) is calculated using measured results from both the SEM/EDS and the Stereo/PLM. The asbestos content estimated by SEM of the fine non-platy soil, $(SEM)_f$, and the platy structures, $(SEM)_p$, is determined as follows:

The average field of view asbestos percentage shall be calculated for each magnification used in the analysis. This is done for both the fine and platy portions of the sample if necessary. These average percentage composition values will them be summed and a mean value determined.

$$\%_{(SEM)f} = \frac{M_1 + M_2 + M_3 + M_4}{4}$$

$$\%_{(SEM)p} = \frac{M_1 + M_2 + M_3 + M_4}{4}$$

Where:
M_1 = Mean percent of asbestos at 10X
M_2 = Mean percent of asbestos at 200X
M_3= Mean percent of asbestos at 2,000X
M_4 = Mean percent of asbestos at 10,000X

(Note: It the opinion of EMSL that this simple summing and averaging of percentage estimate may overestimate analyte content.)

This calculation is based on the assumption that an asbestos area percent approximates the asbestos content in mass percent. This assumption is cautiously reasonable when the fine soil layer that is mounted onto the SEM stub tends towards a thickness of one-particle, is reasonably uniform, and if the asbestos concentration on the surface of the plates is representative of the asbestos concentration within the plates.

The percent of asbestos estimated in the coarse section with the Stereo/PLM {$\%_{(PLM)}$} is then integrated to that found with the SEM {$\%_{(SEM)}$}.

$$\%_{ASB} = \frac{\{(\%_{(PLM)})(W_c)\} + \{(\%_{(SEM)f})(F)(W_f)\} + \{\%_{(SEM)p}(W_p)\}}{W_c + W_f + W_p}$$

Where:

W_c = Weight of coarse portion
W_f = Weight of fine material
W_p = Weight of platy material
F = Fraction of residue remaining after the final step in sample preparation (i.e., ashing, acid treatment)

9.2 Quantifying Fiber Size Distribution

All asbestos fibers will be sized and recorded on the Data Collection Logsheet (Attachment 1) when necessitated by the quantification method selected ***(methods B, B_p, C, and C_p***.

9.3 Determination of the Number of Fibers Per Gram of Soil

When Total Fiber Size Distribution or Protocol Fiber Size Distribution analysis is required (methods B, B_p, C and C_p), then the number of fibers per gram of soil is determined as follows:

$\frac{\text{Total Number of Fibers}}{\text{gram of soil}} =$	$\frac{A}{M}\sum_{i=10x}^{10,000x} fi_{\substack{fine \\ soil}} +$	$\frac{A}{M}\sum_{i=10x}^{10000x} fi_{Plate.}$

$$\frac{\text{Total Number of Protocol Fibers}}{\text{gram of soil}} = \frac{A\ N}{M\ na} \quad \text{(Magnification 10.000X only)}$$

Where:

A = Area of the entire sample on the stub
M = Mass of the entire sample on the stub
$f_i = \frac{N}{(n_i)(a_i)}$
a_i = Area of one field of view in the SEM at each magnification
n_i = Number of fields analyzed at each magnification
N_i = Number of fibers counted in the n fields at each magnification

Asbestos fibers that have been freed from the vermiculite plates or are attached to the surface of the vermiculite plate are visible with SEM and are counted during the analysis. However, any fibers that remain trapped within the vermiculite will not be seen (and counted) by the analyst, resulting in an underestimated fiber count or asbestos content estimate. The higher the content of vermiculite plates in the soil, the larger the number of fibers which will escape detection. Thus, the number of fibers per gram of soil reported should be interpreted as a minimum number.

10.0 QUALITY ASSURANCE/QUALITY CONTROL

This section summarizes activities required to ensure that all sample preparation and analysis activities are of the highest achievable quality.

10.1 Precision

Precision is defined as the agreement between a set of replicate measurements without assumption or knowledge of the true value. It is a measure of agreement among individual measurements of the same attributes under prescribed similar conditions. Agreement is often

expressed as the relative standard deviation (RSD) for a set of replicate measurements. During method development, analytical precision was assessed by preparing and analyzing the same sample twice by the same analyst. The RSDs for each fiber type are presented in Table 3. These values should be used to assess analytical precision.

> *The appropriate table will be provided after results from the Performance Evaluation Study (EPA 2000) are available. Additionally, the table will be updated as new precision data are generated.*

Field Duplicates: Field duplicates are co-located soil samples that are collected by the field personnel, but the laboratory is unaware that the samples are duplicates. These samples serve to test the precision of the both the field sampling and the laboratory's sample preparation and analysis. A field duplicate should be performed at a frequency of 5% of all field samples prepared for analysis (1 laboratory duplicate for every 20 field samples) or 1 per preparation batch, whichever is more frequent. Consult the site-specific Project Plan for actual frequency requirements. The RSD for laboratory duplicates must not exceed values presented in Table 3. Failure to meet acceptance criteria may result in re-analysis and/or re-preparation and re-analysis of all samples in the analytical batch. If corrective action procedures are not already outlined in the site-specific Project Plan, contact the oversight agency to discuss the appropriate corrective action steps.

Laboratory Duplicates: Laboratory duplicates are splits of a well-homogenized sample that is prepared by laboratory personnel (sample preparation technicians). Because the laboratory is aware that the samples are duplicates, these samples serve to test the precision of the laboratory's sample preparation and analysis. A laboratory duplicate should be performed at a frequency of 5% of all field samples prepared for analysis (1 laboratory duplicate for every 20 field samples) or 1 per preparation batch, whichever is more frequent. Consult the site-specific Project Plan for actual frequency requirements. The RSD for laboratory duplicates must not exceed values presented in Table 3. Failure to meet acceptance criteria may result in re-analysis and/or re-preparation and re-analysis of all samples in the analytical batch. If corrective action procedures are not already outlined in the site-specific Project Plan, contact the oversight agency to discuss the appropriate corrective action steps.

10.2 Accuracy

Accuracy is a measure of the closeness of individual measurements to the "true" value. Accuracy usually is expressed as a percentage of the true value. During method development, analytical accuracy was assessed by preparing and analyzing replicate LCSs and counting fibers. The

"percent recovery" for each fiber type is presented in Table 3. These values should be used to assess accuracy.

> *The appropriate table will be provided after results from the Performance Evaluation Study (EPA 2000) are available. Additionally, the table will be updated as new accuracy data are generated.*

Laboratory Control Sample: Laboratory control samples are certified reference standards (independent from the calibration standards) consisting of several asbestiforms. Because LCSs are independent of the calibration standards, they are analyzed to verify the accuracy of the standards used to calibrate the instrument. A LCS must be analyzed with each analytical batch or once a day, whichever is more frequent. An analytical batch is comprised of 20 field samples. The LCS will be evaluated based on two parameters and it must meet the acceptance criteria for both to be considered acceptable. These parameters are: 1) accurate asbestiform identification; and 2) accurate fiber counting and sizing. Acceptance criteria for the LCS are presented in Table 3. Failure to meet acceptance criteria results in re-analysis and/or re-preparation and re-analysis of all samples in the analytical batch. If corrective action procedures are not already outlined in the site-specific Project Plan, contact the oversight agency to discuss the appropriate corrective action steps.

Method Blanks: An instrument blank is composed of the field sample matrix that is free of the analyte(s) of interest (e.g., asbestos-free soil). Method blanks are put through the same sample preparation steps as field samples and are used to discern if laboratory-induced contamination is present. These samples must be inserted in the analysis stream at a frequency of 5% of samples, at minimum. Detection of a single asbestos fiber suggests that laboratory-induced contamination may be present. All associated samples may require re-preparation and re-analysis. If corrective action procedures are not already outlined in the site-specific Project Plan, contact the oversight agency to discuss the appropriate corrective action steps.

10.3 Method Proficiency

Each laboratory must demonstrate the ability to accurately and precisely execute the selected analytical method(s) (Methods A, A_p, B, B_p, C and/or C_p). Analysts and technicians performing sample preparation or analysis, must participate in a method sensitivity study and proficiency test before analysis of any field samples may proceed. The laboratory must also repeat this demonstration whenever new staff members are trained or significant changes in instrumentation

are made. Proficiency tests must be updated yearly, unless staffing or instrumentation changes require more frequent testing.

To evaluate the performance of the total analytical process, certified reference samples or performance evaluation samples must be handled in exactly the same manner as actual field samples. The following steps must be performed. Prepare and analyze at least 4 replicate aliquots of each well-mixed reference sample. Reference samples contain various asbestiforms in a blank or sufficiently characterized soil matrix. Identify and quantify the asbestos using the same procedures and using the same instrumentation planned to analyze actual field samples. Calculate the average and the standard deviation of the measured values for each asbestiform using the 4 results. Compare the reported standard deviation with the range of acceptable values provided in Table 4. Report the results to the oversight agency.

> *The appropriate table will be provided after results from the Performance Evaluation Study (EPA 2000) are available. Additionally, the table will be updated as new precision and accuracy data are generated.*

11.0 DECONTAMINATION

Decontamination (decon) procedures are performed using standard laboratory decontamination procedures.

12.0 RECORDS

12.1 Analytical Data Forms

Analysts will record information on the initial instrument calibration, and the results of all field and quality control samples. The size of all asbestos fibers counted must be recorded in analytical logbook. An example logsheet is attached (Figure 4).

12.2 Photomicrographs

Photomicrographs and EDS spectrographs must be taken for each sample at a rate of 1 photomicrograph and spectrograph per each type of asbestos found per SDG analyzed (Refer to Section 7.7). Asbestiforms selected for photography must be recorded in a logbook. Each photo

and EDS spectrograph will be identified by the unique laboratory identification number. Any opportunistic photographs taken are also numbered, but must accompany a brief explanation of the reason for taking the picture. An example logsheet is attached (Figure 5).

12.3 Instrument Maintenance Logbook

An individual instrument maintenance logbook should be kept for each piece of equipment in use at the laboratory. All maintenance activities must be recorded in the appropriate logbook. An example logsheet is attached (Figure 6).

12.4.1 Data storage and Archival

Electronic Data. Each day of data acquisition, all electronic files will be saved onto two separate media. For example, the data may be saved onto a computer hard drive, but must also be backed up onto a type of portable media such as CD-ROM, floppy disc, or tape. Portable media will be maintained in a single location with limited access.

Hardcopy Data. All data sheets or logbooks, photographs, and spectrographs must be stored in a secured location with limited access (e.g., locking file cabinet) when not in use.

Copies (hardcopy and electronic) of the raw analytical data will be submitted to the oversight agency for archival.

13.0 REFERENCES

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Tables

Figures

Figure 1

Richterite Site Standard (Libby, MO)

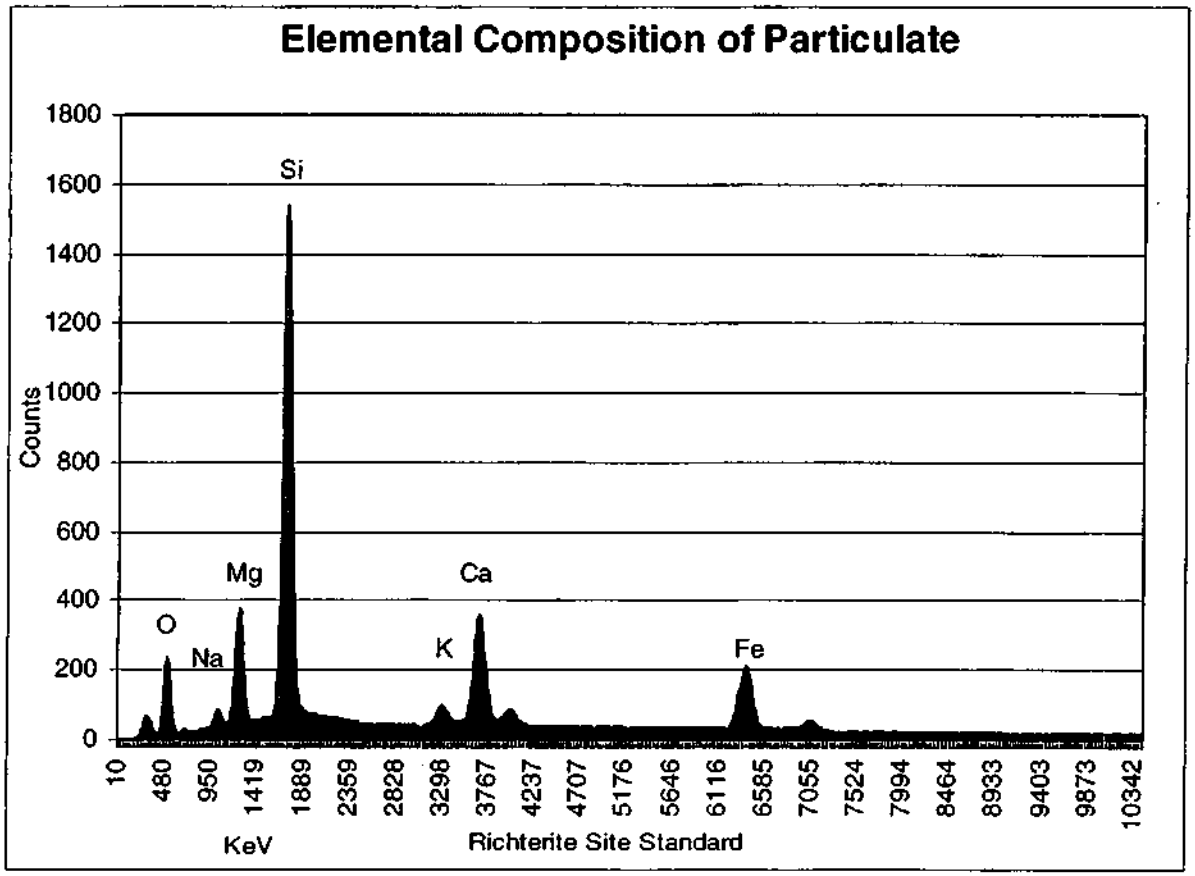


Figure 2

Tremolite Standard (NIST)

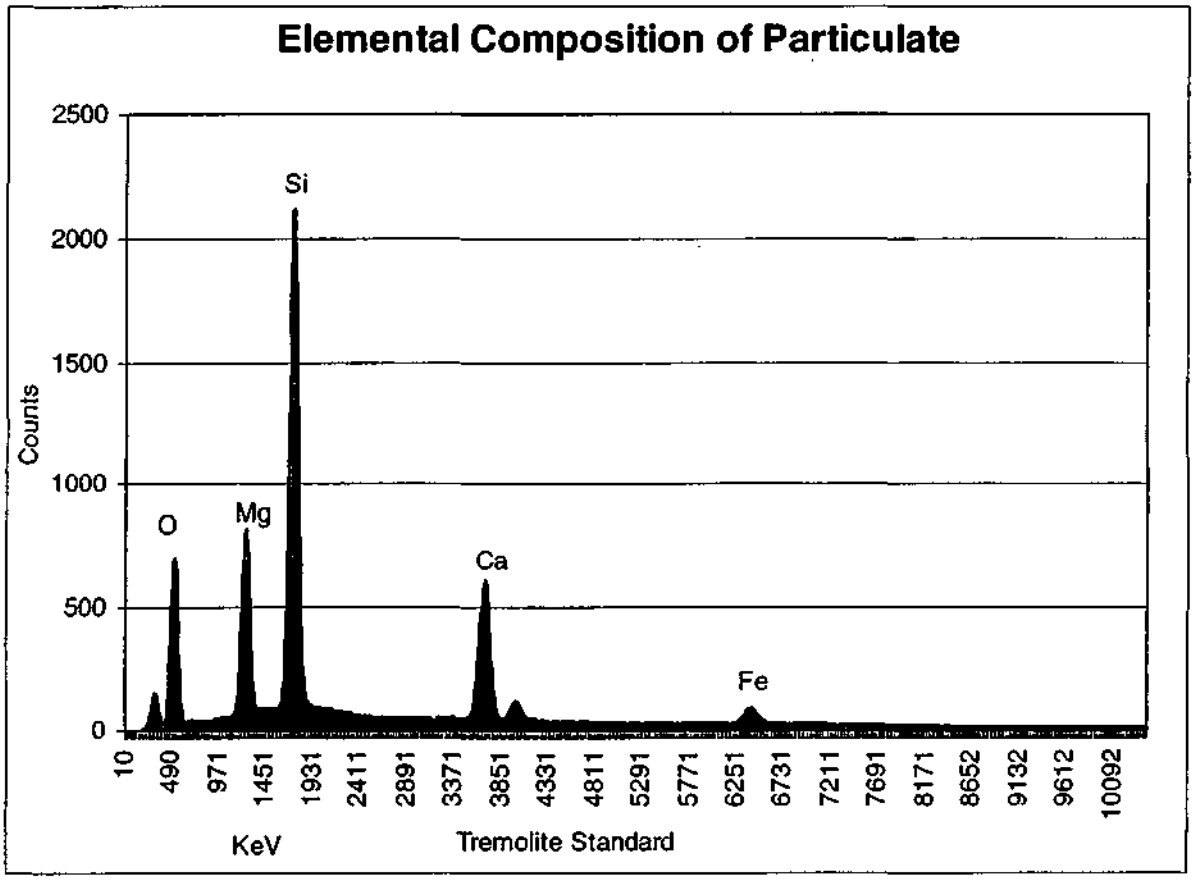
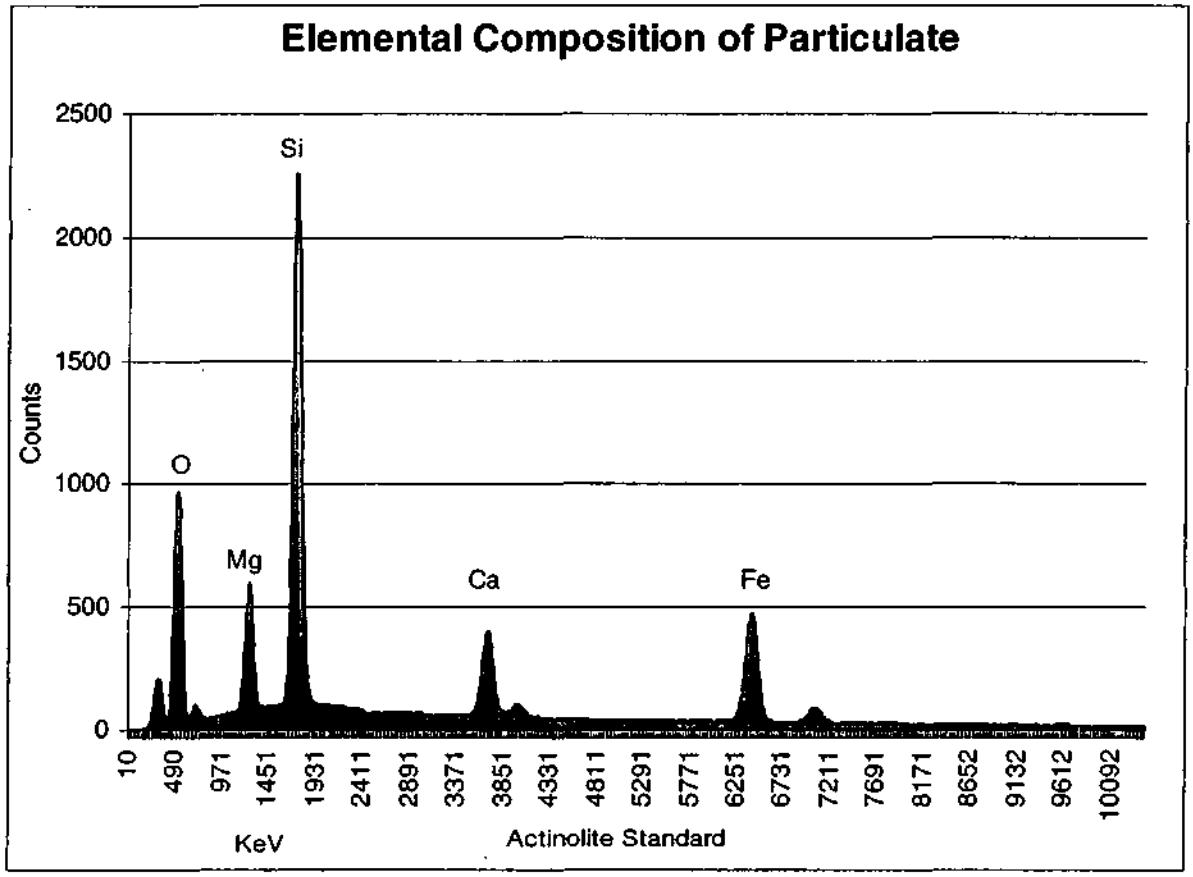


Figure 3

Actinolite Standard (NIST)



Attachment 1

Attachment 2

DRAFT 28 August 2002

Attachment 3

Attachment 4

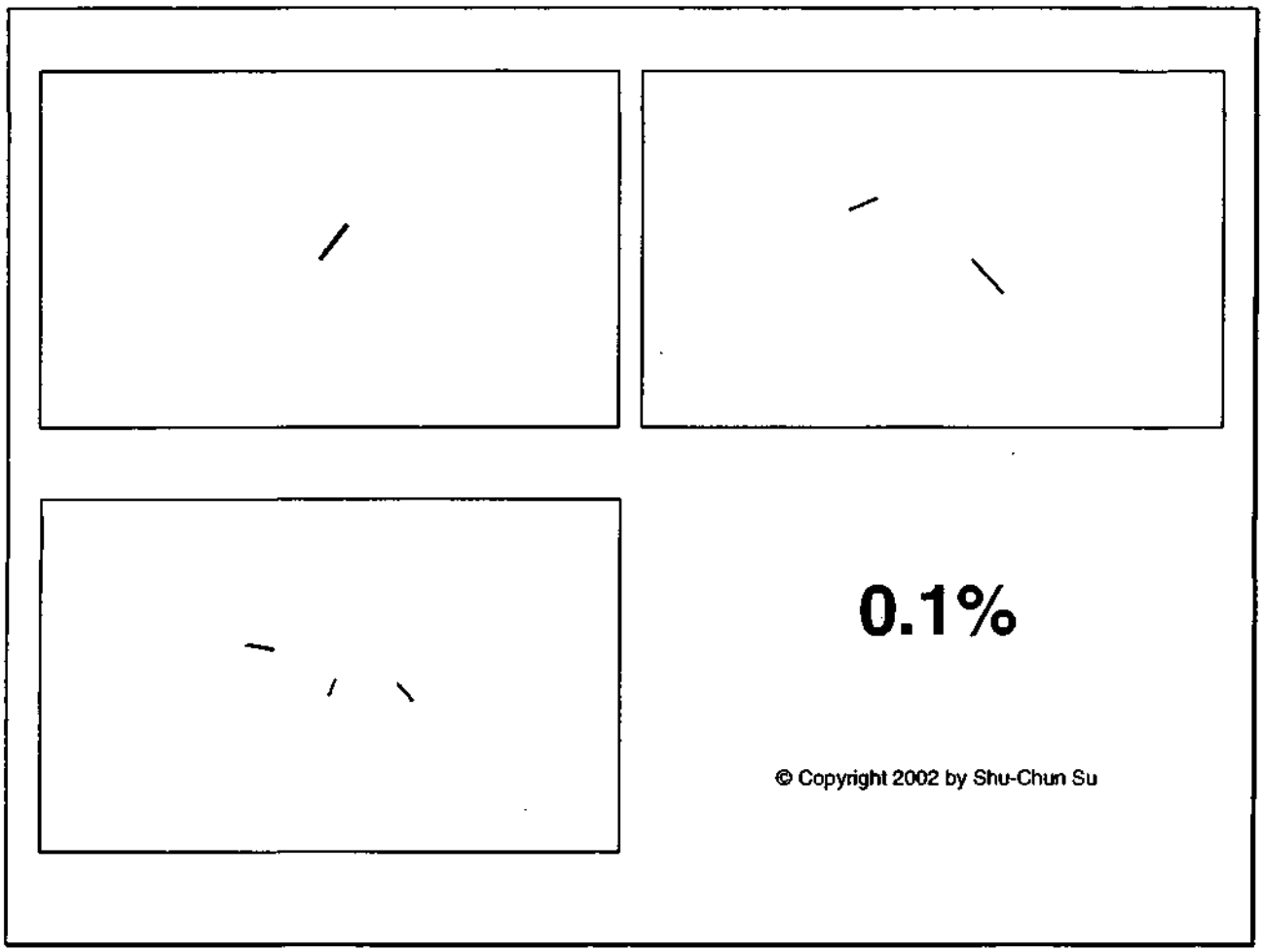
DRAFT 28 August 2002

Reference Charts for Visual Estimate of Area Percentage in SEM Analysis

(For Monitor Screen with Aspect Ratio 1.64)

Shu-Chun Su, Ph.D.
S & L Consulting International
2002

- **These charts were specifically created to aid the visual estimation of fibrous structures in SEM images for the specified screen aspect ratio.**
- **The aspect ratios of the fibrous objects used in these charts range from 12:1 to 20:1, centering around 16:1, to mimic Libby amphiboles.**
- **For each percentage, three charts were created with different numbers of fibrous objects to approximate real-world situations.**
- **The area percentages of fibrous objects in all charts have been analyzed using Image-Pro Plus 4.5 and confirmed to be accurate within ± 0.005% of the specified values.**
- **For each percentage, one set of positives and one set of negatives are provided so that an analyst can choose the one that suits his or her vision.**
- **As shown by the charts of 0.5%, 0.8%, 1.0% and 1.5%, it is not easy to visually ascertain whether the total percentage of asbestos structures in an FOV (field of view) is above or below 1.0%.**
- **As shown by the charts of 20% and 30%, mental grouping structures together without the aid of appropriate reference charts tends to overestimate the actual concentration.**
- **Comments and suggestions to improve these charts are welcome. Please contact the author at 302-995-3498 or *scsu@delanet.com.***


0.1%
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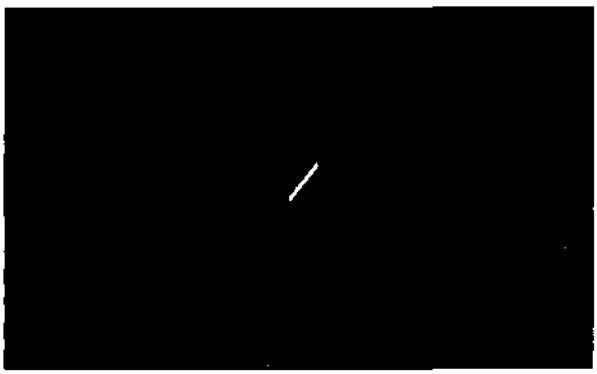

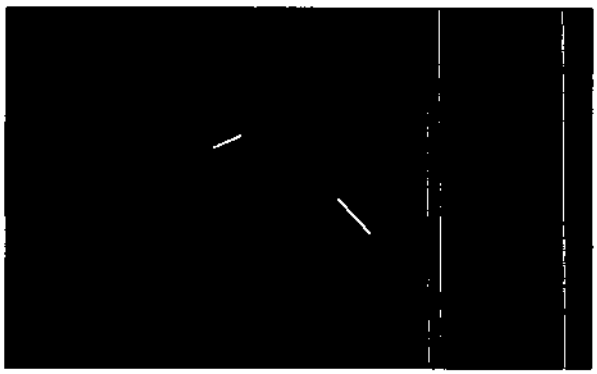

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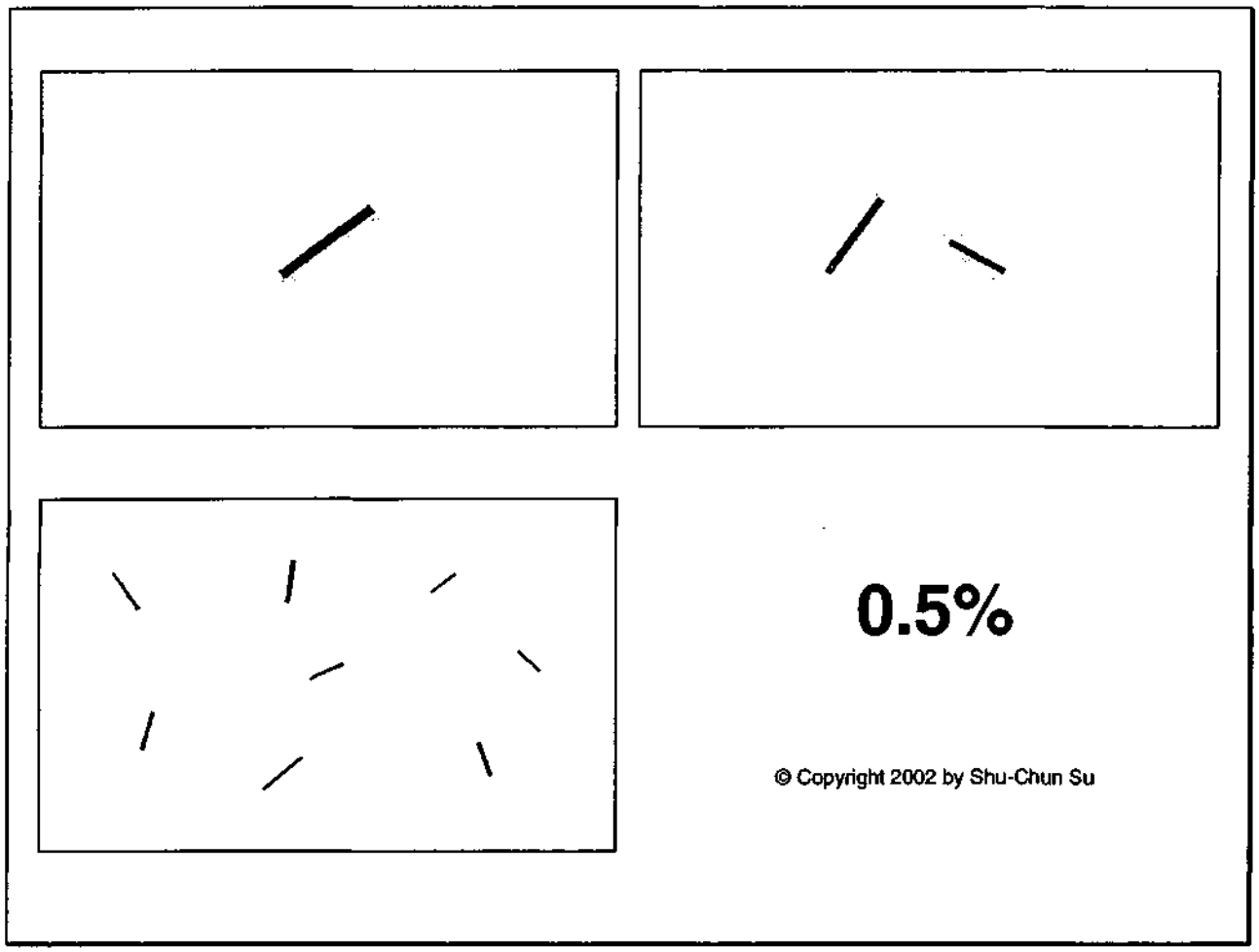
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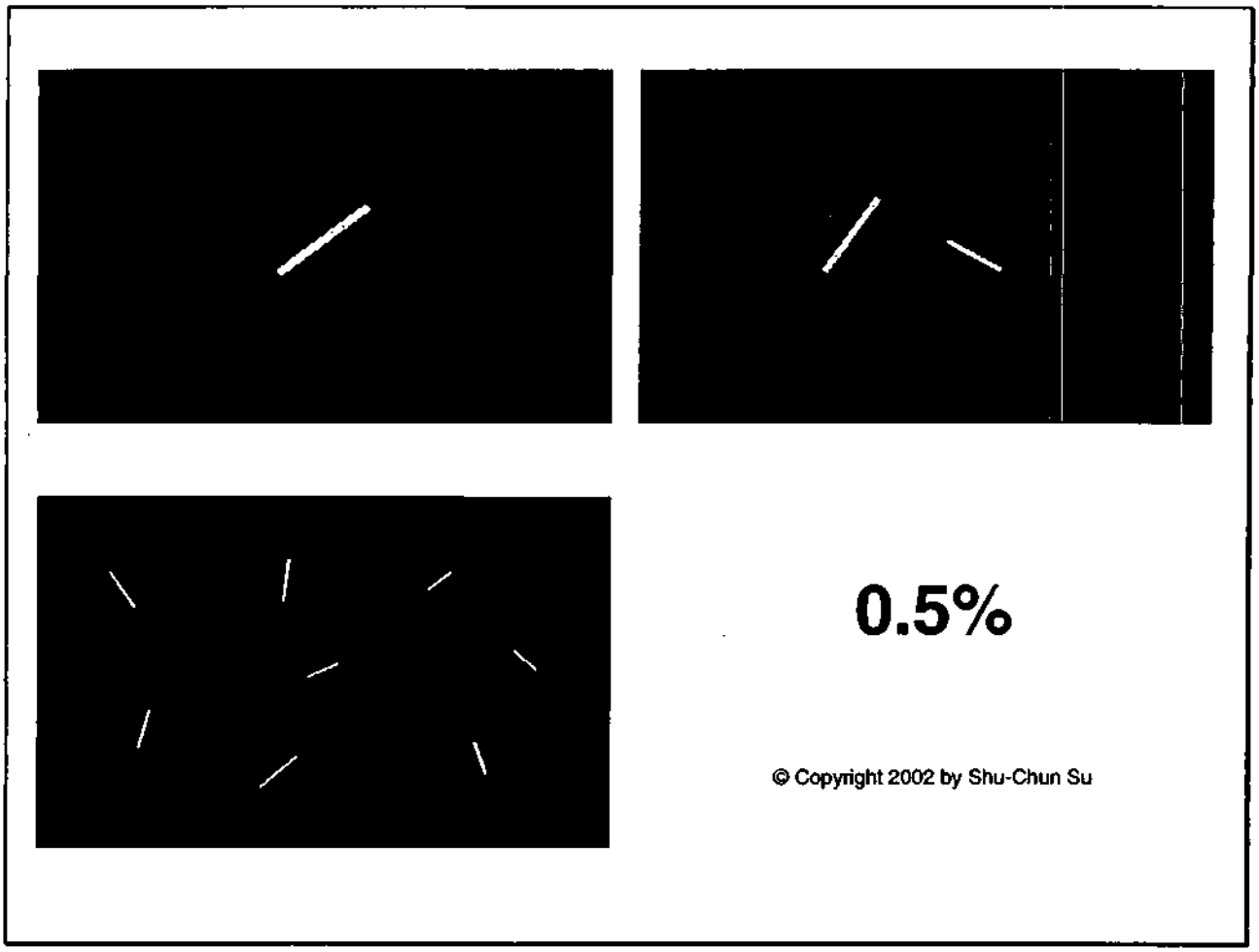
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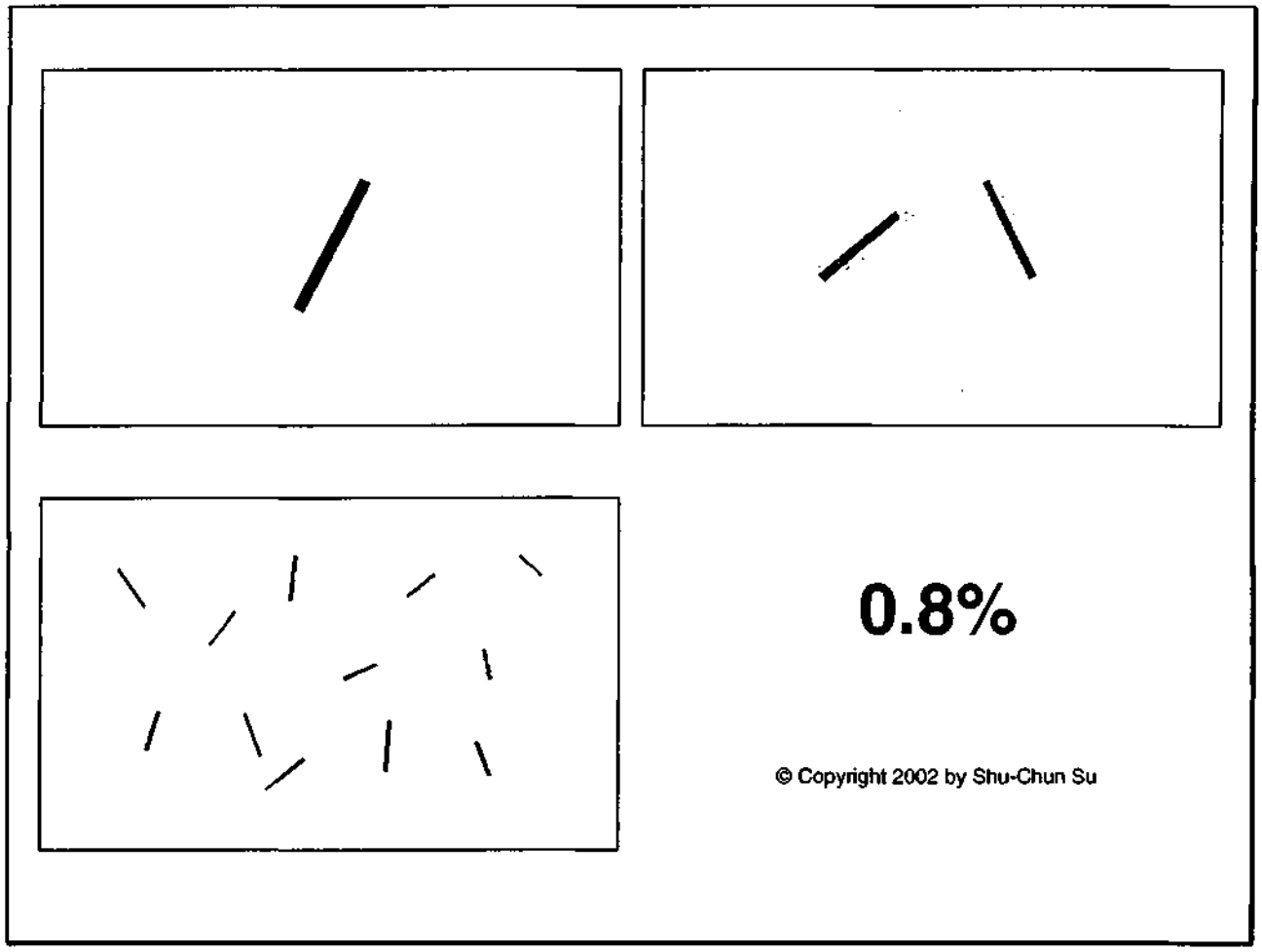
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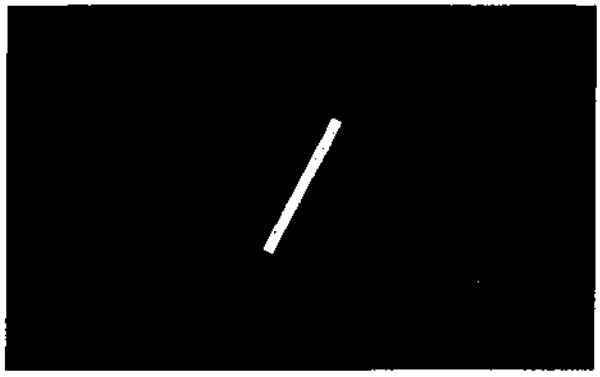

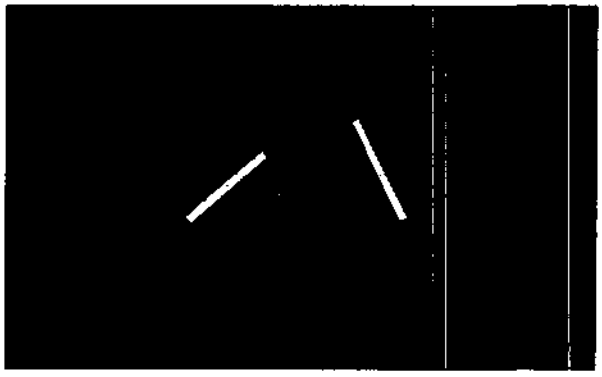

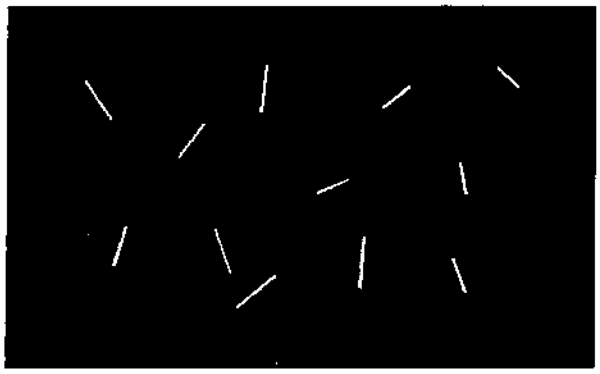

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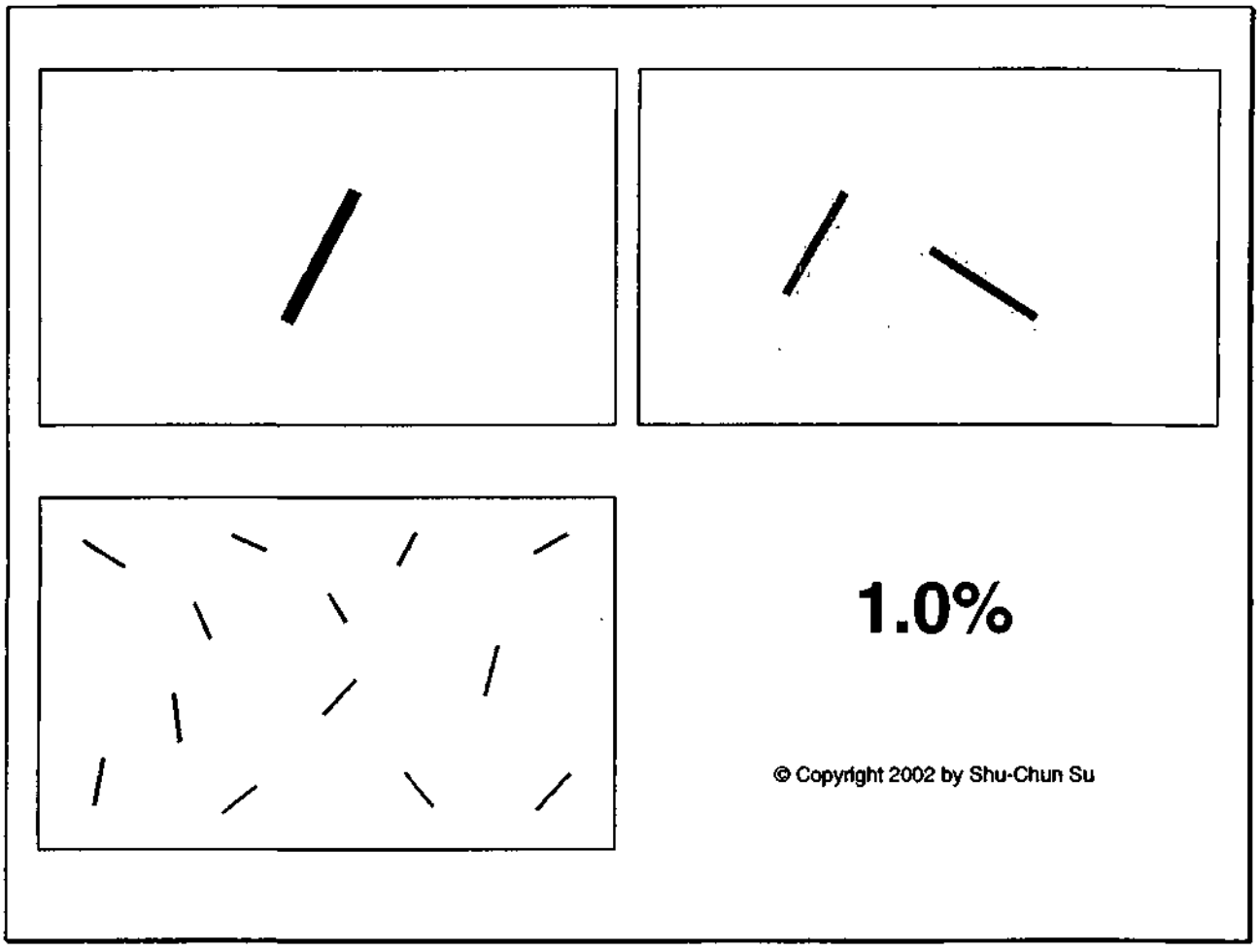
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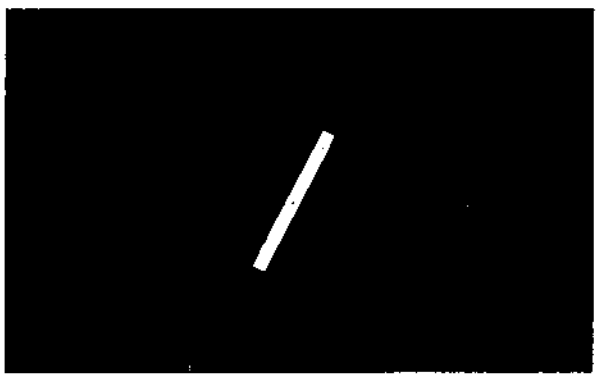

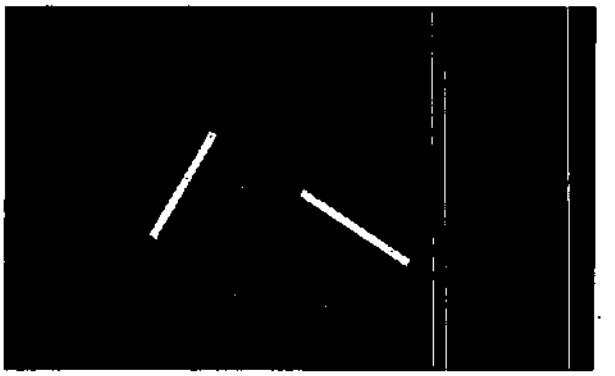

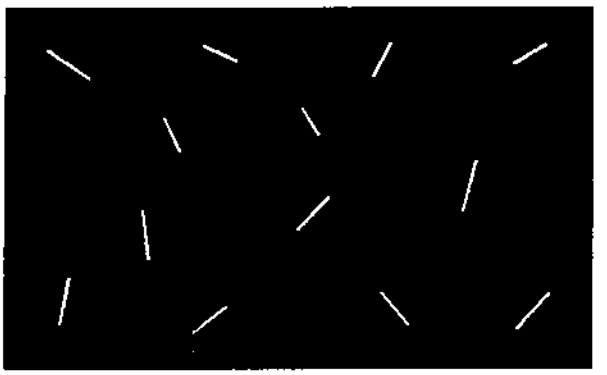

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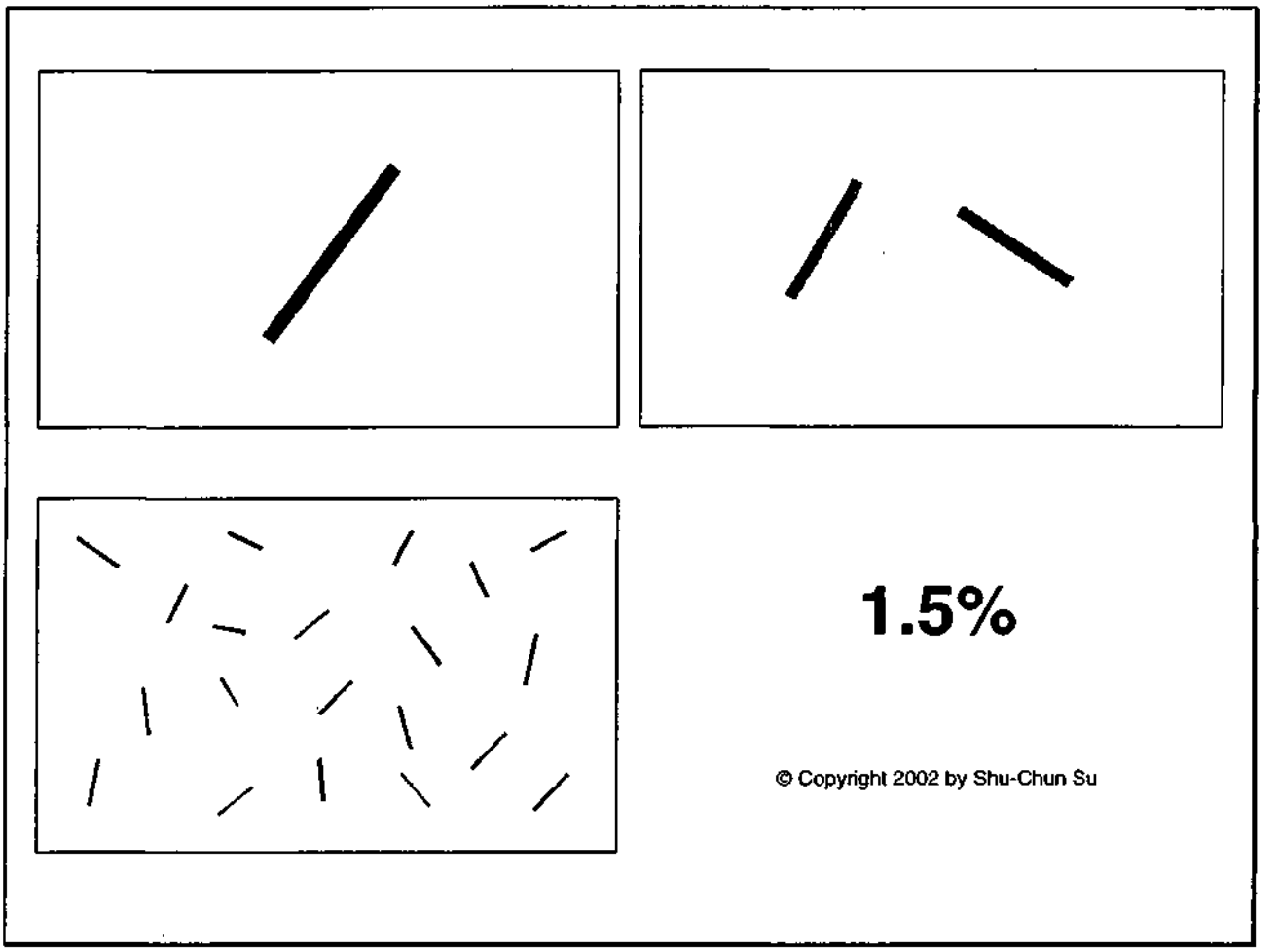
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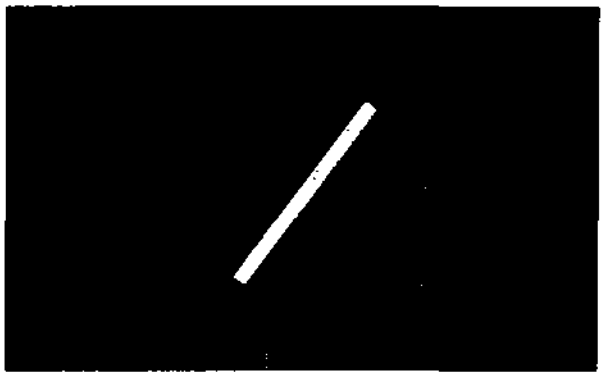

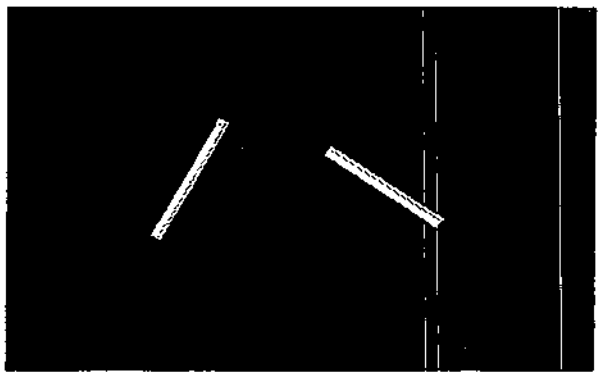

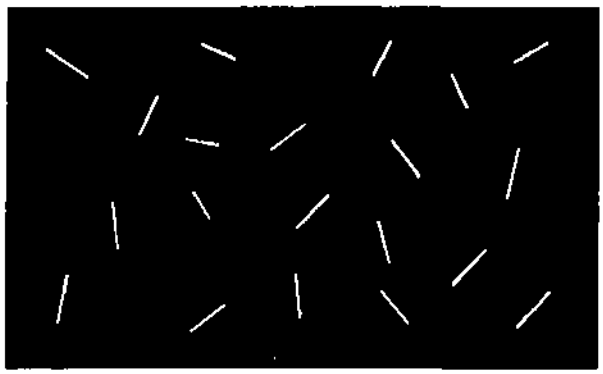

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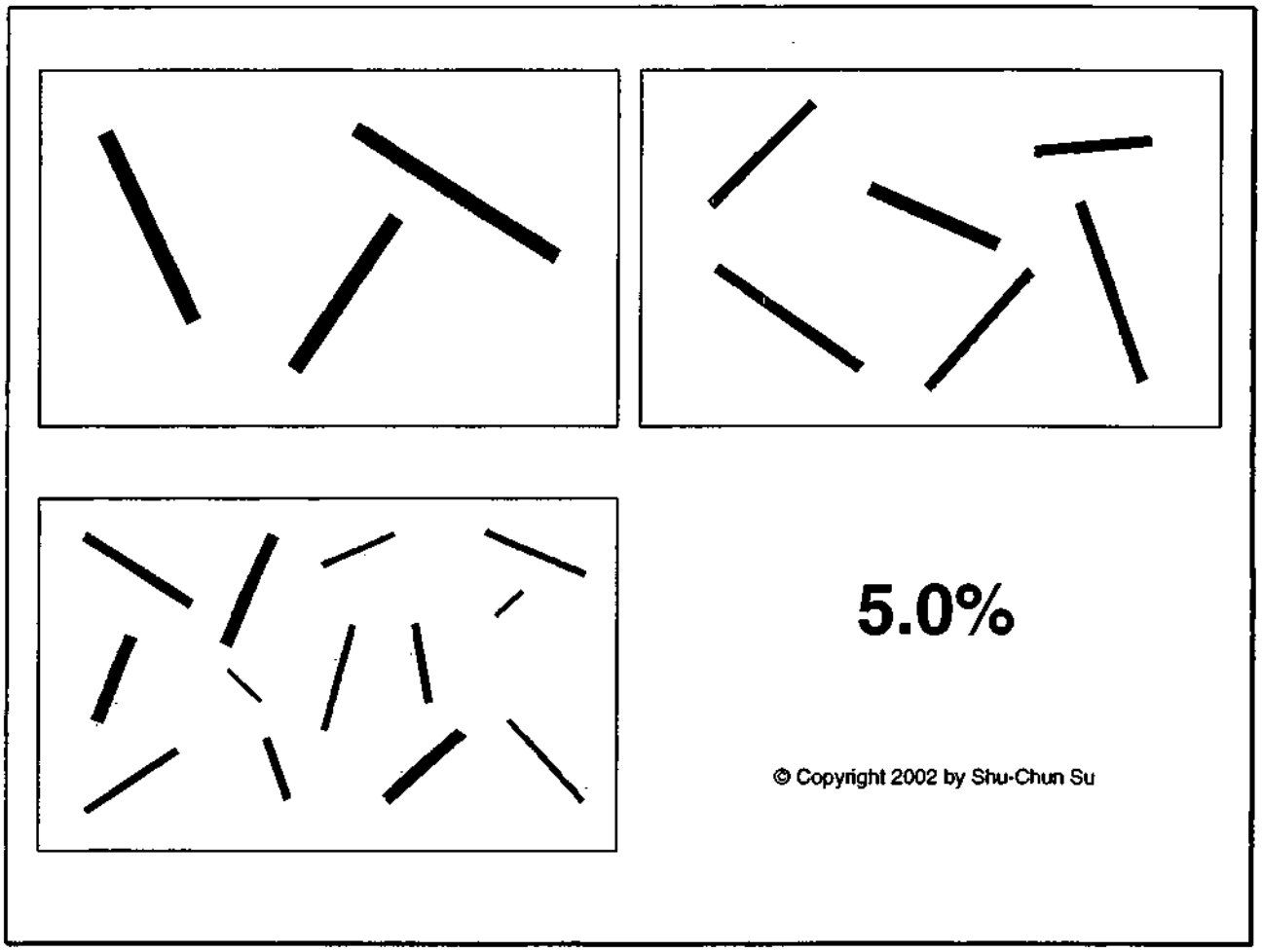
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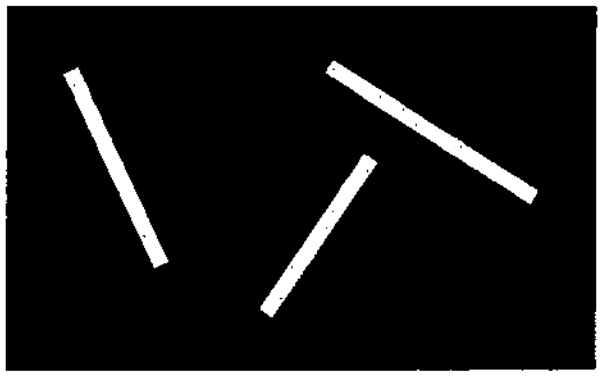

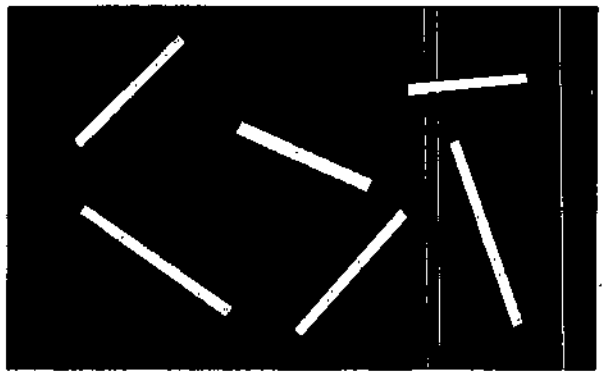

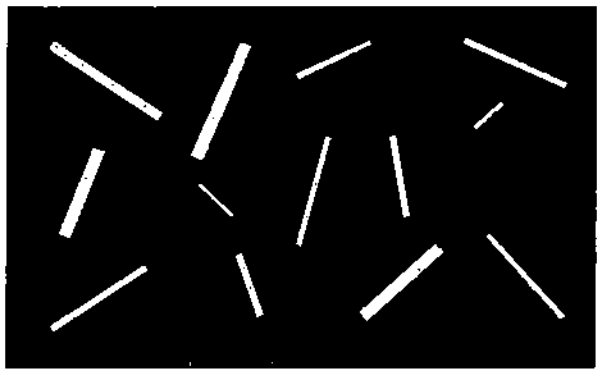

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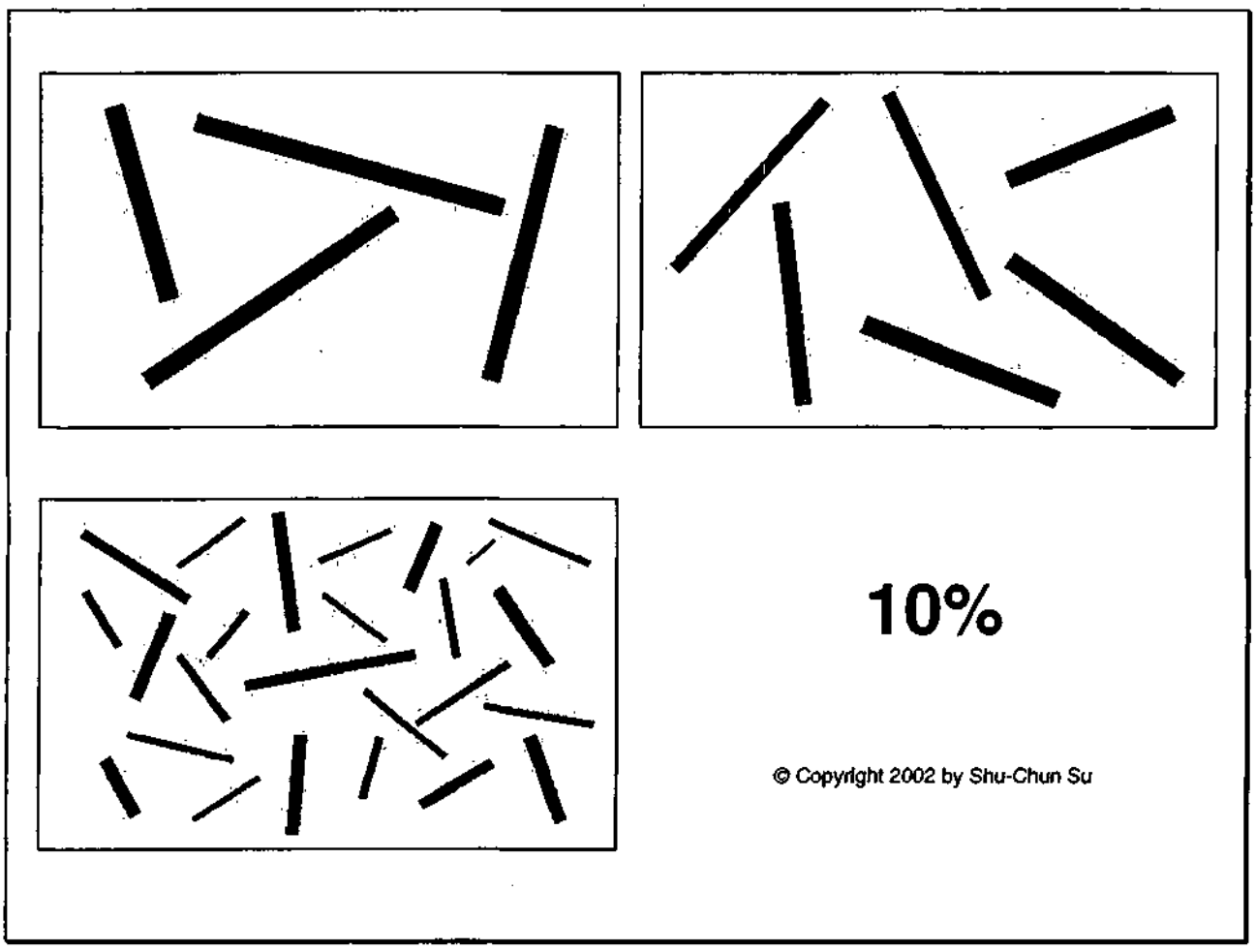
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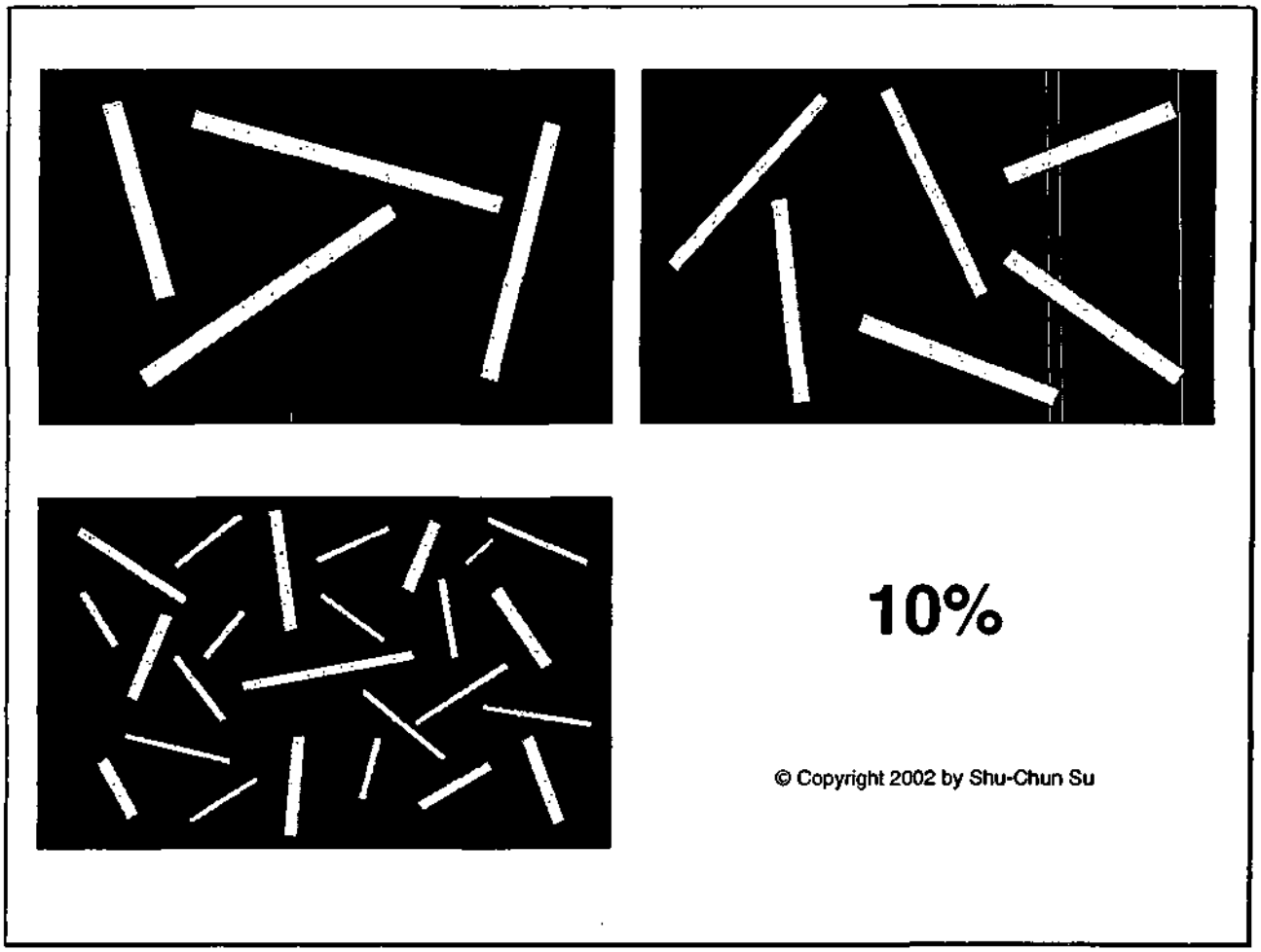
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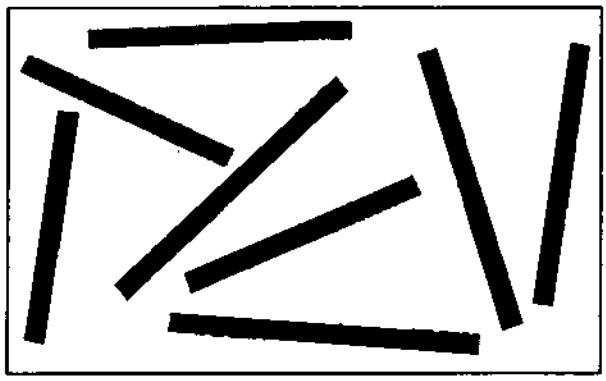

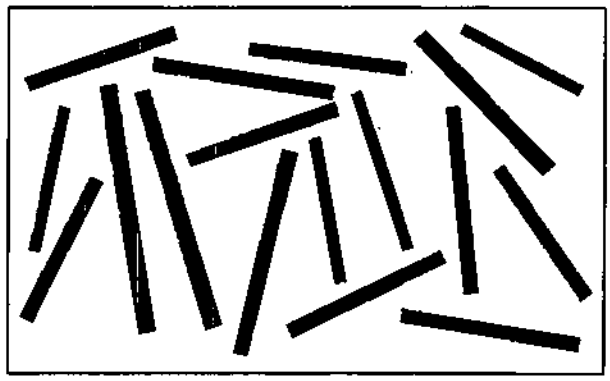

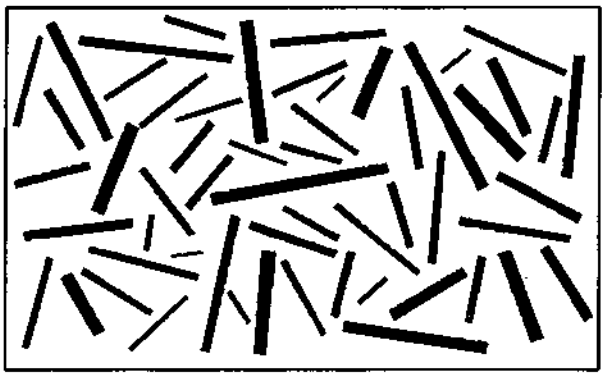

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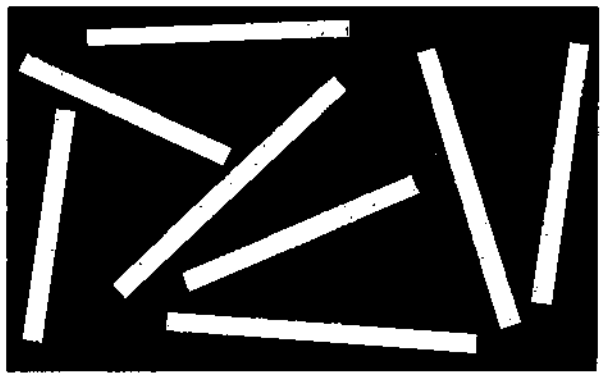

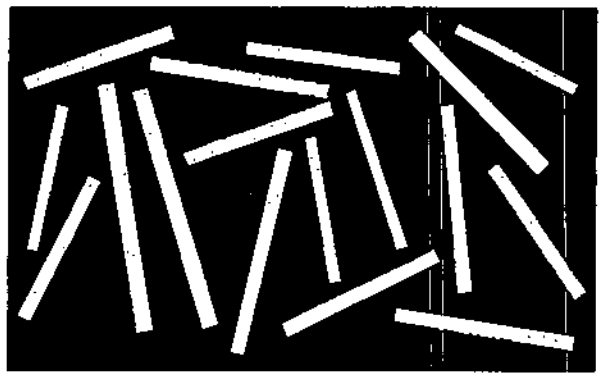

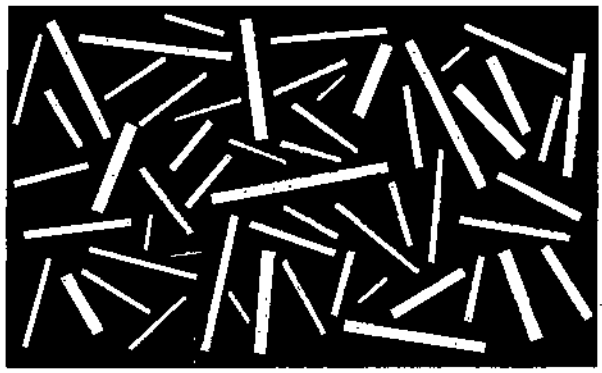

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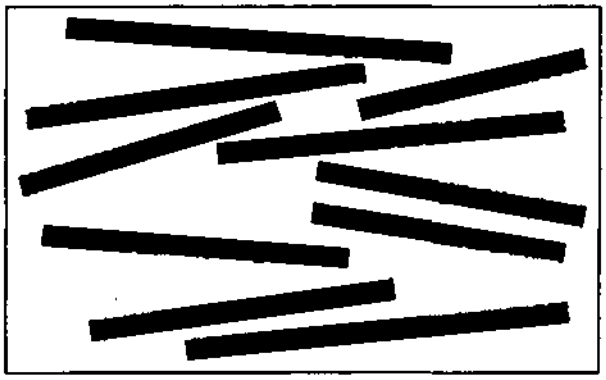

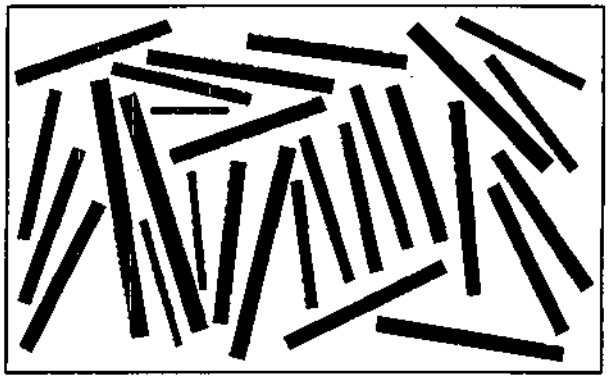

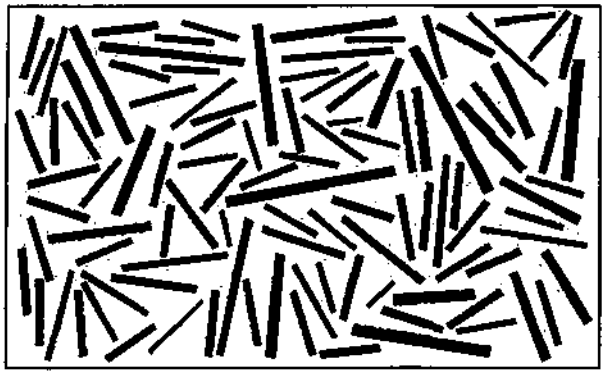

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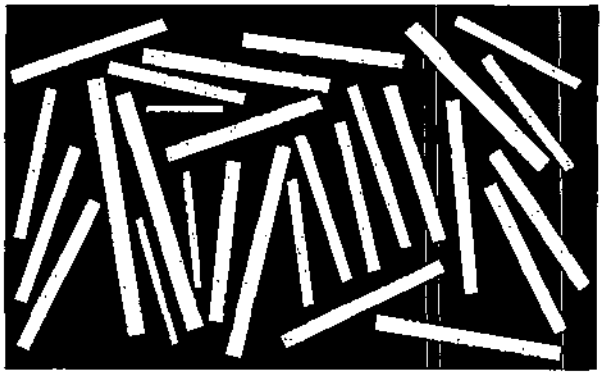

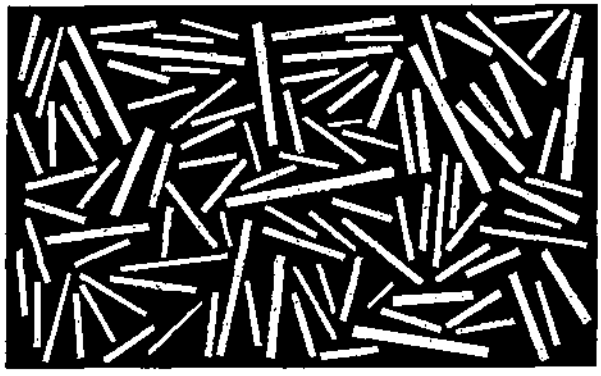

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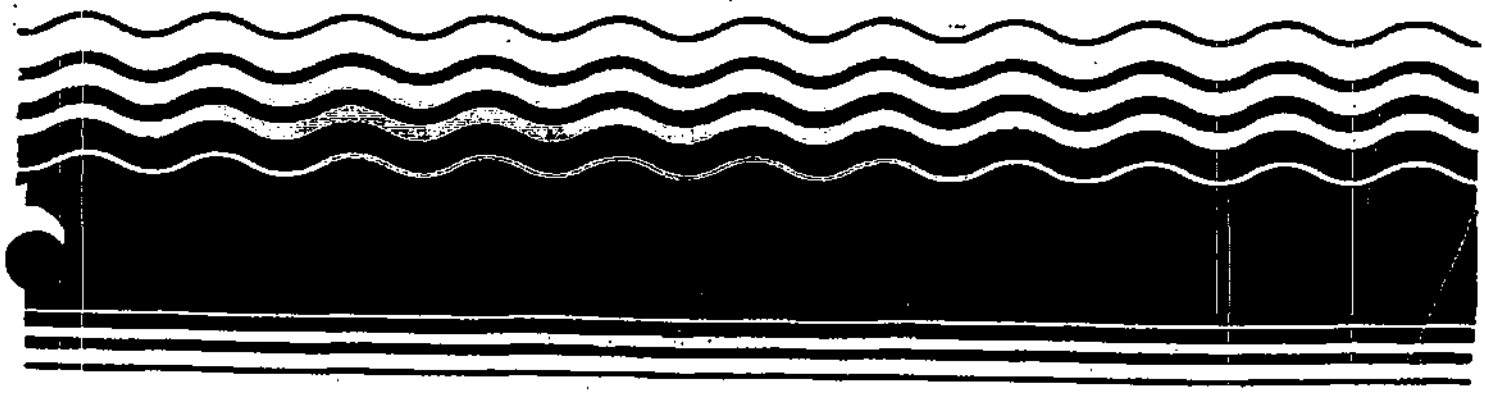
SUPERFUND METHOD FOR THE DETERMINATION OF RELEASABLE ASBESTOS IN SOILS AND BULK MATERIALS



SUPERFUND METHOD FOR THE DETERMINATION OF RELEASABLE ASBESTOS IN SOILS AND BULK MATERIALS

INTERIM VERSION

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A series of conceptual design figures are included in this method to assist users with their own design and construction of an appropriate dust generator for supporting this method. However, these figures are *not* to be construed as formal construction drawings and ICF Technology will accept *no* liability for their reference or use.

TABLE OF CONTENTS

		Page
1.0	INTRODUCTION	1-1
2.0	BACKGROUND	2-1
2.1	REQUIREMENTS FOR A METHOD DESIGNED TO SUPPORT RISK ASSESSMENT	2-1
2.1.1	Sensitivity	2-2
2.1.2	Precision	2-2
2.1.3	Asbestos Characteristics	2-3
2.1.4	Reporting Requirements	2-4
2.2	ASBESTOS CONTAINING MATERIALS TYPICALLY ENCOUNTERED AT SUPERFUND SITES	2-5
2.3	REQUIREMENTS FOR FACILITATING REPRODUCIBILITY BETWEEN LABORATORIES	2-6
2.4	COST CONSIDERATIONS	2-7
3.0	OVERVIEW OF METHOD	3-1
4.0	DEFINITIONS	4-1
5.0	SYMBOLS AND ABBREVIATIONS	5-1
5.1	SYMBOLS	5-1
5.2	ABBREVIATIONS	5-4
6.0	FACILITIES AND EQUIPMENT	6-1
6.1	SAMPLE COLLECTION EQUIPMENT AND CONSUMABLE SUPPLIES	6-1
6.2	LABORATORY FACILITIES	6-2
6.3	THE DUST GENERATOR AND APPURTENANT EQUIPMENT	6-2
6.4	SPECIMEN PREPARATION EQUIPMENT	6-3
6.5	OTHER LABORATORY EQUIPMENT	6-3
6.6	CONSUMABLE/REUSABLE LABORATORY SUPPLIES	6-3
7.0	REAGENTS	7-1
8.0	SOIL OR BULK SAMPLE COLLECTION	8-1
8.1	SAMPLE COLLECTION	8-1
8.1.1	Sampling to Derive Estimates of Asbestos Concentrations in a Road Surface	8-2
8.1.2	Sampling a Mine Tailings Pile to Derive Estimates of Asbestos Concentrations Within the Pile	8-2
8.2	FIELD PREPARATION	8-3
8.2.1	Weighing	8-3
8.2.2	Size Reduction	8-5
8.2.3	Sample Homogenization and Splitting	8-5
8.3	COMPOSITING SAMPLES (OPTIONAL)	8-15
8.4	SAMPLE HANDLING AND SHIPMENT	8-16

TABLE OF CONTENTS (cont)

Page

9.0 SAMPLE PREPARATION BY DUST GENERATION 9-1
9.1 SAMPLE RECEIVING AND STORAGE 9-1
9.2 SAMPLE HOMOGENIZATION AND SPLITTING IN THE LABORATORY ... 9-2
9.3 DUST GENERATOR SETUP 9-2
9.3.1 Conditioning a Stock of Filters 9-2
9.3.2 Initiating Humidity Control 9-3
9.3.3 Priming the Scrubber 9-3
9.3.4 Adjusting Initial Air Flow 9-3
9.4 DUST GENERATOR OPERATION 9-6
9.4.1 Loading the Tumbler 9-6
9.4.2 Conditioning the Sample 9-7
9.4.3 Initiating a Run 9-7
9.4.4 Monitoring the Rate of Respirable Dust Generation 9-8
9.4.5 Generating Appropriately Loaded Filters for Asbestos Analysis 9-9
9.4.6 Obtaining Asbestos Samples from the Scrubber 9-11
9.5 CLEANING THE DUST GENERATOR 9-12

10.0 PREPARATION OF SPECIMEN GRIDS FOR TEM ANALYSIS 10-1
10.1 PREPARATION OF SPECIMEN GRIDS FROM FILTERED ALIQUOTS OF THE SCRUBBER SUSPENSION 10-1
10.2 SPECIMEN GRID PREPARATION FROM FILTERS COLLECTED OVER THE IST OPENING OF THE ELUTRIATOR 10-1
10.2.1 Specimen Grid Preparation Using a Direct Transfer Technique 10-2
10.3 Specimen Grid Preparation Using an Indirect Transfer Technique 10-2

11.0 PROCEDURES FOR ASBESTOS AND DUST ANALYSIS 11-1
11.1 PROCEDURES FOR ASBESTOS ANALYSIS 11-1
11.1.1 Analysis of Specimen Grids Prepared from Filters Collected Over the IST Opening of the Elutriator 11-1
11.1.2 Analysis of Specimen Grids Prepared from Filtered Scrubber Suspension 11-3
11.2 EVALUATING THE RATE OF RELEASE OF RESPIRABLE DUST 11-5
11.3 DETERMINING THE CONTENT OF RESPIRABLE DUST 11-8
11.4 DETERMINING THE CONTENT OF ASBESTOS 11-10
11.4.1 Based on Directly Prepared Filters Collected Over the IST Opening of the Elutriator 11-10
11.4.2 Based on Specimens Prepared from Scrubber Water 11-12
11.4.3 Procedure for Adjusting Asbestos Concentrations to Account for the Presence of Coarse Material in the Sampled Matrix 11-13

12.0 PERFORMANCE CHARACTERISTICS AND QUALITY CONTROL/QUALITY ASSURANCE REQUIREMENTS 12-1
12.1 METHOD PERFORMANCE 12-1

TABLE OF CONTENTS (cont)

		Page
12.1.1	Analytical Sensitivity	12-1
12.1.2	Precision	12-1
12.1.3	Accuracy	12-2
12.1.4	Asbestos Characteristics	12-3
12.1.5	Reporting Requirements	12-3
12.2	QUALITY CONTROL REQUIREMENTS	12-3
12.2.1	Blanks	12-3
12.2.2	Duplicates/Replicates	12-5
13.0	REPORTING REQUIREMENTS	13-1
13.1	FIELD AND LABORATORY NOTEBOOKS	13-1
13.1.1	Field Notebooks	13-1
13.1.2	Laboratory Notebooks	13-2
13.2	FIELD ACTIVITIES REPORT	13-3
13.3	SAMPLE ANALYSIS REPORT	13-4
13.4	SAMPLE BATCH REPORTS	13-6
14.0	REFERENCES	14-1

LIST OF FIGURES

		Page
Figure 3-1	Sample Collection and Field Preparation	3-2
Figure 3-2	Laboratory Preparation and Analysis	3-3
Figure 8-1	Weighing a Soil Sample on a Field Scale	8-4
Figure 8-2	Weighing a Sample Split on a Field Scale	8-6
Figure 8-3	Sieving a Soil Sample	8-7
Figure 8-4	A Riffle Splitter	8-8
Figure 8-5	Using a Riffle Splitter to Homogenize/Split Samples	8-9
Figure 8-6	Loading a Sample Split into a Sample Bottle	8-11
Figure 8-7	Transferring Sample from a Mixer to a Plate for Coning and Quartering	8-13
Figure 8-8	Coning and Quartering	8-14
Figure 11-1	Typical Cumulative Mass Release Versus Time Curve for a 30 RPM Run	11-6
Figure 11-2	Typical Cumulative Mass Release Versus Time Curve for a 60 RPM Run	11-7
Figure 11-3	Illustration of the Optimization of the Estimate of Initial Mass "M_o"	11-9
Figure 13-1	Format for the Field Activities Report	13-5
Figure 13-2	Sample Analysis Report Format	13-7
Figure 13-3	Sample Batch Report Format	13-10
Figure A-1	Schematic of Dust Generator	A-2
Figure A-2	The Dust Generator	A-3
Figure A-3	Tumbler in Constant Humidity Chamber	A-4
Figure A-4	Tumbler Assembly	A-5
Figure A-5	Main Body of Vertical Elutriator	A-8
Figure A-6	Bottom Assembly of Vertical Elutriator	A-9
Figure A-7	Top Assembly of Vertical Elutriator	A-10
Figure A-8	Dust Collection System	A-12
Figure A-9	Filter Mounts	A-13
Figure A-10	Tubing Connections for the Scrubber of the Dust Generator	A-15
Figure A-11	Tubing Connections for Filter Cassettes Mounted on the Elutriator of the Dust Generator	A-16
Figure A-12	Detail of Tubing Connections at the Top of the Vertical Elutriator	A-17
Figure A-13	Overall Prototype Dust Generator Assembly	A-23
Figure A-14	Prototype Tumbler Assembly Detail	A-24
Figure A-15	Prototype Vertical Elutriator Detail	A-26
Figure A-16	Section of Elutriator Top with Extension Tube	A-27
Figure A-17	Longitudinal and Transverse Sections of Isokinetic Sampling Tube	A-28
Figure A-18	Prototype Slide Mechanism Detail	A-29
Figure A-19	Detail of Prototype Filter Cassette Mounts	A-30

1.0 INTRODUCTION

This is a sampling and analysis method for the determination of releasable asbestos in soils and bulk materials. Samples are collected in a manner suitable for providing representative measurements of the releasable fraction of asbestos in the matrix sampled, prepared using a dust generator, and analyzed by transmission electron microscopy (TEM). Guidelines for constructing the required dust generator are also included.

During dust generation, the respirable fraction of the dust generated from the sample is collected on filters. The filters are then weighed and the cumulative mass of dust collected is plotted against time to determine the rate of release of dust. Results are extrapolated to provide an estimate of the total mass of respirable dust in the original sample. The asbestos released during dust generation is either collected on a filter or in the suspension of a scrubber (or both), depending on the configuration under which the dust generator is operated. Filters may be prepared for TEM analysis by either a direct or an indirect transfer technique. Preparation of the scrubber suspension for TEM analysis is equivalent to an indirect transfer procedure. Depending on the intended use of the data, the results of asbestos analyses from this method may be reported either in terms of the number of structures per unit mass of respirable dust generated from the sample or the number of structures per unit mass of the original sample.

The method allows for the determination of the mineralogical type(s) of asbestos that is present in the sample and for distinguishing asbestos structures from non-asbestos structures. In this method, asbestos structures are characterized as fibers, bundles, clusters, or matrices and the length and width of each asbestos structure are measured. Although the method is designed specifically to provide results suitable for supporting risk assessments at Superfund sites, it may be applicable to a wider range of studies.

As reported in this document, the method focuses on requirements for the collection, preparation, and analysis of samples obtained from individual locations. During a site investigation, samples will typically be collected from multiple locations that are arranged in an array designed to provide measurements suitable for deriving a representative (i.e. unbiased) estimate of the concentration of releasable asbestos over the sampled matrix as a whole. Thus, proper design of a comprehensive sampling strategy, which includes the detailed design of the array of sampling locations, is also critical to the success of an investigation. However, design of a sampling strategy is necessarily site specific and site-specific considerations are beyond the scope of this document. For further guidance on developing appropriate sampling strategies, see Berman and Chesson (undated).

This method has not yet been validated. Validation requires completion of a field study in which airborne exposure concentrations of asbestos caused by the release and transport of asbestos from soils or bulk materials (under specific conditions) are related to the bulk measurements of asbestos derived from this method. However, while such a study is in progress, this method has already been successfully tested in the laboratory. Such tests have demonstrated that the method is capable of achieving adequate sensitivity and precision to support risk assessment. The method also provides asbestos measurements that preserve the information on the sizes and shapes of asbestos structures that are required to assess risks. Thus, the principle features of the method are well enough established to allow it to be

employed in current field investigations. At the same time, until the formal validation study is completed, this should be considered an interim method.

NOTE

This document is intended to serve several audiences including site project managers, field sampling teams, data reviewers, and laboratory analysts. The document may be separated into segments so that individuals may focus on the sections of most interest to their particular roles on a project.

2.0 BACKGROUND

This method was developed specifically to satisfy the needs of the Superfund program including:

- the need to provide results suitable for supporting risk assessment;
- the need to be applicable to the types of asbestos-containing materials commonly encountered at Superfund sites; and
- the need to facilitate reproducibility within and between laboratories that may offer the method commercially.

An additional consideration addressed during the development of this method is the need to control sampling and analysis costs.

The first item listed above is what distinguishes sampling and analysis methods adapted for use in the Superfund program from methods used in other programs. This is because the *statutory requirements* of the Superfund program mandate that risk management decisions be based on risk assessment. Risk assessment requires that analytical data be relatable to health effects. Although the remaining requirements listed above are also important, the first item is a central feature of the Superfund program.

Because this method is designed for supporting risk assessment, the results of analyses from this method are intended to be used as inputs to release and transport models to predict airborne asbestos exposure concentrations (see Section 2.2). This is a very different objective from existing bulk asbestos methods, which are designed as qualitative tools for determining whether asbestos is present in a particular matrix in excess of a defined, regulatory limit. For such methods, the regulatory limits are defined operationally as functions of the methods themselves and, therefore, do not necessarily relate in any direct fashion to the potential for asbestos to be released and contribute to risk.

2.1 REQUIREMENTS FOR A METHOD DESIGNED TO SUPPORT RISK ASSESSMENT

A feasibility study (Berman 1990) was completed both to identify the requirements of a method for the determination of asbestos in soils and bulk materials that could be used to support risk assessment and to evaluate existing sampling and analysis technologies to determine whether such a method might be readily developed. Results of the feasibility study indicate that, to support risk assessment under Superfund, the method must:

- achieve sufficient analytical sensitivity to adequately measure asbestos over the entire range of concentrations that might potentially pose an unacceptable risk;
- provide adequate precision over the range of asbestos concentrations of interest;
- provide measurements of the complete range of the sizes and shapes of asbestos structures that are believed to contribute to health effects;

- provide measurements that are representative of the fraction of asbestos that is readily releasable[1] from the matrix of interest; and

- provide results reported in units that are amenable for use as inputs to fate and transport models that can be used to relate (bulk) source concentrations of asbestos to (airborne) exposure point concentrations of asbestos.

2.1.1 Sensitivity

Based on calculations presented in the feasibility study completed for this method (Berman 1990), asbestos concentrations in soil or a bulk environmental matrix that are on the order of $3x10^7$ long asbestos s/g_{solid} (i.e. 30 million asbestos structures longer than 5 μm per gram of solid) or $5x10^8$ total asbestos s/g_{solid} (i.e. 0.5 billion total asbestos structures per gram of solid) may potentially pose a risk exceeding 1×10^{-6}. This is based on evaluation of a series of scenarios in which asbestos is released from the solid matrix due to any of several types of disturbance (i.e. vehicular traffic on the surface, agricultural tilling, or natural weathering). Once released, asbestos is dispersed into the air, where exposure may occur. Note that, for a typical chrysotile matrix, the concentrations reported above for the two different size ranges of structures are expected to be approximately equivalent (i.e. concentrations observed for each of the two size ranges in a sample containing common chrysotile are expected to be approximately proportional to the two values given so that a sample observed to exceed one will also likely exceed the other). Therefore, either concentration might serve as an adequate target sensitivity for chrysotile. However, such may not be the case for all asbestos-containing matrices, particularly those containing amphibole asbestos.

To assure that negative results from a soil-bulk method do not mask potential problems, ideally, the analytical sensitivity for the method should be set at one tenth of the concentrations potentially capable of producing risks that exceed 1×10^{-6}. Therefore, the target analytical sensitivities for this method have been set at:

- $3x10^6$ long asbestos s/g_{solid} (i.e. 3 million asbestos structures longer than 5 μm per gram of solid); or

- $5x10^7$ total asbestos s/g_{solid} (i.e. 50 million total asbestos structures per gram of solid).

The analytical sensitivity is reported for the two different size ranges of asbestos structures to allow flexibility in the application of the method.

2.1.2 Precision

The only precision data currently available for this method is from the recently described pilot study (Berman et al. 1994a). To establish a reasonable goal for the precision of this method, a study of the precision achievable by commercial laboratories performing the method would be required. Because data from commercial laboratories are not available, the data from the

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[1] As used here, "readily releasable" means particles that have already been separated into respirable size and that are available in a pool of loose material that can be released directly during some type of disturbance. This is distinguished from particles that may be aggregated with others and that may be separated from the aggregate for future release.

pilot study (Berman et al. 1994a) were evaluated to provide a rough estimate of the level of precision potentially achievable once the method is commercialized (see Section 12.1.2). Results suggest that a relative percent difference of 50% should be easily achievable for sample splits performed by a *single* laboratory.

The USEPA has set informal precision guidelines for each of the analytical methods employed in the contract laboratory program (CLP), under which the majority of sample analyses have been conducted for the Superfund program. These goals tend to be defined as achievable relative percent differences for sample splits performed within a laboratory and they range between 10% and 35% for the analysis of inorganic chemicals in soils and up to 50% for the analysis of organic chemicals in soils. Therefore, given the above, a guideline of 50% for the relative percent difference between sample splits performed within a laboratory is proposed for this method.

2.1.3 Asbestos Characteristics

Based on what is known about the biological activity of asbestos (for a critical review of the extensive literature on this subject, see Berman and Crump 1989), if asbestos measurements are to be related to risk, it is necessary to characterize the sizes, shapes, and mineralogy of the asbestos structures in each sample. This involves enumeration of individual structures within certain size categories with particular emphasis on the longest and thinnest structures. Although the range of dimensions over which asbestos structures contribute to biological activity has yet to be precisely defined, this method is designed to provide a detailed characterization of structures encompassing the entire range of potential importance.

Results of the feasibility study for this method (Berman 1990) indicate that transmission electron microscopy (TEM) is the only analytical tool capable of characterizing asbestos structures over the entire range of sizes and shapes that potentially contribute to risk. Consequently, the asbestos derived from soil or bulk samples is analyzed using TEM in this method.

When evaluating detailed asbestos size characterizations, it is important to consider the effects of sample preparation. The dust generator incorporated into this method was developed because its use eliminates the need to employ other preparation techniques (such as crushing or grinding) that potentially alter the distribution of respirable asbestos structure sizes and shapes found in the sample. The dust generation employed in this method is a gentle process; it is expected to preserve the distribution of asbestos structure sizes and shapes that may be released to the air when asbestos-containing media are disturbed in the environment.

The size distribution of asbestos structures found in the dusts generated using this method may also vary depending on whether TEM specimen grids are prepared from the dust samples using a direct or an indirect transfer technique. Existing risk factors are based largely on studies incorporating the equivalent of direct transfer techniques,[2] while indirect transfer techniques are expected to provide increased precision (see, for example, Berman

[2] Most of the epidemiology studies (from which estimates of asbestos potency are derived) employed either phase contrast microscopy (PCM) for the analysis of asbestos concentrations in work place air or converted other types of measurements to PCM equivalents (Berman and Crump 1989). PCM analyses are performed directly on sample filters after the filter material has been rendered transparent by a suitable solvent. Since the fibers are observed as originally deposited, this corresponds closely to a direct transfer technique for TEM analysis.

and Chatfield 1990). Hence, this method incorporates a procedure by which the majority of samples are prepared by an indirect technique with a subset prepared in tandem by a direct technique. This facilitates evaluation of the relationship between structure counts derived from samples prepared, respectively, by each technique.

2.1.4 Reporting Requirements

It is anticipated that soil or bulk samples to be analyzed using this method will typically be collected to determine the asbestos content of a potential source from which asbestos may be released (via a particular mechanism) and transported to the air (where exposure may occur). It is also expected that asbestos release and transport will be modeled and that one of the critical inputs to such models will be the concentration of releasable asbestos in the source matrix.

Results of the feasibility study for this method (Berman 1990) indicate that most of the available models that predict releases to the air from soil or some other bulk matrix (in association with specific release mechanisms) were designed to predict the release and transport of respirable dust. Only a very limited number of such models have actually been developed specifically for asbestos. The dust models may be used to predict asbestos release and transport with minimal modification, however, provided that the appropriate types of asbestos measurements are available. Such adaptations rely on the following assumptions:

- the rate of settling of respirable asbestos particles is no more rapid than the average settling rate for respirable dust; and
- the release of asbestos and the release of respirable dust from a source matrix are highly correlated (i.e. are proportional), at least over the long term.

The former assumption is expected to be true because fibers tend to settle more slowly in the air than spheres of comparable mass. The latter assumption appears reasonable because, if it were false, one would expect the source matrix to become either enriched or depleted in asbestos over time. However, such effects are not generally observed.

Many of the release models developed for respirable dust require the mass fraction of silt in the source matrix as an input parameter. For such models, substituting the fiber concentration of asbestos (per unit mass of source matrix) for the mass fraction of silt should allow the model to be used to predict asbestos release (with no additional modifications). Under such circumstances, a dimensional analysis of the model should indicate that the outputs would now be expressed in terms of the number of *asbestos structures* released from a defined area (or mass) of the source matrix per unit time (rather than the mass of *respirable dust* released from a defined area or mass of the source matrix per unit time). Such outputs are then typically combined with air dispersion models to predict airborne concentrations at locations (i.e. points of exposure) of interest.

As an alternative, it may be useful to multiply the respirable dust release rates (that are predicted by a model) by a factor representing the number of asbestos structures (of a size range of interest) per unit mass of respirable dust released from the sample. For certain models, this approach for converting a dust model to an asbestos release model may prove easier than the approach discussed above.

This method is designed to provide results that can be reported as the number of asbestos structures (of a size range of interest) per unit mass of source matrix with an option allowing the reporting of the number of asbestos structures per unit mass of the respirable dust released from the sample. The appropriate reporting option should be selected based on the specific models with which the measurements from this method are anticipated to be used for a specific project.

NOTE

This method may also be used to provide an independent (qualitative) check of the predictions of specific release models in some cases. In one of the intermediate steps of this method, the rate of release of respirable dust from the sample is determined. This can be compared to the rate of release predicted by a release model. The relative magnitude of the release rate observed using this method and the release rate predicted by a model should be consistent with that expected based on the relative aggressiveness of the type of disturbance applied to the sample during measurement using this method and the type of disturbance associated with the field activity (i.e. vehicular traffic, excavation, wind entrainment, etc.) represented by the model.

2.2 ASBESTOS CONTAINING MATERIALS TYPICALLY ENCOUNTERED AT SUPERFUND SITES

Asbestos containing materials commonly encountered at Superfund Sites include:

- natural rocks that contain asbestos;
- soils containing natural asbestos generated from weathered rock;
- soils containing asbestos introduced by transport from other locations;
- mine or mill tailings (i.e. fractured or depleted rock);
- discarded asbestos wastes, including (for example):
  - asbestos/cement pipe;
  - roofing materials;
  - insulation materials; and
- soils containing discarded asbestos wastes.

This method is designed specifically to handle the above materials (with the exception of unfractured rock[3]) and may be applied generally to samples of any unconsolidated or friable[4] matrix.

2.3 REQUIREMENTS FOR FACILITATING REPRODUCIBILITY BETWEEN LABORATORIES

Results of the feasibility study for this method (Berman 1990) indicate that, for a method for the determination of asbestos in soils and bulk materials to adequately facilitate reproducibility between laboratories that might offer the method commercially, the following requirements must be satisfied:

- preparation steps in the procedure have to be kept simple and must be standardized and documented sufficiently to allow technicians in different laboratories to perform them invariably (i.e. objectively); and

- the representativeness of a sample (i.e. the degree with which the sample retains the characteristics of the matrix from which it is derived) has to be preserved throughout all stages of handling, preparation, and sub-sampling that are incorporated into the method.

To address each of the above requirements, the ability to homogenize the sample has to be maintained throughout all stages of sample preparation and handling, and dust generation satisfies these requirements. In addition, dust generation is incorporated into this method as the means of extracting the respirable fraction of releasable asbestos from bulk samples in a manner that can be performed invariably; it is a mechanical process that can be controlled objectively by specifying the design and the operation of the equipment to be used for dust generation.

Use of a dust generator eliminates most of the sample manipulation steps typically performed manually (and subjectively) for other bulk asbestos methods. More importantly, it eliminates the need for sub-sampling of amounts smaller than approximately 100 g, which is a mass that is large enough to be sub-sampled reproducibly (by following specified procedures). A 100 g sample is also sufficiently large to retain representative characteristics of all components of a sampled matrix in which the particles are smaller than approximately 1 cm in diameter.

Results of the feasibility study (Berman 1990) indicate that the ability to homogenize an unconsolidated bulk sample (in preparation for sub-sampling) is a direct function of the largest particle in the distribution of component particles; to allow representative sub-sampling of such a matrix, the largest particle must represent no more than a few percent of the total mass of the sample. Therefore, because the final sub-sample to be extracted in this method is on the order of 100 g, the largest particles that can remain in the sample prior to sub

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[3] It is assumed that any asbestos imbedded in unfractured rock can be considered to be non-releasable. If there is a desire to evaluate the release of asbestos from the surface of such rock (or the potential for release of asbestos from the rock as it becomes fractured in the future due to aging or disturbance), conceivably, a sample of the rock might be crushed to particles no larger than 1 cm in diameter (see text) and a sample of the crushed rock might then be analyzed using this method. However, no formal protocol has been developed for this procedure at this time.

[4] As used here, the term *friable* is intended to mean any material that can be crushed or deformed with the hand with the attendant release of fibers.

sampling must be no larger than 2 or 3 g. Assuming a typical density for silica type materials (i.e. 2.6 g/cc), the largest particle that can be retained while allowing 100 g samples to remain representative of the sampled matrix is approximately 1 cm (3/8th inch) in diameter ($4/3\pi r^3$*density = 1.4 g). Consequently, this method incorporates a step in which samples are sieved to remove particles larger than 1 cm in diameter[5].

It must be emphasized that the particles larger than 1 cm in diameter that are removed from the sample by sieving in this method are *not* discarded. Rather, both the fraction passed by the sieve and the fraction retained by the sieve are weighed and the weights recorded so that the asbestos content measured can ultimately be reported as a function of the *total* mass of the initial sample (i.e. the asbestos concentration measured in the method is multiplied by the ratio of the total mass of the original sample collected and the mass of the fraction of the sample analyzed). The larger particles do not need to be carried through the entire analysis, however, because it is unlikely that significant amounts of the releasable asbestos in a sample reside in the coarse fraction.

2.4 COST CONSIDERATIONS

To be useful, it was determined that the cost of an individual analysis using this method would have to be competitive with other methods that might be used to derive comparable information.[6] In fact, once a laboratory invests in the construction of a dust generator to support the method and procures the required ancillary equipment, it is expected that the cost of analysis using this method will be very competitive with other soil or bulk methods (that might be designed to provide similar information). This is because use of the dust generator effectively concentrates asbestos from the sample (by removing the non-respirable component). Such concentration allows higher loadings on specimen grids so that smaller areas need to be scanned with the TEM to complete an analysis.

It is expected that sample preparation using the dust generator will cost approximately $400 (twice what it costs to complete the preparation of an air sample using an indirect transfer technique). This cost, however, is expected to be more than compensated by the corresponding reduction in cost for TEM scanning time, which is reduced due to the ability to scan more highly concentrated samples (see above). It is therefore expected that the total cost of an analysis using this method may run between $900 and $1,500, which should be competitive with any other soil or bulk method that is designed to provide comparable information. This cost is approximately 10 to 15% higher than that for the analysis of an air sample in which comparable structure size information is recorded.

Incorporated into this method (as an option) is a compositing procedure that can be used to reduce significantly the number of bulk analyses that might otherwise be required to adequately characterize a source matrix from which asbestos may be released (Section 8.3). Recognizing that airborne exposure due to emissions from a source matrix tend to be the result of average emissions over relatively large areas, compositing of samples is a

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5 The dust generator designed for this method best handles samples up to a maximum of approximately 80 g. However, the practical difference between 80 and 100 g samples, in terms of the maximum size of the particles that can be retained (while assuring the ability to homogenize the sample), is not significant.

6 This assumes analysis using TEM in which comparable size and mineralogy information is recorded; methods in which analysis is performed by polarized light microscopy (PLM) are not capable of providing information over the complete range of structure sizes and shapes that are believed to relate to risk.

particularly powerful tool that can be used in tandem with this method. By reducing the number of sample analyses required to adequately characterize a particular source, the cost of a particular investigation can be reduced correspondingly.

An additional cost saving measure incorporated into this method is completion of the early stages of sample preparation in the field. This simplifies the handling and preparation performed by the laboratory and limits the size (mass) of each sample that has to be stored or disposed by the laboratory.

Handling and preparation of bulk samples in the laboratory are dusty operations that require protective enclosures. The larger the sizes of the samples, the larger the protective enclosures required. Additionally, asbestos containing samples handled by a laboratory must be disposed as asbestos wastes. Clearly, the larger the mass of such samples handled by the laboratory, the greater the cost of disposal.

3.0 OVERVIEW OF METHOD

Samples are collected in the field according to a pre-defined sampling plan identifying the number of samples to be collected and the locations from which samples are to be collected. Procedures for designing such a plan are beyond the scope of this document but are reported elsewhere (see, for example, Berman and Chesson, undated).

Any of a variety of commercially available sampling equipment (i.e. trowels, shovels, augers, corers, etc.) may be used to collect samples for this method. However, they must have been specified in the pre-defined sampling plan based on the nature of the material being sampled and the depths over which samples are to be collected. Whatever sampling technique is employed, the *minimum* size sample to be collected at each location shall be 1 kg.

Once collected, each sample is brought to a central location for field preparation. Field preparation steps are listed in Figure 3-1 and discussed in detail in Chapter 8. Each sample is first weighed (Section 8.2.1). Then the sample is sieved using a screen with 3/8th in. (1 cm) openings to separate a coarse and fine fraction. The material placed on the sieve is worked with gloved hands to assure that all friable components pass through the screen (Section 8.2.2).

The coarse fraction, composed of material that is retained by the screen, is transferred to a bucket and weighed prior to discarding on site. The fine fraction is also weighed. As indicated in Figure 3-1, the fine fraction is then homogenized. The procedure recommended in this method for homogenization is repetitive splitting using a riffle splitter with the split halves of the sample being re-combined at the end of each split (Section 8.2.3). Studies indicate that five to seven iterations are typically sufficient to achieve adequate homogenization.

Once homogenized, the fine fraction is then sub-sampled using the riffle splitter (Figure 3-1). During sub-sampling, the one-half of the sample from one of the two receiving trays is discarded after each split (Section 8.2.3) and the second half of the sample is then re-split. The process is repeated until sub-samples weighing between 50 and 80 g are produced in each of the two receiving trays. The material in each tray is then transferred quantitatively to a sample bottle, packaged and shipped to the laboratory.

Sample handling, preparation, and analysis in the laboratory is depicted in Figure 3-2 and described in detail in Chapter 9. Once sub-samples weighing between 50 and 80 g are obtained, they can be separately prepared and analyzed (Section 9.2).

To prepare samples, as indicated in Figure 3-2, first load the sample into the tumbler of a dust generator. The design, construction, and operation of a dust generator suitable for use with this method is provided in appendix A. The sample is then conditioned by flowing humidity-controlled air through the tumbler and over the sample for several hours (Section 9.4.2).

Once the sample is conditioned, the tumbler of the dust generator is started and a sample run is initiated (Section 9.4.3). During each run, a series of filters is collected continuously from the top of one of the openings of the dust generator and these are weighed to plot the cumulative dust loss from the sample (Section 9.4.4 and the right side pathway of Figure 3-2).

FIGURE 3-1

SAMPLE COLLECTION AND FIELD PREPARATION

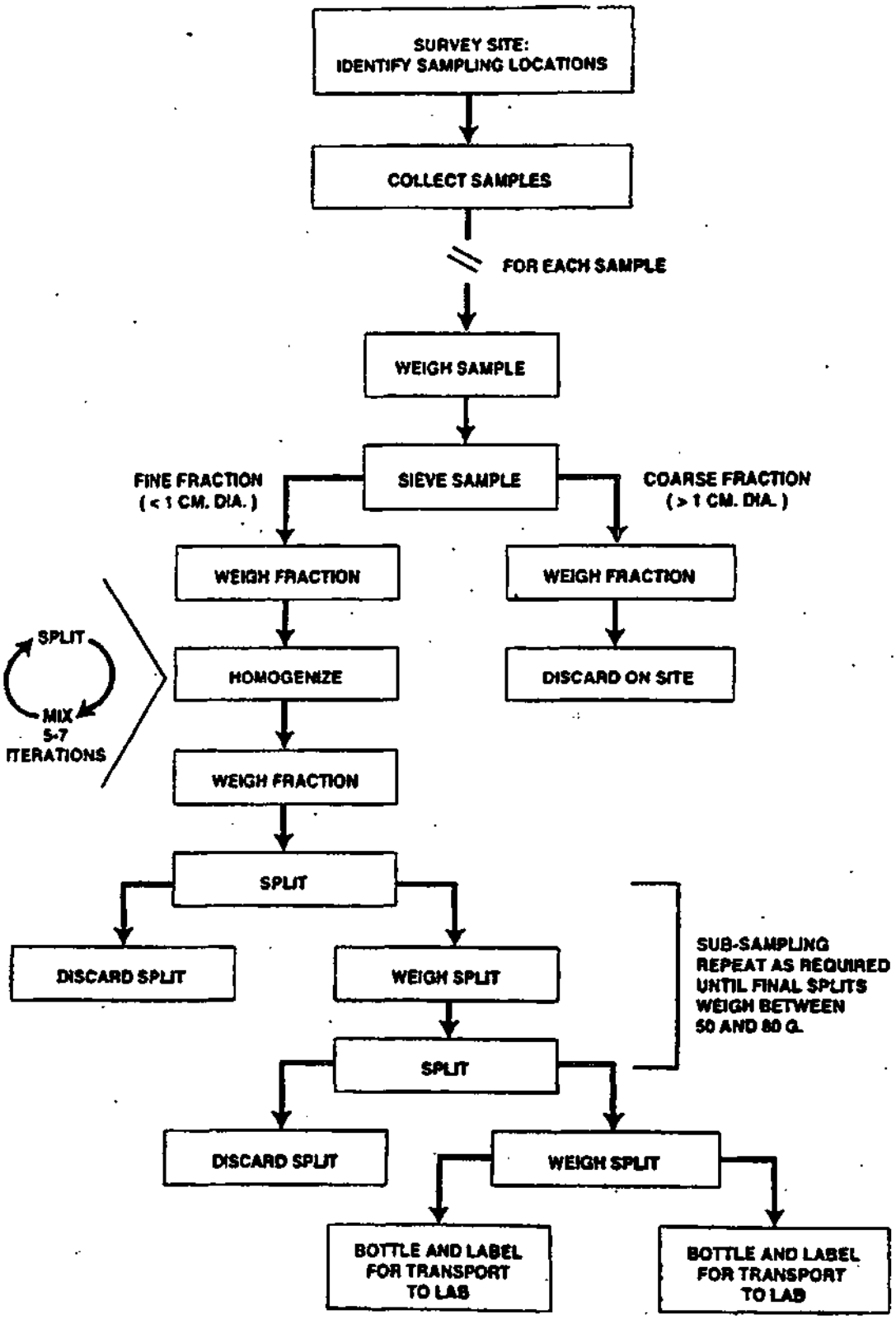
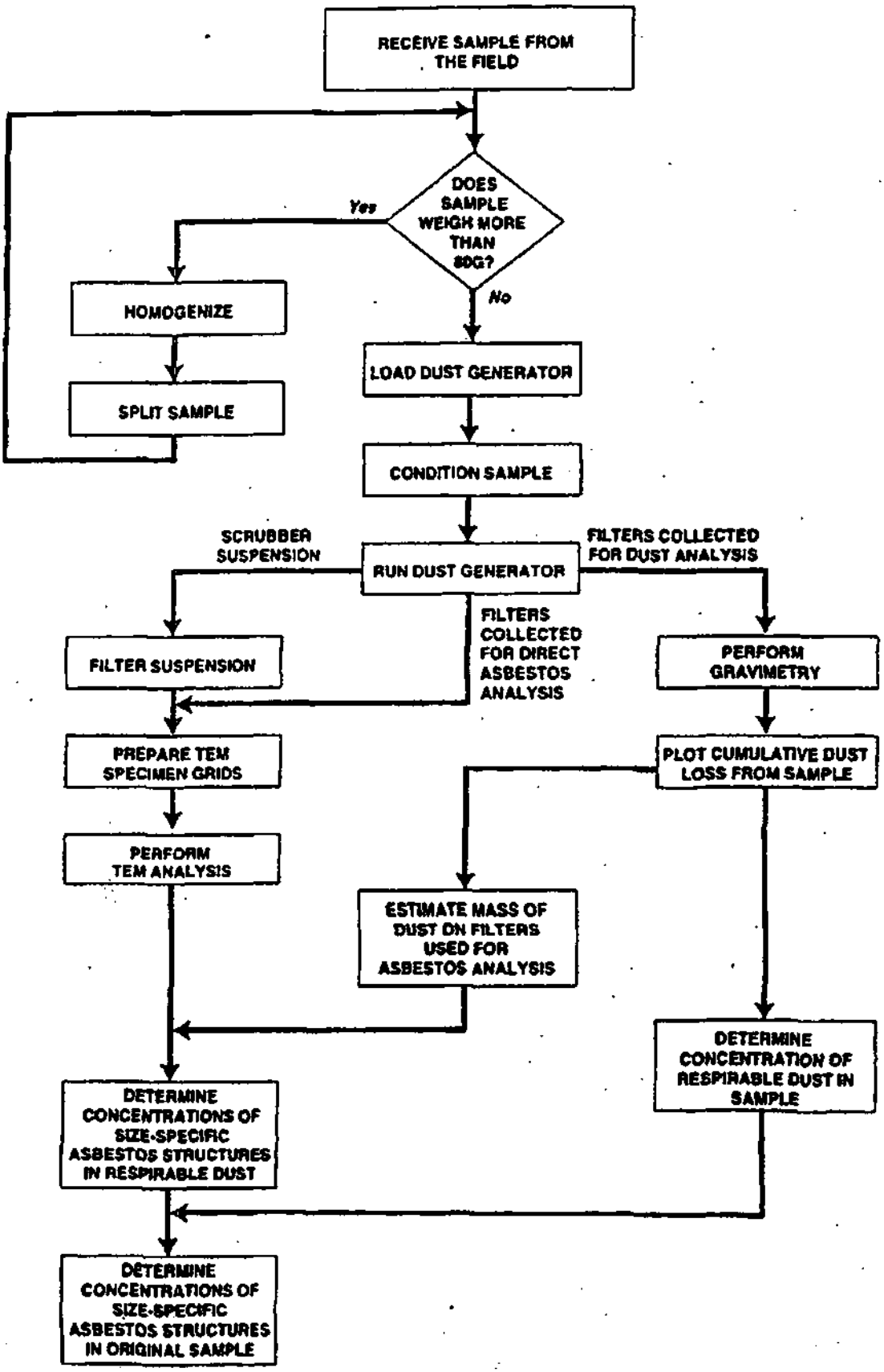


FIGURE 3-2

LABORATORY PREPARATION AND ANALYSIS



While the dust generator is operating, a second set of filters is also collected over the opening of the dust generator that articulates with an isokinetic sampling tube (the center pathway of Figure 3-2). These are collected such that loading is appropriate for specimen grid preparation using a direct transfer technique (Section 9.4.5).

Asbestos structures are also trapped in the suspension of a scrubber during each run of the dust generator (the left side pathway of Figure 3-2). The suspension is then diluted appropriately and filtered to create an additional set of filters from which specimen grids will be prepared for asbestos analysis (Section 9.4.6). However, because asbestos structures derived from this process will have been suspended in the aqueous environment of the scrubber suspension, preparation of grid specimens from filtered scrubber suspension are considered to have been prepared in a manner that is equivalent to an indirect transfer technique.

Next, as indicated in Figure 3-2, TEM specimen grids are prepared using a direct transfer technique from the filters collected either from atop the isokinetic sampling tube of the elutriator or from filtering scrubber suspension (Sections 10.1 and 10.2). Specimen grids are then analyzed using the counting and identification rules of the International Standards Organization (ISO) Method for the determination of asbestos in air using an indirect transfer technique (Chatfield 1993) with the stopping rules modified as indicated in Section 11.1.

Calculations are performed from plots of the cumulative dust loss (Section 11.2) to estimate both the mass of dust co-collected with asbestos on the filters prepared for asbestos analysis and the total mass of respirable dust in the original sample. Dust estimates are then combined with asbestos counts to allow reporting of both the concentration of asbestos structures per unit mass of respirable dust in the sample and the concentration of asbestos structures per unit mass of the original sample (Figure 3-2)[7]. Typically, asbestos concentrations will be reported from this method for a specific size range of asbestos structures of interest.

[7] When asbestos concentrations are to be reported as a function of the mass of the original sample, the concentration calculated in the laboratory, which represents the concentration of asbestos in the fine fraction of the original sample, must ultimately be adjusted to account for the mass of the coarse fraction of the sample as well (Section 11.4.3).

4.0 DEFINITIONS

Amphibole: a group of rock-forming ferromagnesium silicate minerals, closely related in crystal form and composition, and having the nominal formula:

$$A_{0-1}B_2C_5T_8O_{22}(OH,F,Cl)_2$$

where:

A = K, Na;

B = Fe^{2+}, Mn, Mg, Ca, Na;

C = Al, Cr, Ti, Fe^{3+}, Mg, Fe^{2+};

T = Si, Al, Cr, Fe^{3+}, Ti.

In some varieties of amphibole, these elements can be partially substituted by Li, Pb, or Zn. Amphibole is characterized by a cross-linked double chain of Si-O tetrahedra with a silicon: oxygen ratio of 4:11, by columnar or fibrous prismatic crystals and by good prismatic cleavage in two directions parallel to the crystal faces and intersecting at angles of about 56° and 124° (see Chatfield 1993).

Amphibole Asbestos: amphibole in an asbestiform habit.

Analytical Sensitivity: the calculated asbestos concentration in soil or a bulk matrix, in asbestos structures/g, equivalent to counting of one asbestos structure in the analysis.

Asbestiform: a specific type of mineral fibrosity in which the fibers and fibrils possess high tensile strength and flexibility.

Asbestos: a term applied to a group of fibrous silicate minerals that readily separate into thin, strong fibers that are flexible, heat resistant and chemically inert.

Asbestos Component: a term applied to any individually identifiable asbestos sub-structure that is part of a larger asbestos structure.

Asbestos Structure: a term applied to any contiguous grouping of asbestos fibers, with or without equant particles.

Aspect Ratio: the ratio of the length to width of a particle.

Blank: a fiber count made on TEM specimen grids prepared from an unused filter (or a filter through which asbestos-free water has been passed), to determine the background measurement. Blanks consist of filter blanks, field blanks and laboratory blanks. Laboratory blanks for this method may include scrubber blanks.

Bundle: a fiber composed of parallel, smaller diameter fibers attached along their lengths (see Chatfield 1993).

Chrysotile: the asbestiform habit of a mineral of the serpentine group that has the nominal composition:

$$Mg_3Si_2O_5(OH)_4$$

In some varieties of chrysotile, the silicon may be partially substituted by Al or less commonly by Fe. The magnesium may be partially substituted by Fe, Ni, Mn or Co. Some varieties contain Na, Cl or both. Chrysotile is a highly fibrous and silky variety and constitutes the most prevalent type of asbestos (see Chatfield 1993).

Cluster: an assembly of randomly oriented fibers (see Chatfield 1993).

Component Count: for any sample, a tally that includes the individually identified components of complex asbestos structures and each single asbestos structure with no identifiable components.

Elutriator: a device in which differential flow through a fluid (gas or liquid) against an opposing force (i.e. gravity) is employed to separate particles by size.

Equant Particle: as used in this document, a non-asbestos particle bound to, or overlapping with, asbestos structures observed on a TEM specimen grid.

Fiber: an elongated particle that has parallel or stepped sides. In this method, a fiber is defined to have an aspect ratio equal to or greater than 5:1 (see Chatfield 1993).

Fibril: a single fiber of asbestos that cannot be further separated longitudinally into smaller components without losing its fibrous properties or appearance.

Fibrous Structure: a contiguous grouping of fibers, with or without equant particles.

Field Blank: a filter cassette that has been taken to the sampling site, opened, and then closed. Such a filter is analyzed to determine the background asbestos structure count for measurement and to document the treatment of the filter from sample collection through analysis.

Filter Blank: an unused filter that is analyzed to determine the background asbestos structure count on the filter matrix.

Friable: as used in this document, capable of being crushed or deformed with the hand with the attendant release of fibers.

Habit: the characteristic crystal form or combination of forms of a mineral, including characteristic irregularities.

Identify: during asbestos analysis, the use of a sequential set of procedures to determine and confirm the mineralogy of a structure.

Isokinetic Sampling: sampling air in such a manner so as not to disturb the direction or velocity of air flow at the point sampled.

Isokinetic Sampling Tube: a tube placed in the air flow of the vertical elutriator portion of the dust generator used in this method, which samples the air at the top of the elutriator isokinetically.

Laboratory Blank: an unused filter that is analyzed along with sample filters to determine the background asbestos structure count in the laboratory.

Matrix:[8] A connected assembly of asbestos fibers with particles of another species (non-asbestos) (see Chatfield 1993).

PCM Equivalent Structure: A structure of aspect ratio greater than or equal to 3:1, longer than 5 μm, and which has a mean diameter between 0.2 μm and 3.0 μm for a part of its length greater than 5 μm. In this method, PCME structures also must contain at least one asbestos component (see Chatfield 1993).

Riffle Splitter: a device composed of a hopper and multiple, uniform, parallel chutes that alternately feed from the hopper to opposing receiving trays.

Scrubber: a device for removing particles from an air stream by passing the air stream through a super-saturated vapor in which the particles serve as nucleation centers for condensation and are thus captured. The resulting droplets (containing the trapped particles) then fall back into a central reservoir of boiling liquid.

Serpentine: a group of common rock-forming minerals having the nominal formula:

$$Mg_3Si_2O_5(OH)_4$$

Serpentine deposits often contain chrysotile asbestos (which is serpentine in an asbestiform habit).

Structure Count: for any sample, a tally of each individually identified asbestos structure regardless of whether the structure contains identifiable components. This is equivalent to a count of the total number of separate asbestos entities encountered on the sample.

Vertical Elutriator: see Elutriator.

Tumbler: a device that is rotated to provide continuous agitation to a bulk material placed inside. In the dust generator employed in this method, air is blown through a tumbler containing sample to carry away the dust generated during agitation by the tumbler.

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[8] When used to describe an asbestos structure. The term is also used in this document to describe a heterogeneous bulk solid.

5.0 SYMBOLS AND ABBREVIATIONS

5.1 SYMBOLS

A_f - the area of a filter from which a specimen grid is prepared (mm^2).

A_{go} - the average area of a specimen grid opening (mm^2).

C_{dust} - the concentration of asbestos structures (of a defined size and type) in the respirable dust from a sample (s/g_{dust}).

C_{mtrx} - the concentration of asbestos structures (of a defined size and type) in the original field matrix that was sampled for analysis using this method.

C_{smpl} - the concentration of asbestos structures (of a defined size and type) in a soil or bulk sample (s/g).

CF - the coarseness adjustment factor representing the ratio of the mass of the fine fraction to the total mass of a matrix that is sampled in the field.

cm - centimeter (10^{-2} meter).

cm^2 - square centimeter.

cm^3 - cubic centimeter.

cm^3/min - cubic centimeter per minute.

d - the density of a particle (g/cm^3).

DF - the dilution factor representing the ratio of the scrubber suspension volume to the aliquot that is filtered to prepare specimen grids.

^oC - degrees centigrade.

^oK - degrees Kelvin.

ΔM_f - the mass of respirable dust collected on a single filter during the interval Δt (g).

ΔM_s - the mass of respirable dust released from the sample during the interval Δt (g).

Δt - a short time interval (no more than 10 minutes).

η - the dynamic viscosity of air (g/cm*s).

eV - electron volt.

F_c - the rate of airflow (i.e. the volumetric flow rate) through the top exit (ME) opening of the elutriator that does not pass through the isokinetic sampling tube (cm^3/s).

F_d - the rate of airflow (i.e. the volumetric flow rate) through the top exit (IST) opening of the elutriator that passes through the isokinetic sampling tube (cm^3/s).

F_s - the rate of airflow (i.e. the volumetric flow rate) through the scrubber (cm^3/s).

ft - foot.

g - gram.

g - the acceleration due to gravity (cm/s^2), when used as a variable in an equation.

g/L - gram per liter.

g/cm^3 - gram per cubic centimeter.

hp - horsepower.

k - the first order rate constant (s^{-1}).

kg - kilogram (10^3 gram).

kV - kilovolt.

in - inch.

L - liter.

L/min - liters per minute.

M_{coarse} - the mass of the coarse fraction of a matrix sampled in the field.

M_t - the cumulative mass of respirable dust collected on filters from the start of a run to time, t (g).

M_{t30} - the cumulative mass of respirable dust collected on filters during an entire 30 rpm run (g).

M_{fine} - the mass of the fine fraction of a matrix sampled in the field.

M_o - the mass of respirable dust in a sample at the start of a run (g).

M_r - the cumulative mass of respirable dust released from a sample from the start of a run to time, t (g).

M_s - the mass of respirable dust remaining in a sample during a run but after time, t (g).

M_{scrbr} - the mass of respirable dust collected in the scrubber during a run (g).

M_{sample} - the mass of a sample introduced into the dust generator (g).

M_{tot} - the total mass of respirable dust estimated to reside in a sample (g).

ml - milliliter (10^{-3} L).

mm - millimeter (10^{-3} meters).

mm^2 - square millimeter.

µg - microgram (10^{-6} grams).

µm - micrometer (10^{-6} meters).

N_{go} - the number of grid openings counted during a scan (#).

N_{goh} - the number of grid openings counted during a high magnification scan (#).

N_{gol} - the number of grid openings counted during a low magnification scan (#).

nm - nanometer (10^{-9} meter).

P_f - the pressure measured at a flowmeter (torr).

P_t - the pressure estimated at an elutriator opening (torr).

%RD - the mass percent of respirable dust in a sample (%).

r - the radius of a particle (cm).

r^2 - the coefficient of determination (also defined as the correlation coefficient squared).

R_f - the flow reading from a flowmeter (cm/s).

S_{ch} - the number of asbestos structures (of a defined size and type) counted during a high magnification scan (#).

S_{cl} - the number of asbestos structures (of a defined size and type) counted during a low magnification scan (#).

S_d - the number of asbestos structures that must be detected during a TEM scan for asbestos to be defined as detected (#).

S_{smpl} - the required analytical sensitivity for this method (defined separately for total and long asbestos structures) (s/g).

s - second.

S/g - structures per gram.

S/g_{dust} - structures per gram of dust.

S/L - structures per liter.

S/mm^2 - structures per square millimeter.

t - time (s).

T_f - the temperature at a flowmeter (^oK).

T_t - the temperature at an exit opening of the elutriator (^oK).

V_{a1} - the volume of the first aliquot collected from the scrubber suspension to be used for further dilution (ml).

V_{a2} - the volume of the final aliquot collected from V_d, which is filtered for the preparation of specimen grids for TEM analysis (ml).

V_d - the volume into which the first aliquot from the scrubber suspension is diluted (ml).

V_l - linear air flow rate (cm/s).

V_s - the volume of scrubber suspension generated from a run (ml).

V_v - the volumetric air flow rate (cm^3/s).

W - watt.

5.2 ABBREVIATIONS

ED - Electron diffraction

EDXA - Energy dispersive X-ray analysis

FWHM - Full width at half maximum

HEPA - High efficiency particle absolute

IST - refers to the opening at the top of the elutriator that is associated with the *isokinetic sampling tube*

MCE - Mixed cellulose ester

ME	-	refers to the *main exit* opening at the top of the elutriator, which is *not* associated with the isokinetic sampling tube
PCM	-	Phase contrast optical microscopy
PCME	-	Phase contrast microscopy equivalent
PLM	-	Polarized light microscopy
RPM	-	Revolutions per minute
SAED	-	Selected area electron diffraction
TEM	-	Transmission electron microscopy
TSP	-	Total suspended particulate
UICC	-	Union Internationale Contre le Cancer

6.0 FACILITIES AND EQUIPMENT

6.1 SAMPLE COLLECTION EQUIPMENT AND CONSUMABLE SUPPLIES

To complete field sampling per this method, the following field equipment is mandatory:

- survey equipment appropriate to the manner in which sample locations are to be defined per the sampling plan;
- appropriate trowels, shovels, augers, or corers for sample collection per the sampling plan;
- (when sampling surface materials) a 12 in square aluminum template with an 8 in square hole in the center;
- a minimum of three 3-gal plastic buckets;
- a brass or steel sieve with 3/8 in. (1 cm) openings;
- a field balance (with a capacity of 40 kg and capable of achieving a precision of ± 10 g);
- a field balance (with a capacity of 2 kg and capable of achieving a precision of ± 0.2 g)[9]
- a riffle splitter with a minimum of 24, 3/4 in. (minimum size) chutes and three sample trays;
- one L plastic sample containers;
- sufficient plastic coolers to store and ship samples at ice temperature;
- equipment for cleaning sampling tools, including:
  - large buckets and tubs;
  - a container of asbestos-free water;
  - garden sprayers;
  - bio-degradable detergent;
  - assorted asbestos-free rags, sponges, etc.;
  - an air compressor with HEPA filter (optional, for drying equipment);
- field logbook and appropriate custody forms and sample labels;
- assorted garbage bags, paper towels, and tape;
- Tyvek suits and protective gloves; and

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[9] If appropriate equipment is available, it is advantageous to use a single field balance to achieve both sets of capacity and precision requirements for field weighing.

- appropriate equipment for respiratory protection.

6.2 LABORATORY FACILITIES

Laboratories wishing to adopt this method must develop and maintain the following facilities:

- a properly ventilated room for bulk sample handling that is entirely isolated from other room(s) in which air samples are handled and asbestos samples are analyzed. All such facilities must be sufficiently well ventilated to allow preparation of blanks that yield background determinations satisfying the requirements of Section 10.6 of the Superfund air method (Chatfield and Berman 1990);

- a glove box or equivalent isolation chamber of sufficient size to house a riffle splitter (or other equipment) required for the homogenization and sub-sampling of samples for this method. The glove box or isolation chamber must provide ample room for handling kg size soil or bulk samples while maintaining background concentrations in the outside room air at levels considered acceptable as defined in Section 10.6 of Chatfield and Berman (1990);

- a dust generator constructed per the specifications provided in Appendix A;

- a TEM operating at an accelerating potential of 80-120 kV, with a resolution better than 1.0 nm and a magnification range of approximately 300 to 100,000. The ability to obtain a direct screen magnification of about 100,000 is necessary for inspection of fiber morphology; this magnification may be obtained by supplementary optical enlargement of the screen image by use of a binocular if it cannot be obtained directly. The TEM shall also be equipped with an energy dispersive X-ray analyzer capable of achieving a resolution better than 175 eV (FWHM) on the MnK_{α} peak. For requirements concerning screen calibration and SAED and ED performance, see Chatfield and Berman (1990); and

- a computer system for recording analytical results. As indicated in the section addressing reporting requirements (see Chapter 13), analytical results are to be provided on computer disk (either 3.5 inch or 5.25 inch in double sided or high density format) in a file format that is compatible with LOTUS™. ASCII files are acceptable.

6.3 THE DUST GENERATOR AND APPURTENANT EQUIPMENT

The dust generator is to be constructed per the design drawings and specifications provided in Appendix A. Appurtenant equipment required to support the dust generator includes:

- a 129 hp DC motor (rated for 0 to 139 rpm) to drive the tumbler;

- two vacuum pumps capable of drawing 20 L/min at minimum load (will be run at 1 to 2 L/min);

- two variable area flowmeters capable of reading volumetric airflow velocities up to 1500 ml/min and one variable area flowmeter capable of reading airflow up to 250 ml/min;
- a heating mantle and variable voltage transformer suitable for maintaining water at a boil in a 1 L round bottom flask; and
- an immersion pump and cooler (or equivalent system) of sufficient capacity to provide 0^{0} C water at a rate of 1 to 2 L/min.

6.4 SPECIMEN PREPARATION EQUIPMENT

As defined in the ISO Method for the determination of asbestos in air using an indirect transfer technique (Chatfield 1993).

6.5 OTHER LABORATORY EQUIPMENT

As defined in the ISO Method for the determination of asbestos in air using an indirect transfer technique (Chatfield 1993).

6.6 CONSUMABLE/REUSABLE LABORATORY SUPPLIES

For each run of a sample using the dust generator:

- a lot of fifty MCE filters (0.45 µm pore size, 25 mm) that exhibit no more than 10 s/mm^2 asbestos as background[10]; and
- forty plastic petri dishes for storing 25 mm filters.

Also, other items as defined in the ISO Method for the determination of asbestos in air using an indirect transfer technique (Chatfield 1993)[11].

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[10] This value is selected to assure that detection of a single structure in 4 grid openings is more likely than not to constitute asbestos from a sample.

[11] This includes a supply of MCE filters (0.22 µm pore size) for filtering scrubber suspension.

7.0 REAGENTS

To support use of the dust generator, the following reagents are required:

- asbestos-free water (a regular supply of freshly distilled, filtered water must be available);
- potassium carbonate dihydrate (analytical grade)[12]; and
- sodium hexametaphosphate (analytical grade).

Also, reagents required to support asbestos analysis are defined in the ISO Method for the determination of asbestos in air using an indirect transfer technique (Chattfield 1993).

WARNING - USE ALL REAGENTS IN ACCORDANCE WITH THE APPROPRIATE HEALTH AND SAFETY REGULATIONS.

[12] This salt is required for loading into the constant humidity chamber and the desiccators to be used for conditioning filters under the recommended default conditions for running the dust generator. A supply of alternate salt (analytical grade) may be employed for studies in which dust generation is to be performed at a relative humidity other than the default recommendation (see Section 9.3.2).

8.0 SOIL OR BULK SAMPLE COLLECTION

Sample collection procedures adopted for this method are flexible to allow adequate sampling of a broad variety of matrices. The method also incorporates several field preparation steps that are designed to preserve sample representativeness while reducing the mass of the samples sent to a laboratory for analysis. Controlling the mass of the samples sent to a laboratory from the field is a cost saving measure (see Section 8.2).

WARNING:

MOST OF THE SAMPLE COLLECTION PROCEDURES AND FIELD PREPARATION PROCEDURES DISCUSSED IN THIS DOCUMENT ARE INHERENTLY DUSTY OPERATIONS. THEREFORE, WHEN HANDLING SOILS OR BULK MATERIALS THAT ARE KNOWN TO CONTAIN OR POTENTIALLY CONTAIN ASBESTOS, IT IS IMPERATIVE THAT PROPER RESPIRATORY PROTECTION BE WORN WHILE CONDUCTING THESE PROCEDURES.

8.1 SAMPLE COLLECTION

Any variety of commercially available field sampling equipment (trowels, shovels, augers, corers, etc.) may be used to collect samples for this method. The equipment and procedure(s) selected should be based on the nature of the material being sampled and the depths over which samples are to be collected. Two common examples are presented below. Whatever equipment and procedures are chosen, however, shall be applied consistently and invariably at each sampling location[13].

Whatever technique is chosen, the *minimum* size sample that shall be collected at each sampling location shall be 1 kg. Larger samples may be required, however, if particularly large (i.e. larger than a 4 or 5 cm in diameter) rocks or debris are present in the material being sampled. To assure representativeness, the largest component sampled should occupy no more than a few percent of the volume of the sample collected (Section 2.3). If samples are to be composited (Section 8.2), they shall be of similar mass (i.e. differences in mass between composited samples shall be no larger than 10% of the mass of the smallest sample).

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[13] Locations from which soil or bulk samples are to be collected shall be selected formally as part of a comprehensive strategy that is designed to provide a representative (unbiased) set of measurements for characterizing the releases of asbestos from the entire source matrix of interest. Procedures for designing such a strategy are beyond the scope of this document but are available elsewhere (see, for example, Berman and Chesson undated).

It is particularly critical, when collecting samples for soil or bulk asbestos analysis, to minimize field decisions that might alter the locations for sample collection that have been selected as part of a formal strategy. Such locations should be representative of the variation of *all* characteristics of the sampled matrix that might affect asbestos release. It is inappropriate, for example, to adjust the location of a sample just because a large rock happens to be located within the footprint over which the sample is supposed to be collected; the presence of that rock as part of that sample helps to represent the fraction of the surface of the sampled matrix from which asbestos release *cannot* occur.

When used to represent the central characteristics of a large matrix, random or systematic sampling schemes depend on faithfully preserving the consequences associated with the choice of *each* sampling location. In general, therefore, it is not appropriate to alter the selected locations even if collection of a sample at a specific location is impossible. Rather than altering such a location, any difficulties or interference that may hinder sample collection at a *defined* location shall be noted in detail in the field log book.

All sampling equipment shall be washed thoroughly with water and detergent between collection of each sample. Sampling equipment shall then be rinsed thoroughly with filtered, distilled water and allowed to air dry. Forced air may be used to expedite drying. If forced air is to be used to facilitate drying, however, such air must be passed through a HEPA filter to prevent delivery of any potential contamination.

Record the identification number, the date, time, and method of collection for each sample in a field notebook. Record the locations from which each sample is collected in the field notebook. Note in the logbook any changes between the sampling locations proposed in the sampling strategy and the actual locations sampled. As indicated previously, such changes are to be avoided to the extent possible. If changes are absolutely necessary, clearly document the rationale behind each change.

Supplement written documentation with photographs of each sampling location. This is particularly important if the sampling locations are not laid out on a formal, documented sampling grid that is tied to a permanent field marker.

8.1.1 Sampling to Derive Estimates of Asbestos Concentrations in a Road Surface

To illustrate the important features of sampling for this method, assume that sampling is to be performed to determine the concentration of releasable asbestos in the material of a serpentine-covered road. In this case, it is assumed that measured asbestos concentrations are to be related to current emissions so that it is only the actual surface layer of the road that is of interest.

Sampling a road surface shall be conducted using a metal template to define the bounds of each sample and a trowel (or other digging device) to remove material within the template to a uniform depth of 0.5 in. (approximately 1.5 cm). To assure that the samples collected exceed 1 kg in mass (assuming a density for unconsolidated serpentine of 2.2 g/cm^3), the sample volume should be a minimum of 450 cm^3 (28 in^3). Assuming, as indicated above, that each sample will be excavated to a uniform depth of 0.5 in., an 8 in. square hole in a 12 in. aluminum template works well.

At *each* selected sampling location, center the template on the defined location and press it firmly against the ground surface. Carefully excavate the material within the template to a uniform depth of 0.5 in. and place the material in a clean, pre-weighed bucket (see Section 8.2.1)[14]. Transport the sample to a central location on the site where field preparation (Section 8.2) will be performed.

8.1.2 Sampling a Mine Tailings Pile to Derive Estimates of Asbestos Concentrations Within the Pile

As a second illustration of sampling to support this method, assume that sampling is to be performed to determine the concentration of releasable asbestos in the material of a mine

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[14] Depending on conditions encountered, it may be necessary to remove surface debris (such as leaves or foreign dust) from an area prior to sampling. The determination as to whether or to what extent surface debris needs to be removed prior to sampling should be based on careful inspection of the sampling location and review of the motivation for sampling. Surface debris should generally *not* be removed unless such material is clearly distinguished visually from the matrix material to be sampled.

tailings pile. Assume further that the goal is to predict long-term emissions from the pile based on the asbestos concentrations measured. In this case, it is the concentration of asbestos within the volume of the entire pile that is of interest.

For the assumed tailings pile, conduct sampling using a hand auger (if the pile is no thicker than a few feet) or a power auger or other drilling equipment (if the pile is significantly thicker than a few feet). A coring cylinder that is 12 in. long and 2 in. in diameter (assuming an average density for the pile material of 2.2 g/cm^3) yields a sample of approximately 1.4 kg. Such a cylinder shall be collected from each location (defined in 3-dimensions: longitude, latitude, and depth) that is selected for sampling within the pile.

Cores shall be driven until their mid-point overlies the proposed sampling location. Collect the core carefully from each selected location, being sure that the entire core is extracted from the pile. Transport the sample to a central location on the site where field preparation (Section 8.2) will be performed.

8.2 FIELD PREPARATION

Although the following activities might conceivably be conducted in the laboratory (following collection and shipment of kg size samples), to minimize the mass of samples sent to the laboratory and thereby reduce costs, the following field preparation activities are incorporated into this method. For special cases, assuming that the laboratory of choice has access to the required equipment in the required protective enclosures, these activities may be conducted in the laboratory rather than in the field.

Per the instructions in the following sections, samples that are collected as defined in Section 8.1 are to be:

- weighed;
- sieved to separate a coarse and fine fraction;
- the coarse and fine fractions are to be weighed;
- the fine fraction is to be homogenized and split; and
- each of the two final sub-sample splits of the fine fraction are to be weighed, packaged, and shipped to the laboratory (as paired duplicates).

8.2.1 Weighing

As indicated in Section 8.1, samples shall be transported in clean, pre-weighed buckets from the locations at which they were collected to a central location for field preparation. The first step of the process shall be to weigh each sample.

Kg size samples are to be weighed using a field scale capable of reading mass with a minimum precision of ± 10 g. If necessary, wipe the outside surface of each sample bucket with a clean, dry (asbestos free) cloth before placing it on the scale (Figure 8-1). Record the mass measured for each sample (along with its identification number) in a field notebook and subtract the tare weight of the bucket to derive the net weight of the sample.

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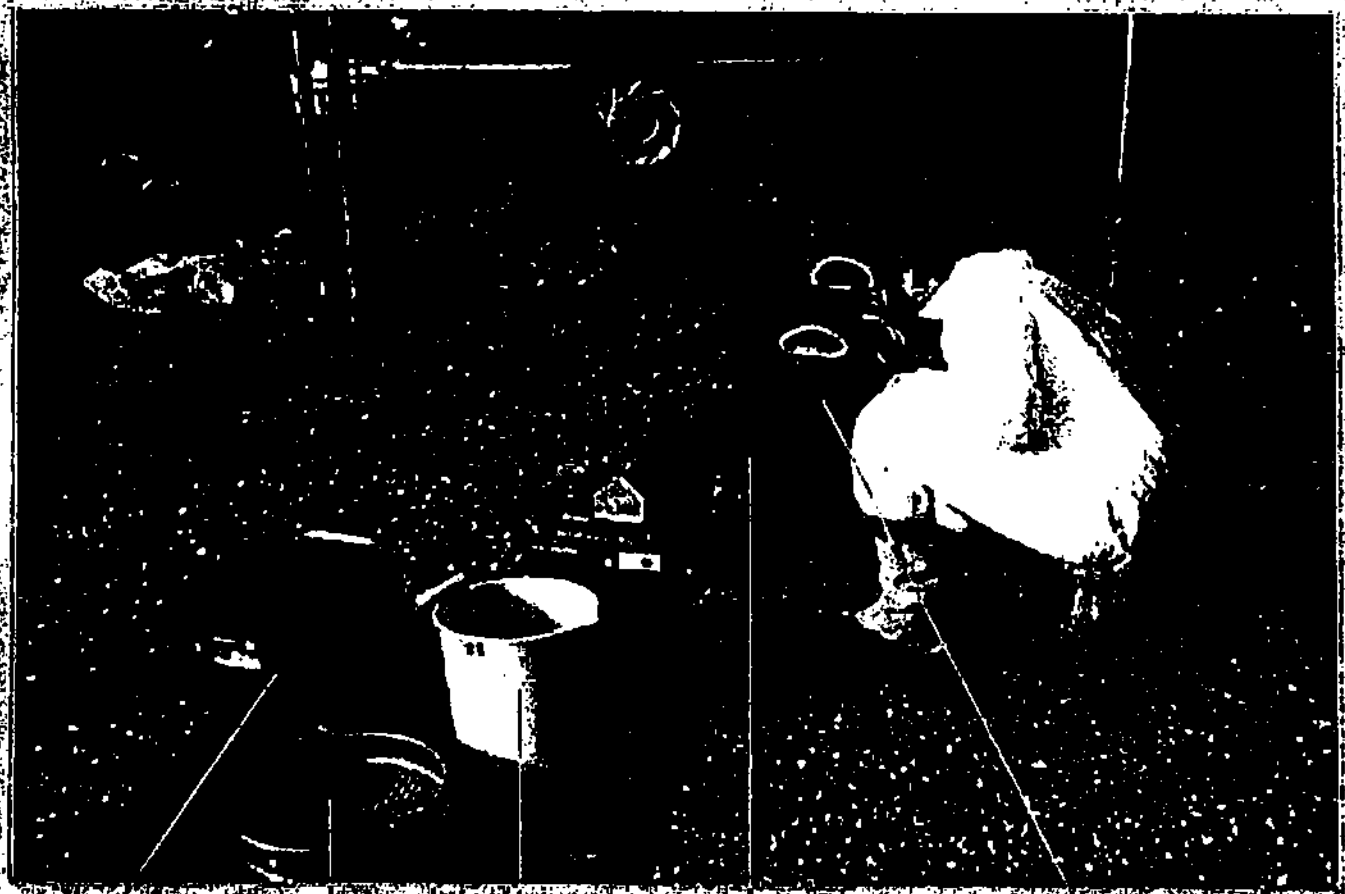
FIGURE 8-1

WEIGHING A SOIL SAMPLE ON A FIELD SCALE



Following size reduction (Section 8.2.2), both the coarse and fine fractions of each sample are to be weighed again. If necessary, wipe the outside surfaces of the buckets containing each size fraction with a clean, dry (asbestos free) cloth before placing it on the scale. Record the measured weight for each fraction in the field notebook under the appropriate sample identification number. Subtract the tare weight of each bucket holding, respectively, the coarse and fine fractions from the sample and record the net weights of each in the field notebook.

Following homogenization and splitting (Section 8.2.3), sub-samples that are to be sent to the laboratory are to be weighed yet again. In this case, sample weights are expected to range between approximately 50 and 80 g (see Section 9.1) and will need to be measured on a scale that can achieve a precision of ± 0.2 g. Depending on the types of equipment available to the sampling team, this may or may not be the same scale that is used for weighing the initial (heavier) samples. When sending 50 to 80 g splits to the laboratory, both halves of the final sample split shall be sent to the laboratory as a duplicate pair.

Wipe the outside surface of each sample container with a clean, dry (asbestos free) cloth before placing it on the scale. Record the weight, identification number, and pedigree of each sample split of each duplicate pair sent to the laboratory in the field notebook (Figure 8.2). Subtract the tare weight of each sample container from each sample and record the net weight of each sample in the field notebook next to the appropriate sample identifier. Package and send each sample to the laboratory as described in Section 8.4.

8.2.2 Size Reduction

After collection and the initial weighing (described above), pass each sample through a clean, wire-mesh sieve with 1 cm (3/8th in) openings. Reduce all clods and soft aggregates by hand and force all reducible material through the sieve into a clean, pre-weighed bucket (Figure 8-3). Stones and debris retained by the sieve that cannot be hand crushed shall be placed in a separate pre-weighed bucket. Once separated, the coarse and fine fractions from each sample are to be weighed separately (Section 8.2.1).

Clean the sieve with detergent and rinse with filtered, distilled water between samples. Dry the sieve with an asbestos free cloth or with appropriately filtered, forced air before each use.

8.2.3 Sample Homogenization and Splitting

The fine fraction of samples collected for this method may be homogenized and split by either of two procedures.

FIGURE 8-2

WEIGHING A SAMPLE SPLIT ON A FIELD SCALE

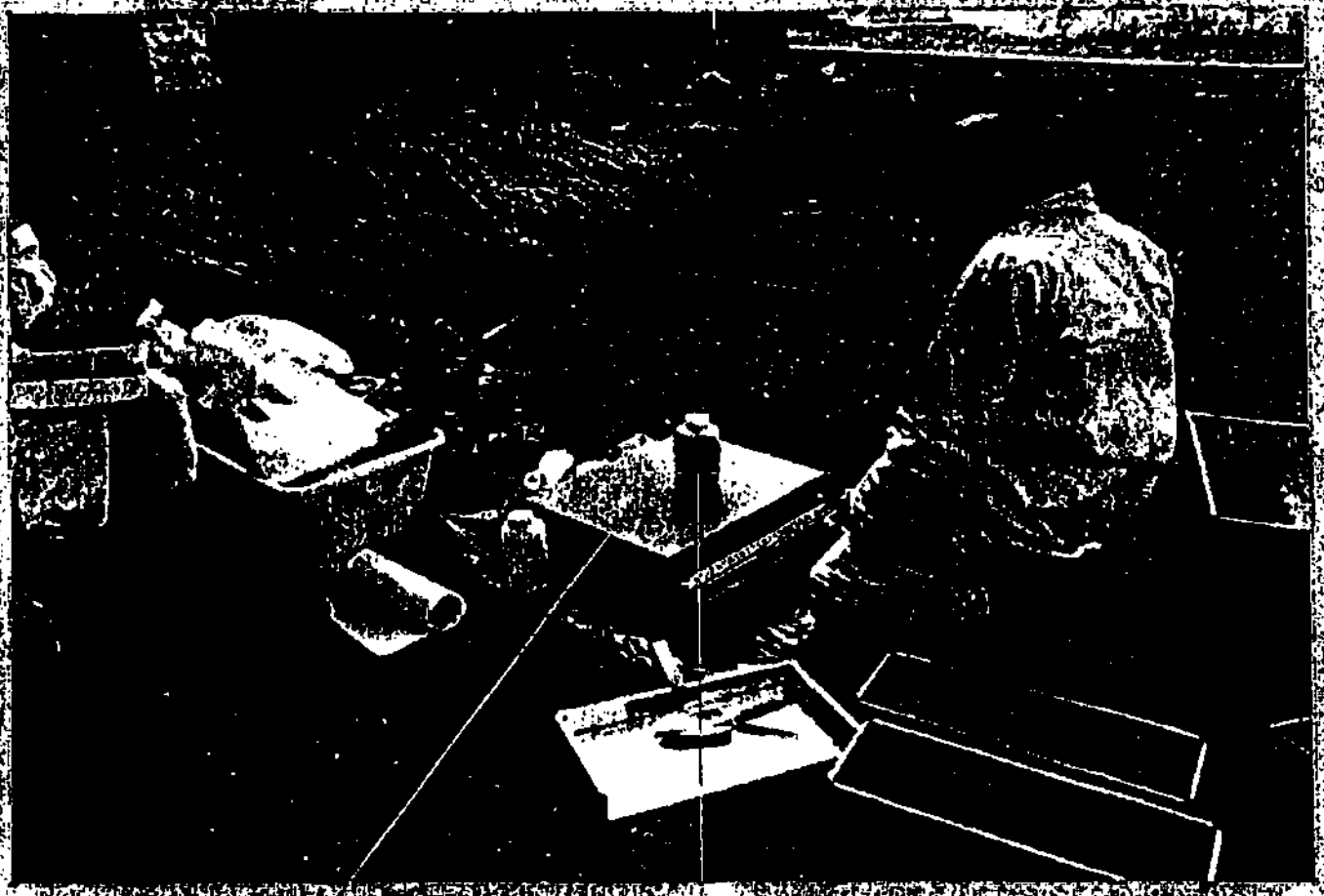


FIGURE 8-3
SIEVING A SOIL SAMPLE

Option 1: use of a riffle splitter[15]. Set a clean, dry riffle splitter with 3/4 to 1 in. chutes (Figure 8-4) on its stand on flat ground and place two receiving trays under the splitter so that they will each catch material that falls through one of the two sets of chutes (Figure 8-5). Place the sample to be homogenized in a third splitter tray (which must be clean and dry). Shake the splitter tray gently, until the sample is evenly distributed within the entire tray.

Place the long lip of the tray containing the sample against the inside of the long lip of the splitter hopper and slowly rotate the tray along an access defined by its lip so that the sample slowly empties into the splitter and slides down the near wall of the hopper to the chutes (Figure 8-5). Continue to rotate the tray until it lies entirely inverted over the top of the hopper of the splitter.

Tap the tray vigorously several times to free any remaining material and remove the emptied tray from the splitter. Tap the splitter vigorously several times to facilitate the flow of all material through the chutes into the receiving trays. If necessary, any sample remaining along any of the soldered corners and nooks of the splitter may be freed with a clean, coarse nylon brush. When brushing is completed, tap the brush vigorously against the splitter wall to free any material clinging to the brush[16]. Remove the two receiving trays (each containing half of the sample) from the splitter.

What is to be done next depends on whether the goal is to homogenize the sample or to split the sample. If the sample is in the process of being homogenized, combine the half of the sample from each receiving tray back into the third tray from the splitter. Be sure to tap each tray vigorously to assure quantitative transfer of the sample material. Replace the two *empty* receiving trays under the splitter and repeat the process of splitting the sample (in the manner described above). The sample should be subjected to a minimum of five cycles to assure adequate homogenization.

If the goal is to split (rather than homogenize) the sample, pour the material in one of the receiving trays from the splitter into a spare bucket and tap the tray vigorously to assure quantitative transfer. The remaining tray that still contains sample material now becomes the new sample tray and the original sample tray (now empty), along with the just emptied receiving tray, should be placed under the splitter as the new receiving trays.

Repeat the process of dispersing the remaining sample material (containing half the mass of the original sample) by shaking the sample tray so that it is uniformly distributed. Repeat the procedure described above for splitting the sample, discarding the material in one of the two receiving trays each time, until the mass of the material in each receiving tray at the end of one cycle falls in the range of 50 to 80 g. At that point, carefully transfer the material from

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[15] Results obtained as part of the pilot study for this method (Berman and Kolk 1994) suggest that some respirable dust may be lost each time a sample is passed through a riffle splitter. If the process is conducted carefully, however, such loss may be kept sufficiently small so that the multiple passes required to homogenize and split a sample properly will not significantly alter the estimated concentration of dust and asbestos derived using this method: losses should be less than an absolute maximum of 10 to 15% of the total respirable dust in the sample after as many as 10 passes. Because the estimate of the magnitude of loss was necessarily based on measurements of samples that had to be suspended in water, however, actual losses for most cases are expected to be much smaller.

One important consideration: avoid using the splitter in the field on windy days (i.e. when wind velocities exceed approximately 5 mph) unless an effective wind screen can be devised.

[16] The brush will have to be washed, rinsed, and dried thoroughly before use on another sample.

FIGURE 8-4

A RIFFLE SPLITTER

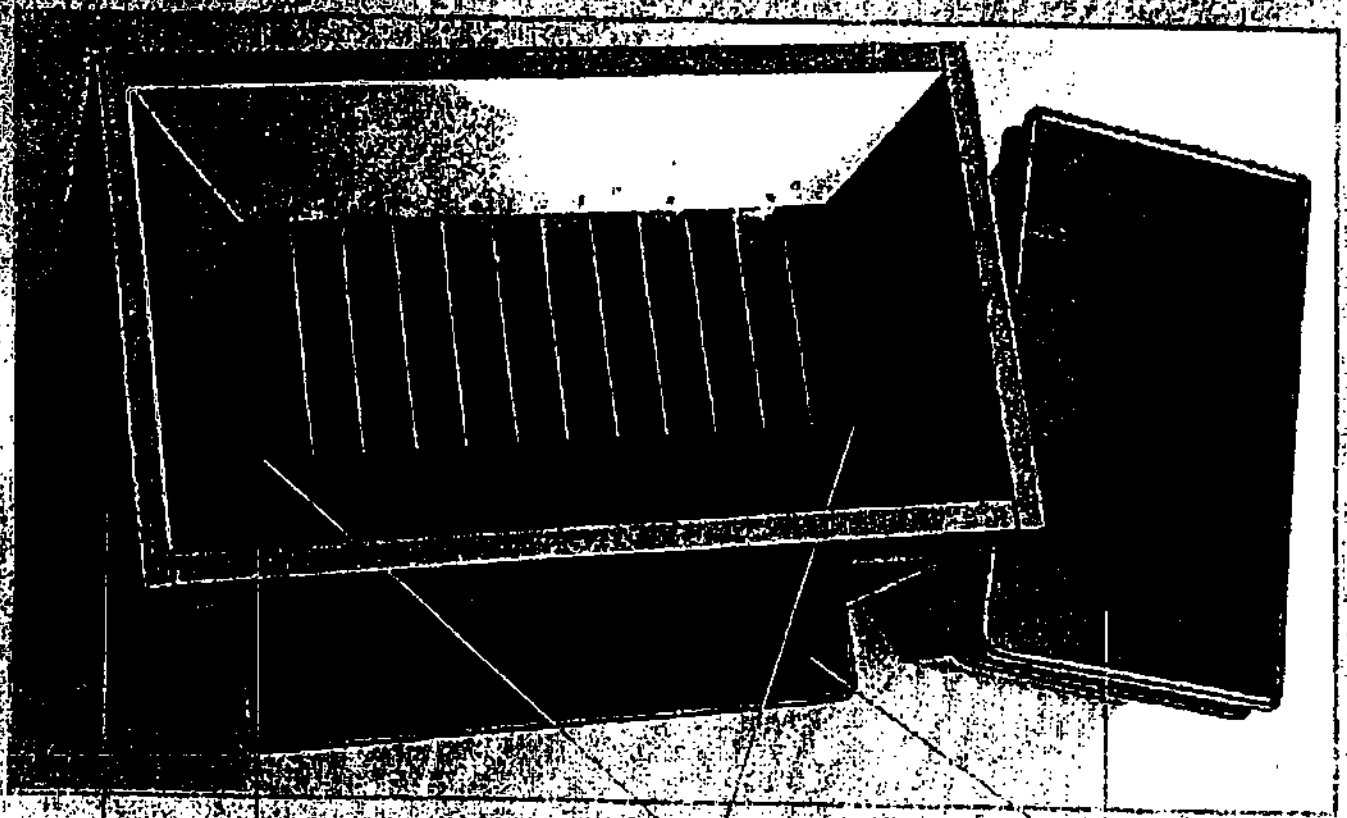
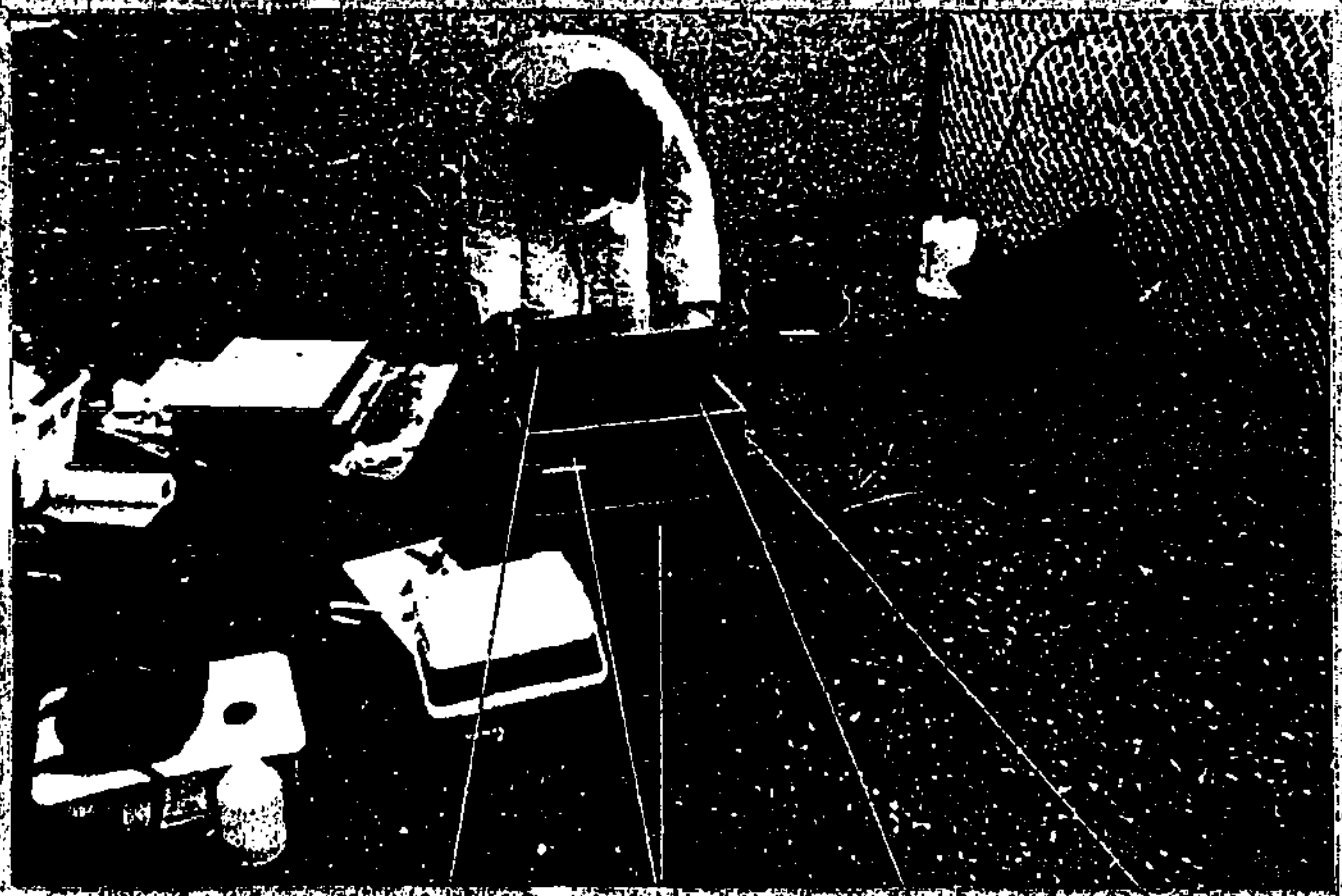


FIGURE 8-5

USING A RIFFLE SPLITTER TO HOMOGENIZE/SPLIT SAMPLES



Sample Tray Receiving Trays Splitter Hopper Splitter Stand

each tray into a clean, pre-weighed sample bottle (Figure 8-6) to be weighed (Section 8.2.1) and packaged for shipment to the laboratory (Section 8.4). Be sure that samples are transferred quantitatively from each tray.

NOTE

***NEVER* MAKE A PARTIAL TRANSFER OF MATERIAL FROM A SPLITTER TRAY TO A RECEIVING CONTAINER. THIS WOULD DEFEAT THE PURPOSE FOR HOMOGENIZATION AND SPLITTING BECAUSE IT IS IMPOSSIBLE TO ASSURE THAT ALL OF THE SIZE COMPONENTS OF THE SAMPLE ARE TRANSFERRED PROPORTIONALLY UNDER SUCH CIRCUMSTANCES.**

Clean the body of the splitter, all trays, and any appurtenant equipment (such as a nylon brush) between samples (but *not* between splits of the same sample) with a detergent wash followed by thorough rinsing with distilled, filtered water. Be sure that the splitter, trays, and appurtenant equipment are completely dry before use. These may be dried with forced air that is properly filtered to be free of asbestos.

Option 2: Use of a mixer with coning and quartering. Samples may also be homogenized for this method using any of various sealed, rotating mixers (tumblers). The mixers should contain internal baffles to promote mixing. Such mixers must be sufficiently large to accommodate the largest sample to be homogenized[17] with adequate room to spare so that tumbling is facilitated. The mixers must be sealable to prevent the loss of fines during mixing.

Place the fine fraction of the sample to be homogenized in a clean, dry mixer. Seal the mixer. Tumble the mixer at the manufacturers recommended speed for an amount of time recommended by the manufacturer to assure adequate homogenization. Stop the mixer and allow ample time (approximately 15 minutes) for the fines to settle. Disconnect the mixing container from the rest of the mixer. Open the mixer.

Under this option, a procedure termed coning and quartering is used to split a homogenized sample. Lay out a clean, aluminum plate on a flat surface. Hold the mixer immediately over the center of the plate and rotate the mixer around an axis represented by the lip on one side of its mouth so that the sample material *slowly* pours onto the metal plate forming a symmetrical cone (Figure 8-7). Keep the point at which the poured material impacts the cone at the same spot and slowly raise the mixer as the pouring continues to keep the distance between the mixer lip and the top of the cone approximately constant. When the mixer is fully inverted, tap it vigorously to complete the quantitative transfer.

To halve the cone, hold a second (clean, dry) aluminum plate directly over the apex (top center) of the cone at an angle that is perpendicular to the aluminum plate on which the cone lies. Slowly lower the second plate so that it splits the cone precisely in half (Figure 8-8a). While holding the two plates steady, push one half of the cone off of the original plate and away from the rest of the sample (Figure 8-8b). Brush the area from which this material is

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[17] If samples are to be composited as described in Section 8.3, the mixer may have to be capable of handling samples that range up 40 kg in size.

FIGURE 8-6

LOADING A SAMPLE SPLIT INTO A SAMPLE BOTTLE

Scale | Sample Bottle | Receiving Trays From Splitter | Riffle Splitter

FIGURE 8-7
TRANSFERING SAMPLE FROM A MIXER TO A PLATE
FOR CONING AND QUARTERING

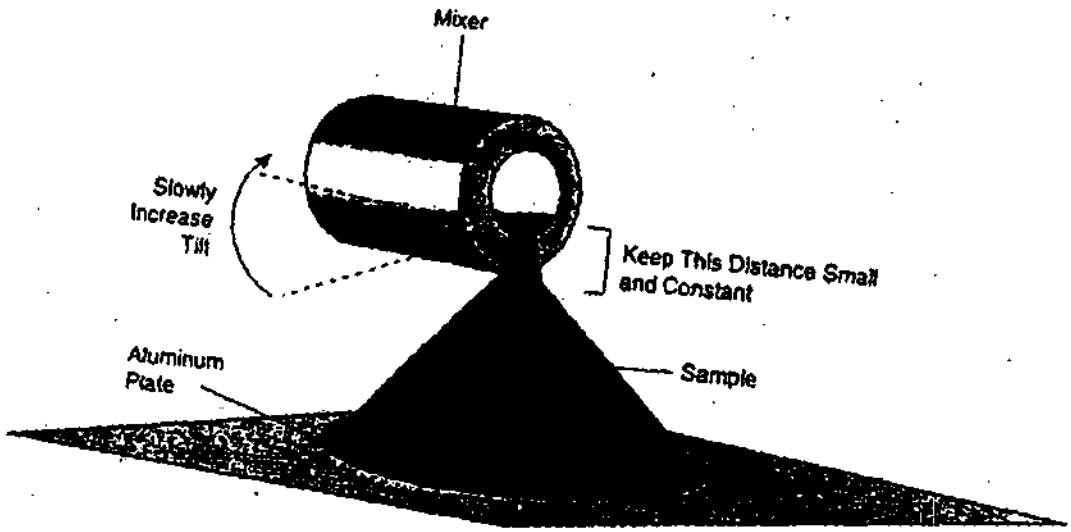
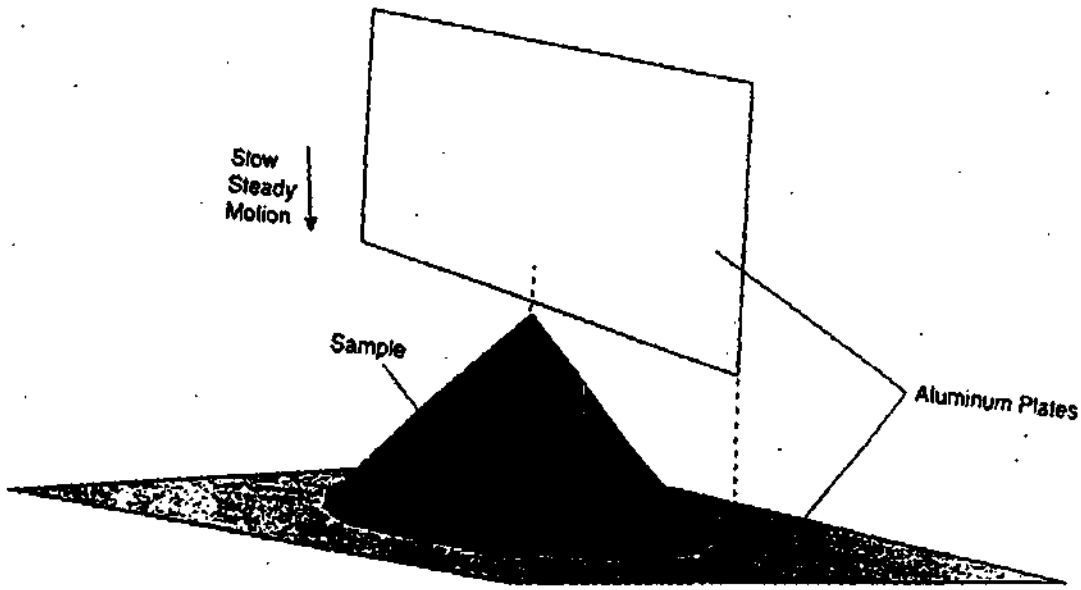
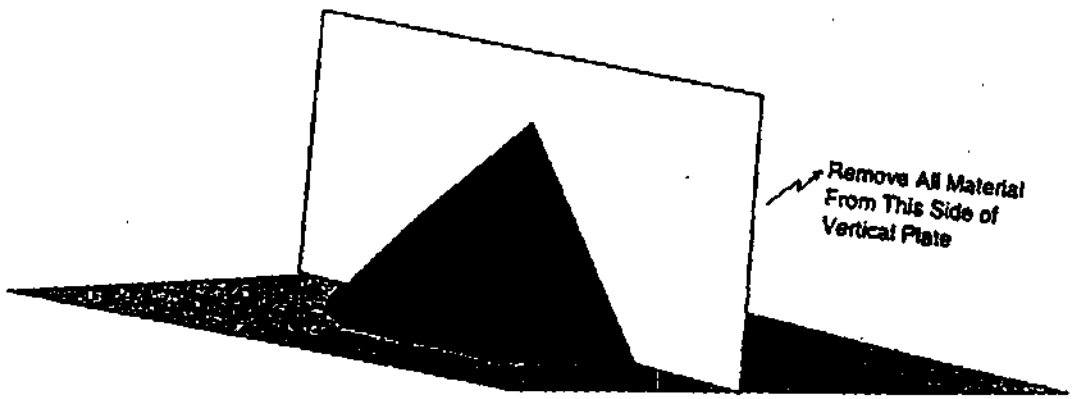


FIGURE 8-6

CONING AND QUARTERING



A. POSITION OF ALUMINUM PLATES IN PREPRATION FOR HALVING SAMPLE



B. POSITION OF ALUMINUM PLATES AFTER HALVING SAMPLE

removed to complete a quantitative transfer, leaving only clean metal. Once half of the sample material has been removed, withdraw the vertical aluminum plate slowly by pulling it upward vertically.

To complete quartering of the cone, rotate the vertical aluminum plate above the cone 90° on a vertical axis. Lower the plate slowly once again so that it splits the remaining portion of the sample cone evenly into two new halves. Once again, quantitatively remove half of the sample material (i.e. remove all of the material from one side of the vertical plate). This process may be repeated by quantitatively transferring the remaining sample material into a clean bucket and pouring the sample onto the clean aluminum plate to form a new cone.

Repeat the coning and quartering process until the remaining quarters (or halves) of the sample at the end of one cycle falls in the range of 50 to 80 g. At that point, carefully transfer the material from each quarter (or half) into a clean, pre-weighed sample bottle to be weighed (Section 8.2.1) and packaged for shipment to the laboratory (Section 8.4).

NOTE

NEVER MAKE A PARTIAL TRANSFER OF MATERIAL FROM ANY PORTION OF THE CONE THAT DOES NOT INCLUDE A WEDGED-SHAPE SLICE THROUGH THE CENTER OF THE CONE OVER ITS ENTIRE THICKNESS (DEPTH). THIS WOULD DEFEAT THE PURPOSE FOR FORMAL CONING AND QUARTERING BECAUSE IT IS IMPOSSIBLE TO ASSURE THAT ALL OF THE SIZE COMPONENTS, WHICH WILL *NOT* BE HOMOGENEOUSLY DISTRIBUTED VERTICALLY THROUGHOUT THE CONE OF THE SAMPLE, ARE TRANSFERRED PROPORTIONALLY.

8.3 COMPOSITING SAMPLES (OPTIONAL)

In many cases, there may be interest in limiting the number of analyses required to characterize a matrix that serves as a potential source *without* sacrificing representativeness. One procedure that may be employed for this purpose is to composite samples in the field. Note however, while compositing can reduce the cost of analysis by reducing the number of samples requiring analysis, also lost is information concerning the spatial variability of the sampled matrix. Therefore, if such information is desired for any particular reason, compositing is not recommended.

Only minor adjustments to the field preparation procedures described above are required to incorporate compositing into this method. First, during planning, group the samples to be collected in the field into sets that are to be composited. For example, there may be a desire to combine all samples from the eastern part of a road into a composite representing the east end of the road. Similarly, samples from the west end might be combined into a west end composite. Alternately, all samples from the road may be combined into a single composite, representing the road as a whole. As another alternative, the composite road sample might also be split into duplicate pairs to allow determination the variability contributed by sample preparation and analysis. Such decisions shall all be determined during planning.

NOTE

It is expected that the same set of locations will be selected for sample collection whether or not compositing is employed; compositing only changes the number of analyses required. This is because, the same number of samples collected from a set of locations selected using the same formal procedures are still required to adequately characterize the sampled matrix, whether or not samples are composited prior to analysis.

When brought to the central location where field preparation is conducted, after the initial weighing, the samples collected from a set that is to be composited can be combined in a common bucket. Modify the procedures described in Sections 8.2.1 and 8.2.2 as follows:

- transport each sample (of a set to be composited) from the point of collection to a central location in a clean bucket;

- weigh the sample and record the weight along with the appropriate sample identifier. Subtract the tare weight of the bucket and record the net weight of the sample;

- sieve the sample and collect the fine fraction from each sample in the set to be composited in a common bucket. Weigh the bucket containing the fines following the addition of the contributions of each sample and subtract the previous weight of the bucket to determine the net weight contributed by each sample. Record the weight with the proper identifier in the field notebook; and

- transfer the coarse fraction from each sample in the set to be composited to a common bucket. Weigh the bucket containing the coarse fraction following the addition of the contributions from each sample and subtract the previous weight of the bucket to determine the net weight contributed by each sample. Record the weight of the coarse fraction with the proper sample identifier in the field notebook.

Once all the samples of the set to be composited have been collected and added, the combined fine fraction from all of the samples (which resides in a common bucket) shall be given a separate identifier representing the intended composite. Record the new identifier in the field notebook. This material can now be treated as a single, composite sample for all remaining steps of field preparation and sample handling, packaging, and shipment to the laboratory. Thus, homogenize and split the sample as described in Section 8.2.3 and package and ship the sample to the laboratory as described in Section 8.4. Record the appropriate weights of the samples to be shipped as described in Section 8.2.1.

8.4 SAMPLE HANDLING AND SHIPMENT

Once their weights and identifiers are recorded, the samples to be shipped to the laboratory must be sealed and labeled. Fill out and apply appropriate labels to each sample bottle. Record the date and time that each sample was created on both the label and the field notebook and be sure that the identification numbers on the label and field notebook match.

Fill out the appropriate chain of custody forms and seal each sample cap with a breakaway label. As indicated in Section 12.2, be sure to complete the field activities report and to include this report in the package with samples sent to the laboratory.

Wipe each sample to be shipped to the laboratory with a clean, asbestos-free cloth and place it in a cooler. Ship samples to the laboratory in a cooler with ice to limit biological growth during shipment. Sufficient ice must be provided to assure that samples remain cold until received and processed by the laboratory.

NOTE

When matrices that are sampled contain a significant fraction of coarse material (i.e. more than 10% by mass), the final determination of the concentration of asbestos in that matrix must be adjusted to account for the fraction of coarse material. This requires determining the ratio of the mass of *fine* material in the sampled matrix to the total mass of material in the sampled matrix to generate a "coarseness adjustment factor". The concentration of asbestos determined for samples sent to the laboratory must then be multiplied by this coarseness adjustment factor to determine the concentration of asbestos in the sampled matrix.

Equations for deriving and using the coarseness adjustment factor are provided in Section 11.4.3. The weights of the coarse and fine fractions of each sample are to be included (along with the appropriate sample identifiers) as part of the field activities report that is to be shipped with the sample to the laboratory (Section 12.2). This assures that individuals responsible for estimating the concentration of asbestos for the project have access to the required field information.

9.0 SAMPLE PREPARATION BY DUST GENERATION

The primary purpose for sample preparation by dust generation that is described in this section is to generate dust-laden filters that can be suitably prepared for analysis by an appropriate method for the determination of asbestos in air[18]. The rate of generation of total respirable dust is also monitored and is used both to estimate the total mass of respirable dust in the original sample and to tie asbestos structure concentrations determined from filters to the mass of the original sample. Such information might also be used in some studies to characterize the releasibility of asbestos (or total dust) from particular sample types.

A detailed description of the apparatus employed for dust generation and its theory of operation is provided in Appendix A. Specifications and construction drawings are also provided.

9.1 SAMPLE RECEIVING AND STORAGE

All samples received from the field are to be wiped clean with a damp cloth prior to storage or other handling. Samples shall be stored at ice temperature (to minimize biological growth) until sample preparation is initiated. To minimize complications from biological agents, once initiated, sample preparation shall be completed expeditiously. In any case, sample preparation shall be completed within 48 hours.

Samples to be prepared using the dust generator are to be inspected for the presence of free water. If a sample contains free water or if the sample appears visibly moist, it shall be dried at low temperature. If time permits, place the sample in an open, shallow container and store it for several days in a desiccator containing *moist* potassium carbonate dihydrate or another salt that corresponds to the salt selected for humidity control (see Section 9.3.2). The type of salt placed in the desiccator is chosen deliberately; rather than to dry the sample completely, the goal is to bring the moisture content of the sample into equilibrium with conditions that will prevail in the dust generator.

If sufficient time is not available to dry the sample in a desiccator, the sample may be oven dried. Dry the sample in an open, shallow container in an oven that is maintained at a temperature *below* 60° C until the sample comes to constant weight. Note that oven-dried samples may require additional time for conditioning (Section 9.4.2) because the moisture content of the sample will need to be increased to bring it into equilibrium with conditions prevailing in the dust generator.

Once dry, samples smaller than 80 g can be loaded directly into the tumbler of the dust generator (Section 9.4.1). Larger samples must be homogenized and split, as described in Section 9.2, prior to being placed in the tumbler of the dust generator.

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[18] The ISO method for the determination of asbestos in air (using either an indirect or a direct filter preparation technique - - Chatfield 1993) is the default method recommended for use in tandem with this method.

9.2 SAMPLE HOMOGENIZATION AND SPLITTING IN THE LABORATORY

Samples received from the field that are larger than approximately 80 g must be dried, as described above, and then homogenized and split as described in this section.

WARNING:

BECAUSE ASBESTOS CONTAINING DUSTS MAY BE GENERATED FROM THE HANDLING AND PREPARATION OF BULK SAMPLES, ALL OF THE FOLLOWING PREPARATION STEPS SHALL BE PERFORMED IN A PROTECTIVE ENCLOSURE (I.E. A HEPA FILTERED GLOVE BOX OR AN APPROVED FUME HOOD THAT IS DESIGNED TO MINIMIZE EXPOSURE TO LABORATORY PERSONNEL[18]). IT IS ALSO NECESSARY THAT ALL HANDLING OF BULK SAMPLES BE CONDUCTED IN A SEPARATE ROOM THAT IS PHYSICALLY ISOLATED FROM THE ROOM(S) IN WHICH AIR SAMPLES ARE HANDLED AND ASBESTOS ANALYSIS IS PERFORMED.

As with field homogenization and splitting (Section 8.2.3), either of two options may be selected for homogenization and splitting in the laboratory. When performed in the laboratory, however, such equipment must fit within an appropriately designed, protective enclosure, which is why field preparation may be cost-effective.

Homogenize large samples in precisely the same manner as described in Section 8.2.3. Once samples are homogenized, split samples in precisely the same manner as described in Section 8.2.3. Continue splitting until a paired set of samples are produced that each contain between 50 and 80 g of material. Record in a laboratory notebook the final weights and identification numbers of the samples homogenized and split.

9.3 DUST GENERATOR SETUP

Prior to using the dust generator, a supply of at least 35 MCE filters must be conditioned and stored for use, the constant humidity chamber must be loaded with the appropriate solution, the scrubber must be primed, and air flow within the dust generator must be calibrated and adjusted.

9.3.1 Conditioning a Stock of Filters

A stock of at least 35 filters (0.45 µm pore size, 25 mm diameter), all from the same filter lot, must be conditioned in a desiccator overnight to bring them into equilibrium with the relative humidity at which they will be used during a run. Place the 35 MCE filters in a desiccator containing *moist* salt of the same variety as that selected to fill the pans in the humidity control chamber of the dust generator (Section 9.3.2). For most applications, this will be potassium carbonate dihydrate (see Appendix A).

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[18] The work should be performed in a Class II biohazard hood as per the specifications of Standard #49 of the National Sanitation Foundation.

After storing the filters overnight in the desiccator, pre-weigh each filter to a minimum precision of ± 0.0002 g[20]. Each filter shall then be placed in a separate, covered Petri dish with its weight marked on the top of the container. The lids shall also be numbered sequentially and the filters shall all be used during the run in the order numbered.

9.3.2 Initiating Humidity Control

Use asbestos-free (filtered, distilled) water to make a 2 L solution of saturated salt. As indicated previously, for most applications, use potassium carbonate dihydrate (to achieve a relative humidity of 43%), but other salts may be used for specific applications (see Appendix A)[21].

Prepare the solutions by placing 1000 g of the anhydrous salt into a one L container and adding distilled water to fill the container. The container should be capped and additional water and salt added as necessary the next day. No water or salt should be added within a day of using the mixture since some time is needed to saturate the salt solution and form a hydrate when adding anhydrous potassium carbonate. However, sufficient salt shall have been added previously to assure that sufficient undissolved potassium carbonate dihydrate has precipitated in the container to form a closely spaced layer of the material on the bottom of the shallow pans that will be covered with a thin layer of the solution (next paragraph).

Open the top of the humidity control chamber and remove the two pans. Fill each pan with the saturated salt solution being sure that a small quantity of excess (undissolved) salt is also transferred to each pan. Replace the pans and seal the top of the plastic enclosure; air should enter the enclosure primarily from the front opening.

9.3.3 Priming the Scrubber

Fill the round bottom flask of the scrubber to about one third full with asbestos-free (i.e. filtered, distilled) water. Initiate the flow of ice water through the entrance and exit condensers. Adjust the variable voltage transformer on the heating mantle so that water in the round bottom flask boils and the rate at which condensate drops back into the flask from the condensers is approximately equal to one drop per second.

9.3.4 Adjusting Initial Air Flow

The air flow within the various components of the dust generator must be adjusted so that flow within the vertical elutriator will properly separate and pass only respirable particles. Based on the discussion presented in Appendix A (Section A.2.3), the proper linear flow rate in the elutriator shall be set at 0.31 cm/s, which is 5% greater than the Stokes' velocity estimated for the largest spherical, respirable particles (i.e. those with a radius of 5 μm).

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[20] Filters to be used to collect samples over the isokinetic sampling tube of the elutriator (see Section 9.4.5) must be weighed to a minimum precision of ± 0.00002 g.

[21] Note that potassium carbonate dihydrate is not the usual form of potassium carbonate sold commercially. The usual commercial forms are the anhydrous and the sesquihydrate. The dihydrate can be made by allowing either of the commercial varieties of the salt to stand in their saturated solution for some extended period of time with some temperature cycling (Berman and Kolk 1994). A week appears to be sufficient but the process can be accelerated by augmenting the temperature cycling.

Next, calculate the required volumetric air flow, V_v, within the elutriator using Equation 9-1:

$$V_v = 81.1 * V_l \quad (9\text{-}1)$$

where:

V_l is the estimated linear flow rate required to separate respirable particles (i.e. 0.31 cm/s); and

V_v is the corresponding volumetric flow rate (cm^3/s) through the elutriator.

The coefficient, 81.1, in Equation 9-1 corresponds to the cross-sectional area of the elutriator (in cm^2).

To adjust the initial flow valve settings on the pumps, first connect one of the flowmeters such that air flows directly from the exit opening at the top of the elutriator that is *not* articulated with the isokinetic sampling tube[22]. This is the exit that opens directly into the top, tapering portion of the elutriator (see Appendix A) and is labeled the "ME" opening in Figure A-1. A filter cassette containing a filter from the batch of filters to be used for the run shall be placed between the flowmeter and the pump when adjusting the flow valve on this pump.

NOTE

The easiest way to directly connect a flow line to one of the top openings of the elutriator (the ME or the IST opening) is to align the appropriate slide mechanism so that a filter mount is directly over the opening, mount a cassette *without* a filter into the mount, and connect the air flow line to the exit side of the filterless cassette (see Section A.1.6 of Appendix A). During calibration, such a line would then feed sequentially into a flowmeter, a filter-containing cassette, and a pump.

Connect a second flowmeter directly to the exit line of the scrubber (the entrance line of which should already be attached to the two side exit openings on the elutriator) and place a filter cassette containing a filter from the batch of filters to be used for the run between this flowmeter and the pump for the scrubber.

Adjust the flow control valves on both the scrubber pump and the pump to be connected to the ME opening of the elutriator so that flow in both lines are equal and that each flow is set at $0.48 * V_v$.

Connect a third flowmeter to the exit opening on the top of the elutriator that articulates with the isokinetic sampling tube. This is labeled the "IST" opening in Figure A-1.

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[22] To access this opening, it will be necessary to dismount any filter cassettes from the appropriate slide mechanism and to align one of the two openings in the appropriate slide mechanism over the desired opening in the elutriator (see Appendix A).

The flowmeter attached to the IST opening shall also be backed by a filter cassette containing a filter from the batch to be used during the run and adjust the flow in this line so that it is equal to $0.047*V_v$[23]. Due to the low flow required on this line, an auxiliary low flow valve is also attached to this pump and must be adjusted to achieve the desired flow. To optimize conditions, it may be necessary to adjust the flow control valves on the three air lines that exit the elutriator iteratively.

To prepare for a run using the dust generator, disconnect the flowmeters from the slide mechanisms over the top openings of the elutriator (i.e. the ME and IST openings) and mount filters in each of the four cassette holders on the two slide mechanisms that cover the two elutriator openings. Connect one of the air flow lines coming off of the "T" from each pump to each of the two filters on the same slide mechanism for the appropriate opening of the elutriator (i.e. the opening for which the pump had been calibrated). Then adjust each slide so that one filter cassette is aligned directly over each elutriator opening. Be sure that the valves on each "T" are configured so that flow is directed from the filter cassette that is aligned directly over the elutriator opening (see Section A.1.6 of Appendix A).

NOTE

The connections between the exit side of the filter cassettes mounted on the slide mechanisms and the flow control valves on the pumps should now be direct; there should be no second filter cassette in the line.

The flowmeter attached to the exit line of the scrubber may remain attached during a run to monitor air flow through the scrubber. If there is a desire also to monitor airflow through one or both of the filter cassettes mounted over the top openings of the elutriator during a run, flowmeters may now be attached to the downstream side of the filter cassettes (i.e. between the filter cassettes and the pumps). Due to the pressure drop across the filters, however, the readings from these flowmeters must be adjusted using the following equation to provide estimates of the true flow through each elutriator opening:

$$F_t = R_f*(P_f*T_t/P_t*T_f) \qquad (9\text{-}2)$$

where:

F_t is the true flow rate through the elutriator opening (cm/s);

R_f is the flow reading from the flowmeter (cm/s);

P_f is the pressure at the flowmeter (torr);

T_f is the *absolute* temperature at the flowmeter (°K);

[23] The coefficient, 0.047, used to estimate the volumetric flow rate for air passing through the isokinetic sampler represents the fraction of the cross-sectional area of the elutriator that is subtended by the isokinetic sampling tube and therefore represents the fraction of the total flow that should pass through the tube, assuming that flow in the elutriator has been properly set.

P_1 is the pressure at the elutriator opening (torr); and

T_1 is the *absolute* temperature at the elutriator opening (^oK).

To use this equation, P_f and P_t will have to have been measured during flow calibration, prior to a run, using mercury manometers or other appropriate pressure measuring devices. Generally, T_t and T_f can be considered equal and will drop out of the equation. However, Equation 9-2 can also be used to adjust flow readings between calibrations and runs that are conducted on different days, such that temperatures may vary between the time during which the calibration was conducted and the time that the run is performed.[24]

9.4 DUST GENERATOR OPERATION

To prepare asbestos samples using the dust generator, load the tumbler, condition the bulk sample, begin the run, monitor the rate of dust generation, and collect appropriately loaded filters for asbestos analysis. Asbestos is also collected in the scrubber. Prior to use, be sure that the dust generator is clean (see Section 9.5).

9.4.1 Loading the Tumbler

Detach the tumbler from its drive motor and the vertical elutriator and remove it from the plastic enclosure at the bottom of the dust generator (see Appendix A). Place the tumbler on a flat surface and open the top for loading. Be sure that the tumbler is clean prior to loading.

Introduce a sample[25] by holding the sample container against the inner lip of the tumbler and tilting the container so that the sample pours smoothly into the tumbler. Move the sample container back and forth along the length of the tumbler to facilitate uniform deposition of the sample in the tumbler. When pouring is complete, tap the sample container vigorously so that the quantitative transfer is complete. The masses of samples introduced into the tumbler shall range between 50 and 80 g. Larger samples shall be homogenized and split prior to loading as described in Section 9.2.

Shake the tumbler gently to assure uniform deposition of the sample within the tumbler, which should be no more than about one third full. Be sure that the rubber gasket on the tumbler is in good repair and properly seated. Replace the gasket if it is worn. Secure the top of the tumbler with 10 screws and replace the tumbler within the plastic enclosure at the bottom of the dust generator. Reattach the elutriator entrance tube and D.C. motor to the tumbler (see Appendix A).

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[24] Because the viscosity of air is somewhat temperature dependent, when runs are to be conducted at temperatures that differ by more than a few degrees from room temperature (nominally 20° C), Equation A.10 (in Appendix A) may have to be adjusted to account for the varying viscosity (see Equation A.9) so that the correct flow regime can be established at the new temperature to assure that the elutriator of the dust generator passes only respirable particles.

[25] Samples to be introduced into the tumbler shall have been dried per Section 9.1.

9.4.2 Conditioning the Sample

Before conditioning the sample, be sure that the dust generator has been properly set up. This means, check that:

- the pans in the constant humidity chamber have been filled with saturated solution;
- filters have been mounted on each of the four cassette mounts on the slide mechanisms atop the elutriator;
- water is boiling in the scrubber;
- the air flow valves have been properly set; and
- all air lines between the dust generator, flow valves, and pumps are properly configured (see Section A.1.6 of Appendix A).

To condition the sample, turn on all pumps and begin the flow of air through the dust generator. ***DO NOT TURN ON THE TUMBLER MOTOR.*** Allow the flow of air to continue for a minimum of two hours before beginning a run. If the sample was oven dried rather than equilibrated with an appropriate salt in a desiccator (Section 9.1), the sample should be conditioned for a minimum of four hours prior to initiating a run.

9.4.3 Initiating a Run

Once the sample has been conditioned, set the tumbler drive motor to 30 rpm and turn it on. Simultaneously, move the two slide mechanisms at the top of the elutriator so that new, clean filters are now aligned over both the ME and IST openings of the elutriator. Be sure to change the valve orientations on the lines leading to the filters so that air flow is directed through the filter cassettes that are newly aligned with the elutriator openings.

Replace the filters originally aligned over the elutriator openings (but no longer aligned) with clean filters and weigh and store the old filters in labelled Petri dishes. These filters are equipment blanks. After five minutes,[26] move the sliding mechanism again to bring new, clean filters over the ME and IST openings of the elutriator. Immediately after the filters are brought out of alignment, dismount the cassette and turn the potentially dust-laden side of the filter face up before halting the flow of air through the filter (by turning the appropriate valves). Once flow from the dismounted cassettes has been halted (correspondingly, flow will have been re-directed to the cassettes that are currently aligned over the elutriator openings), replace the dismounted filters with clean filters and weigh and store the dismounted filters in labelled Petri dishes. These filters are run blanks.

As the run proceeds, record the times that air flow was started and stopped for each filter, the initial and final weights of each filter, and the identifier of each filter in a log book.

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[26] This interval is selected because, in the absence of channeling, five minutes is just less than the time over which the fastest particles are expected to reach the filter.

9.4.4 Monitoring the Rate of Respirable Dust Generation

The rate of respirable dust generation is monitored during a run by recording the weights of a set of filters that are sequentially changed out of the filter mounts over the ME opening of the elutriator at defined, regular intervals.

Initially, change the filter that is aligned over the ME opening of the elutriator at intervals of five to eight minutes. The change is accomplished by moving the slide mechanism to switch a new filter into alignment at the same time that the old filter is switched out of alignment. Immediately after the filter is brought out of alignment, dismount the cassette and turn the dust-laden side of the filter face up before halting the flow of air through this filter (i.e. by turning the appropriate valves to re-direct air flow to the filter that is newly aligned over the ME opening). Exchange the old filter for a new filter.

NOTE

Because the time during which air flow is directed through a dismounted cassette (rather than the filter that is aligned over the elutriator opening) results in a disturbance in the otherwise smooth flow of air through the elutriator, the changing of filters shall be performed expeditiously. As long as this interval is not more than a few seconds, however, studies indicate that this effect is not significant (Berman and Kolk 1994).

Along with the proper identifier, record the times during which air flow is started and halted for each filter. Weigh each filter after dismounting. Record the initial and final weights of the filter and the net weight of dust deposited on the filter (i.e. the difference between the initial and final weight).

After exchange of the first two or three filters, the interval over which dust is collected on each filter may be optimized. The ideal weight of dust to be deposited on each filter is between 0.01 and 0.03 g (Berman and Kolk 1994). Based on the rate of dust deposition on the first two or three filters, estimate the interval of time required to deposit approximately 0.02 g and exchange later filters at this rate (see Section A.2.2 of Appendix A, Equation A-8)[27]. However, it is important that time be adjusted so that no more than 0.03 g be deposited on each filter because the possibility that a portion of the deposit accidently drops from the filter increases as the weight of the deposit on the filter increases.

NOTE

Until there is a need for generating filters for asbestos analysis (see Section 9.4.5), the filters that get aligned over the IST opening of the elutriator need to be changed only one fifth to one tenth as often as the filters over the ME opening of the elutriator. These filters shall be changed at this lower rate, however, to prevent the potential for a heavy deposit to drop off of the filter and fall back into the elutriator.

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[27] At the beginning of a run the rate of dust deposition on the filters has been observed to be nearly constant with time.

Continue the run at 30 rpm (with continuing exchange of filters) for approximately two hours; this time interval has generally been observed as sufficient to define the rate of respirable particle release at this rotation rate (Berman et al 1994a). Generally, the plot of the release of respirable dust versus time at 30 rpm shows almost no curvature (see Section 11.2).

After completing the run at 30 rpm, select a new, higher rotation rate to continue the run. Generally, the new rotation rate selected shall be 60 rpm, unless the rate of release at 30 rpm was noticeably low in comparison with prior runs on other samples, in which case 90 or 120 rpm shall be used.

NOTE

Use of the highest rotation rates should generally be avoided, unless there is compelling evidence for their efficacy, because they tend to facilitate the transport of non-respirable particles from the tumbler into the bottom of the elutriator and, if such transport is heavy, this may affect results (see Section A.1.3 of Appendix A).

Continue the run at the higher rate of rotation by collecting a minimum of eight additional dust-laden filters. The same procedures outlined above should be continued for collecting data during the run at this higher rotation rate except that the interval between the exchange of filters must be adjusted downward to assure that deposits on these latter filters do not exceed 0.03 g (see Section A.2.2 of Appendix A, Equation A-8, but note that the rate constant, k, is dependent on the rotation rate for the tumbler so that Equation A-8 cannot be extrapolated across runs).

When the run is complete, turn off the tumbler motor but allow the air flow to continue for ten or fifteen additional minutes to empty the elutriator. Be sure to continue the exchange of filters, if necessary to prevent overloading. The air flow pumps may now be shut off.

9.4.5 Generating Appropriately Loaded Filters for Asbestos Analysis

The primary purpose for collecting dust on filters mounted over the exit of the isokinetic sampling tube of the elutriator (the opening labeled "IST" in Figure A-1) is to obtain samples suitable for asbestos analysis using a direct transfer technique, although use of an indirect transfer technique is not precluded. This is an option built into the design of the dust generator as an alternative to preparation of a specimen for asbestos analysis using the liquid from the scrubber, which necessarily mimics an indirect transfer technique because (in the scrubber) the asbestos is captured and suspended in water.

Collect filters for asbestos analysis near the end of each of the two runs (i.e. one run at each of two rotational speeds for the tumbler) that is described in the previous section of this chapter. Filters to be used for asbestos analysis shall be collected at the end of the runs both because this will be the period when the rate of asbestos emission is the lowest and because sufficient time will have elapsed over each run to allow a steady-state distribution of particle sizes to have developed in the dust traversing the elutriator. At the beginning of a run, only the smallest (fastest) respirable particles reach the filters and it takes time for the larger (slower) respirable particles in the air stream to begin reaching the filters in numbers that are proportional to their rate of emission from the tumbler. It takes several tens of minutes for transport of a steady state distribution to develop.

Collect multiple filters during each run that bracket the estimated time during which an optimal loading for analysis of a directly prepared specimen is expected to be achieved. Mount, exchange, dismount, weigh, record, and store filters precisely in the manner described in Section 9.4.4.

Estimate the time required for achieving an optimum loading as follows. The optimal mass loading on a filter to be prepared by a direct transfer technique lies between 1 and 10 μg (see, for example, Berman and Chatfield 1990). Assume a target of 5 μg. However, this may have to be adjusted based on experience with the dust generator. Equation A-7 (Appendix A) can then be re-arranged to estimate the time required to collect 5 μg (or some other defined mass) of dust:

$$\Delta t = \Delta M_f / 0.047 * k * M_s \qquad (9\text{-}3)$$

where:

M_s is the mass of respirable dust remaining in the sample at time "t" after the beginning of the run, but it is assumed constant over the short interval of time "Δt" (g);

ΔM_f is the mass of respirable dust collected on a filter over the IST opening during the short time interval "Δt" (as indicated above, assume a target of 5 μg or 5×10^{-6} g);

Δt is a short time interval (no more than several minutes) during which the release of dust is being estimated (s); and

k is the first-order rate constant for the release that is derived from the dust measurements collected during the run (s^{-1}).

The mass of respirable dust remaining in the sample during the time interval of interest (K_s) is estimated using a rearrangement to Equation A-2 (Appendix A):

$$M_s = M_o * \exp(-kt) \qquad (9\text{-}4)$$

where:

M_s is the mass of respirable dust remaining in the sample at time "t" (g);

M_o is the mass of respirable dust in the sample at the start of the run (i.e. at time t = 0) (g).

t is the time from the start of the run to the beginning of the time interval "Δt" (s); and

k is the first-order rate constant for the release (s^{-1}).

Based on the recently completed pilot study for this method (Berman et al. 1994a), a typical rate constant for dust emission from the tumbler is 0.004 min^{-1} (6.7 x 10^{-5} s^{-1}). Results from this study also suggest that the range in respirable dust content likely to be encountered for samples typically run using the dust generator may vary between 0.5% and 2%. Therefore, given a typical sample mass of 70 g, M_o for the 30 rpm run likely ranges between approximately 0.17 and 0.68 g. Given that a typical 30 rpm run lasts for approximately 3 hours (1.1 x 10^4 s) and substituting these values for M_o, k, and t into Equation 9-4, and then substituting the subsequent estimate of M_s into Equation 9-3, it appears that between 2.3 and 9 seconds would be required at the end of the 30 rpm run to collect 5 µg of material on a filter loaded over the isokinetic sampler.

Similarly, assuming that the initial mass of respirable dust in a sample at the beginning of a 60 rpm run, $M_{o\text{-}60}$, is equal to the remaining mass at the end of the 30 rpm run (i.e. the "M_s" calculated above), remembering that a 60 rpm run typically lasts 2 hours (7.2 x 10^3 s), and once again noting the typical value for k indicated above, Equation 9-4 is used to estimate an appropriate M_s for the end of a 60 rpm run. Substituting this new value into Equation 9-3, it appears that between 4 and 15 seconds may be required to collect 5 µg of material on a filter that is loaded over the isokinetic sampler at the end of a 60 rpm run.

Given the above, to properly bracket the optimal loading for a filter to be employed for asbestos analysis using a (direct transfer technique), collect filters over the IST opening of the elutriator that are exposed for periods of 3, 10, and 20 seconds (at both the end of the 30 rpm run and the end of the 60 rpm run).

9.4.6 Obtaining Asbestos Samples from the Scrubber

At the end of all runs for a particular sample (after all pumps have been shut off), turn off the heating mantle to the scrubber and let it cool for 10 to 15 minutes before discontinuing the flow of ice water to the condensers. Disconnect the outlet lines from the elutriator to the scrubber at the elutriator. Samples shall be extracted from the scrubber *expeditiously* to minimize losses to the walls of the glassware and to facilitate cleaning.

To minimize loss, before disconnecting the transfer lines and condensers from the round-bottom flask of the scrubber, pour approximately 100 ml of asbestos-free (filtered, distilled) water down the exit condenser and another 100 ml down the transfer lines and entrance condenser. Such rinsing should be performed in multiple stages, approximately 20 ml at a time. Swirl each condenser (and the transfer lines) as the water drains into the flask. Tap each condenser (and the transfer lines) several times after rinsing to assure a reasonably quantitative transfer.

Detach and remove the round-bottom flask from the condensers and its stand and pour the contents of the flask into a clean, pre-weighed, wide-mouthed 1 L plastic container. Rinse the round bottom flask several times with additional asbestos-free water to assure a quantitative transfer of any residual solids. Reweigh the container and record the net weight as the total weight of suspension. If necessary, the sample may then be stored at ice temperature until it can be prepared. However, preparation shall not be delayed for more than 48 hours.

Immediately prior to filter preparation, add 1.5 g/L of sodium hexametaphosphate to the suspension in the plastic container. Shake the suspension vigorously and divide it

approximately evenly into two (or, if necessary, three) 500 ml Erlenmeyer flasks. Place the flasks on a laboratory shake table for approximately three hours. Quickly re-combine the contents of the two (or three) flasks into a clean, plastic container (with minimal flushing) and place the container in a sonicator. Sonicate the suspension for approximately 1 minute (with the power of the sonicator set at no more than 0.1 W/ml). Withdraw one ml with a disposable pipette from the center of the volume of the suspension in the plastic container and dilute this with asbestos-free water to 100 ml in a clean, volumetric flask.

The mass of respirable dust collected in the scrubber should be equal to the sum of the cumulative mass of dust measured on the filters collected above the ME opening of the elutriator over the entire run(s) during which the scrubber suspension was collected. Use this estimated mass (and account for the 100-fold dilution performed as described in the last paragraph) to estimate the size of aliquots required to produce filterable suspensions containing: 0.5, 2, and 5 µg of respirable material. Dilute each aliquot to a minimum total volume of 20 ml and filter each aliquot in the manner described in Sections 10.34 and 10.35 of the ISO Method for the determination of asbestos in air using an indirect transfer technique (Chatfield 1993)[28].

The filtered aliquots shall all be prepared as described in Section 10.1 and scanned briefly at low magnification in the TEM to select the optimally loaded specimen for detailed analysis (see Section 11.1.2).

9.5 CLEANING THE DUST GENERATOR

The dust generator is designed for quick and easy assembly and disassembly to facilitate cleaning. Most of the joints are simple friction couplings or ring clamp couplings. To clean the dust generator, disconnect and disassemble the tumbler, remove the bottom cup and dust collector system from the elutriator, decouple the two halves of the elutriator tube, disassemble the slide mechanisms of the dust collector and disconnect the transfer lines to the scrubber. The metal pieces of the dust generator may then be washed with biodegradable detergent, rinsed with asbestos-free water, sonicated briefly, and rinsed again. The pieces may then be left to dry in room air or may be dried with a forced, HEPA-filtered air stream.

The glassware of the scrubber shall also be washed with biodegradable detergent and asbestos-free water, rinsed liberally, and dried in room air or dried with a forced, HEPA-filtered air stream. It is recommend that new transfer lines between the elutriator and the scrubber (constructed of 1.00 in. I.d. Tygon tubing) be cut and installed after each cleaning.

[28] As indicated in Chatfield (1993), filters to be employed for filtering scrubber suspensions are of a different type than those employed in dust generator mounts. Filters used to filter scrubber suspension are to be the 0.22 µm pore size variety

10.0 PREPARATION OF SPECIMEN GRIDS FOR TEM ANALYSIS

For Superfund applications of this method, asbestos analysis of all samples prepared using the dust generator are to be performed on specimen grids prepared from aliquots of the scrubber suspension. In addition, for a minimum of a subset of 5% (preferably 10%) or 10 samples (whichever is greater), asbestos analysis is also to be performed on specimen grids prepared by a direct transfer technique from filters collected over the IST opening of the elutriator (i.e. the opening over the isokinetic sampling tube). These analyses are then paired with the analyses of asbestos from scrubber suspension collected during the same runs to provide a link between samples prepared by each technique.

The primary reason for preparing 100% of samples from the scrubber suspension is to facilitate identification of distinctions in sample characteristics; samples prepared in this manner are expected to exhibit the best precision among the options for this method. At the same time, when comparing results to published slope factors, the apparent need for normalizing asbestos analyses to counts derived from directly prepared specimens (see, for example, Berman and Crump 1989) is satisfied by providing a subset of samples prepared both ways to allow a regression to be performed linking results from the scrubber suspension samples to specimens prepared by a direct technique. As indicated previously (Section 2.1.3), the recommended procedure is based on a compromise allowing optimum precision for distinguishing among relative measurements (and relative risks) while excepting a small reduction in the precision of estimates of absolute risk.[29]

10.1 PREPARATION OF SPECIMEN GRIDS FROM FILTERED ALIQUOTS OF THE SCRUBBER SUSPENSION

Filters generated from aliquots of the scrubber suspension (as described in Section 9.4.6) shall be prepared using the direct transfer technique that is described in Sections 10.5 of the ISO Method (Chatfield 1993). As indicated previously, multiple aliquots representing a sequence of dilutions are to be prepared to allow selection of the optimally loaded filters (and corresponding set of specimen grids) for final, detailed analysis.

From each filter, prepare a minimum of three specimen grids: one from near the center of the filter, one from a location that is half the distance between the center and the outer edge, and one from near the outer edge of the filter.

10.2 SPECIMEN GRID PREPARATION FROM FILTERS COLLECTED OVER THE IST OPENING OF THE ELUTRIATOR

Although this method specifies that filters collected over the IST opening of the elutriator shall be prepared using a direct preparation technique, an indirect preparation technique is also described, as an option for non-Superfund applications.

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[29] For other applications of this method, options might include preparation of 100% of samples from filters collected over the IST opening of the elutriator and/or preparation of filters collected over this opening using an indirect transfer technique. The method is designed to be flexible.

10.2.1 Specimen Grid Preparation Using a Direct Transfer Technique

Filters collected over the IST opening of the elutriator (as described in Section 9.4.5) shall be prepared using the direct transfer technique that is described in Section 10.5 of the ISO Method (Chatfield 1993). As indicated previously, sections of multiple filters representing a range of loadings are to be prepared to allow selection of the optimally loaded specimen grids for final, detailed analysis.

From each filter that has been collected over the IST opening, prepare four specimen grids from locations on the filter that are each separated by 90° radially. Select two of the locations (from opposing sides of the filter) at points that are about two thirds of the distance from the center to the edge of the filter. The remaining two locations shall be selected at points that are about one third of the distance from the center to the edge of the filter. Such an arrangement will eliminate any effects potentially associated with a linear gradient across the filter that may develop due to the brief time over which the filters are exposed to air flow from the elutriator and, consequently, the potentially significant time during which the filter is being slid in and out of alignment.

10.3 Specimen Grid Preparation Using an Indirect Transfer Technique

As an option to the procedure described in Section 10.2.1 above (for non-Superfund applications *only*), filters collected over the IST opening of the elutriator (as described in Section 9.4.5) may also be prepared using the indirect transfer technique that is described in Sections 10.3 to 10.5 of the ISO Method (Chatfield 1993). For this option, multiple sections of the most highly loaded filter obtained from the dust generator shall be prepared using a range of dilutions to allow selection of the optimally loaded specimen grids for final, detailed analysis.

11.0 PROCEDURES FOR ASBESTOS AND DUST ANALYSIS

11.1 PROCEDURES FOR ASBESTOS ANALYSIS

Specimen grids prepared as described in Chapter 10 are to be analyzed using transmission electron microscopy (TEM). Follow the procedures for analysis described in the ISO Method (Chatfield 1993) including procedures for:

- examining specimen grids to determine acceptability for analysis;
- structure counting by TEM (except determination of the stopping point);
- structure morphological classification;
- structure mineralogical identification; and
- blank and quality control determinations.

The stopping points for the analyses conducted in support of this method are a function of the required sensitivity for the method and are defined in Sections 11.1.1 and 11.1.2 below.

Begin by examining one of each set of specimen grids derived for a defined loading from a particular run of the dust generator and select the optimally loaded set for analysis. Use the criteria for determining the acceptability of specimen grids (from the ISO Method) to define optimal loading.

When performing detailed analysis, be sure to distribute asbestos counts evenly over the entire set of specimen grids prepared from a particular filter at a defined (optimal) loading. Record the morphology and mineral type of asbestos structures as described in the ISO Method (Chatfield 1993). Also as described in the ISO Method, complete separate scans for counts of total structures and, at lower magnification, for counts of structures longer than 5 μm.

11.1.1 Analysis of Specimen Grids Prepared from Filters Collected Over the IST Opening of the Elutriator

Prior to initiating a detailed analysis of specimen grids, the stopping rules for the analysis must be defined. Assuming these specimen grids have been prepared using a direct transfer technique (as discussed in Section 10.2), define the stopping rules for the detailed analysis as follows.

First, calculate the maximum number of grid openings that will have to be scanned during the analysis from the relationship:

$$N_{go} = S_d * A_f * \%RD / (S_{smpl} * A_{go} * 100 * \Delta M_f) \qquad (11\text{-}1)$$

where:

N_{go} is the maximum number of grid openings to be scanned;

S_d is the number of structures required to define detection using the analysis (defined here as 1);

A_f is the total area of the filter from which the specimen grids were prepared (mm^2);

%RD is the mass percent of respirable dust in the sample and is defined using Equation 11-9;

S_{smpl} is the required analytical sensitivity for the method (s/g);

A_{go} is the area of a single grid opening (mm^2); and

ΔM_f is the mass of respirable dust collected on the filter from which the specimen grids were prepared. It is defined using Equation A-7 of Section A.2.2 of Appendix A (g).

The following are typical values for the above parameters:

S_d = 1;
S_{smpl} = 5×10^7 (for total structures) or
= 3×10^6 (for structures longer than 5 µm)[30];
A_f = 382 mm^2;
A_{go} = 8.1×10^{-3} mm^2;
%RD = between 0.5% and 2%[31]; and
ΔM_f = 5 µg [32];

Given the above values, it is estimated using Equation 11-1 that between 1 and 4 grid openings will typically need to be scanned to derive a count of total structures. However, a minimum of 4 grid openings shall be scanned during any analysis. Based on the above, similarly, between 15 and 62 grid openings will typically need to be scanned to derive a count of structures longer than 5 µm.

The number of grid openings to be scanned for specific analyses shall be determined by substituting case-specific values for the above listed parameters into Equation 11-1.

Stop the counting, characterization, identification, and recording of asbestos structures on a particular analysis when one of the following obtains:

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[30] See Section 2.1.1.

[31] This is the range of values observed for a diverse variety of samples tested during the pilot study for this method (Berman et al. 1994a).

[32] See Sections 9.4.5 and A.2.2 of Appendix A.

- the scan is completed for the grid opening on which the 50th asbestos structure is counted; or

- either 4 grid openings or the maximum number of grid openings (estimated as defined above), whichever is greater, are scanned completely.

These rules are to be applied separately to the scan for total structures and the scan for long structures (i.e. longer than 5 μm) that are described in the ISO Method.

11.1.2 Analysis of Specimen Grids Prepared from Filtered Scrubber Suspension

Prior to initiating a detailed analysis of specimen grids, the stopping rules for the analysis must also be defined in this case. Given that these specimen grids have been prepared as described in Section 10.1, define the stopping rules for the detailed analysis as follows.

First, calculate the maximum number of grid openings that will have to be scanned during the analysis from the relationship:

$$N_{go} = S_d * A_f * \%RD * DF / (S_{smpl} * A_{go} * 100 * M_{scrbr}) \quad (11\text{-}2)$$

where:

N_{go} is the maximum number of grid openings to be scanned;

S_d is the number of structures required to define detection using the analysis (defined here as 1);

A_f is the total area of the filter from which the specimen grids were prepared (mm^2);

%RD is the mass percent of respirable dust in the sample and is defined using Equation 11-9;

S_{smpl} is the required analytical sensitivity for the method (s/g);

A_{go} is the area of a single grid opening (mm^2);

DF is the dilution factor by which the scrubber suspension had to be diluted to prepare specimen grids that are suitably loaded for analysis; and

M_{scrbr} is the mass of respirable dust collected in the scrubber suspension during the run.

The dilution factor, DF, is simply the product of the individual dilution factors for the two sequential dilutions performed to derive the final volume that is ultimately filtered (per the procedure defined in Section 9.4.6):

$$DF = (V_s/V_{a1})*(V_d/V_{a2}) \quad (11\text{-}3)$$

where:

V_s is the volume of the initial scrubber suspension (which is estimated from the recorded weight of the supernatant assuming a density of 1 g/cm^3) (ml);

V_{a1} is the volume of the first aliquot collected from the scrubber suspension for further preparation (the default defined in Section 9.4.6 is 1 ml);

V_d is the volume into which the first aliquot from the suspension is diluted (the default defined in Section 9.4.6 is 100 ml); and

V_{a2} is the volume of the final aliquot collected from V_d that is ultimately filtered for preparation of the optimally loaded specimen grids (ml).

The mass of respirable dust collected in the scrubber suspension, M_{scrbr}, is derived from the cumulative mass of dust collected during the run on filters that are mounted over the ME opening of the elutriator (defined below as M_f). These differ by the ratio of the air flow into the filters and the air flow into the scrubber:

$$M_{scrbr} = M_f*(F_s/F_c) \quad (11\text{-}4)$$

where:

M_{scrbr} is the mass of respirable dust collected in the scrubber during the run (g);

M_f is the cumulative mass of dust collected on the filters (mounted over the ME opening of the elutriator) during the same run (calculated from Equation A-4 of Section A.2.1 of Appendix A);

F_s is the volumetric air flow rate into the scrubber (cm^3/s); and

F_c is the volumetric air flow rate into the filters over the ME opening of the elutriator (cm^3/s).

Assuming air flow in the elutriator is setup as described in Section 9.3.4, F_s and F_f are equal so that M_{scrbr} simply equals M_f.

Given the typical values for the corresponding parameters provided in Section 11.1.1, assuming a typical value for DF [33] of between 2×10^4 and 4×10^4, and selecting a range of typical values for M_f (i.e. between 0.1 and 0.2 g) from among the range observed during the pilot study (Berman et al. 1994a), it is estimated using Equation 11-2 that between 1 and 8 grid openings will need to be scanned to derive counts of total structures. Similarly, it is estimated that between 8 and 133 grid openings will need to be scanned to derive counts of long structures (i.e. longer than 5 µm).

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[33] This assumes typical values for M_f (between 0.1 and 0.2 g) from among the range of values observed among diverse samples during the pilot study (Berman and Kolk 1994) and further assumes that the dilution factor is selected so as to produce a loading of 5 µg on the filter.

Determine the actual number of grid openings required for a specific analysis by substituting case-specific values for the above parameters into Equations 11-2, 11-3, and 11-4.

Stop the counting, characterization, identification, and recording of asbestos structures on a particular analysis when one of the following obtains:

- the scan is completed for the grid opening on which the 50th asbestos structure is counted; or
- either 4 grid openings or the maximum number of grid openings (estimated as defined above), whichever is greater, are scanned completely.

These rules are to be applied separately to the scan for total structures and the scan for long structures (i.e. longer than 5 μm) that are described in the ISO Method (Chatfield 1993).

11.2 EVALUATING THE RATE OF RELEASE OF RESPIRABLE DUST

The rate of release of respirable dust from a sample prepared using the dust generator is estimated from measurements of the mass of dust collected over time on the set of filters mounted over the ME opening of the elutriator. The measurements used specifically are from those filters that are collected while the tumbler is operating at the *highest* rotation rate employed for the sample (see Section A.2.1 of Appendix A).

Begin by plotting the cumulative mass collected on the filters as a function of time. To derive the cumulative mass for a particular time interval, add the mass of dust measured on the filter collected from that time interval to the sum of the masses measured on the set of filters collected earlier in the run. Typical curves are depicted in Figures 11-1 and 11-2. Next, calculate the cumulative mass released from the sample over time from the cumulative mass collected on filters over time using the relationship developed in Section A.2.1 of Appendix A:

$$M_r = 2.1 * M_f \qquad (11\text{-}5)$$

where:

M_r is the cumulative mass of dust released from a sample between the start of a run and time "t" (g); and

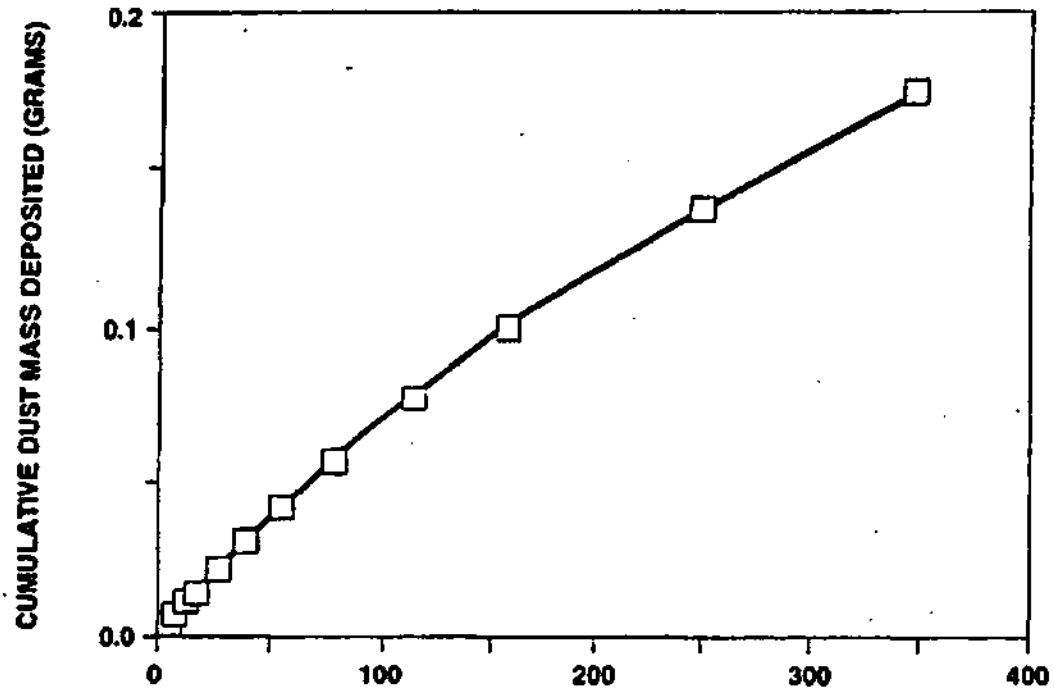
M_f is the cumulative mass collected on filters[34] between the start of a run and time "t" (g).

Equation 11-5 is appropriate to use to relate the mass of dust collected on filters to the mass released from the sample when air flow in the dust generator is setup as indicated in Section 9.3.4. If different air flow conditions are established for a particular experiment, the relationship between M_r and M_f will have to be derived using Equation A-4 from Appendix A.

[34] These are the filters that are mounted over the ME opening of the elutriator.

FIGURE 11-1

TYPICAL CUMULATIVE MASS RELEASE VERSUS TIME CURVE FOR 30 RPM RUN[a]



KEY:

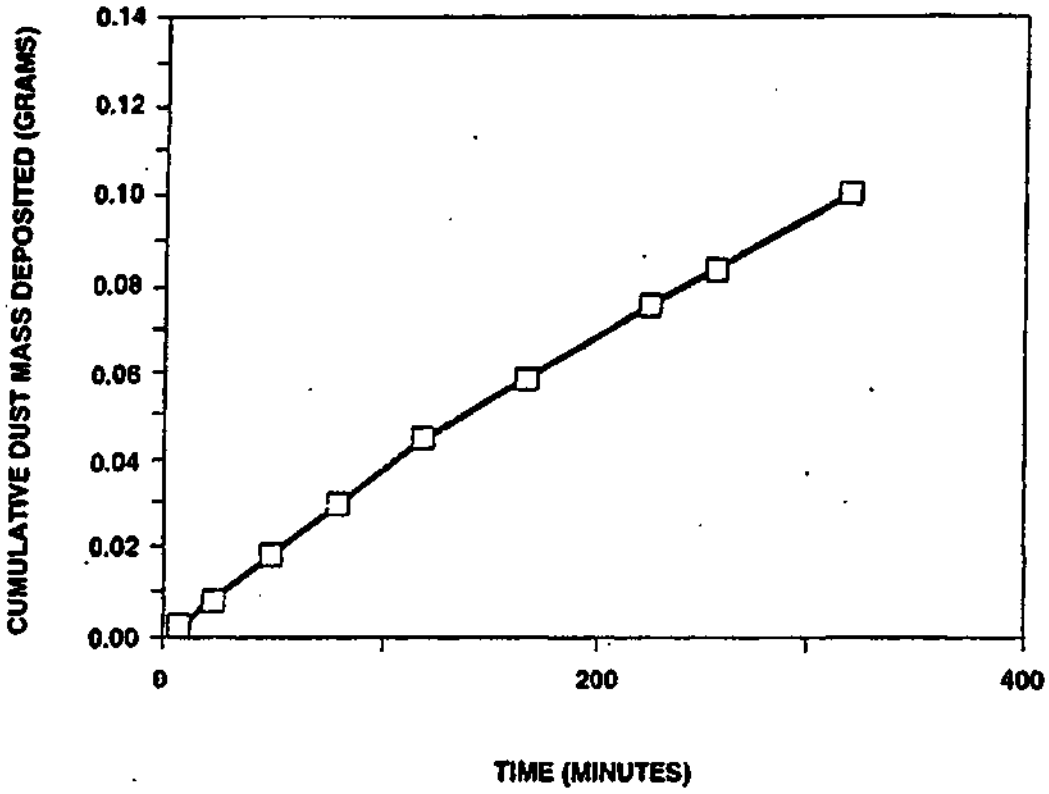
□ DUST SAMPLE MEASUREMENT

—— BEST FIT LINE

[a]FROM THE 30-RPM RUN ON SAMPLE 1-100, WHICH WAS COMPLETED ON 8/27/93 DURING THE PILOT STUDY (BERMAN ET. AL. 1994a).

FIGURE 11-2

TYPICAL CUMULATIVE MASS RELEASE VERSUS TIME CURVE FOR 60 RPM RUN[a]



KEY:

□ DUST SAMPLE MEASUREMENT

—— BEST FIT LINE

[a]FROM THE 60-RPM RUN ON SAMPLE 1-100, WHICH WAS COMPLETED ON 8/27/93 DURING THE PILOT STUDY (BERMAN ET. AL. 1994a).

The total mass of dust in the sample at the beginning of the run must next be estimated using the relationship developed in Appendix A. Based on the relationship (see Section A.2.1):

$$\ln(M_o - M_t) = \ln(M_o) - kt \qquad (11\text{-}6)$$

where:

M_o is the mass of dust in the sample at the start of the run (g);

k is the first-order rate constant for the release of dust from the sample (s^{-1}); and

t is the time since the start of the run (s);

a plot of $\ln(M_o - M_t)$ versus t should be a straight line with a slope equal to the rate constant for the release of dust from the sample and an intercept equal to the natural logarithm of the mass of dust in the sample at the start of the run. Derive estimates of "M_o" and "k" by programming Equation 11-6 into a spreadsheet and running a regression[35].

Input a range of guesses for the value of M_o into the spreadsheet and run a regression to fit a value for k and to calculate a value for the regression coefficient, "r^2" for each value of M_o. Plot the regression coefficient, "r^2" as a function of M_o. An example of such a plot is presented in Figure 11-3. The value of M_o that provides the fit with the largest regression coefficient (i.e. with r^2 closest to 1) shall be reported as the correct value for the mass of dust in the sample at the start of the run and shall be reported with the corresponding k value as the estimated rate constant for dust release from the sample during the run.

11.3 DETERMINING THE CONTENT OF RESPIRABLE DUST

To determine the mass percent of respirable dust in the original sample, first determine the total mass of respirable dust in the sample *at the start of a run* for the last run completed on the sample, which is derived as described in the last section.

Because the dust generator run analyzed as described in Section 11.2 will generally have been preceded by a run with the tumbler speed set at 30 rpm (see Section 9.4.4), to estimate the total mass of dust present in the sample, it is necessary to include the mass released during this first run.

Sum the masses of dust measured on each of the filters collected during the 30 rpm run and designate this sum, "M_{f30}", which is the cumulative mass of dust collected during the 30 rpm run. Using the following equation, estimate the total mass of dust *released* from the sample during the 30 rpm run, "M_{r30}," based on the mass of dust *collected* during that run (see Section A.2.1 of Appendix A):

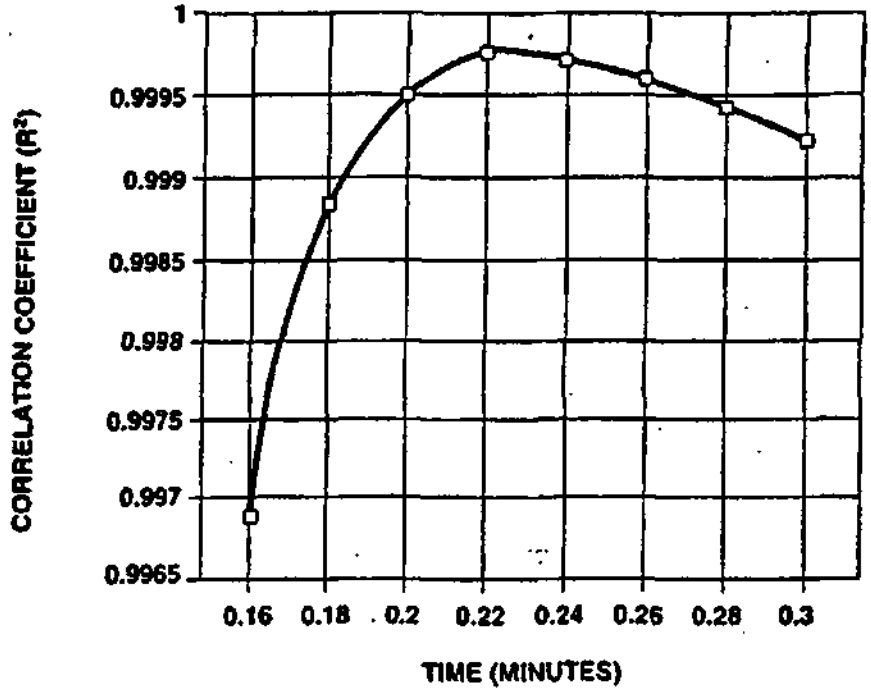
$$M_{r30} = 2.08 * M_{f30} \qquad (11\text{-}7)$$

[35] Any of several commercial spreadsheet programs (including, for example, LOTUS™) contain the necessary capabilities and may be employed to derive optimum values for "M_o" and "k."

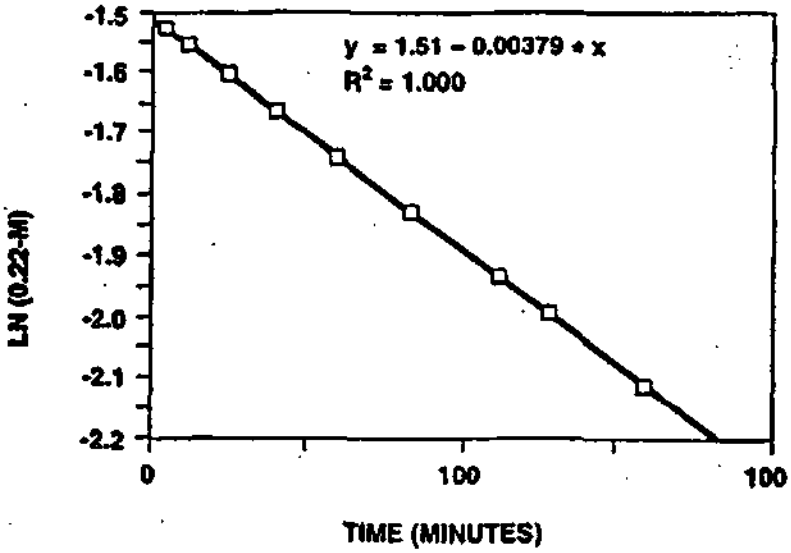
FIGURE 11-3

ILLUSTRATION OF THE OPTIMIZATION
OF THE ESTIMATE OF INITIAL MASS "M_0"

A. TYPICAL PLOT OF CORRELATION COEFFICIENT VERSUS INTITIAL MASS FOR A DUST GENERATOR RUN[a]



B. TYPICAL PLOT OF THE OPTIMIZED FIRST ORDER RATE EQUATION FOR A DUST GENERATOR RUN[a]



[a]FROM THE 60-RPM RUN ON SAMPLE 1-100, WHICH WAS COMPLETED ON 8/27/93 DURING THE PILOT STUDY (BERMAN ET. AL. 1994a).

Calculate the total mass of dust originally present in the sample, M_{tot}, by summing the mass released during the 30 rpm run with the mass of dust estimated to have resided in the sample at the beginning of the higher rpm run, M_o (this is equal to the mass of dust remaining in the sample at the end of the 30 rpm run). M_o will have been derived as described in Section 11.2:

$$M_{tot} = M_{r30} + M_o \qquad (11\text{-}8).$$

Estimate the mass percent of respirable dust in a sample as follows:

$$\%RD = 100 * M_{tot}/M_{sample} \qquad (11\text{-}9)$$

where:

%RD is the mass percent of respirable dust in the sample (%); and

M_{sample} is the mass of the original sample placed in the tumbler (g).

11.4 DETERMINING THE CONTENT OF ASBESTOS

The concentration of asbestos in a sample is determined differently depending on whether asbestos is determined from sampling grids prepared from filters collected over the IST opening of the elutriator or from scrubber suspension.

NOTE

For samples originally containing a significant fraction of coarse material (see Section 8.4), the concentration of asbestos reported as a function of the mass of the sample (specified in this section) must be adjusted for the quantity of coarse material originally measured in the matrix sampled in the field before it can be considered representative of that matrix. A procedure for adjusting asbestos concentrations to account for the coarse fraction of an environmental matrix is presented in Section 11.4.3.

11.4.1 Based on Directly Prepared Filters Collected Over the IST Opening of the Elutriator

Procedures for determining the concentrations of asbestos structures in a sample differ slightly depending on whether the structures of interest are longer or shorter than 5 µm.

Structures that are shorter than 5 µm in length are derived *only* from the high magnification scan of an analysis. Calculate and report the concentration of short asbestos in the original sample based on the counts of asbestos structures that are derived as defined in Section 11.1.1 using the following relationship:

$$C_{smpl} = S_{ch} * A_f * \%RD / (N_{goh} * A_{go} * 100 * \Delta M_f) \quad (11\text{-}10)$$

where:

C_{smpl} is the concentration of asbestos structures (of a defined size range or type) in the original sample (s/g);

S_{ch} is the number of structures (of the defined size range or type of interest) counted during the high magnification scan of the analysis;

A_f is the total area of the filter from which the specimen grids were prepared (mm^2);

%RD is the mass percent of respirable dust in the sample and is defined using Equation 11-9 (%);

N_{goh} is the number of grid openings scanned during the high magnification scan of the analysis;

A_{go} is the area of a single grid opening (mm^2); and

ΔM_f is the mass of respirable dust collected on the filter from which the specimen grids were prepared. It is defined using Equation A-7 (see Section A.2.2 of Appendix A) (g).

Structures that are longer than 5 µm in length are derived from combined counts collected during both the high and low magnification scans of an analysis. Calculate and report the concentration of long asbestos in the original sample based on the counts of asbestos structures that are derived as defined in Section 11.1.1 using the following relationship:

$$C_{smpl} = (S_{ch} + S_{cl}) * A_f * \%RD / [(N_{goh} + N_{gol}) * A_{go} * 100 * \Delta M_f] \quad (11\text{-}11)$$

where:

C_{smpl} is the concentration of asbestos structures (of a defined size range or type) in the original sample (s/g);

S_{cl} is the number of structures (of the defined size range or type of interest) counted during the low magnification scan of the analysis; and

N_{gol} is the number of grid openings scanned during the low magnification scan of the analysis.

Thus, for long structures, it is the total counts of structures observed over both the high and low magnification scans and the total area scanned (over both the low and high magnification scans) that are used to determine concentration.

As an option, asbestos concentrations may also be reported as a function of the mass of respirable dust in a sample using the following relationships. For short structures, use:

$$C_{dust} = S_{ch} * A_f / (N_{goh} * A_{go} * \Delta M_f) \quad (11\text{-}12)$$

where:

C_{dust} is the concentration of asbestos structures (of any defined size range or type) in the respirable dust of the sample (s/g_{dust}); and all other parameters are defined as described above.

Similarly, for long structures, use:

$$C_{dust} = (S_{ch} + S_{cl}) * A_f / [(N_{goh} + N_{gol}) * A_{go} * \Delta M_f] \quad (11\text{-}13)$$

11.4.2 Based on Specimens Prepared from Scrubber Water

As described in Section 11.4.1, procedures for determining the concentrations of asbestos structures in a sample differ slightly depending on whether the structures of interest are longer or shorter than 5 μm.

Structures that are shorter than 5 μm in length are derived only from the high magnification scan of an analysis. Calculate and report the concentration of short asbestos in the original sample based on the counts of short asbestos structures that are derived from the scrubber suspension as defined in Section 11.1.2 using the following relationship:

$$C_{smpl} = S_{ch} * A_f * \%RD * DF / (N_{goh} * A_{go} * 100 * M_{scrbr}) \quad (11\text{-}14)$$

where:

C_{smpl} is the concentration of asbestos structures (of a defined size range or type) in the original sample (s/g);

S_{ch} is the number of structures (of the defined size range or type of interest) counted during the high magnification scan of the analysis;

A_f is the total area of the filter from which the specimen grids were prepared (mm^2);

%RD is the mass percent of respirable dust in the sample and is defined using Equation 11-9 (%);

N_{goh} is the number of grid openings scanned during the high magnification scan of the analysis;

A_{go} is the area of a single grid opening (mm^2);

DF is the dilution factor by which the scrubber suspension had to be diluted to prepare specimen grids for analysis (derived as defined in Equation 11-3); and

M_{scrbr} is the mass of respirable dust collected in the scrubber suspension during the run (derived as defined in Equation 11-4).

Structures that are longer than 5 µm in length are derived from combined counts collected during both the high and low magnification scans of an analysis. Calculate and report the concentration of long asbestos in the original sample based on the counts of long asbestos structures that are derived from scrubber suspension as defined in Section 11.1.2 using the following relationship:

$$C_{smpl} = (S_{ch} + S_{cl})*A_f*\%RD*DF/[(N_{goh} + N_{gol})*A_{go}*100*M_{scrbr}] \quad (11\text{-}15)$$

where:

C_{smpl} is the concentration of asbestos structures (of a defined size range or type) in the original sample (s/g);

S_{cl} is the number of structures (of the defined size range or type of interest) counted during the low magnification scan of the analysis; and

N_{gol} is the number of grid openings scanned during the low magnification scan of the analysis.

As an option for analysis of the scrubber suspension, asbestos concentrations may also be reported as a function of the mass of respirable dust in a sample using the following relationships. For short structures, use:

$$C_{dust} = S_{ch}*A_f*DF/(N_{goh}*A_{go}*M_{scrbr}) \quad (11\text{-}16)$$

where:

C_{dust} is the concentration of asbestos structures (of any defined size range or type) in the respirable dust of the sample (s/g_{dust}); and all other parameters are defined as described above.

Similarly, for long structures, use:

$$C_{dust} = (S_{ch} + S_{cl})*A_f*DF/[(N_{goh} + N_{gol})*A_{go}*M_{scrbr}] \quad (11\text{-}17)$$

11.4.3 Procedure for Adjusting Asbestos Concentrations to Account for the Presence of Coarse Material in the Sampled Matrix

As indicated in Section 8.4, due to the need to incorporate field data into this calculation, a formal protocol designating who is to perform this calculation and how that individual is to obtain the needed field information must be defined at the start of a study using this method.

To provide a representative measure of the concentration of asbestos in an environmental matrix in which significant coarse material is found (i.e. more than 10% by weight), first derive an appropriate coarseness adjustment factor:

$$CF = M_{fine}/(M_{fine} + M_{coarse}) \quad (11\text{-}18)$$

where:

CF is the coarseness adjustment factor for a particular sample (dimensionless);

M_{fine} is the mass of fine material measured immediately after sieving the sample in the field (g); and

M_{coarse} is the mass of coarse material measured immediately after sieving the sample in the field (g).

Then, to determine the concentration of asbestos in the environmental matrix that was sampled, perform the following adjustment:

$$C_{mtrx} = CF * C_{smpl} \quad (11\text{-}19)$$

where:

CF is the coarseness adjustment factor for a particular sample (derived as defined above);

C_{mtrx} is the concentration of asbestos structures (of a defined size range or type) in the field matrix sampled (s/g); and

C_{smpl} is the concentration of asbestos structures (of a defined size range or type) in the sample sent to the laboratory (s/g). This is determined as described in Sections 11.4.1 and 11.4.2 above.

12.0 PERFORMANCE CHARACTERISTICS AND QUALITY CONTROL/QUALITY ASSURANCE REQUIREMENTS

12.1 METHOD PERFORMANCE

The method defined in this document achieves the target performance requirements defined in Section 2.1 at a cost that should be competitive with other procedures that might be designed to produce comparable information (Section 2.4).

12.1.1 Analytical Sensitivity

As indicated in Section 11.1, depending on sample characteristics, between 1 and 8 grid openings will likely have to be scanned at high magnification (20,000x) to achieve the target analytical sensitivity for total asbestos structures of $5x10^7$ s/g_{solid}. Similarly, between 15 and 133 grid openings will likely have to be scanned at low magnification (10,000x) to achieve the target analytical sensitivity for long asbestos structures (longer than 5 μm) of $3x10^6$ s/g_{solid}.

The defined sensitivities for total and long structures were easily achieved during the pilot study for the method (Berman et al 1994a).

12.1.2 Precision

Results of the pilot study for this method (Berman et al 1994a) indicate that, when 50 structures are counted, the average relative percent difference observed among eight sets of duplicate samples (four sets of duplicate samples analyzed by each of two laboratories) is 20%.

The precision of an asbestos measurement (in this case, expressed as the relative percent difference) should be inversely proportional to the square root of the number of structures counted. Given that 10 structures are likely to be counted at the target concentrations for this method (see Section 2.1.1) and, based on the precision observed in the pilot study when 50 structures are counted, it is expected that the average relative percent difference achievable for this method should be 43% at the target concentrations listed in Section 2.1.1. Thus, given the analytical sensitivities defined in Section 2.1.1 and the stopping rules defined in Section 11.1, this method is capable of achieving a level of precision that is comparable with the guidelines recommended in the CLP for the analysis of other analytes in soils and defined for this method in Section 2.1.2.

The precision observed during the pilot study for this method is based on specimen grids prepared from the suspension collected in the scrubber of the elutriator (Appendix A). It is expected that the precision achieved for analyses derived from scans of specimen grids derived by direct preparation of filters collected over the isokinetic sampling tube of the dust generator (see Appendix A) will be significantly worse. Procedures are therefore incorporated into this method in which the majority of samples will be analyzed based on preparation of specimen grids from filtered scrubber suspension with a subset of 5 to 10% simultaneously analyzed using specimen grids prepared by a direct transfer technique from filters collected over the isokinetic sampling tube of the elutriator (see Chapter 10). The latter is required to develop a regression relating these two types of preparations so that measurements can be evaluated using existing dose-response factors for asbestos (see Section 2.1.3); existing

dose-response factors tend to be based on the analysis of samples whose preparation corresponds most closely to a direct preparation.

NOTE

It is anticipated that a direct, linear relationship will be observed between asbestos concentrations derived, respectively, from scrubber suspension and from filters collected over the IST opening of the elutriator for samples collected from the same environmental matrix. However, there is little reason to expect that such a relationship will hold across samples collected from different environmental matrices. Therefore, samples to be employed to determine the relationship between asbestos measured from scrubber suspension and from filters mounted over the IST opening of the elutriator should be selected and evaluated separately for each environmental matrix sampled during a study for analysis using this method.

It must be recognized that compromises are required when developing a method of this type. The proposed reporting of samples prepared primarily by an indirect technique with a small subset of directly prepared samples (the latter used primarily to provide a link between the reported measurements and existing dose-response factors) represents such a compromise.

The proposed procedure allows for the determination of *relative* concentrations with maximum precision so that arbitrarily small differences in concentrations can be easily distinguished. Among other things, this will facilitate distinguishing upwind and downwind concentrations by a source. At the same time, somewhat lower precision is considered acceptable for estimating the *absolute* risk associated with any particular measurement.

NOTE

The uncertainty of the proposed regression to link results from directly and indirectly prepared samples is expected to be attributable almost exclusively to the limited precision of the directly prepared samples. Therefore, use of the proposed regression for estimating risks may not significantly increase the uncertainty of such a risk analysis beyond what would be associated with assigning an estimated risk to an asbestos concentration derived from a directly prepared sample in any case.

It is also assumed that a sufficient number of samples (i.e. a minimum of approximately 10) will be co-prepared and analyzed to allow reasonable confidence in the results of the regression.

12.1.3 Accuracy

Based on the results of the pilot study (Berman et al. 1994a), there appears to be a problem with laboratory bias during the counting of asbestos structures in support of this method. The bias is introduced specifically during the analysis of specimen grids because duplicate samples prepared by the same laboratory using identical procedures nonetheless yield significantly different counts by analysts from each of two laboratories.

Given that analysts within a single laboratory appear to be capable of achieving good agreement on counting (also demonstrated in the pilot study), the problem of between-laboratory bias will need to be addressed by the implementation of an aggressive inter-laboratory quality control (QC) program in which samples are regularly shared among laboratories and a procedure for verified counting is instituted. It may also be useful to promote meetings with discussion sessions in which analysts from different laboratories discuss the interpretation of structures viewed simultaneously (either directly from a microscope or from a video).

12.1.4 Asbestos Characteristics

As was observed during the pilot study (Berman et al. 1994a), this method is easily capable of preserving information concerning the distribution of the sizes and shapes of asbestos structures that are likely to be released from environmental matrices that are disturbed by natural or anthropogenic forces. As indicated in Section 2.1.3, such information is critical to evaluating the potential health effects of the asbestos dusts generated by releases from asbestos-containing matrices (see, for example, Berman and Crump 1989).

Interferences and limitations concerning the ability to identify and characterize asbestos structures using the counting and identification rules of the ISO Method, as adopted for this method (Section 11.1), are described in the ISO Method (Chatfield 1993).

12.1.5 Reporting Requirements

As indicated in Section 11.4, the concentrations of asbestos structures (of any defined size or type) that are measured using this method can be easily reported either as a function of the mass of the sampled material or as a function of the mass of respirable dust in the sampled material. This should allow sufficient flexibility to facilitate use of results from this method in concert with any of the fate and transport models that may be employed to predict exposure (Section 2.1.4).

12.2 QUALITY CONTROL REQUIREMENTS

The quality assurance/quality control (QA/QC) requirements indicated in the ISO Method (Chatfield 1993) shall be considered relevant and appropriate when using this method. In addition, the following blank and duplicate/replicate schedule shall be employed when running samples using this method.

12.2.1 Blanks

The following blanks shall be collected routinely in concert with use of this method:

- lot blanks or filter blanks. Two filters from each lot of 50 filters obtained from the manufacturer shall be prepared using a direct transfer procedure and analyzed to assure that background contamination on the filters does not exceed 10s/mm^2 (Section 6.6). Only filters from lots whose blanks pass the defined criterion shall be used in support of this method;

- laboratory blanks. A sufficient number of laboratory blanks shall be collected, prepared using a direct transfer technique and analyzed to show that the room in

which bulk samples are handled and prepared satisfy the requirements defined in Section 10.6 of Chatfield and Berman (1990). When laboratory blanks indicate that room air is out of compliance with the stated criterion, use of this method is to cease until appropriate corrective actions are completed;

- field blanks. Field blanks shall be collected during any sample collection activities performed in association with use of this method. The number of such blanks to be collected and the schedule for their analysis shall be determined based on the complexity of the anticipated sampling scheme and shall be defined as part of the sampling plan for the site. QC criteria for field blanks will also be set as part of the planning for the study;

- equipment blanks. Equipment blanks are collected at the beginning of each run of the dust generator, as described in Section 9.4.3. Equipment blanks do not generally need to be analyzed on a regular basis but shall be stored in case their use is required to help determine the source of contamination that may be discovered by some other means;

- run blanks. Run blanks are also collected at the beginning of each run of the dust generator, as described in Section 9.4.3. One run blank shall be analyzed routinely for the first run completed on any particular sample. The remaining run blanks generated in association with a particular sample shall be stored in case their use is required to help determine the source of contamination that may be discovered by some other means.

  Should asbestos structure counts on run blanks exceed the target criterion of 10 s/mm^2, use of this method shall cease until appropriate corrective actions have been completed and new run blanks are shown to achieve the stated criterion; and

- scrubber blanks. Scrubber blanks shall be derived by collecting a 1 ml aliquot of scrubber liquid (after the scrubber is loaded and assembled for a run but before heating of the scrubber is initiated), diluting the aliquot to 10 ml, and completing the preparation of the diluted aliquot as described in Section 9.4.6. Scrubber blanks do not need to be analyzed routinely but shall be stored in

  case their use is required to help determine the source of contamination that may be discovered by some other means.

In addition to the above listed blanks that must be collected routinely in association with use of this method, the following may prove helpful for identifying the source of any contamination that might be detected in association with use of the dust generator:

- modified run and scrubber blanks. Modified run and scrubber blanks may be generated by setting up and operating a clean dust generator *without* sample. Filters may be collected from either the ME or the IST opening of the dust generator at any point of such a run. Similarly, aliquots of scrubber liquid may be withdrawn from the scrubber at any point. Such blanks may prove useful for determining whether contamination is being introduced by any of the components of an operating dust generator (including, for example, the constant

humidity solution, the rotating tumbler, the elutriator tube, the air transfer lines, and/or the glassware or liquid of the boiling scrubber); and

- post-run scrubber blanks. A post-run scrubber blank may be generated by repeating the rinse of the transfer lines, condensers, and scrubber flask immediately after the quantitative rinse conducted for a particular sample (see Section 9.4.6). The resulting liquid must then be weighed, diluted quantitatively, and prepared in the same manner as described for the scrubber suspension of a sample (Section 9.4.6). The normalized concentration of asbestos structures found in such a blank shall represent no more than 10% of the concentration of asbestos structures observed in the sample prepared immediately prior to collecting the post-run blank. Higher blank concentrations shall be considered unacceptable. If blank concentrations are observed, procedures for quantitative rinsing shall be reviewed, modified, and tested until losses can be shown to be acceptable.

NOTE

Asbestos observed in post-run blanks constitute asbestos that is lost from a sample during preparation.

12.2.2 Duplicates/Replicates

A fixed fraction (5 to 20%) of the samples collected in the field in support of this method shall be collected as spatial duplicates (two samples collected at immediately adjacent locations). These shall be labeled and sent to the laboratory in such a manner so as to assure that laboratory personnel cannot identify them as duplicates. The frequency of collection of spatial duplicates shall be defined as part of the sampling plan for the site. Comparison of the results of the analysis of such samples provides a measure of all of the components of total precision except population variability.

As indicated previously (Section 8.2.1), 100% of the samples shipped from the field are to be shipped as duplicate pairs. The laboratory shall randomly select 10% of the duplicate samples shipped from the field and shall analyze both samples of the pairs so selected. Comparison of the results of the analysis of such samples, which are homogenized splits of the same sample, provides an indication of the precision achieved by sample preparation and analysis.

Should analysis of duplicate pairs indicate an unacceptable degree of variability (i.e. a relative percent difference greater than 50%), replicate counts shall be performed on designated samples by multiple analysts in the laboratory (or by the same analyst on different days). Laboratory management shall assign such counts so as to assure that analysts cannot determine which counts are replicates. Results of such replicate counts shall serve to distinguish whether the major source of variability observed among duplicate pairs is due to analysis or to sample preparation. Appropriate corrective actions may then be devised.

13.0 REPORTING REQUIREMENTS

13.1 FIELD AND LABORATORY NOTEBOOKS

Over the course of the project, information critical to the proper reporting and interpretation of each sample analysis will be developed both in the field and in the laboratory. Formal procedures are required to preserve such information and to allow for the documentation of attendant information that, while not employed directly in the calculation of results, may provide insight into the interpretation of such results.

13.1.1 Field Notebooks

During sample collection, detailed notes will be kept in a standard field or laboratory notebook that is bound and pre-paginated. All entries to the notebook are to be dated and initialed. Information to be recorded during sample collection should include (but is not limited to):

(a) reference to this method;

(b) the project title, identification of the site, and the names and titles of field personnel participating in the sampling effort;

(c) identification of each sample or fraction of each sample handled in the field;

(d) the date and time that each sample was collected and the time interval during which each sample was prepared in the field;

(e) the location from which each sample was collected and the manner in which sample locations were selected (including reference to the sampling plan under which sampling was conducted);

(f) a general description of the physical appearance of the materials sampled;

(g) the types of equipment and the procedures employed to collect each sample;

(h) a summary of the procedure employed for field preparation of each sample (including, for example, the number of passes through a riffle splitter employed for homogenization, and the number of splits required to reduce the sample to the size required);

(i) the relevant identities and weights of each fraction of sample handled during field preparation (including specifically the weights of the coarse and fine fractions separated from each sample during sieving);

(j) other relevant field observations (including, for example, the meteorological conditions under which sampling was conducted); and

(k) the identification, weight, and intended destination of each sample shipped from the field.

13.1.2 Laboratory Notebooks

During sample handling, preparation, and analysis, detailed notes will be kept in a laboratory notebook that is bound and pre-paginated. All entries to the notebook are to be dated and initialed. Information to be recorded during sample preparation and analysis should include (but is not limited to):

(a) reference to this method;

(b) identification of each sample or fraction of each sample received from the field and the date and time that each sample was received;

(c) identification of all laboratory personnel who participate in the preparation and analysis of samples and the specific operations performed by each;

(d) identification of reagents, equipment, and supplies employed during sample handling, preparation, and analysis;

(e) relevant material weights and/or volumes;

(f) the lot number and manufacturer of the filters employed during sample preparation;

(g) a summary of the procedure employed for laboratory preparation of each sample (including, for example, the number of passes through a riffle splitter employed for homogenization and the number of splits required to reduce the sample to the size required);

(h) the relevant identities and weights of each fraction of sample handled during laboratory preparation;

(i) the setup conditions employed for the dust generator (including, for example, the identity of the salt employed for humidity control, air flow conditions, and the setting on the variable voltage transformer employed for the scrubber);

(j) the date and starting time, description (including, primarily, the tumbler rotational speed), and sample identification for any run conducted on the dust generator;

(k) the weight and identity of the sub-sample placed in the dust generator;

(l) the identities, time intervals of collection, the starting and ending weights, and the net weights of filters collected during a run on the dust generator that are to be used to determine the rate of dust generation;

(m) the figures and calculations employed to determine the rate of dust generation and the quantity of dust in the sample;

(n) the identity, time interval of collection, starting and ending weights, and the net weights of filters collected during a run on the dust generator that are to be used to generate specimen grids for asbestos analysis;

(o) the identity, time interval of collection, the weight/volume, dilution factor, aliquot identification, and filter identification for the collection, handling, and filtering of scrubber suspension;

(p) the identity of all filter sections used for preparation of specimen grids and the identity of the specimen grids (include a description of the sector and radial distance from the center of the filter represented by each section);

(q) energy levels and settings for instruments employed for analysis;

(r) flow rates, pressures, temperatures, and other relevant physical parameters that potentially impact procedures;

(s) room conditions (i.e. temperature, relative humidity, and ventilation rates) prevailing during sample preparation;

(t) other relevant observations (including, for example, any difficulties encountered during preparation and analysis and any procedural changes incorporated into the method that are necessitated by such difficulties);

(u) documentation of all calculations performed in support of preparation and analysis;

(v) all relevant QA/QC measurements (including the results of the analysis of all blanks, duplicates, and replicates–see Chapter 12); and

(w) a detailed time log of events including the time that particular procedures are initiated and completed for each sample.

13.2 FIELD ACTIVITIES REPORT

To assure that the field information required to complete estimation of dust and asbestos concentrations and release rates are provided to the data users, a field activities report must be completed and must be submitted to the laboratory along with the corresponding samples. Laboratory personnel are then to attach this report directly to their batch report, which shall cover the corresponding batch of samples.

The field activities report shall include the following for each sample batch, at a minimum:

(a) the project title and the identification of the site;

(b) reference to this method;

(c) reference to the sampling and analysis plan under which samples were collected;

(d) a brief description of the objectives for sampling;

(e) a brief description of the procedures employed for selecting sampling locations and the motivation for employing such procedures;

(f) for each sample in the batch accompanying the report:

- the identifier for each sample fraction submitted to the laboratory;
- the identifier for the original sample from which each submitted sample fraction was derived;
- the type of equipment and reference to (or a brief description of) the procedures employed for collection of the original sample;
- the coordinates at which each original sample was collected[36];
- the total mass of the original sample from which each sample fraction originated and the masses of the coarse and fine fractions separated during sieving of the sample in the field;
- a brief description of the procedures employed for sample homogenization and for sample splitting;
- the date and time that each sample was collected and the date and time that each sample fraction was prepared in the field; and
- a brief discussion of any deviation from the sampling and analysis plan not covered in e.

An example of the format to be employed for a field activities report is provided in Figure 13-1.

13.3 SAMPLE ANALYSIS REPORT

The sample analysis report for each sample shall include the following at a minimum:

(a) reference to this method;

(b) reference to the sample identification and batch number for the sample;

(c) the date and site from which the sample was collected;

(d) the weights and identities of the coarse and fine fractions of the sample and the sub-sample of the fine fraction sent for analysis;

(e) the weights and identities of any splits or other fractions of the sample generated during laboratory preparation;

(f) the weight and identity of the sub-sample placed in the dust generator;

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[36] If the submitted sample fraction is a sub-sample of a composite, what should be described here is the specific area of concern (or portion thereof) that is intended to be represented by the composite).

FIGURE 13 - 1
FORMAT FOR THE FIELD ACTIVITIES REPORT

Name of field activities contractor
Address of contractor
Contact Name
Telephone Number

Report Title:
Report Number:

Date

PROJECT/SITE:
Name
Address

METHODS AND PROCEDURES:
Field Investigation Design: *(Reference the sampling plan)*
Sample Collection and Handling: *(Reference this method)*

SAMPLING OBJECTIVES:
(Complete a brief description here)

SAMPLING LOCATION SELECTION PROCEDURE:
(Complete a brief description here)

SAMPLE DATA

Field Sample Number	Location Identifiers	Mass of Sample	Mass Fine Fraction	Mass Coarse Fraction	Mass Sample Split	Mass Duplicate Split	Sample Split ID	Duplicate Split ID	Date Sampled	Time Sampled	Comments: *(include identification of all field sampling and field preparation procedures employed)*

An example of the format to be employed for a sample analysis report is presented in Figure 13-2.

13.4 SAMPLE BATCH REPORTS

In addition to the sample analysis report for each sample, provide a summary page for each batch of samples representing an entire project. The summary sheet shall include:

(a) the project title;

(b) reference to this method;

(c) the date that samples were collected, the date they were received by the laboratory, and the date they were analyzed;

(d) a summary listing of sample results including:

- the sample number;
- the estimated concentration of respirable dust in the sample;
- the analytical sensitivity achieved for each size/type category of interest;
- the total number of structures of each size/type category of interest counted;
- the concentration of asbestos structures of each size/type category of interest in the sample and in the respirable dust of the sample (both reported along with corresponding 95% confidence limits); and
- the concentration of asbestos structures of each size/type category of interest estimated for the environmental matrix that was sampled in the field (reported along with corresponding 95% confidence limits).

An example of the format to be employed for sample batch reports is presented in Figure 13-3.

FIGURE 13-2
SAMPLE ANALYSIS REPORT FORMAT

Laboratory Name
Laboratory Address
Laboratory Contact
Telephone Number

Report Date
Project Name *(Optional)*

METHODS:
(reference this method)

Date Analysis Started (M/D/Yr)
Date Analysis Completed (M/D/Yr)
Analyst(s) Initials

Laboratory Sample No.
Field Sub-Sample Identification No.
Field Preparation Technique (Attach a Copy of the Relevant Field Activities Report)
Additional Laboratory Preparation Procedures *(describe any employed)*
Sample Drying
Sample Splitting
Other

TEM Analysis:
Effective Area of Analytical Filter (sq mm)
(Indicate whether from Scrubber or from IST Opening)
Magnification
Grid Opening Area (sq mm)
Number of G.O. Scanned
Asbestos Structure Size and Type Categories of Interest *(see Chatfield 1993)*
Minimum Acceptable Structure Identification Category *(see Chatfield 1993)*

Dust Generator
Mass of Sample Tumbled (g)
Air Flow Rate Through ME Opening of Dust Generator (ml/min)
Air Flow Rate Through IST Opening of Dust Generator (ml/min)
Air Flow Rate Through Scrubber (ml/min)
Estimated Total Air Flow Rate Through Elutriator (ml/min)

Total Mass of Dust Collected on Dust Filters (g)
Time of Dust Collection *(24 hour clock)* at 30 rpm
Start:
Stop:
Time of Dust Collection *(24 hour clock)* at 60 rpm
Start:
Stop:
Estimated first-order rate constants for dust generation (min^{-1})
At 30 rpm:
At 60 rpm:
Estimated Starting Mass of Respirable Dust in Sample (g)
(Attach time plots and calculations).

Samples from the Scrubber Suspension
Total Volume of Scrubber Suspension (ml)
Estimated Mass of Dust Collected in Scrubber Suspension (g)
Volume of Aliquot Withdrawn from Scrubber Suspension (ml)
Volume into which Scrubber Aliquot Diluted (ml)
Dilution Factor (dimensionless)
Volume of Aliquot Filtered *(from Diluted Suspension)* (ml)

Samples from the Isokinetic Sampling Tube (IST) Opening of the Dust Generator
(indicate whether 30 or 60 rpm run)
Time of Collection *(24 hour clock)*
Start:
Stop:
Estimated Mass of Dust Collected on Filter

FIGURE 13-2
SAMPLE ANALYSIS REPORT FORMAT (Cont.)

Laboratory Name

Report Date

Laboratory Sample No.

	Low Magnification	High Magnification
Chrysotile Asbestos Analysis Results:		
No. of Total Chrysotile Asbestos Structures	XXX	
No. of Long (> 5 um) Chrysotile Asbestos Structures		
No. of Total Chrysotile Asbestos Fibers/Bundles	XXX	
No. of Long (> 5 um) Chrysotile Asbestos Fibers/Bundles		

	Low Magnification	High Magnification
Amphibole Asbestos Analysis Results:		
No. of Total Amphibole Asbestos Structures	XXX	
No. of Long (> 5 um) Amphibole Asbestos Structures		
No. of Total Amphibole Asbestos Fibers/Bundles	XXX	
No. of Long (> 5 um) Amphibole Asbestos Fibers/Bundles		

(Indicate Amphibole Mineral Type)

ESTIMATED CONCENTRATIONS OF RELEASABLE ASBESTOS IN SAMPLE

	Conc.	95%UCL
Total Chrysotile Structures per g Sample:		
Total Amphibole Structures per g Sample:		
Total Asbestos Structures per g Sample:		
Long Chrysotile Structures per g Sample:		
Long Amphibole Structures per g Sample:		
Long Asbestos Structures per g Sample:		

Estimated Analytical Sensitivity: *(structures/g sample)*

ESTIMATED CONCENTRATIONS OF RELEASABLE ASBESTOS IN RESPIRABLE DUST OF SAMPLE

	Conc.	95%UCL
Total Chrysotile Structures per g Dust:		
Total Amphibole Structures per g Dust:		
Total Asbestos Structures per g Dust:		
Long Chrysotile Structures per g Dust:		
Long Amphibole Structures per g Dust:		
Long Asbestos Structures per g Dust:		

Estimated Analytical Sensitivity: *(structures/g dust)*

(Attach a Copy of the TEM Raw Data Sheets)

FIGURE 13–3
SAMPLE BATCH REPORT FORMAT

Laboratory Name
Laboratory Address
Laboratory Contact
Telephone Number

Report Date
Project Name *(Optional)*

METHODS:
(reference this method)

RELEASABLE ASBESTOS IN RESPIRABLE DUST

Laboratory Sample I.D.	Respirable Dust Conc (g/g smpl)	Total Asbestos Analytical Sensitivity (s/g dust)	Total Asbestos Conc (s/g dust)	Total Asbestos 95% UCL (s/g dust)	Long Asbestos Analytical Sensitivity (s/g dust)	Long Asbestos Conc (s/g dust)	Long Asbestos 95% UCL (s/g dust)	Dust Generation Rate (at 30 rpm) (min^{-1})	Dust Generation Rate (at 60 rpm) (min^{-1})	Comments:

RELEASABLE ASBESTOS IN LABORATORY SAMPLES

Laboratory Sample I.D.	Respirable Dust Conc (g/g smpl)	Total Asbestos Analytical Sensitivity (s/g smpl)	Total Asbestos Conc (s/g smpl)	Total Asbestos 95% UCL (s/g smpl)	Long Asbestos Analytical Sensitivity (s/g smpl)	Long Asbestos Conc (s/g smpl)	Long Asbestos 95% UCL (s/g smpl)	Comments:

RELEASABLE ASBESTOS IN FIELD SAMPLE MATRICES

Laboratory Sample I.D.	Field Sample I.D.	Mass Fine Fraction (g)	Mass Coarse Fraction (g)	Adjusted Respirable Dust Conc (g/g mtrx)	Adjusted Total Asbestos Conc (s/g mtrx)	Adjusted Total Asbestos 95% UCL (s/g mtrx)	Adjusted Long Asbestos Conc (s/g mtrx)	Adjusted Long Asbestos 95% UCL (s/g mtrx)	Comments:

14.0 REFERENCES

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APPENDIX A:

CONSTRUCTION AND OPERATION OF A DUST GENERATOR

APPENDIX A:
CONSTRUCTION AND OPERATION OF A DUST GENERATOR FOR ISOLATING THE RESPIRABLE FRACTION OF MATERIAL FROM SOILS OR OTHER BULK SAMPLES

The dust generator incorporated into this method for the determination of asbestos in soils and bulk materials is designed to isolate the respirable fraction of material (including the releasable fraction of respirable asbestos) that is present within the matrix of the parent sample. A description of the apparatus is provided below along with a brief discussion of the theory of its operation. Figures depicting design of the prototype are also provided in the last section of this appendix to facilitate design and construction of similar equipment.

A.1 DUST GENERATOR DESCRIPTION

The dust generator is composed of a tumbler, a vertical elutriator, a dust collection system, and a scrubber. A schematic diagram of the apparatus is presented in Figure A-1 and a photograph of the apparatus is shown in Figure A-2. The appurtenant equipment required to operate the dust generator is also shown in Figure A-2. This includes:

- a DC motor to drive the tumbler;
- a constant humidity chamber to control the humidity of the air that flows into the dust generator. This is the clear plastic box enclosing the tumbler in the photograph;
- the pumps required to create an air flow through the dust generator and the flow controllers required to monitor and apportion air flow through the various filters and the scrubber; and
- the heating mantle, variable voltage transformer, and cooling towers required for the scrubber.

A.1.1 The Tumbler

The tumbler is located in the clear plastic enclosure at the bottom of the dust generator (Figures A-2 and A-3). It is a long shallow tube of square cross section that is approximately 1 and 1/8 inches in height and width and has an overall length of 10 inches (Figure A-4).

The tumbler is driven by a variable speed DC motor, which rotates the tumbler around its long axis. The tumbler's rotation rate can be varied over the range of 10 to 150 revolutions per minute (rpm). The DC motor is attached to the tumbler using a slip-on type flexible coupling so that they can be readily detached simply by pulling the motor away from the tumbler. The tumbler can also be readily detached from the connection tube to the elutriator; they are connected with a slip fit over the outer race of the ball bearing assembly that is welded to the end of the tumbler (Figure A-4).

FIGURE A-1

SCHEMATIC OF DUST GENERATOR

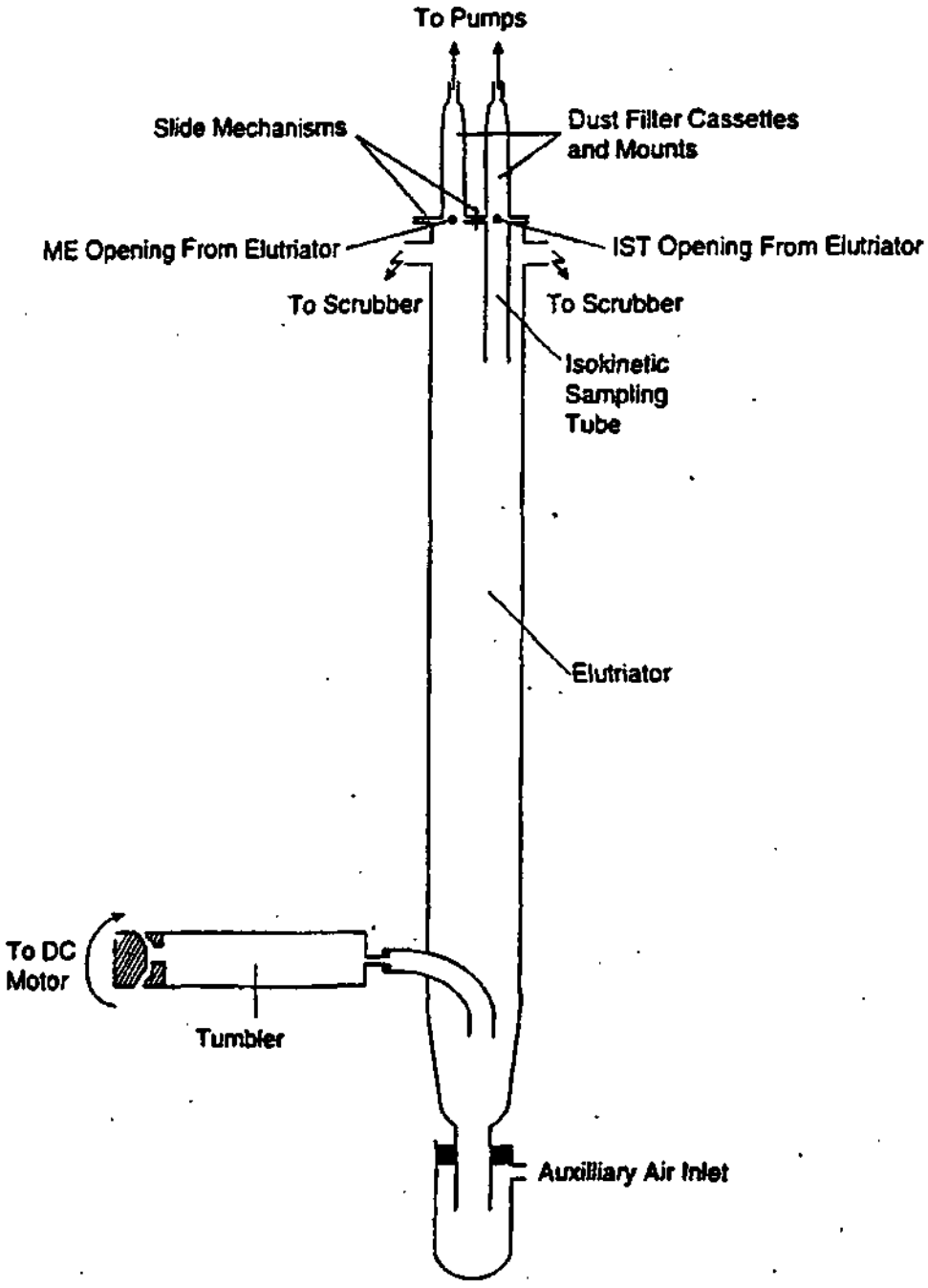


FIGURE A-2

THE DUST GENERATOR

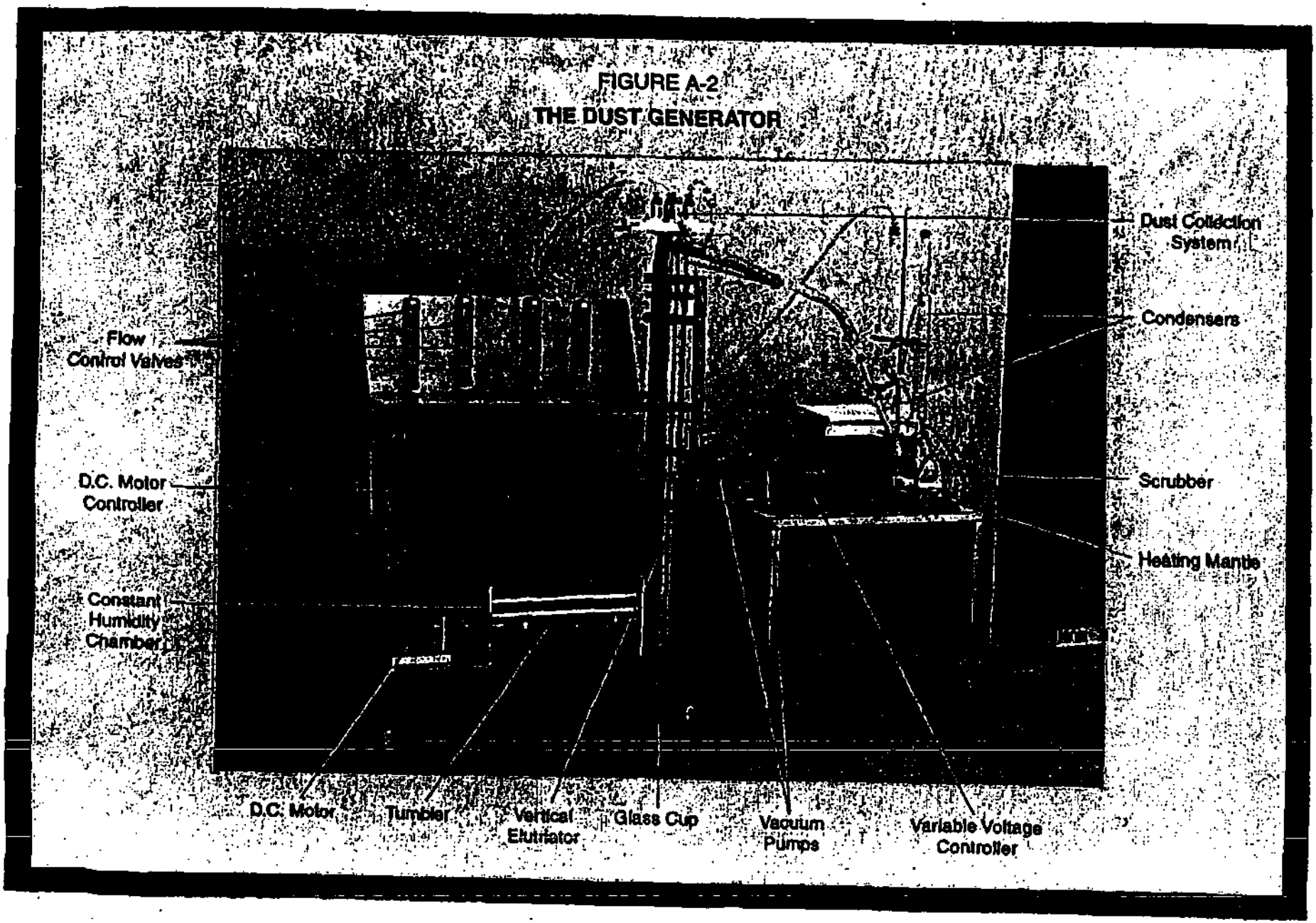


FIGURE A-3

TUMBLER IN CONSTANT HUMIDITY CHAMBER

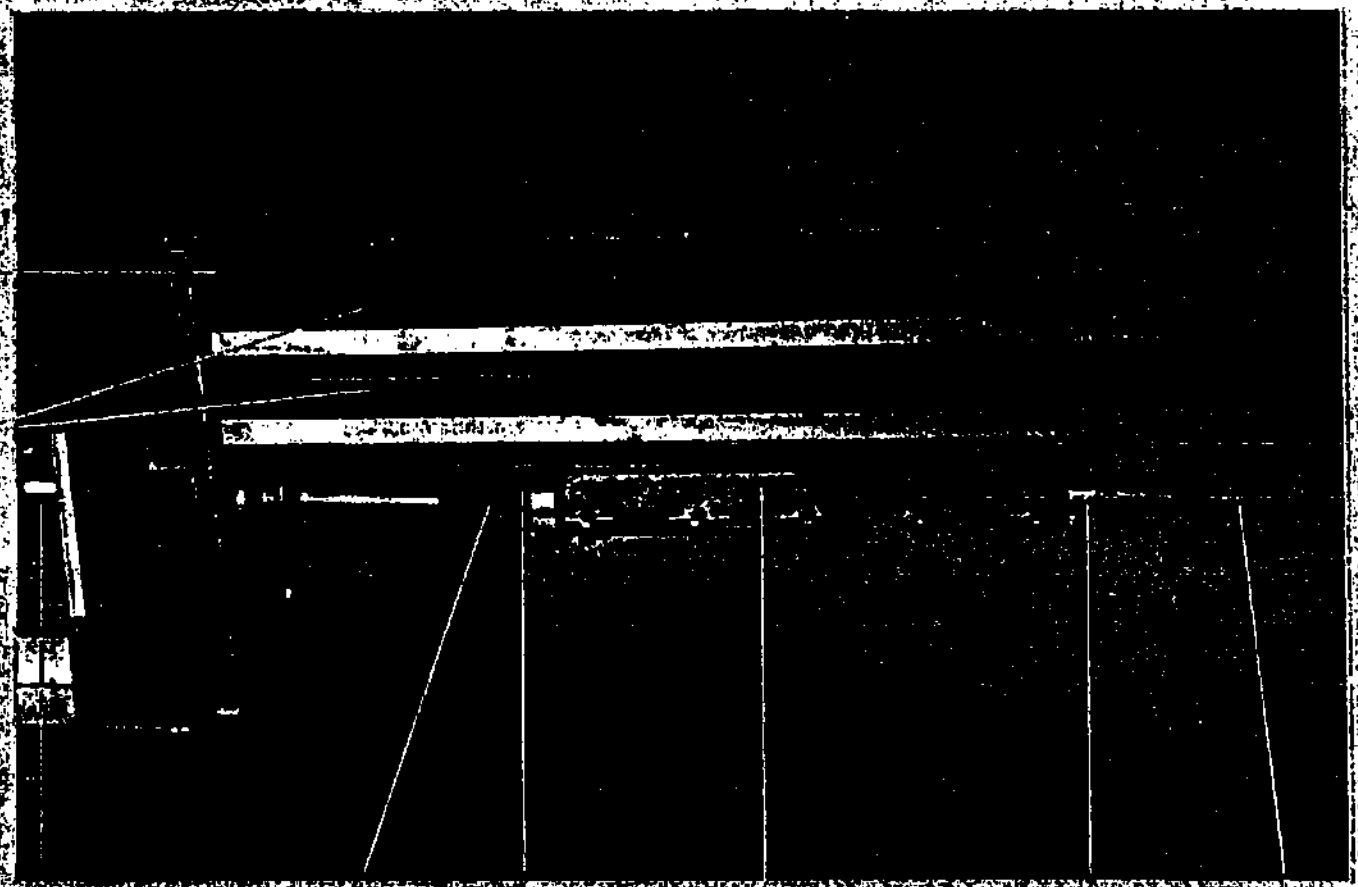
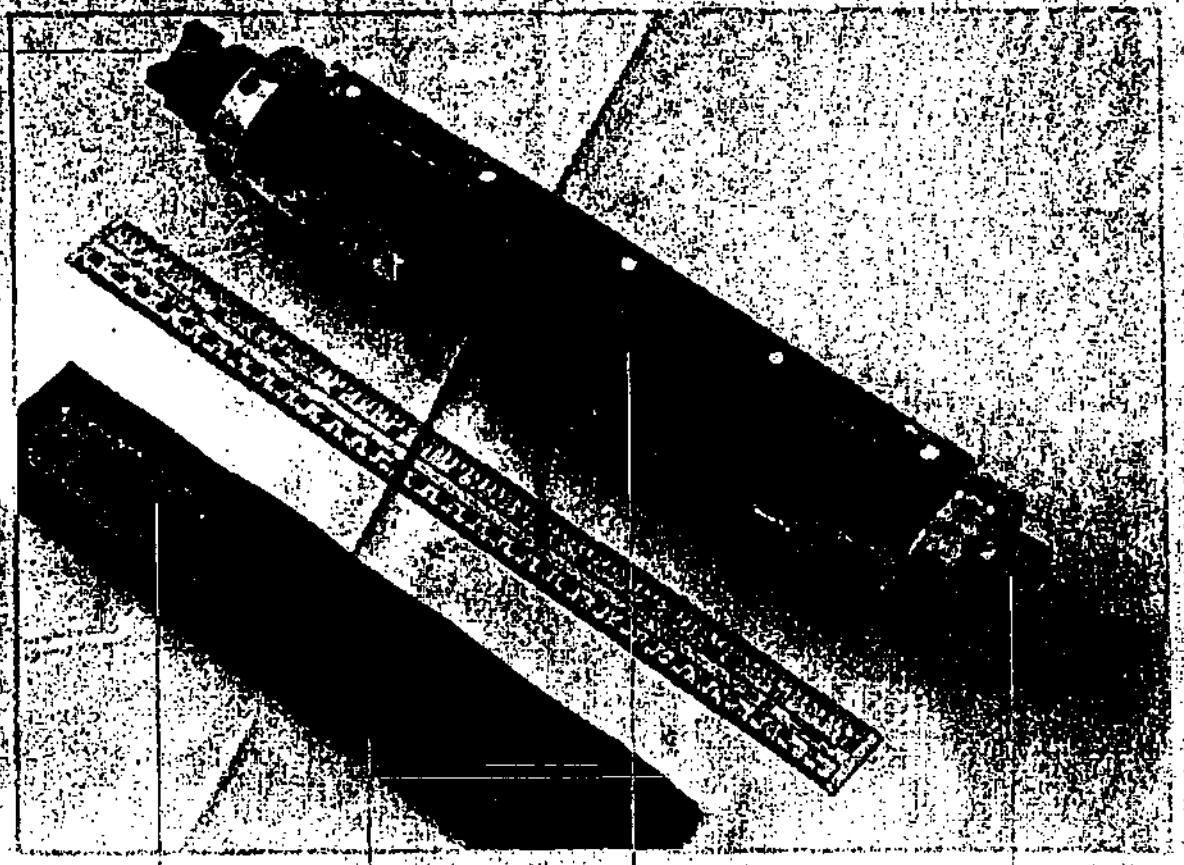


FIGURE A-4

TUMBLER ASSEMBLY



NOTE

The seal between the tumbler and elutriator need not be air tight. In fact, a *small* leak at this fitting will reduce the chance that particles discharged from the tumbler will settle in the entrance tube to the elutriator. However, such a leak must not be more than 10% of the total airflow through the tumbler.

Once the tumbler has been detached from the motor and elutriator, it can simply be lifted out of the constant humidity chamber.

The top cover of the tumbler is secured with 10 screws and can be removed so that the tumbler can be loaded or cleaned (Figure A-4). A rubber gasket is employed to assure an airtight seal between the tumbler cover and body.

A.1.2 The Constant Humidity Chamber

The plastic enclosure that houses the tumbler and isolates it from outside air, except for an opening at the top front of the enclosure, is designed as a constant humidity chamber (Figures A-2 and A-3). Air drawn into the front of the enclosure flows successively over two trays (located on shelves at the top of the enclosure) before being drawn into the tumbler. The trays are designed to hold a saturated salt solution that is selected to exhibit a vapor pressure equal to the relative humidity desired for running the dust generator. The path length over the two trays is designed to assure that air drawn through the device will come to equilibrium at a relative humidity within a few percent of the desired value (assuming that the outside air exhibits a starting humidity within the range common to Pasadena, California[1]); the path length must be sufficient to allow adequate exchange of vapor between the salt solution and the moving air.

For most studies, a relative humidity of approximately 50% should be employed because this is the humidity at which emission of asbestos is expected to be maximum (Zimon, A.D. 1982). At higher humidities, air moisture tends to wet surfaces so that aggregation decreases particle release. At lower humidities, electrostatic effects tend also to cause aggregation and, therefore, also to decrease emissions. A saturated solution of potassium carbonate dihydrate is recommended for most applications because such a solution maintains equilibrium with air at 43% relative humidity at room temperature (20° C) with less than 1% change in humidity over a range of temperatures varying by several degrees on either side of room temperature.

For special applications of this method, where dust generation is to be run at a different relative humidity than that produced over a saturated solution of potassium carbonate dihydrate, the conditioning pans in the dust generator may be filled with a saturated solution of another salt. Such salts may be selected to yield any of a broad range of conditions. For example, the International Critical Tables provides a list of saturated salt solutions that maintain equilibrium with a broad range of relative humidities in air.

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[1] The prototype device was constructed and tested in Pasadena. The design of the constant humidity chamber may have to be modified slightly (e.g. by increasing the number of trays) for operation in other locales.

A.1.3 The Vertical Elutriator

The vertical elutriator is the tall metal cylinder visible in Figure A-2. Its dimensions were selected to assure that the path length traversed by particles in the elutriator is at least 10 times the diameter of the elutriator (in the direction perpendicular to the air flow). Thus, channeling is expected to be minimized (Dennis, R 1976).

The elutriator separates respirable from non-respirable particles and respirable particles are passed on to dust collection filters and the scrubber. Air flow within the elutriator is adjusted to a velocity such that only those particles in the respirable size range will be lifted to the top. The velocity of air selected for the elutriator determines the rate of air flow through all of the other parts of the dust generator.

The main body of the elutriator is composed of two, 20 in. sections of 4-in. i.d. stainless steel tubing with a tapered, o-ring flange welded to each end of each tube (Figure A-5a and b). These flanges allow the two halves of the main body to be connected to each other and to the top and bottom assemblies of the elutriator using quick-release, pressure clamps (Figure A-5a).

The entrance tube to the elutriator (from the tumbler) is pointed downward at its exit point in the bottom of the elutriator (Figures A-1 and A-5b). This geometry was selected to prevent introduction of a large concentration of particles into the bottom center of the elutriator. This geometry should also promote a flatter velocity profile in the air traveling up the elutriator. It is expected that respirable particles will make the upward turn because the taper at the bottom of the elutriator promotes a higher velocity profile at this location than in the main body of the elutriator (where the air velocity is just sufficient to lift particles in the respirable size range but no larger).

The bottom assembly of the elutriator has also been designed to allow the removal of coarse particles while assuring that respirable particles remain in the elutriator air stream (Figure A-6a and b). This is to minimize buildup of a zone at the bottom of the elutriator of a high concentration of coarse particles through which respirable particles would have to pass before moving up the elutriator to the dust collection system. Thus, it is expected that collisions between respirable particles and coarse particles (with subsequent loss of respirable particles due to aggregation) are minimized.

An opening at the bottom of the elutriator allows coarse particles to pass into a removable, glass cup (Figures A-1 and A-6a and b). The glass cup has a small side arm that is connected to a two ft. piece of one quarter in. i.d. Tygon tubing. The far end of the Tygon tube is open to the air. It has been found that, as long the Tygon tube is not crimped or otherwise blocked, air drawn through this tube is just sufficient to prevent respirable particles from passing through the bottom opening of the dust generator into the glass cup while allowing larger particles to do so.

There are four openings where air may exit from the top assembly of the elutriator (Figures A-1 and A-7a and b). The two side openings lead into the scrubber (described below). The two top openings lead into the air filter cassettes of the dust collection system through two different paths; one draws air isokinetically from the uniform portion of the elutriator through a long, thin-walled tube and the other draws air from the top, tapered part of the elutriator (Figures A-1 and A-7a and b). For ease of reference, the opening in the elutriator that draws

FIGURE A-5

MAIN BODY OF VERTRICAL ELUTRIATOR

a. Top Section

b. Bottom Section

FIGURE A-6

BOTTOM ASSEMBLY OF VERTICAL ELUTRIATOR

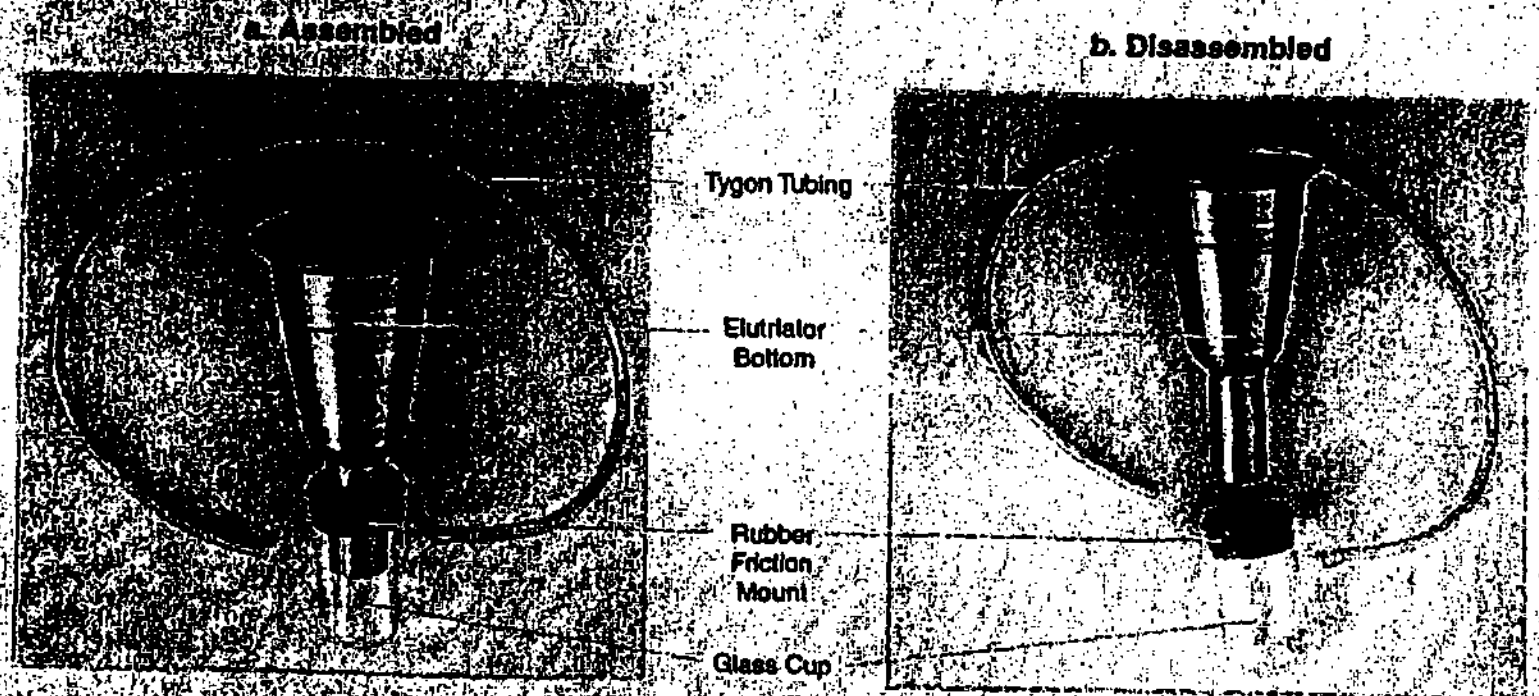
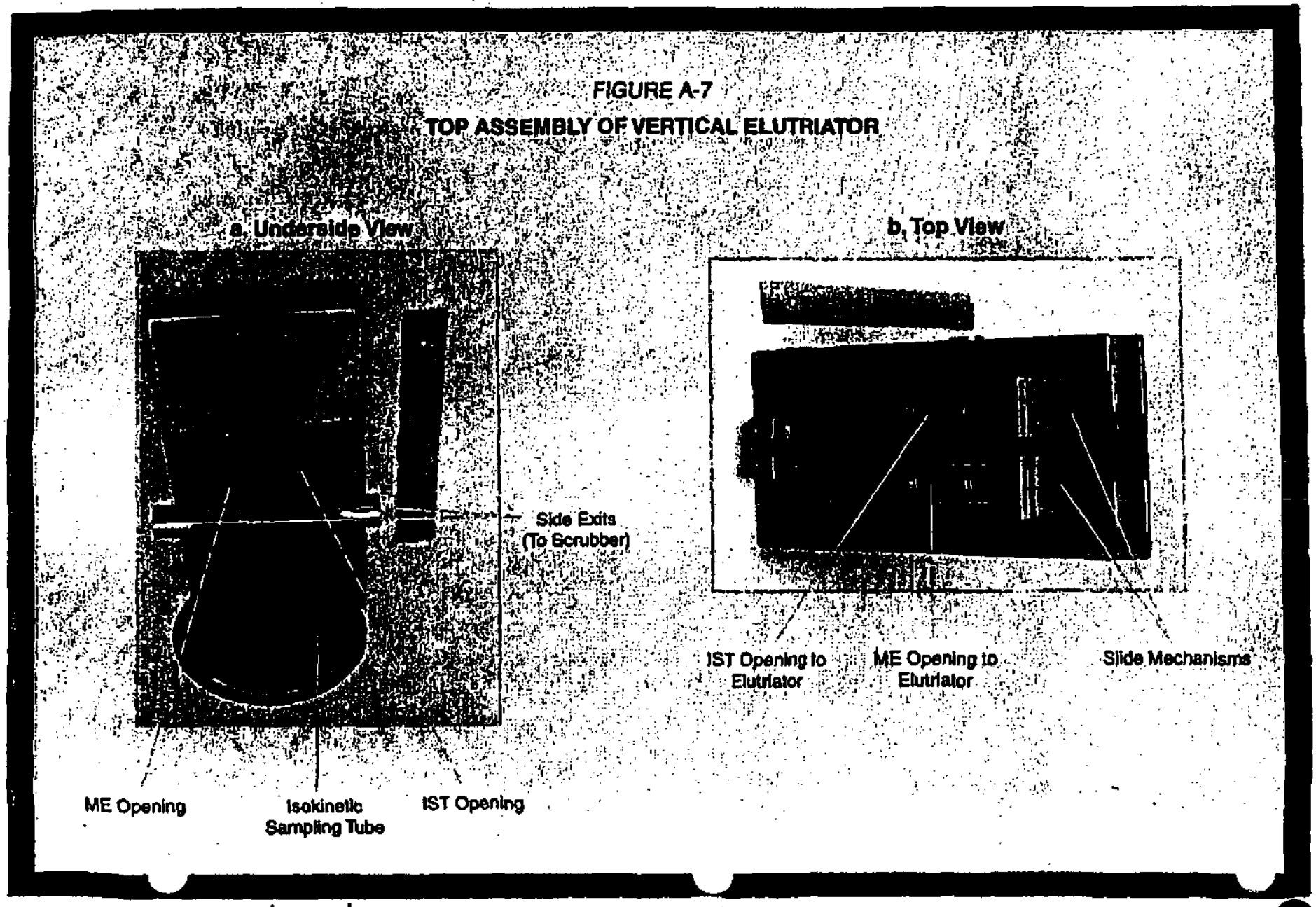


FIGURE A-7

TOP ASSEMBLY OF VERTICAL ELUTRIATOR



air through the isokinetic sampling tube is labeled the "IST" opening in this document. The opening that draws air from the top of the main body of the elutriator is labeled the "ME" opening. The dust collection system mounted over both of the top openings of the elutriator is described below.

A.1.4 The Dust Collection System

The dust collection system consists of two mounts for filter cassettes welded to each of two sliding mechanisms, which in turn are mounted directly over the exit openings of the top assembly of the vertical elutriator (Figure A-8a and b). An underside view of the tapered top piece of the elutriator was also shown in Figure A-7a in which the isokinetic sampling tube is visible.

The sliding mechanisms permit the filter cassettes aligned over the ME and the IST openings of the elutriator, respectively, to be changed with minimal disturbance of the air flow; at either of the two extreme positions in the travel of each slide mechanism, one of the two cassette mounts are aligned over the corresponding exit opening of the elutriator and the other is effectively isolated from the air flow. Details of the sliding mechanisms and filter mounts are shown in Figures A-7b and A-8a and b.

NOTE

The slides are sealed against the elutriator with o-rings. These must be inspected periodically for wear and replaced if worn.

The design of the filter mounts in the dust collection system proved to be important to the performance of the dust generator; it is critical that these mounts be leak tight. The design that was ultimately adopted is depicted in Figure A-9a and b. The filter mounts each consist of a tapered aluminum base that is glued to the bottom half of a commercially available filter cassette.

The aluminum base of a filter mount is sealed in each of the openings of the slide mechanisms with the o-rings that are visible in Figure A-7b. The bottom half of the plastic, 25 mm filter cassette is glued into the inside taper of the aluminum base. Filters are mounted in the traditional manner between the top and bottom halves of the plastic filter cassette, which are then pressure sealed. To further assure a good seal, pressure tape is also applied to the outside of each filter cassette at the seam where the filters are mounted.

A.1.5 The Scrubber

The scrubber, which can be seen sitting on the table to the right of the main body of the dust generator in Figure A-2, is constructed from ordinary laboratory glassware. In the scrubber, water is boiled in the bottom of a 1 liter, round bottom flask (shown seated within a heating mantle). A straight-jacketed, cold-water condenser is incorporated along the entrance line to the scrubber and a spiral condenser is incorporated along the exit line from the scrubber. An immersion pump circulates water between a cooler containing a water-ice mix and the condensers. Water flowing into the condensers is maintained at approximately 0° C to prevent moisture from leaving the scrubber.

FIGURE A-8

DUST COLLECTION SYSTEM

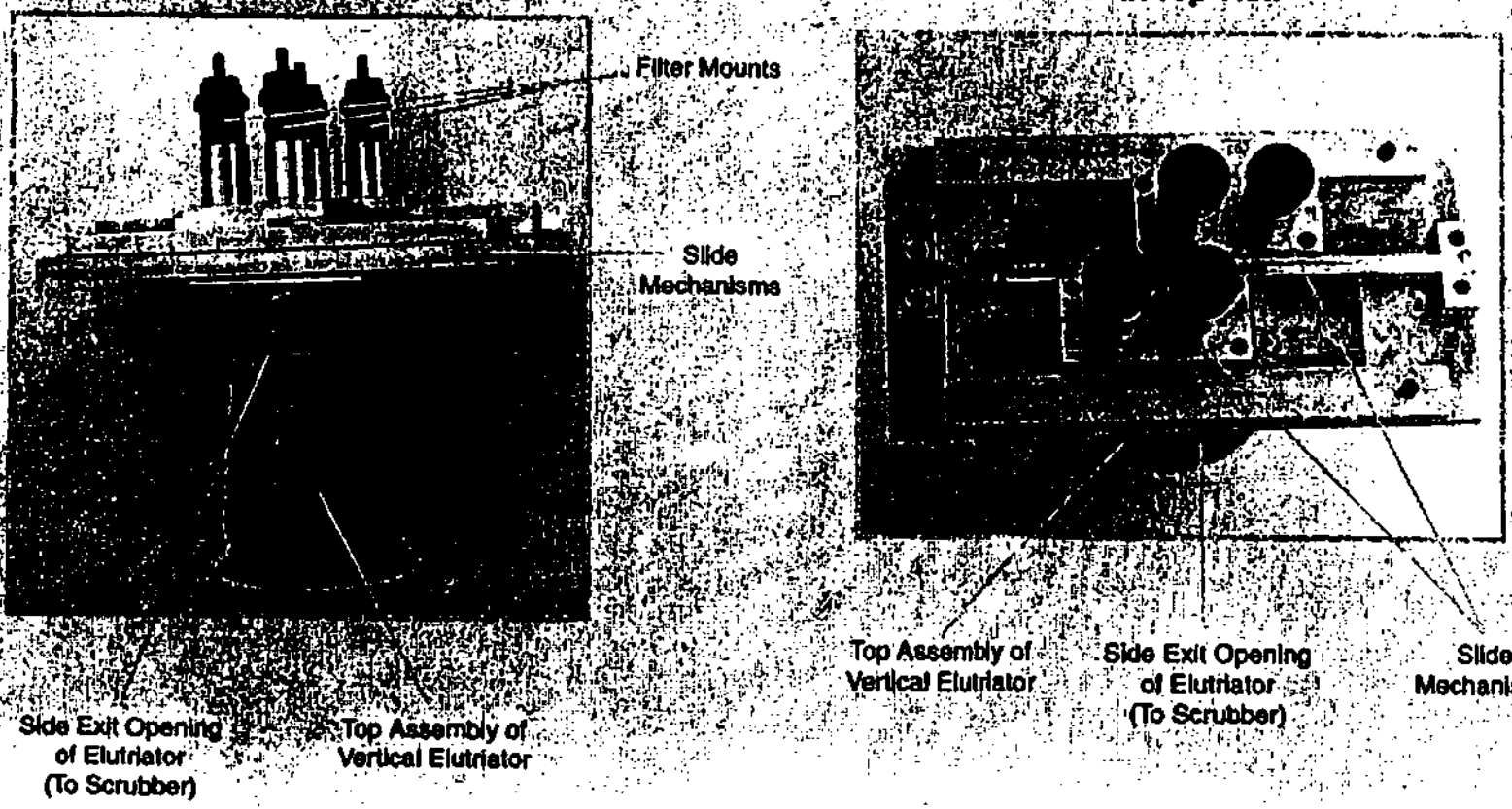
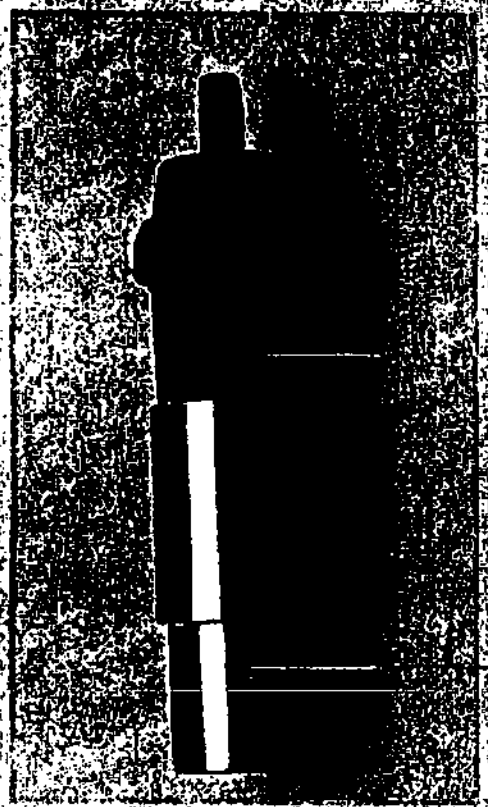


FIGURE A-9

FILTER MOUNTS

a. Assembled Filter Mount



Commercially Available 25mm Filter Cassette

Aluminum Base

b. Disassembled Filter Mount

Particles in the air stream entering the scrubber serve as nucleation centers around which the steam in the scrubber condenses. The resulting water droplets eventually fall back into the water reservoir at the bottom of the scrubber so that the trapped particles are collected in this reservoir.

A flowmeter and a filter cassette are also placed in the air flow line that exits the scrubber (between the scrubber and the vacuum pump). The primary reason for including the filter cassette is to make the pressure drop through this path approximately equal to the pressure drop through the other filter cassettes mounted on top of the elutriator (the dust collection system). The pressure drop through the elutriator and scrubber is small enough not to affect the flowmeter operation.

A.1.6 The Configuration of Air Transfer Lines

A schematic indicating the configuration of air transfer lines leading to and from the scrubber is presented in Figure A-10. As indicated in the figure, the two side exits from the elutriator are each attached to one ft sections of 1.00 in. i.d. Tygon tubing, which then join at a glass "Y" connector. The stem end of the "Y" connector is attached (with a one ft section of 1.00 in. i.d. Tygon tubing) to a diameter reducing piece of glass that feeds into the entrance condenser to the scrubber through a rubber stopper. The large tubing is held in place at connections by ring clamps (Figure A-2).

Also as indicated in Figures A-10 and A-2, the exit condenser of the scrubber is connected to a flow control valve with 0.25 in. i.d. Tygon tubing. The exit side of the flow control valve is connected first to a 25 mm filter cassette and then to a vacuum pump. Both of these connections also use 0.25 in. i.d. Tygon tubing.

A schematic indicating the configuration of air transfer lines leading from the filter cassette mounts of the dust collection system is depicted in Figure A-11. A photograph of the transfer line connections is also presented in Figure A-12. As indicated in the figures, a 0.25 in. i.d. Tygon line leads from the exit side of each filter cassette to a plastic, stop cock valve. Another Tygon line then leads from each valve to one of two plastic "T" connectors so that the pair of filter cassettes mounted on the slide mechanism over the IST opening of the elutriator are joined (beyond the stop cock valves) and the pair of filter cassettes mounted on the slide mechanism over the ME opening are also joined (beyond the stop cock valves). The common line from the exit side of each "T" connector is then connected first to a flowcontrol valve and then to a vacuum pump. All such connections use 0.025 in. i.d. Tygon tubing.

NOTE

This is the configuration of air transfer lines that is appropriate during the operation of the dust generator. The configuration that is appropriate during calibration of air flow is discussed in Section 9.3.4 of the main text.

As indicated previously, the side arm on the bottom cup of the elutriator is connected to a two ft section of 0.25 in. i.d. Tygon tubing that is simply allowed to hang free (Figure A-6a).

FIGURE A-10

TUBING CONNECTIONS FOR THE SCRUBBER
OF THE DUST GENERATOR

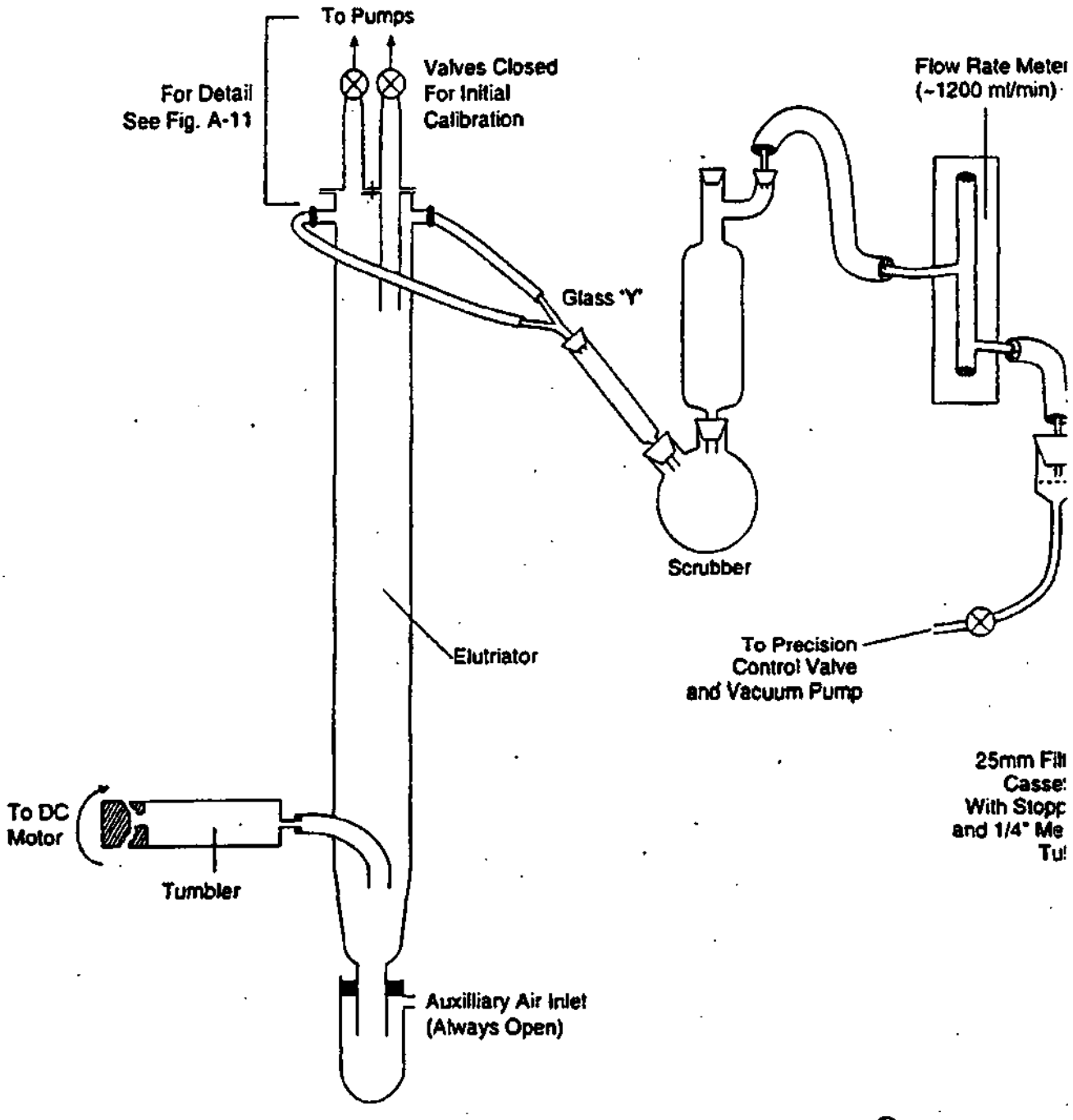
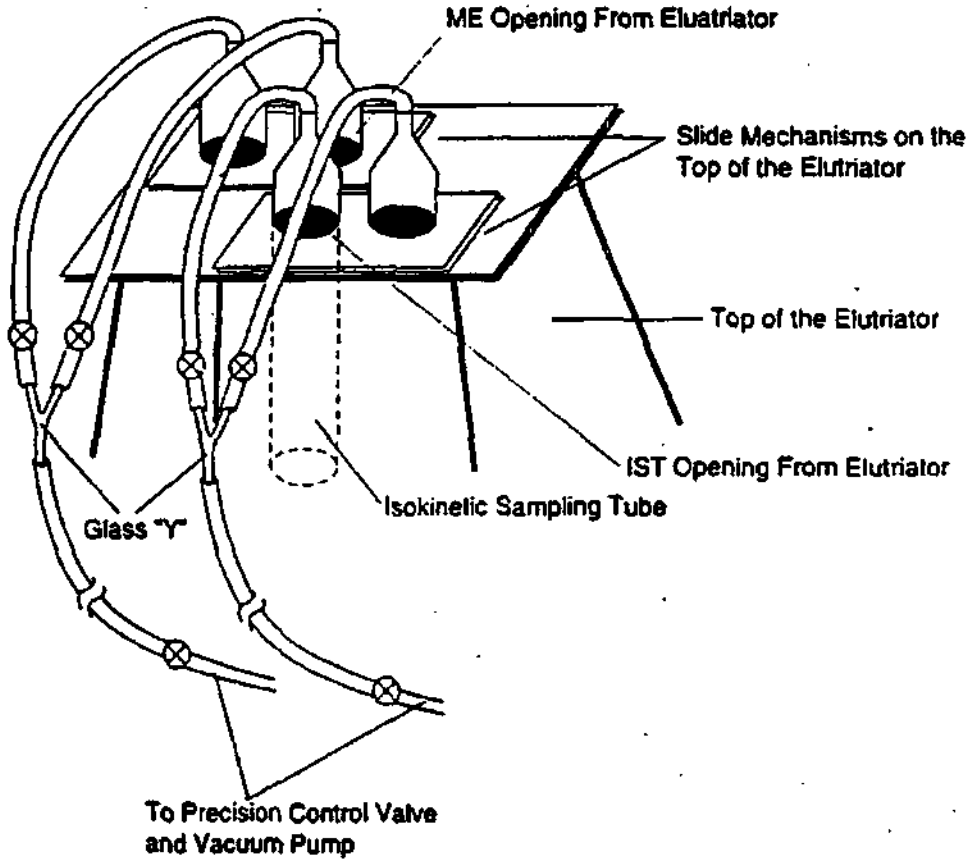


FIGURE A-11

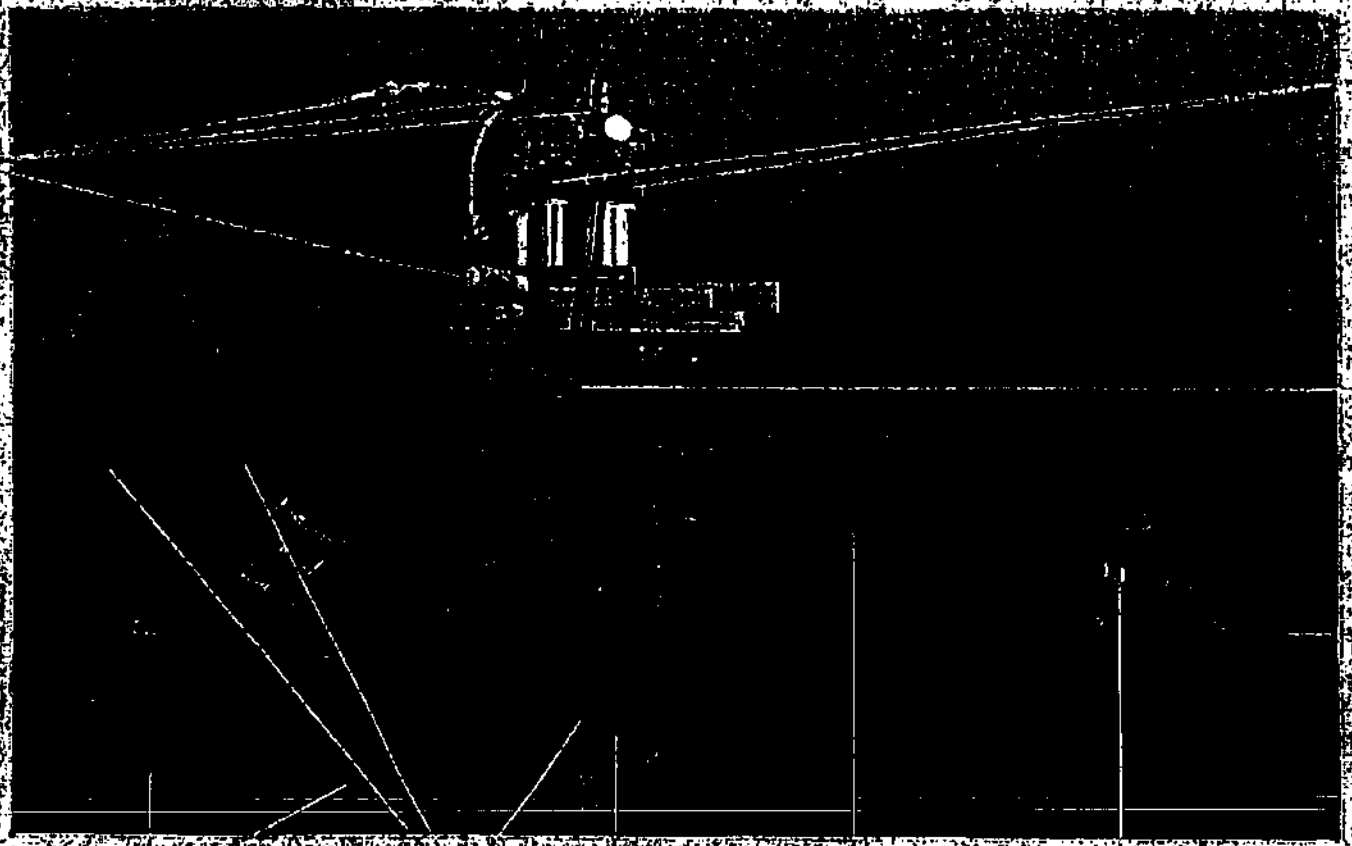
TUBING CONNECTIONS FOR FILTER CASSETTES MOUNTED ON THE ELUTRIATOR OF THE DUST GENERATOR



⊗ Stop Cock Valves

FIGURE A-12

DETAIL OF TUBING CONNECTIONS AT THE TOP OF THE VERTICAL ELUTRIATOR



A.2 THEORY OF OPERATION

A.2.1 The Dynamics of Dust Generation

The dynamics of the release of dust from a sample during a run using the dust generator have been evaluated so that the rate of release and mass of dust in the sample can be derived from measurements of the mass of dust deposited over time on the set of filters collected over the ME opening of the elutriator. Analysis of data obtained from several different types of samples during the pilot study for this method (Berman et al., 1994) indicate that the rate of release of mass from a sample in the dust generator is well described by a first-order rate equation:

$$-dM_s/dt = k*M_s \qquad \text{(A-1)}$$

where:

M_s is the mass of respirable dust remaining in the sample at time "t" (g);

t is the time since the start of the run (s); and

k is the first-order rate constant for the release (s^{-1}).

The minus sign in this equation indicates that mass is lost with time.

Equation A-1 can be integrated to yield:

$$\ln(M_s) = \ln(M_o) - kt \qquad \text{(A-2)}$$

where:

M_o is the mass of respirable dust in the sample at the start of the run (i.e. at time t = 0) (g).

Given that "M_s" can also be expressed as the difference between "M_o" and "M_r" the cumulative mass released up to time "t," Equation A-2 can also be expressed as:

$$\ln(M_o - M_r) = \ln(M_o) - kt \qquad \text{(A-3)}$$

where:

M_r is the cumulative mass released between the start of a run and time "t" (g).

The relationship presented in Equation A-3 indicates that a plot of the natural logarithm of the quantity ($M_o - M_r$) versus time should be a straight line with a slope equal to the rate constant for dust release, k, and an intercept equal to the initial mass of dust in the sample at the start

of the run, M_o. The cumulative mass of dust released from the sample over time, M_r, can be derived from measurements of dust collected on filters during the run. However, because M_o also appears as part of one of the parameters that must be plotted to evaluate the relationship expressed in Equation A-3, the value of M_o must be optimized using regression, as described in Section 11.2 of the main text of this method.

The cumulative mass released from a sample at time "t" during a run, "M_r" is directly proportional to the cumulative mass measured on filters collected during the run:

$$M_r = M_f*(F_s + F_d + F_c)/F_c \qquad \text{(A-4)}$$

where:

M_f is the cumulative mass measured on filters collected from filters mounted over the top of the elutriator up to time "t" (g);

F_s is the rate of airflow through the scrubber (cm^3/s);

F_d is the rate of airflow through the IST opening of the elutriator (cm^3/s); and

F_c is the rate of airflow through the ME opening of the elutriator (cm^3/s).

Because F_s and F_c will typically have been set to $0.48*V_v$ and F_d will typically have been set to $0.047*V_v$ during the initial setup of the dust generator (see Section 9.3.4), for most applications, Equation A-4 reduces to:

$$M_r = 2.1*M_f \qquad \text{(A-5)}$$

As indicated above, values for M_o must be derived by performing a regression analysis of the relationship described by Equation A-3. This can be accomplished by using any of several commercially available spreadsheet programs (such as, for example, LOTUS™). The procedure to be followed to derive estimates of M_o and k are described in Section 11.2.

A.2.2 The Time Dependence of Dust Collection

As indicated in Section A.2.1 above, the generation of dust from the tumbler is well described by the first order rate equation:

$$-dM_s/dt = k*M_s \qquad \text{(A-1)}$$

However, experience gained during the pilot study for this method (Berman et al. 1994) further indicates that the rate of change of M_s, is sufficiently slow in most cases such that, for periods of no more than 5 to 10 minutes, M_s can be considered constant. Thus, for

estimating such things as the time required to load individual filters in the dust generator, a simpler form of Equation A-1 can be used (in which M_s is considered constant):

$$\Delta M_s = k * M_s * \Delta t \qquad \text{(A-6)}$$

where:

M_s is still the mass of respirable dust remaining in the sample at time "t" but it is assumed constant over the short interval of time "Δt" (g);

ΔM_s is the mass of respirable dust released from the sample over the short time interval "Δt" (g);

Δt is a relatively short time interval (no more than ten minutes) during which the release of dust is being estimated (s); and

k is still the first-order rate constant for the release (s^{-1}).

Based on Equation A-6, the mass of respirable dust deposited on a filter in the dust generator, call this ΔM_f, is simply the product of the dust released from the sample, ΔM_s, and the fraction of the flow through the dust generator that is also directed through that filter. Thus, for filters collected over the isokinetic sampling tube (the IST opening of the elutriator):

$$\Delta M_f = 0.047 * k * M_s * \Delta t \qquad \text{(A-7)}$$

or for filters collected over the ME opening of the elutriator:

$$\Delta M_f = 0.48 * k * M_s * \Delta t \qquad \text{(A-8)}$$

The correct value for M_s to be used with Equations A-7 and A-8 is the value estimated by the relationship provided in Equation A-2 of Section A.2.1 where the "t" in Equation A-2 is the time that has elapsed from the beginning of the run to the start of the interval of interest, "Δt".

NOTE

In deriving Equations A-7 and A-8, it was assumed that air flow within the dust generator was setup as described in Section 9.3.4.

A.2.3 Size Separation Using the Vertical Elutriator

Separation of the respirable fraction of a particulate matrix can be accomplished by exploiting differences in the settling velocities of particles of different sizes when such particles are suspended in either a liquid or gaseous medium. However, air was selected as the medium into which samples would be suspended in this method to avoid changes in the characteristics of asbestos that typically occur when asbestos samples are placed in water[2]. The force on a particle suspended in a moving fluid is given by Stoke's Law (Fuchs, N.A. 1964). When such a particle is suspended in a fluid that is moving upward such that the force of the fluid and the force of gravity on the particle just balance and the particle remains motionless, Stoke's Law indicates that the following relationship holds:

$$4/3*\pi*r^3*d*g = -6*\pi*\eta*r*V_l \qquad \text{(A-9)}$$

where:

- r is the radius of the particle (cm);
- d is the density of the particle (g/cm^3);
- g is the acceleration due to gravity (cm/s^2);
- η is the dynamic viscosity of the fluid ($g/cm*s$); and
- V_l is the linear velocity of the fluid (cm/s).

The velocity estimated in Equation A-9 is termed the Stoke's velocity, V_s.

By substituting the viscosity of air at room temperature (i.e. 20° C) and the acceleration due to gravity into Equation A-9, the value of the Stoke's velocity of a particle is estimated as follows:

$$V_s = 1.18*10^6*r^2*d \qquad \text{(A-10)}$$

where "r" is the radius of the particle and "d" is the density of the particle. Because respirable particles are generally defined as those exhibiting an "aerodynamic equivalent diameter" of 10 µm, where an aerodynamic equivalent diameter is the diameter of a particle of unit density that settles at the same rate as the particle of interest, Equation A-10 can be used to find the Stoke's velocity of the largest respirable particles in the elutriator (i.e. by substituting a radius of 5 µm and assuming a density of 1):

$$V_s = 0.295 \text{ cm/s}$$

---

[2] It is well documented that the size distribution of asbestos structures in a sample change when such a sample is placed in water. For an overview of such documentation, see Berman and Chatfield (1990). Typically, the number of small fibers and bundles increases and the number and complexity of clusters and matrices decrease when asbestos samples are placed in water.

The Stoke's velocity for a particle is also equal to the velocity of the fluid stream that will just hold a particle motionless against gravity. Because the goal of the dust generator is to capture all particles that are potentially respirable, the velocity of air within the elutriator should be set so that it is just slightly larger (i.e. by 5%) than the Stoke's velocity estimated above (for the largest respirable particles) so that all respirable particles entering the elutriator will be imparted with an upward velocity and will be carried along with the air stream so that they are, ultimately, either deposited on a filter or passed into the scrubber. Therefore, airflow within the dust generator should be set so that the velocity of air within the vertical elutriator is 5% greater than 0.295:

$$V_l = 0.31 \text{ cm/s}.$$

A.3 DUST GENERATOR PROTOTYPE DESIGN FIGURES AND CONSTRUCTION GUIDELINES

The figures of the prototype dust generator that are included in this package are intended to be suggestive and not meant to be followed in exact detail. However, the dimensions of the various cross-sectional areas of different parts of the dust generator, which affect the relative air flows in various places, need to be followed closely for the dust generator to perform as intended. Other design features, such as couplings, clamps, and seals are intended more to be illustrative; alternate designs can be equally effective.

In spite of the low pressure differentials developed during operation in the prototype, performance was found to be very sensitive to leaks, especially leaks occurring in the isokinetic sampler filter mounts. Design features associated with the dust collection system should therefore be selected so as to minimize the potential for leaks in this area. The air flow path in the elutriator of any dust generator that is constructed for use with this method should be tested for leaks when construction is completed and periodically thereafter.

So that dust generator equipment constructed for use with this method will perform adequately, the following requirements must be incorporated into its design:

- the tumbler must be designed to hold a minimum of approximately 100 g of sample (with ample space left over to allow adequate tumbling) so that field homogenization requirements are not compromised. It should also be designed to assure a reasonably long pathlength over which air passes through the sample and baffles (or square corners) should be incorporated to assure adequate tumbling action and thorough mixing of sample and air;

- the pathlength of the elutriator should be a minimum of 10 times its diameter to minimize the possibility of channeling and the diameter should be large enough to assure a cross-sectional area that is at least 10 times that of the tumbler. This latter requirement is to assure adequate air flow in the tumbler that will effect efficient transfer of sample while flow throughout the device is limited to allow flow in the elutriator to be set at 1.05 times the settling velocity of the largest respirable particle of interest;

- the entrance tube and bottom of the elutriator should be shaped so that sufficient air velocities are maintained in this part of the device to assure a smooth transition (with efficient sample transfer) between flow in the tumbler and flow in the elutriator. It is also recommended that the entrance tube to the elutriator be pointed downward as a further hindrance to channeling in the elutriator; and

- filter mounts in the dust collection system need to allow for ready, facile exchange of filters while minimizing the potential for air leaks in this area. A modification incorporated into the prototype device to achieve both requirements was to design aluminum mounts that are tapered such that the shaved bottom half of commercially available filter cassettes fit snugly and can be glued in place (to prevent air leaks at this joint). These can then be fitted with filters and joined to the matching top halves of commercially available cassettes. This feature takes good advantage of the fit between the two plastic halves of a commercial filter cassette, which are designed to join with minimal leakage while allowing for rapid exchange of filters. The bottom half of the aluminum filter mounts are sealed into the slide mechanisms on the prototype apparatus with o-rings.

The following figures are included in this package:

- Figure A-13 indicates the overall assembly of the prototype dust generator;

- Figure A-14 indicates the details of the prototype tumbler assembly;

- Figure A-15 indicates the details of the prototype vertical elutriator;

- Figure A-16 is a vertical cross-section of the top of the prototype vertical elutriator indicating the relationship between the elutriator openings, the isokinetic sampling tube, and the slide mechanisms of the dust collection system;

- Figure A-17 indicates the details of the isokinetic sampling tube;

- Figure A-18 indicates the details of the prototype slide mechanism of the dust collection assembly; and

- Figure A-19 indicates the details of the prototype filter cassette mounts.

FIGURE A-13 OVERALL PROTOTYPE DUST GENERATOR ASSEMBLY

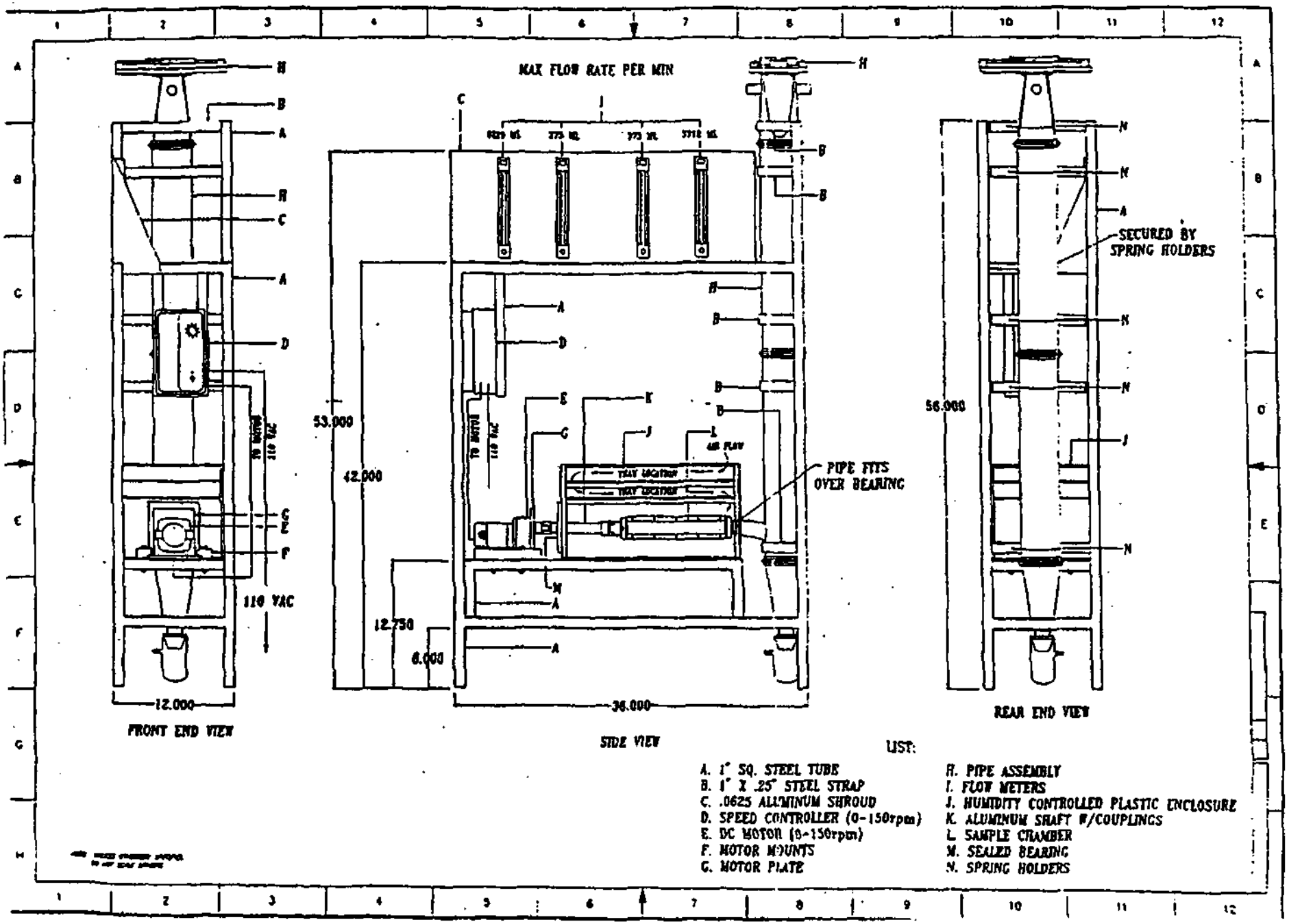


FIGURE A-14 PROTOTYPE TUMBLER ASSEMBLY DETAIL

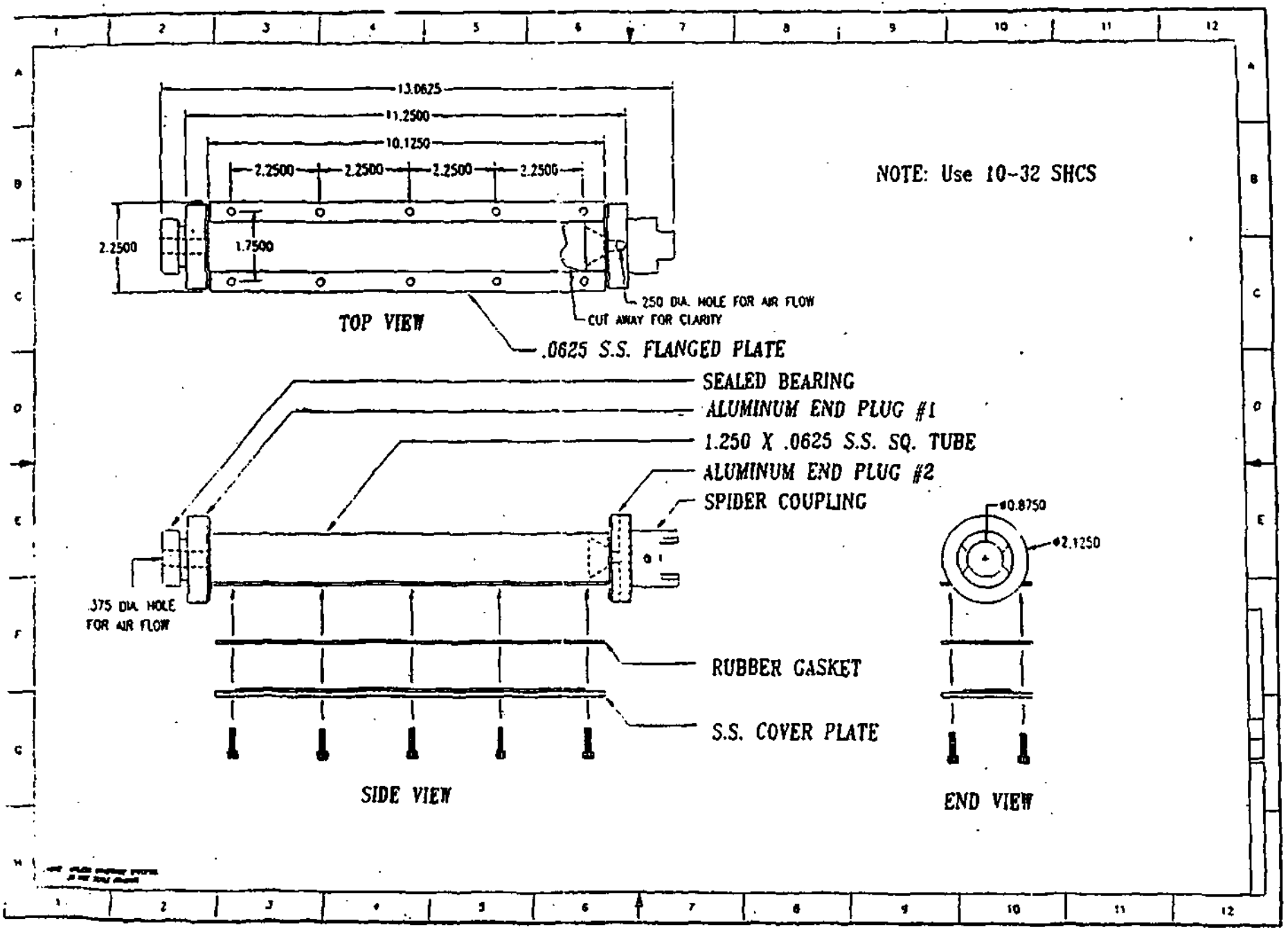


FIGURE A-15 PROTOTYPE VERTICAL ELUTRIATOR DETAIL

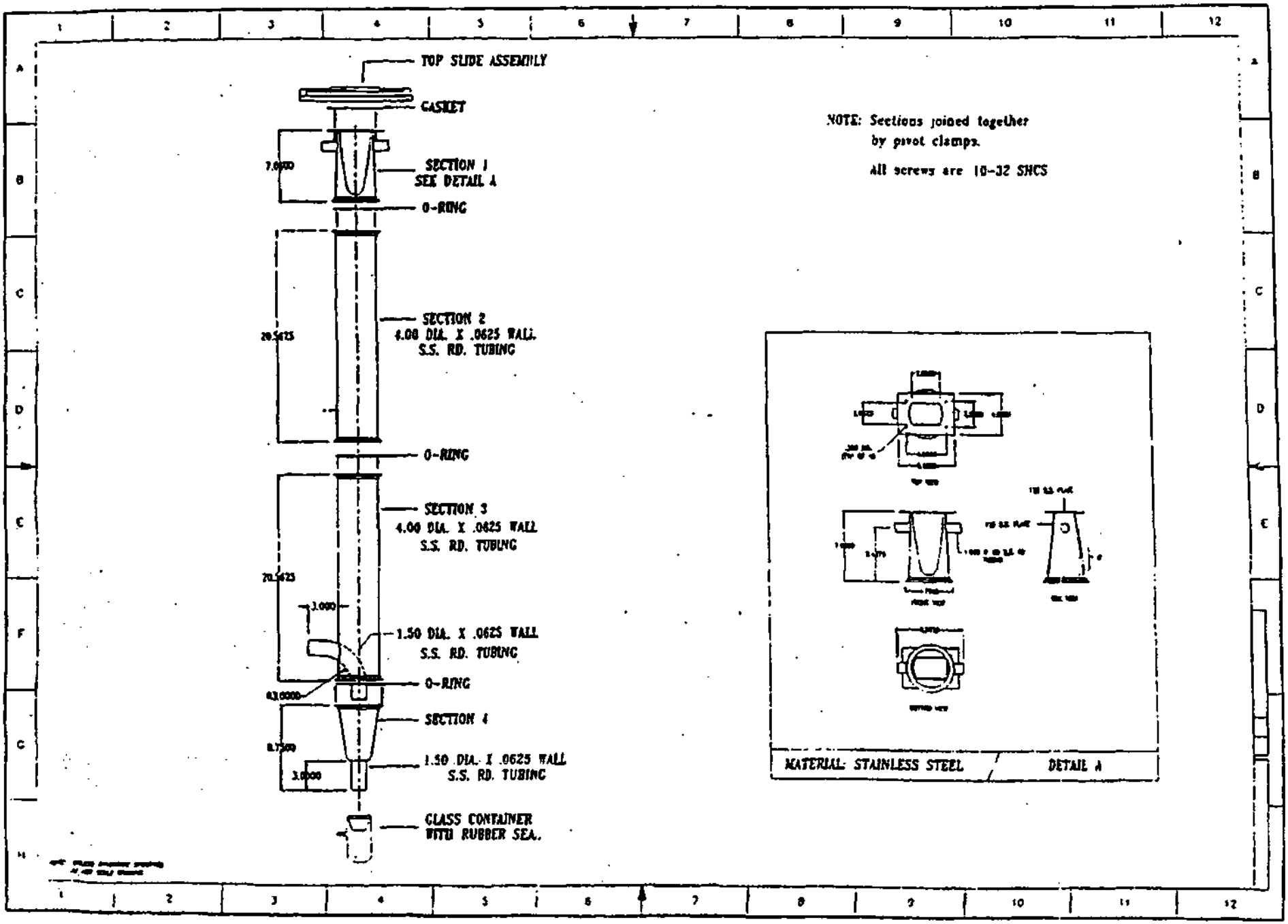


FIGURE A-16

SECTION OF ELUTRIATOR TOP
WITH EXTENSION TUBE

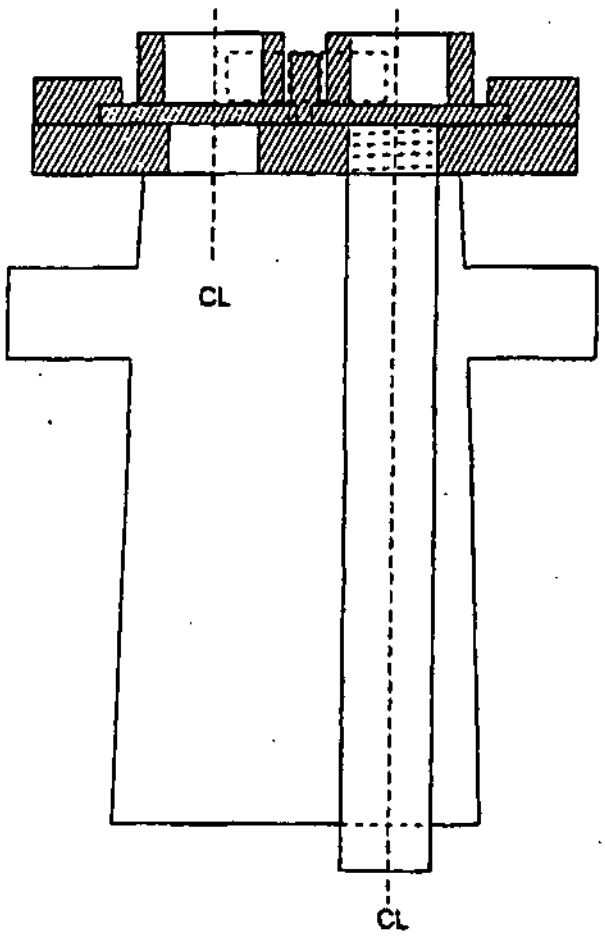


FIGURE A-17

LONGITUDINAL & TRANSVERSE SECTIONS OF
ISOKINETIC SAMPLING TUBE

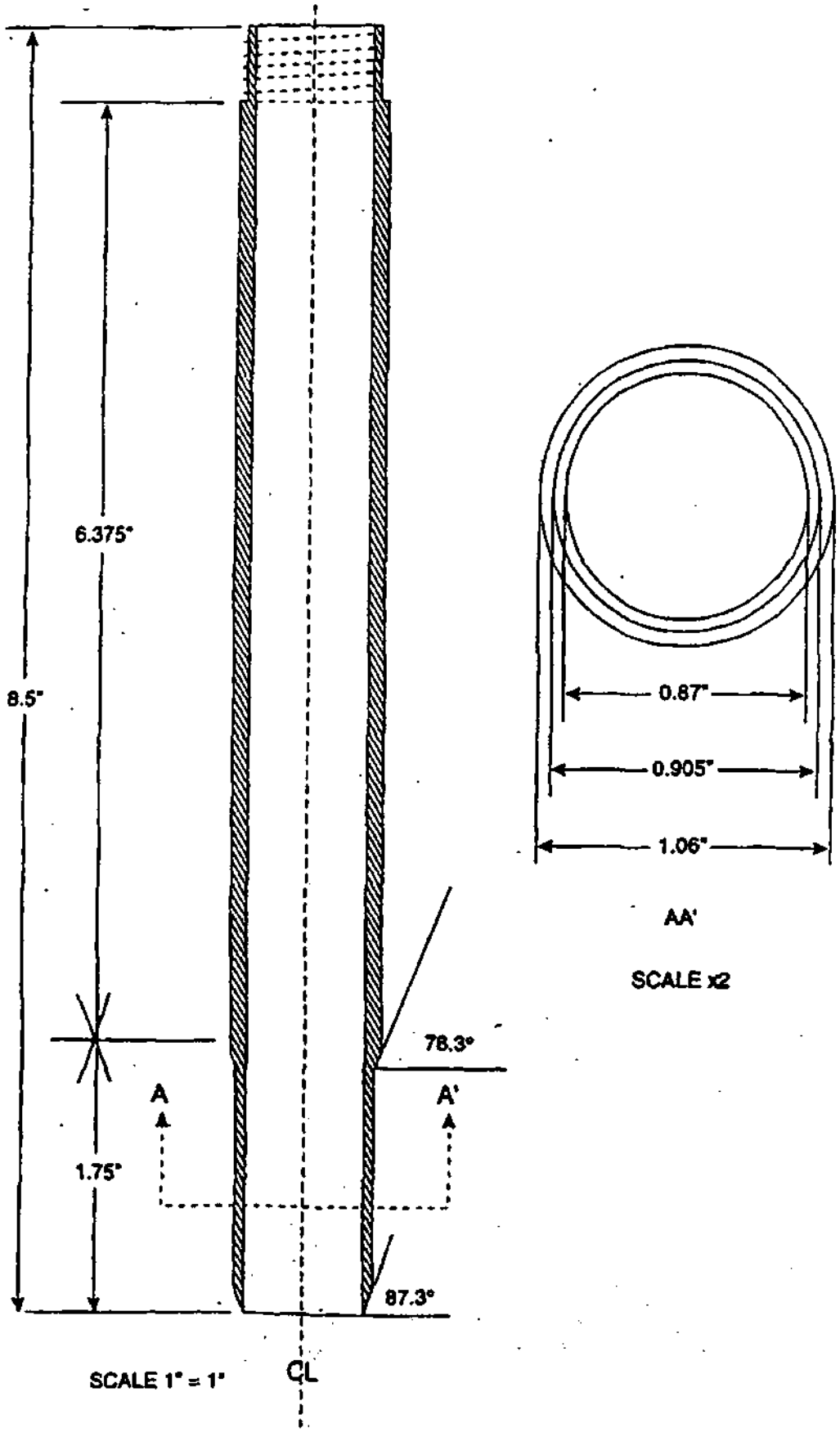
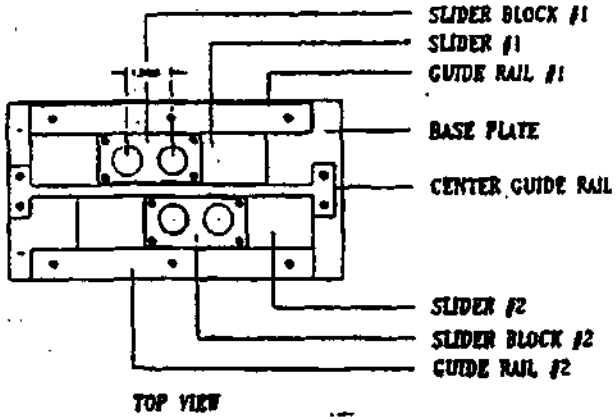
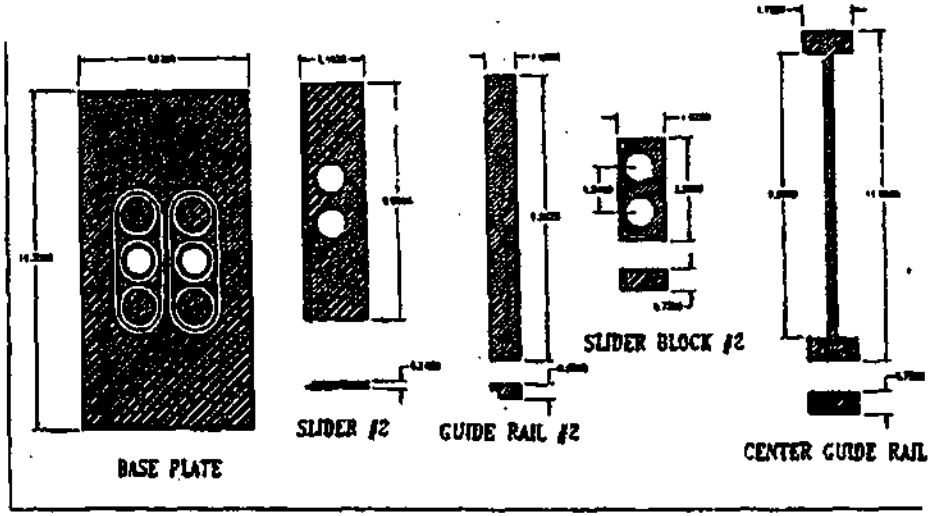
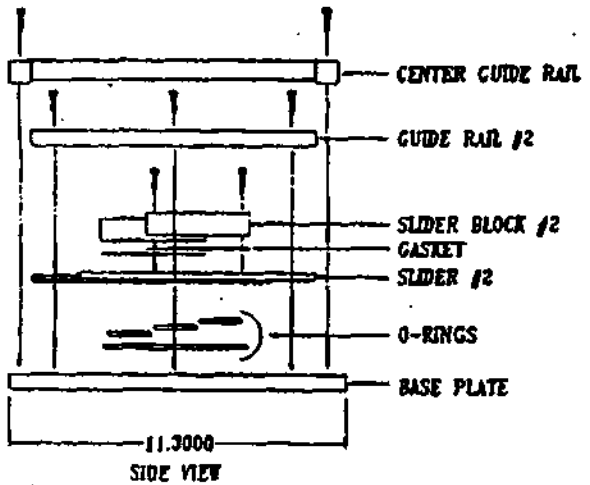
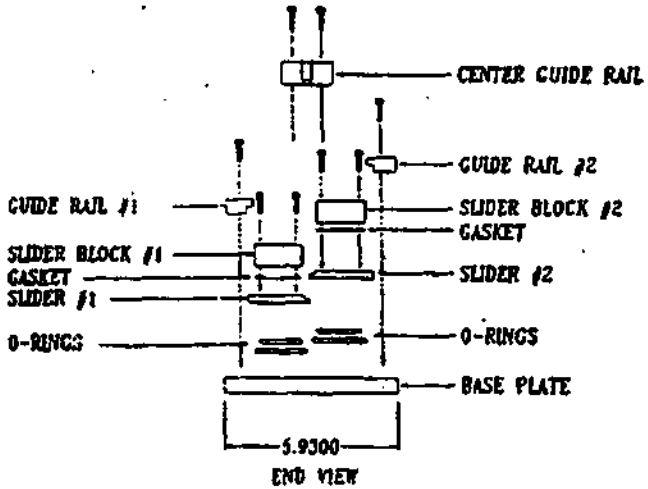


FIGURE A-18 PROTOTYPE SLIDE MECHANISM DETAIL







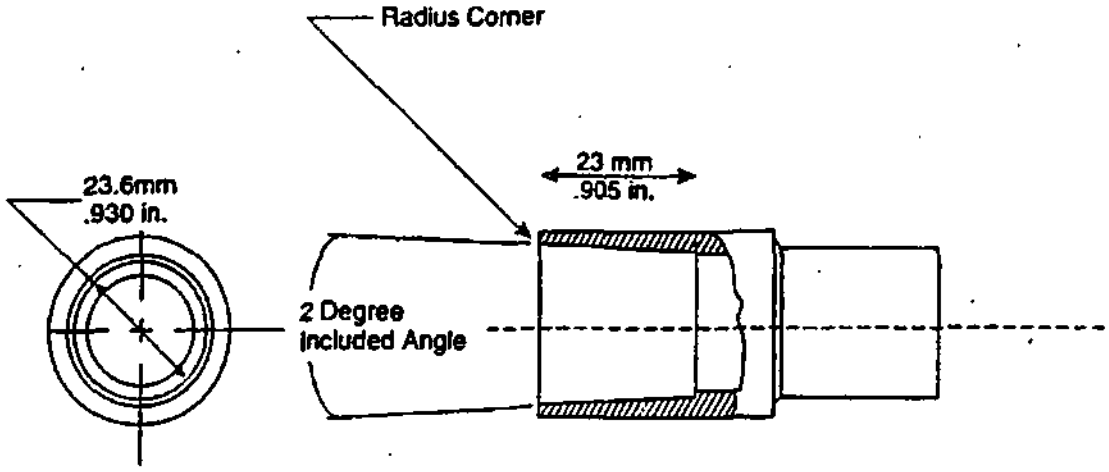


TOP SLIDE ASSEMBLY

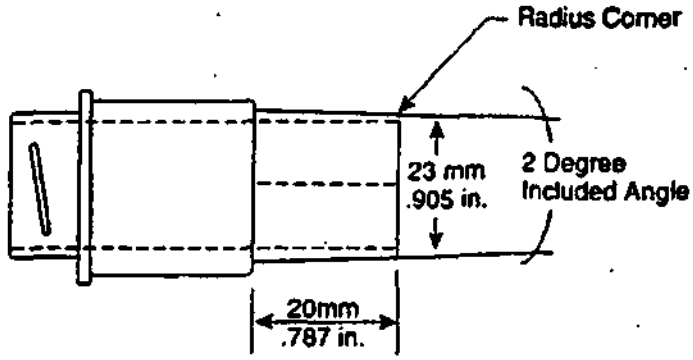
MATERIAL: ALUMINUM

FIGURE A-19

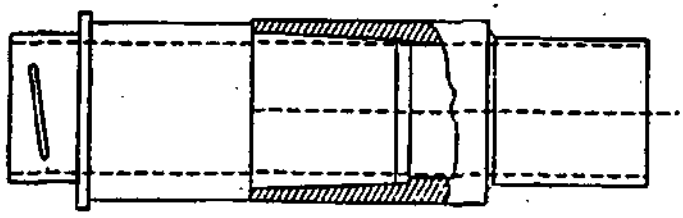
DETAIL OF PROTOTYPE FILTER CASSETTE MOUNTS



ALUMINUM CASSETTE HOLDER, TOP MODIFIED WITH INTERNAL TAPER AS SHOWN



25MM MILLIPORE CASSETTE, BOTTOM MODIFIED WITH EXTERNAL TAPER AS SHOWN



CASSETTE AND HOLDER SHOWN JOINED TO CREATE A TAPER LOCK SEAL

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Tabbed Page: 22

Date: August 10, 2004 SOP No. EPA-LIBBY-03 (Rev. 2)

Title: **ANALYSIS OF ASBESTOS IN SOIL BY TEM**

Author: William Brattin Syracuse Research Corporation

SYNOPSIS: A standardized method for quantifying asbestos in soil using transmission electron microscopy (TEM) is provided. This method is based on EPA Method 600/R-93/116, with project-specific specifications and modifications intended specifically for use at the Libby Superfund Site.

APPROVALS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
USEPA Region 8		8/17/04
Syracuse Research Corp.	W J Brattin	8/10/04

Revision	Date	Principal Changes
0	01/31/03	–
1	02/09/04	Revise soil to water ratio; add text on analytical sensitivity and detection limit; add use of empiric calibration curve as option for data reduction
2	08/10/04	Clarify optimum loading, provide additional details on mineral type identification

1.0 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide a standard approach for quantitative analysis of asbestos in samples of soil or other soil-like materials using transmission electron microscopy (TEM). Results are expressed as weight % (grams of asbestos per 100 grams of soil). This SOP is based on EPA Test Method 600/R-93/116 (*Method for Determination of Asbestos in Bulk Building Materials*), and includes clarifications and modifications specifically intended for application at the Libby Superfund site.

2.0 SCOPE AND APPLICATION

This method is intended for analysis of asbestos in soil or other similar soil-like media. This method is generally appropriate for the analysis of all types of asbestos fibers, including both chrysotile and amphiboles, but it has been optimized for amphiboles that are characteristic of the Libby site. Note that the ashing preparation step may cause decomposition of chrysotile asbestos, so results for chrysotile may be biased low.

This method provides an estimation of asbestos concentration in units of weight % and is designed for soils that have been ground in accord with SOP ISSI-Libby-01. Recommended counting rules generally follow the method defined in the Asbestos Hazard Emergency Response Act (AHERA) (USEPA 1987), except where specifically indicated in this method or appropriate project-specific laboratory modifications.

3.0 RESPONSIBILITIES

It is the responsibility of the laboratory supervisor to ensure that all analyses and quality assurance procedures are performed in accord with this SOP, and to identify and take appropriate corrective action to address any deviations that may occur during sample preparation or analysis. The laboratory supervisor should also communicate with project managers at EPA or their oversight contractors any situations where a change from the SOP may be useful, and must receive approval from EPA for any deviation or modification from the SOP before proceeding with sample preparation and analysis.

4.0 EQUIPMENT

- Analytical balance(s)- for weighing samples and preparations
- Crucibles and cover - for sample ashing
- Spatula or sample thief - for sample transfer from bulk sample container to crucible
- Muffle furnace - for sample ashing
- 25-mm or 47-mm diameter 0.22-µm pore size MCE filters - for filtering after wet grinding and after sonication
- 25-mm or 47-mm diameter 5-µm pore size MCE filter - for backing the 0.22-um MCE filter
- 25-mm or 47-mm filter apparatus (glass or disposable)
- Petri dishes - used as "containment" for filter weighing and labeling
- Mortar and pestle - particle size reduction via wet grinding
- Concentrated Hydrochloric Acid - to dissolve carbonaceous material in the sample
- Filtered deionized (FDI) water - wet grinding and sample transfer to filter apparatus; sample suspension medium for sonication
- Hot plate or drying oven - for drying filters
- 4-ounce wide-mouth bottles - sample container for 100-mL sample suspension
- MicroPipettes, disposable tip (0.050 mL, 0.100 mL, 0.500 mL) - for sample suspension transfer after sonication
- Tweezers - MCE filter preparation for TEM
- Scalpel blade - MCE filter preparation for TEM
- Acetone or dimethylformamide/acetic acid mixture - MCE filter preparation for TEM
- Micropipette with disposal tips - MCE filter preparation for TEM
- Plasma etcher -MCE filter preparation for TEM
- Carbon coater - MCE filter preparation for TEM
- Jaffe washer - MCE filter preparation for TEM
- Acetone vapor generator - MCE filter preparation for TEM
- TEM- 80 to 120 kV transmission electron microscope (TEM), capable of performing electron diffraction with a fluorescent screen inscribed with calibrated gradations, is required. The TEM must be equipped with energy dispersive X-ray spectroscopy (EDS) and it must have a scanning TEM (STEM) attachment or be capable of producing a spot size of less than 250 nm in diameter in crossover. The microscope must be calibrated and maintained according to the requirements described in NVLAP Airborne Asbestos Program.

5.0 METHOD SUMMARY

An aliquot of the soil sample is ashed to eliminate organic material, and then is ground in acid to reduce particle size and dissolve carbonate-containing minerals. The resulting residue is weighed and a measured aliquot is suspended in water and applied to a filter for examination by TEM. The weight percent of asbestos in the sample is calculated either based on the measured number and dimensions of the asbestos particles observed on the filter, or by use of a calibration curve (Libby amphibole only).

6.0 DETAILED METHOD

6.1 Sample Preparation

6.1.1 Ashing

Substantial organic material in the sample can result in interferences for asbestos identification and quantification by TEM. Therefore, all samples[a] are ashed using the following procedure:

1. Weigh the crucible plus cover to the nearest 0.01 grams (g) and record the weight on the Gravimetric Reduction Data Sheet (Attachment 1).

2. Mix each sieved and ground sample by tipping the bag upside down, and then returning it to its original position. Use a clean sample thief to remove a uniform aliquot of the material, and transfer a minimum of 0.5 to 1.0 g of the sample into the pre-weighed crucible. Use as large a sample aliquot as possible so that the aliquot better represents the soil sample, but do not fill the crucible more than about 1/3 full.

3. Cover the crucible and weigh the crucible plus sample to the nearest 0.01 g. Record this weight in the Gravimetric Reduction Data Sheet (Attachment 1). Note: if the sample appears to be moist (e.g., there is a tendency for the sample particles to adhere to each other), the sample should be re-dried by heating at 60°C for 30-60 minutes and then cooled before weighing as described above.

[a] In cases where the sample contains very little organic material, ashing my be omitted after approval from EPA.

4. Place the covered crucible into a muffle furnace.

5. Turn the furnace on and set the temperature to 480°C. [Note: if the furnace can achieve this temperature faster than 2 hours, first turn the temperature to 250°C and hold at this temperature for 1 hour before increasing the temperature to 480°C.] .

6. Hold the sample at 480°C for at least 4 hours.

7. Shut off the furnace and allow to the sample to cool.

8. When the sample has cooled, remove the crucible and weigh to the nearest 0.01 g. Record this final weight in the Gravimetric Reduction Data Sheet (Attachment 1).

9. The weight % of the residue is calculated by dividing the ashed sample weight by the starting sample weight and multiplying by 100. Record on the Gravimetric Reduction Data Sheet (Attachment 1).

Note: It is understood that this ashing procedure might result in some parts of the sample reaching temperatures that may begin to decompose chrysotile asbestos (about 500°C). However, this SOP is intended for the analysis of amphibole asbestos, and amphibole asbestos is not expected to begin decomposition unless temperatures are substantially higher.

6.1.2 Acidification and Grinding

1. Pour the sample from the ashing crucible into a mortar and combine with sufficient concentrated hydrochloric acid (typically only a few drops) to dissolve any reactive carbonaceous materials. This is indicated by cessation of effervescence.

2. Grind the mixture with the pestle for approximately 1 minute. The purpose of the grinding is to ensure that any clumps of soil are fully disaggregated and well mixed with the acid.

3. Wash the pestle by spraying FDI water over the pestle to remove all materials, allowing the rinse water to drip into the mortar.

4. Pour the sample/FDI water suspension from mortar into a filtering funnel containing a pre-weighed 25-mm or 47-mm MCE filter. Rinse the mortar with FDI water and ensure that all material is transferred to the funnel.

5. Rinse down the inside walls of the filter basin and verify that particles are not adhering to the sides of the filter basin.

6. After filtering, place the filter on a hot plate or in a drying oven to dry.

7. When the sample residue and filter are dry, weight the filter and residue and calculate the weight of the residue by subtracting the weight of the filter. Record on the Gravimetric Reduction Data Sheet (Attachment 1).

8. Calculate the gravimetric reduction ratio (GRR) as:

   GRR = final weight / starting weight

9. Decontaminate the mortar and pestle by washing thoroughly with FDI water between samples.

6.1.3 TEM Filter Preparation

1. From the dried sample residue, remove about 100 mg, weighed to the nearest 1.0 mg and record the weight on the Sample Analysis Data Sheet (Attachment 2).

2. Place the sample residue in a 4-ounce wide-mouth bottle and fill the bottle with 100 ± 1 mL of FDI water.

3. Sonicate the sample solution for 15 minutes, using a sonicator that delivers 0.10 ± 0.02 watts per mL of sample fluid. [Note: the sonication time of 15 minutes rather than the 1 hour recommended in EPA 600/R-93/116 is based on laboratory observations that 15 minutes is sufficient to break up the soil from the asbestos].

4. After sonication, thoroughly mix the suspension and remove aliquots for filtration through 25-mm or 47-mm MCE filters. The aliquot volumes should be appropriate to produce a total loading (fiber plus soil matrix) on the filter of about 15-25% (this is

usually about 0.2 to 1.0 mL for 47 mm MCE filters). Prepare at least two grids from each filter for examination by TEM in accord with standard AHERA methods.

6.2 Fiber Counting and Characterization by TEM

Begin by examination of the grids that have been prepared as described above, and identify the pair that has the most nearly optimum total loading (about 15-25%). Attachment 3 provides photomicrographs that illustrate the optimum total loading. If neither pair of grids is suitable, prepare a new filter using an appropriate aliquot volume. If the appropriate volume is smaller than about 0.2 mL, serial dilution should be used to help ensure the sample is representative and to help achieve uniform total loading on the filter.

Record all results using the most recent version of the electronic data recording sheet entitled "EPA 600 TEM Data Sheet.xls" (see Attachment 2). Contact the Volpe project manager to identify the latest version authorized for use.

Counting Rules

Counting rules described below generally follow the AHERA method, except where specifically indicated in this method for use in assessing asbestos fibers in Libby soils or appropriate project-specific laboratory modifications. For example, refer to laboratory modification LB-000031 for site-specific clarification of procedures for measurement and classification of particles. Discussions pertaining to analytical sensitivity and detection limits from which the stopping rules were derived are presented in Section 7.

1. For every asbestos fiber with an aspect ratio ≥3:1, inspect the diffraction pattern and EDS in order to assign the structure into one of three mineral classes:

   Libby Amphibole (LA)
   Other Amphibole (OA)
   Chrysotile (C)

   Examples of EDS that are characteristic of LA particles can be found in USEPA (2001) and USEPA (2004). In addition, because EDS spectra vary between instruments, each laboratory must generate a number of example spectra from authentic samples of LA in order to establish a laboratory-specific reference library.

Key attributes of the electron diffraction pattern that must be satisfied in order to rank a particle as amphibole may be found in the AHERA TEM method (40 CFR Part 763). A more detailed description is provided in Su (2003). As above, each laboratory should establish and utilize a reference library of selected area electron diffraction (SAED) patterns derived from authentic Libby amphibole samples to aid in particle classification.

With regard to classifying asbestos particles as LA, it is understood that LA spans a range of mineral classes (actinolite, tremolite, winchite, richterite, etc.) and that there can be variability in mineralogy between different LA particles and even within a single LA particle. Hence, it is expected that there may be uncertainty in the correct classification of some particles. In general, the "tough calls" (any particles for which the LA identification is uncertain) should not be ranked as LA. However, all such structures should be recorded on the data sheet, being sure to assign a "0" in the Total column. The comment field should be used to indicate that the particle may have been LA and why a definitive assignment was not made. If a "tough call" is ranked as LA, a record of the spectrum and the diffraction pattern should be collected and retained.

It is assumed that most asbestos structures observed in ground and sonicated soil samples will be fibers. If a higher order structure (bundle, cluster or matrix with multiple structures) is encountered, count and record every substructure (fiber) that has an aspect ratio of 3:1 or greater. Identify the particle type as fiber, but add a note to the comment field indicating that the fiber was part of a higher-order structure. In the case of a bundle, all of the sub-structures (fibers) may be recorded as a single unit, giving the dimensions of the bundle rather than the dimensions of each of the individual sub-elements (fibers). Be sure to record the particle type as bundle rather than fiber.

If any structure is observed that the analyst wishes to record but does not intend to be included in the estimation of weight percent, that structure should be assigned an index number of zero in the Total field of the data recording sheet along with a comment in the comment field explaining the importance of the observed structure.

2. Analyze a minimum of 5 grid openings or 50 fibers (whichever occurs first) on each of two grids. Always complete the structure count for the last grid opening evaluated.

3. Terminate fiber count at a minimum of 100 fibers or 10 grid openings (whichever occurs first), providing that an analytical sensitivity of 0.02% has been achieved (see Section 7).

If not, continue until this analytical sensitivity has been achieved. Always complete the structure count for the last grid opening evaluated.

4. Record all data using the hardcopy and electronic data sheets provided in the Sample Analysis Data Sheet (Attachment 2). Record the length, width, and asbestos type for each structure. Make a record of the EDS spectrum and the diffraction pattern and take a photomicrograph for at least one fiber of each asbestos type encountered in each sample.

6.3 Estimation of Weight Percent

The weight percent of asbestos in a sample may be estimated by one or both of two procedures, as described below. For the PE study, both methods will be employed.

Calculation Based on Particle Size Data

Calculate the weight fraction in the residue sample by estimating the total weight of all asbestos structures on the filter and dividing by the total weight of soil on the filter. The total weight of all asbestos structures on the filter is calculated as follows:

$$W_A = \frac{EFA \cdot 10^{-9}}{GO \cdot Ago} \cdot \sum t^2 \cdot l \cdot \delta$$

where:

W_A = weight of asbestos on filter (mg)
EFA = Effective filter area (mm^2)
GO = Number of grid openings counted
Ago = Area of grid opening (mm^2)
t = particle thickness (um)
l = particle length (um)
δ = particle density (g/cm^3)
10^{-9} = Unit conversion factor (10^{-12} $cm^3/um^3 \cdot 10^3$ mg/g)

Note that this calculation assumes that the cross-section of an asbestos particle is approximately square. The density of LA fibers is estimated to be approximately 3.1 g/cm^3 based on typical values for actinolite (3.0-3.2) and tremolite (2.9-3.2) (Dana 1950).

The weight of soil on the filter is calculated as follows:

$$W_S = w \cdot (V1 / V2)$$

where:

W_S = weight of soil on the filter (mg)
w = weight of soil suspended and sonicated in water (mg)
V1 = volume of sonicated suspension applied to filter (mL)
V2 = volume of water (mL) used to suspend and sonicate sample (typically 100 mL)

Thus, the weight fraction (expressed as a percent) in the sample, adjusted to account for loss during gravimetric reduction, is calculated as:

$$WF(\%) = (W_A / W_S) \cdot GRR \cdot 100$$

Estimation Using a Standard Curve

An alternative method for estimating the weight fraction of LA in a soil sample will be assessed as part of the PE study. The method uses an empiric standard curve to relates the frequency of LA structures (expressed as LA structures per gram of soil) to the nominal weight percentage in a series of calibration standards prepared and provided by USGS. For the PE study, the standard curve will be based on at least three replicates of four different concentrations (0.2%, 0.5%, 1%, and 2%) and a control soil. The control soil is a site-specific soil sample that is expected to be free of asbestos at the study target detection limit (TDL). The range of concentrations included must bound the range of concentrations identified in investigate samples. The best fit linear equation through the data is established by ordinary linear regression, resulting in a model of the following form:

$$\text{Weight \% (residue)} = a + b \cdot LA\ (s/g)$$

The final estimate of weight percent in the starting sample is the calculated by adjusting for the gravimetric reduction ratio (GRR) as follows:

$$\text{Final weight \%} = \text{Weight \% (residue)} \cdot GRR$$

Based on the best estimates of the model parameters (intercept = a, slope = b), the weight fraction of any field sample may be calculated from the measured LA particle frequency (s/g) and the GRR.

6.4 Disposal of Investigation Derived Waste

After successful preparation of all necessary filters and grids from a sample, place all of the unused materials into a large container and allow to settle for 1-2 days. Decant the fluid and filter through a 5 um filter in accord with all applicable state and federal regulations. Dispose of the filtered water by pouring down the drain. Dispose of the filter and the residual mud in accord with applicable regulations for asbestos-containing waste.

7.0 LABORATORY-BASED QUALITY ASSURANCE/QUALITY CONTROL

7.1 Analytical Sensitivity

The analytical sensitivity of the method may be estimated as follows:

$$AS = \frac{EFA \cdot \mu}{GO \cdot Ago \cdot w \cdot (V1/V2)} \cdot GRR$$

Typical values for these variables are as follows:

EFA (effective filter area)	= 1295 mm^2
μ (average mass of an asbestos structure)	= 6E-09 mg [b]
GO (number of grid openings counted)	= 10
Ago (area of a grid opening)	= 0.01 mm^2
w (weight of residue suspended in water)	= 0.1 g
V1 (volume of suspension applied to filter)	= 0.5 mL
V2 (volume of suspension water)	= 100 mL
GRR (gravimetric reduction weight ratio)	= 0.9 [c]

[b] This value is based on the average of 344 LA particles that have been evaluated as part of the ISTMs study and preliminary tests using the PE samples.

[c] This value is an initial estimate based on the results of the gravimetric reduction process for several soil samples from Libby.

Based on these typical values, it is expected that the typical analytical sensitivity will be less than 0.02%, which is sufficient for current decision-making requirements. If the a value of 0.02% is not achieved within 10 grid openings (e.g., because the mass of soil applied to the filter is small), or if samples are encountered that are enriched in larger particle sizes (sensitivity is strongly dependent on average particle size), increase the number of grid openings counted until a sensitivity of 0.02% has been achieved. The equation for calculating the number of grid openings needed to achieve a specified analytical sensitivity is as follows:

$$GO = \frac{EFA \cdot \mu}{(Target\ AS) \cdot Ago \cdot w \cdot (V1/V2)} \cdot GRR$$

7.2 Detection Limit

The Method Detection Limit (MDL) depends on the true average rate that countable asbestos structures are observed in filters prepared from soil blanks (ASTM 2000). Because these data have not yet been collected, it is not possible to estimate the method detection limit at this time. After these data are collected, the MDL will be estimated in accord with ASTM Standard Practice D 6620-00 (ASTM 2000). A Target Detection Limit (TDL) may be assigned if defined by project objectives. For the PE study, a target value of 0.2% will be useful in evaluating the efficacy of the method since the lowest concentration on the standard calibration curve is 0.2%. Therefore, a TDL of 0.2% is assigned as an *a priori* guideline. This value is subject to change based upon information gained from the PE Study.

7.3 Precision

Precision is defined as the agreement between a set of replicate measurements without assumption or knowledge of the true value. Agreement is usually expressed as the relative standard deviation (RSD) for a set of replicate measurements, or as the absolute difference between replicates. Each laboratory will obtain a measure of method precision by preparing and analyzing laboratory splits at a rate of 5% (1 laboratory split for every 20 field samples) or 1 per preparation batch, whichever is more frequent.

Data on the precision of this method are not yet available, and one of the goals of the PE study is to assess this parameter. Initial data will be collected from a limited number of highly qualified

laboratories, and these results will be used to establish interim performance criteria for other laboratories. These criteria may be revised as additional data and experience are collected.

Once established, failure to meet acceptance criteria for precision may result in re-analysis and/or re-preparation and re-analysis of all samples in the analytical batch.

7.4 Accuracy

Accuracy is a measure of the closeness of individual measurements to the "true" value. Accuracy usually is expressed as a percentage of the true value. Accuracy is assessed by analysis of Laboratory Control Samples (LCS). For the PE project, the USGS will provide a set of Reference Materials that contain known amounts of Libby amphibole mixed with Libby soil. These site-specific reference materials will be the primary basis for evaluating accuracy. In addition, NIST has several reference standards for asbestiform minerals that are available on filters. Accuracy may also be evaluated based on analysis of these NIST filters with the understanding that they can assess accuracy in fiber counting and asbestos identification, but cannot evaluate the soil sample preparation techniques.

Data on the accuracy of this method are not yet available, and one of the goals of the PE study is to assess this parameter. Initial data will be collected from a limited number of highly qualified laboratories, and these results will be used to establish interim performance criteria for other laboratories. These criteria may be revised as additional data and experience are collected.

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Absence of contamination in the collection and analysis of samples is assessed by analysis of blanks. Two types of blanks will be run with each sample batch to ensure that there are no significant sources of asbestos contamination in the laboratory.

A matrix blank is a sample of field matrix (soil) that is free of the analyte(s) of interest (i.e., asbestos-free soil). Material to be used as matrix blanks will be provided by the

USGS. These samples must be inserted in the analysis stream and prepared in the same way as field samples at a frequency of 1% of samples, at minimum.

A sample blank is prepared by filtering 50 mL of water (the same type as used for sample suspension/sonication) through the same type of filter used to prepare TEM grids. A sample blank should be prepared each time a new batch of filters is opened and each time the filtering unit is cleaned. Blanks will be considered contaminated if they have a running average fiber loading greater than 18 asbestos structures per square millimeter or a maximum loading greater 53 asbestos structures per square millimeter (USEPA 1987). This generally corresponds to three or four asbestos structures found in ten grid openings. The source of the contamination must be found before any further analysis can be performed. Reject samples that are processed along with the contaminated blank samples and prepare new samples after the source of the contamination is found.

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Each laboratory performing sample preparation or analysis under this SOP must demonstrate method proficiency before analysis of field samples. The laboratory must also repeat this demonstration whenever new staff are trained or significant changes in instrumentation are made. Proficiency tests must be updated yearly, unless staffing or instrumentation changes require more frequent testing.

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At present, the acceptance criteria for blind PE samples have not yet been established. These criteria will be developed and applied as work progresses.

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Hardcopy Data. All data sheets and micrographs must be stored in a secured location with limited access (e.g., locking file cabinet) when not in use.

Copies (hardcopy and electronic) of the raw analytical data will be submitted to USEPA for archival.

9.0 REFERENCES

40 CFR 763, Appendix A to Subpart E of Part 763 -- Interim Transmission Electron Microscopy Analytical Methods – Mandatory and Nonmandatory – and Mandatory Section to Determine Completion of Response Actions.

ASTM. 2000. Standard Practice for Asbestos Detection Limit Based on Count. ASTM Designation D 6620-00.

Dana, ES. 1950. A Textbook of Mineralogy (4th edition). John Wiley and Sons, New York.

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USEPA. 1983. Analytical Method for Determination of Asbestos Fibers in Water. E.J. Chatfield and M.J. Dillon. United States Environmental Protection Agency Environmental Research Laboratory. EPA-600/4-83-043.

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USEPA. 1993. Method for the Determination of Asbestos in Bulk Building Materials. United States Environmental Protection Agency, Office of Research and Development. EPA/600/R-93/116. July 1993.

USEPA. 2001. EDS Spectra Characteristic Study for Libby-Type Amphiboles. Report prepared for USEPA Region 8 by Syracuse Research Corporation. July, 2001.

USEPA. 2004. Research Method for sampling and Analysis of Fibrous Amphibole in Vermiculite Attic Insulation. U.S. Environmental protection Agency, Office of Research and Development, National Risk Management Research Laboratory. EPA/600/R-04/004.

ATTACHMENT 1

GRAVIMETRIC REDUCTION DATA SHEET

EPA 600 Gravimetric Reduction Data Sheet.xls
(Check with Volpe or SRC to determine the most recent version number)

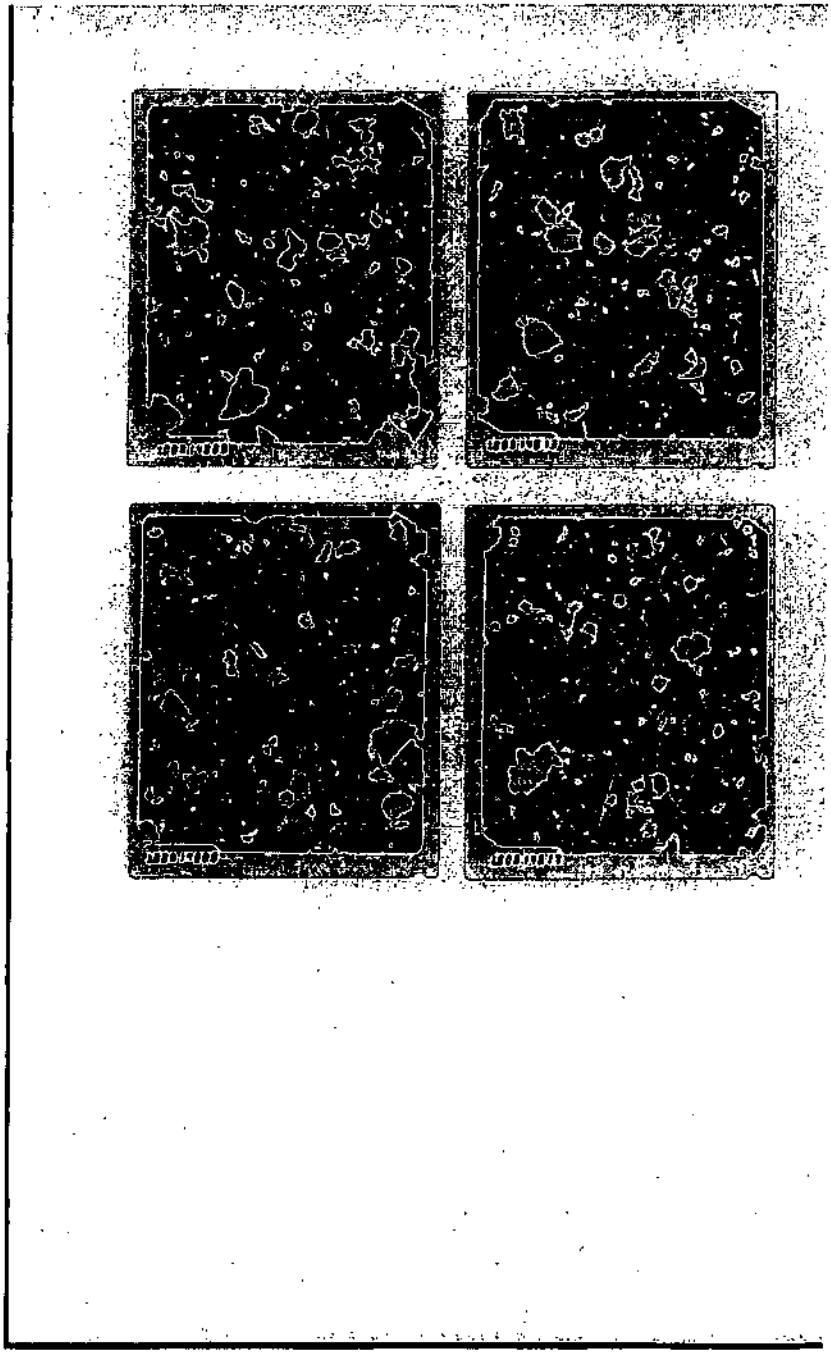
ATTACHMENT 2

SAMPLE ANALYSIS DATA SHEET

EPA 600 TEM Data Sheet.xls
(Check with Volpe or SRC to determine the most recent version number)

ATTACHMENT 3

PHOTOMICROGRAPHS ILLUSTRATING OPTIMAL LOADING



Tabbed Page: 23

Date: August 10, 2004 SOP No. EPA-LIBBY-07 (Revision 4)

Title: **ANALYSIS OF ASBESTOS IN SOIL BY TRANSMISSION ELECTRON MICROSCOPY FOLLOWING WATER SEDIMENTATION FRACTIONATION**

Authors: William Brattin — Syracuse Research Corporation
Jeanne Orr — Reservoir Environmental Services Inc.

SYNOPSIS: A standardized method for quantifying asbestos in soil using transmission electron microscopy is provided. This method uses water sedimentation to help separate soil particles from asbestos particles for easier identification and quantification.

APPROVALS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
USEPA Region 8	[signature]	8/17/04
Syracuse Research Corp.	WJ Brattin	8/10/04

Revision Number	Revision Date	Reason for Revision
0	11/16/01	NA
1	11/12/02	Incorporate changes based on laboratory experience
2	6/24/03	Incorporate changes based on laboratory experience
3	03/03/04	Allow for quantification by two methods
4	08/10/04	Add details on loading and mineral type classification

1.0 PURPOSE

The purpose of this standard operating procedure (SOP) is to specify a method for quantification of asbestos in samples of soil or other soil-like materials soil using transmission electron microscopy (TEM). This SOP is based on methods established previously, including *The Search for a Method Suitable for Supporting Risk Assessment: The Determination of Asbestos in Soils and Bulk Materials. A Feasibility Study* (Berman 1990), and *Superfund Method for the Determination of Releasable Asbestos in Soils and Bulk Materials, EPA 540-R-97-028* (USEPA 1997). This SOP includes clarifications and modifications to these methods specifically intended for application at the Libby Superfund site. This method is still undergoing testing and evaluation, and is not yet approved for application to field samples.

2.0 SCOPE AND APPLICATION

This method is specifically intended for analysis of asbestos in soil or other similar soil-like media that have been ground in accord with SOP ISSI-Libby-01. This method is appropriate for the analysis of all types of asbestos fibers, including both chrysotile and amphiboles, but has been optimized for estimation of amphiboles that are characteristic of the Libby site. These are referred to as Libby amphiboles (LA).

3.0 RESPONSIBILITIES

It is the responsibility of the laboratory supervisor to ensure that all analyses and quality assurance procedures are performed in accord with this SOP, and to identify and take appropriate corrective action to address any deviations that may occur during sample preparation or analysis. The laboratory supervisor should also communicate with project managers at EPA or their oversight contractors any situations where a change from the SOP may be useful, and must receive approval from EPA for any deviation or modification from the SOP before proceeding with sample preparation and analysis.

4.0 EQUIPMENT

- Analytical balance – for weighing samples
- Spatula or sample thief - for sample transfer from bulk sample container
- 25-mm or 47-mm filter apparatus, glass or disposable
- Filtered, deionized (FDI) water - sample suspension medium
- 25-mm or 47-mm diameter cellulose ester (MCE) filters with 0.22 um or smaller pore size - for filtration after fractionation by sedimentation
- Tweezers - MCE filter preparation for TEM

- Scalpel blade - MCE filter preparation for TEM
- Dimethylformamide/acetic acid mixture - MCE filter preparation for TEM
- Micropipette with disposal tips - MCE filter preparation for TEM
- Plasma etcher -MCE filter preparation for TEM
- Carbon coater - MCE filter preparation for TEM
- Jaffe washer - MCE filter preparation for TEM
- Acetone vapor generator- MCE filter preparation for TEM
- TEM- 80 to 120 kV transmission electron microscope (TEM), capable of performing electron diffraction with a fluorescent screen inscribed with calibrated gradations, is required. The TEM must be equipped with energy dispersive X-ray spectroscopy (EDS) and it must have a scanning TEM (STEM) attachment or be capable of producing a spot size of less than 250 nm in diameter in crossover. The microscope must be calibrated and maintained according to the requirements described in NVLAP Airborne Asbestos Program.

5.0 METHOD SUMMARY

A soil sample is suspended in water and allowed to settle under the force of gravity. Soil particles are mainly larger than asbestos particles, so they tend to settle toward the bottom more rapidly than the asbestos particles. Therefore, the upper portion of the water column becomes enriched in the asbestos fraction. A portion of fluid from the upper part of the water column is removed and the remaining particles are collected on an MCE filter by vacuum filtration. This filter is then prepared and examined by TEM. The concentration of asbestos in the sample (expressed as mass percent) may be estimated either from the mass of the asbestos particles observed (this method may tend to be biased low), or from the asbestos particle count using a standard curve developed using site-specific reference materials.

6.0 DETAILED METHOD

6.1 Fractionation by Water Sedimentation

1. Mix the sieved and ground soil sample by tipping the container upside down, and then returning it to its original position. Use a clean sample thief to remove a uniform aliquot (about 1,000 mg) of the material and weigh to the nearest 1 mg.

2. Add the sample though a large-neck funnel into a 100-mL graduated cylinder containing 20-mL of FDI water.

3. Rinse the container and funnel thoroughly with FDI water and add additional FDI water to a final volume of 100 mL.

4. Seal the top of the graduated cylinder with either a glass stopper or parafilm and thoroughly mix and suspend the soil by hand-inverting the cylinder 10 times.

5. Place the cylinder on a level surface and allow the suspension to settle for 30 minutes.

   Note: Based on empiric observations, this time is sufficient to allow most soil particles larger than about 5 um in diameter to settle out of the upper 5-10 cm of the cylinder, with relatively low loss of asbestos fibers.

6. Using a clean pipette, remove a 1.0-mL aliquot from a depth of 5 cm below the meniscus.

   Note: Do this by drawing lines on the cylinder at 4 cm and at 6 cm, and placing the pipette tip between them. Do not allow the tip to go deeper than 6 cm. The distance that particles settle under the force of gravity is not a function of the volume or size of the graduated grass cylinder. Thus, the sample should be collected at a depth of 5 cm from the top regardless of the cylinder size.

7. Place the 1.0-mL aliquot in a 100-mL graduated cylinder and add FDI water to a final volume of 100-mL.

6.2 TEM Filter Preparation

1. Thoroughly mix the 100 mL sample by hand-inverting 10 times.

2. Remove 20 mL and filter through a 47 mm MCE filter (0.22 um or smaller pore size) with a 47 mm MCE filter (5 um pore size) as backing filter using disposable filter funnel.

3. Prepare at least two grids from the filter for examination by TEM in accord with the standard methods described in the Asbestos Hazard Emergency Response Act (AHERA) (USEPA 1987), except where specifically indicated in this method or appropriate project-specific laboratory modifications.

6.3 Fiber Counting and Characterization by TEM

Begin by examination of a grid to evaluate the loading. If the density of fibers is too high (e.g., more than 20-30 per grid opening), or if the density of solid debris is too low (less than about

15%) or too high (e.g., greater than 25% coverage), then estimate the appropriate level of dilution that is needed to achieve an optimal filter preparation (15-25% loading), and re-prepare new TEM grids before proceeding with quantification.

Counting Rules

1. For every asbestos fiber with an aspect ratio ≥3:1, inspect the diffraction pattern and EDS in order to assign the particle into one of three classes:

   Libby Amphibole (LA)
   Other Amphibole (OA)
   Chrysotile (C)

   Examples of EDS that are characteristic of LA particles can be found in USEPA (2001) and USEPA (2004). In addition, because EDS spectra vary between instruments, each laboratory must generate a number of example spectra from authentic samples of LA in order to establish a laboratory-specific reference library.

   Key attributes of the electron diffraction pattern that must be satisfied in order to rank a particle as amphibole may be found in the AHERA TEM method (40 CFR Part 763). A more detailed description is provided in Su (2003). As above, each laboratory should establish and utilize a reference library of selected area electron diffraction (SAED) patterns derived from authentic Libby amphibole samples to aid in particle classification.

   With regard to classifying asbestos particles as LA, it is understood that LA spans a range of mineral classes (actinolite, tremolite, winchite, richterite, etc.) and that there can be variability in mineralogy between different LA particles and even within a single LA particle. Hence, it is expected that there may be uncertainty in the correct classification of some particles. In general, the "tough calls" (any particles for which the LA identification is uncertain) should not be ranked as LA. However, all such structures should be recorded on the data sheet, being sure to assign a "0" in the Total column. The comment field should be used to indicate that the particle may have been LA and why a definitive assignment was not made. If a "tough call" is ranked as LA, a record of the spectrum and the diffraction pattern should be collected and retained.

   It is assumed that most asbestos structures observed in the ground soil samples will be fibers. If a higher order structure (bundle, cluster or matrix with multiple structures) is encountered, count and record every substructure (fiber) that has an aspect ratio of 3:1 or greater. Identify the particle type as fiber, but add a note to the comment field indicating

that the fiber was part of a higher-order structure. In the case of a bundle, all of the sub-structures (fibers) may be recorded as a single unit, giving the dimensions of the bundle rather than the dimensions of each of the individual sub-elements (fibers). Be sure to record the particle type as bundle rather than fiber.

If any structure is observed that the analyst wishes to record but does not intend to be included in the estimation of weight percent, that structure should be assigned an index number of zero in the Total field of the data recording sheet along with a comment in the comment field explaining the importance of the observed structure.

2. Analyze a minimum of 5 grid openings or 50 fibers (whichever occurs first) on the first grid. Analyze the second grid until reaching a total (including the first grid) of at least 100 fibers or 10 grid openings (whichever occurs first).

3. Record all data for asbestos structures using the hardcopy and electronic data sheets provided as Attachment 2. Record the length, width, and asbestos type (LA, OA, C) for each structure. Make a record of the EDS spectrum and the diffraction pattern and take a photomicrograph for one fiber of each asbestos type encountered in each sample. It is not necessary to record data for non-asbestos structures.

6.4 Quantification of Results

The weight percent of asbestos in a sample may be estimated by one or both of two procedures, as described below. For the PE study, both methods will be employed.

Calculation Based on Particle Size Data

Calculate the weight fraction in the residue sample by estimating the total weight of all asbestos structures on the filter and dividing by the total weight of soil on the filter. The total weight of all asbestos structures on the filter is calculated as follows:

$$W_A = \frac{EFA \cdot 10^{-9}}{GO \cdot Ago} \cdot \sum t^2 \cdot l \cdot \delta$$

where:

W_A = weight of asbestos on filter (mg)
EFA = Effective filter area (mm^2)
GO = Number of grid openings counted

Ago = Area of grid opening (mm^2)
t = particle thickness (um)
l = particle length (um)
δ = particle density (g/cm^3)
10^{-9} = Unit conversion factor (10^{-12} $cm^3/um^3 \cdot 10^3$ mg/g)

Note that this calculation assumes that the cross-section of an asbestos particle is approximately square. The density of LA fibers is estimated to be approximately 3.1 g/cm^3 based on typical values for actinolite (3.0-3.2) and tremolite (2.9-3.2).

The weight of soil on the filter is calculated as follows:

$$W_S = (w / V1) \cdot (V2 / V3) \cdot V4$$

where:

W_S = weight of soil on the filter (mg)
w = weight of soil suspended in water (typically about 1000 mg)
V1 = volume of water used in original suspension (typically 100 mL)
V2 = volume of water (mL) removed after settling (typically 1.0 mL)
V3 = volume of water used to dilute V2 (typically 100 mL)
V4 = volume of suspension applied to filter (typically 20 mL)

Thus, the weight fraction (expressed as a percent) in the sample is calculated as:

$$WF(\%) = (W_A / W_S) \cdot 100$$

It is important to note that this method for estimating weight fraction may tend to be biased low. This is because some asbestos may be lost during settling, especially if any large particles of asbestos remain in the sample. Thus, the results derived from this approach should be treated as semi-quantitative, with the expectation that the true value is more likely to be above than below the measured value.

Estimation Using a Standard Curve

An alternative method for estimating the weight fraction of LA in a soil sample will be assessed as part of the PE study. The method uses an empiric standard curve to relates the frequency of LA structures (expressed as LA structures per gram of soil) to the nominal weight percentage in a series of calibration standards prepared and provided by USGS. For the PE study, the standard

curve will be based on at least three replicates of four different concentrations (0.2%, 0.5%, 1%, and 2%) and a control soil. The control soil is a site-specific soil sample that is expected to be free of asbestos at the study target detection limit (TDL). The range of concentrations included must bound the range of concentrations identified in investigate samples. The best fit linear equation through the data is established by ordinary linear regression, resulting in a model of the following form:

Weight % (residue) = a + b·LA (s/g)

Based on the best estimates of the model parameters (intercept = a, slope = b), the weight fraction of any field sample may be calculated from the measured LA particle frequency (s/g).

6.5 Disposal of Investigation Derived Waste

After successful preparation of all necessary filter and grids from a sample, place all of the unused materials into a large container and allow to settle for 1-2 days. Decant the fluid and filter through a 5 um filter as described in applicable State regulations. Dispose of the filtered water by pouring down the drain. Dispose of the filter and the residual mud in accord with applicable regulations for asbestos-containing waste.

7.0 LABORATORY-BASED QUALITY ASSURANCE/QUALITY CONTROL

7.1 Analytical Sensitivity

The analytical sensitivity of the method may be estimated as follows:

$$AS = \frac{EFA \cdot \mu}{GO \cdot Ago \cdot (w / V1) \cdot (V2 / V3) \cdot V4}$$

Typical values for these variables are as follows:

EFA (effective filter area)	= 1295 mm^2
μ (average mass of an asbestos structure)	= 6E-09 mg [1]
GO (number of grid openings counted)	= 10
Ago (area of a grid opening)	= 0.01 mm^2
w (weight of residue suspended in water)	= 1000 mg
V1 (volume of primary suspension)	= 100 mL

[1] This value is based on the average of 344 LA particles that have been evaluated as part of the ISTMs study and preliminary tests using the PE samples.

V2 (volume of suspension taken for analysis)	= 1.0 mL
V3 (volume of secondary suspension)	= 100 mL
V4 (volume applied to filter)	= 20 mL

Based on these typical values, it is expected that the typical analytical sensitivity will be less than 0.01%, which is sufficient for current decision-making requirements. If the a value of 0.01% is not achieved within 10 grid openings (e.g., because the mass of soil applied to the filter is small), or if samples are encountered that are enriched in larger particle sizes (sensitivity is strongly dependent on average particle size), increase the number of grid openings counted until a sensitivity of 0.01% has been achieved. The equation for calculating the number of grid openings needed to achieve a specified analytical sensitivity is as follows:

$$GO = \frac{EFA \cdot \mu}{(Target\ AS) \cdot Ago \cdot (w / V1) \cdot (V2 / V3) \cdot V4}$$

7.2 Detection Limit

The Method Detection Limit (MDL) depends on the true average rate that countable asbestos structures are observed in filters prepared from soil blanks (ASTM 2000). Because these data have not yet been collected, it is not possible to estimate the method detection limit at this time. After these data are collected, the MDL will be estimated in accord with ASTM Standard Practice D 6620-00 (ASTM 2000). A Target Detection Limit (TDL) may be assigned if defined by project objectives. For the PE study, a target value of 0.2% will be useful in evaluating the efficacy of the method since the lowest concentration on the standard calibration curve is 0.2%. Therefore, a TDL of 0.2% is assigned as an *a priori* guideline. This value is subject to change based upon information gained from the PE Study.

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Precision is defined as the agreement between a set of replicate measurements without assumption or knowledge of the true value. Agreement is usually expressed as the relative standard deviation (RSD) for a set of replicate measurements, or as the absolute difference between replicates. Each laboratory will obtain a measure of method precision by preparing and analyzing laboratory splits at a rate of 5% (1 laboratory split for every 20 field samples) or 1 per preparation batch, whichever is more frequent.

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9.0 REFERENCES

Berman DW. 1990. The Search for a Method Suitable for Supporting Risk Assessment: The Determination of Asbestos in Soils and Bulk Materials. A Feasibility Study. Report prepared for USEPA Region IX by D. Wayne Berman (ICF Technologies). February 6, 1990.

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USEPA. 2003. Technical Memo 8. Procedure for Combining Mass Fraction Estimates for Coarse and Fine Fractions of Soil. Prepared by US EPA Region 8 with technical assistance from Syracuse Research Corporation.

ATTACHMENT 1

EPA-LIBBY-07
TEM DATA RECORDING SPREADSHEET

SOP 7 TEM Data Sheet.xls
(Check with Volpe or SRC to determine the most recent version number)

Tabbed Page: 24

SAMPLING AND ANALYSIS PLAN FOR INDOOR DUST

For Use at the Libby, Montana, Superfund Site

Revision 0

August 7, 2003

Prepared by:
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APPROVAL PAGE

This Sampling and Analysis Plan has been prepared by the U.S. Environmental Protection Agency, Region 8, with technical support from CDM Federal Programs Corporation and Syracuse Research Corporation. Study activities addressed in this Plan are approved without condition.

Program Approval — Date: 8/6/03
Jim Christiansen
USEPA Remedial Project Manager
Libby Asbestos Site

Technical Approval — Date: 8/7/03
Mary Goldade
USEPA Regional Chemist

DOCUMENT REVISION LOG

Revision	Date	Major Changes
0	08/07/03	--

TABLE OF CONTENTS

A. PROJECT MANAGEMENT 1
A4. PROJECT/TASK ORGANIZATION 1
A5. PROBLEM DEFINITION and BACKGROUND 1
A6. PROJECT/TASK DESCRIPTION 2
A7. QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA 2

B. MEASUREMENT/DATA ACQUISITION 5
B1. SAMPLING PROCESS DESIGN 5
B2. SAMPLING METHODS REQUIREMENTS 7
B3. SAMPLE DOCUMENTATION, HANDLING AND CUSTODY REQUIREMENT 7
B4. ANALYTICAL METHODS REQUIREMENTS 9
B5. QUALITY CONTROL 11
B6. INSTRUMENT CALIBRATION AND FREQUENCY 12
B7. DATA MANAGEMENT 12

C. ASSESSMENT AND OVERSIGHT 13
C1. ASSESSMENTS AND RESPONSE ACTIONS 13
C2. REPORTS TO MANAGEMENT 13

D. DATA VALIDATION AND USABILITY 13
D1. DATA REVIEW, VERIFICATION, VALIDATION, and EVALUATION 14
D2. VERIFICATION AND EVALUATION METHODS 14
D3. RECONCILIATION WITH DQOs 15

E. REFERENCES 16

LIST OF FIGURES

Figure 1 Probability of Decision Errors 17

LIST OF APPENDICES

Appendix A Field Data Sheet for Dust A1
Appendix B Electronic Data Sheet for Air and Dust B1

LIST OF ACRONYMS AND ABBREVIATIONS

AHERA	Asbestos Hazardous Emergency Response Act
CSS	Contaminant Screening Study
DQA	Data Quality Assessment
EDS	Energy Dispersive Spectroscopy
LA	Libby Amphibole
PE	Performance Evaluation
QA	Quality Assurance
QC	Quality Control
RI	Remedial Investigation
RPD	Relative Percent Difference
RPM	Remedial Project Manager
SAP	Sampling and Analysis Plan
TEM	Transmission Electron Microscopy
USEPA	United States Environmental Protection Agency
VCI	Vermiculite-Containing Insulation

A. PROJECT MANAGEMENT

A4. PROJECT/TASK ORGANIZATION

The project for which this Sampling and Analysis Plan (SAP) has been prepared is being planned and performed by the U.S. Environmental Protection Agency (USEPA) Region 8. The following individuals are the USEPA project personnel with overall responsibility for the design and conduct of the project:

- Jim Christiansen, USEPA Remedial Project Manager (RPM), oversees all remedial activities at the Libby Operable Unit 4 site and is the primary data user and decision-maker at the site.

- Aubrey Miller, USEPA Regional Toxicologist, serves as the technical lead for all scientific and medical aspects of the project related to estimation of human health risk from asbestos exposure.

- Mary Goldade, USEPA Regional Chemist, serves as the technical lead for all aspects of the project related to the collection and analysis of environmental samples for asbestos content.

Responsibility for implementation of the SAP specified in this document has been assigned to the U.S. Department of Transportation Volpe Center and to CDM Federal Programs Corporation. The Volpe Technical Lead will have overall responsibility for coordinating the project and ensuring compliance with this SAP.

A5. PROBLEM DEFINITION and BACKGROUND

Libby, Montana, is a community located near an open pit vermiculite mine which began limited operations in the 1920's and was operated on a larger scale by the W. R Grace Company from approximately 1963 to 1990. Studies at the site have revealed that the vermiculite from the mine contains amphibole-type asbestos. This is referred to in this project as Libby Amphibole (LA) asbestos. Although the mine has ceased operations, concern exists that residual mine-related materials could be serving as on-going sources of LA release to indoor and outdoor air in Libby, and that these exposures could be of health concern to current and future residents and workers in the area.

One potential source of LA exposure in area homes and workplaces is indoor dust. Asbestos in indoor dust may become airborne as a result of disturbances by human activities, leading to the potential for inhalation of asbestos fibers by residents or workers. It is important to note that indoor dust is not a primary source of LA, but may become contaminated with LA from other

sources, including past or present releases from indoor vermiculite insulation, transport of outdoor soil that contains LA, historic transport of LA-containing dust from the mine on workers clothing, etc. Thus, once the pathway from the primary source to dust is interrupted, an eventual decrease in LA levels in indoor dust will occur. However, if the dust has become substantially contaminated with asbestos, human exposure may continue to occur for some time at levels of potential concern even after the primary source(s) is(are) controlled. It is for this reason that a method is needed to evaluate the level of asbestos in indoor dust and a guideline is needed for determining when the asbestos level is high enough to warrant a direct remediation of the dust.

This document provides a detailed description of the program that will be used for collection, analysis and interpretation of asbestos levels in indoor dust at Libby, Montana.

A6. PROJECT/TASK DESCRIPTION

The basic tasks required to develop a SAP that will support risk management decision-making with regard to indoor dust are:

1. Establish a standard method for the selection of indoor dust sampling locations
2. Establish a standard method for the collection of indoor dust samples
3. Establish a standard method for the analysis of indoor dust samples
4. Establish a quality assurance program to assess the reliability of the dust data
5. Establish a standard method for interpreting the results and deciding when dust remediation is needed

A7. QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA

EPA has developed a seven-step Data Quality Objectives (DQO) procedure that is designed to ensure that sampling and analysis plans are carefully thought out and that the plan will be adequate to meet the basic objectives of the program. Application of this seven step procedure to the development of the SAP for indoor dust is presented below.

Step 1. State the Problem

As discussed above, EPA's plan for addressing human exposure to asbestos in Libby focuses on identifying and removing LA sources such as vermiculite insulation and contaminated outdoor soils. However, in some locations, indoor dust may have become sufficiently contaminated that active remediation of dust is needed to protect human health. A standard method for dust collection and asbestos analysis has been established (ASTM D5755-95), but some project-specific clarifications and modifications are needed to ensure the data collected by this method are adequate to identify and quantify LA, and to ensure consistency in approach between laboratories. In addition, this method needs to be incorporated into a larger sampling and

analysis plan that provides a consistent and logical basis for deciding where to sample and how to interpret the results.

Step 2. Identify the Decision

The basic decision to be made is whether indoor dust at a particular location contains LA levels that are high enough that active remediation by EPA of contaminated dust is needed (in addition to direct source control). Although other types of asbestos may occur in some dust samples, all decisions regarding the need for active dust remediation by EPA will be based on LA levels.

Step 3. Identify Inputs to the Decision

Inputs to the decision include reliable data on the concentration of LA in dust. This information, in turn, is obtained by the collection and analysis of dust samples from homes and commercial properties in Libby. Analysis of dust for asbestos is achieved by microscopic examination of the dust sample in order to estimate the number of LA structures present in the sample. Results are generally expressed a structures per cm^2 of surface area sampled.

Step 4. Define the Study Boundaries

The spatial boundary of the project includes all homes and workplaces in Libby that may have been impacted by past or ongoing asbestos contamination. Because it is possible that, within any particular building, dust contamination with LA may vary between levels (floors), each level of a residence or building will be evaluated as a separate unit. Note, however, that because dust is not a primary but a secondary source, dust sampling is not needed in all locations, but only in locations where there is some reason to suspect that contamination of dust may have occurred by one or more LA release or transport mechanisms.

Step 5. Develop a Decision Rule

At present, there is no standard method for establishing a quantitative relationship between the level of LA in dust and the resultant level of health risk to humans. However, screening-level risk calculations indicate that a concentration of approximately 5,000 LA structures per cm^2 may be associated with risks that exceed EPA's usual guidelines and which could pose an unacceptable residual risk to residents (USEPA 2003). On this basis, the decision rule is:

> *If the average concentration of LA in indoor dust on a particular level (floor) of a particular residence or building is greater than 5,000 LA structures per cm^2, EPA will perform active remediation of that contaminated dust.*

If the average indoor dust concentration does not exceed 5,000 LA s/cm^2, active dust remediation will not be taken.

Step 6. Specify Limits on Decision Errors

Because of statistical variation when a dust sample is analyzed, the reported concentration in a sample may not equal the true concentration in that sample. In some cases, the estimate will be too high, while in other cases the estimate will be too low. Because of this statistical variation in analytical results, two types of decision error may occur:

> Type I Error: The true concentration of LA in dust is lower than 5000 s/cm^2, but the estimated value is above 5000 s/cm^2, and active dust remediation is undertaken even though it is not needed.
>
> Type II Error: The true concentration of LA in dust is higher than 5000 s/cm^2, but the estimated value is less than 5000 s/cm^2, and no active dust remediation is undertaken.

Both types of error are of concern to EPA. Occurrence of a Type II error means that humans may be exposed to LA at a level of potential concern, and a Type I error means that time and money resources have been expended that might have been better spent elsewhere.

Figure 1 shows the probability that a decision error will occur, plotted as a function of the true concentration of LA at a location. Because the probability of a decision error depends on the sensitivity of the analysis, three different curves are shown, corresponding to an analytical sensitivity of 500, 1,000, or 2,500 s/cm^2. When the true concentration is less than 5,000 s/cm^2, only Type I errors can occur (the true concentration is less than 5,000 s/cm^2, but the measured value may be above 5,000 s/cm^2). Conversely, when the true concentration is above 5,000 s/cm^2, only a Type II error can occur (the true concentration is above 5,000 s/cm^2, but the measured value may be below 5,000 s/cm^2). As seen, as the sensitivity increases (becomes poorer), the chances of both a Type I and a Type II error increase.

Choosing the maximum acceptable Type I and Type II error rate is a risk management decision. At this site, the error rate goals are as follows:

Error Type	Error Rate Goal
Type I	Less than 10% at a true concentration of 2,500 s/cm^2
Type II	Less than 10% at a true concentration of 10,000 s/cm^2

These error rates are chosen based on the recognition that the decision rule for active dust cleanup by EPA (5,000 LA s/cm^2) is based on a number of assumptions, and hence it is inappropriate to think of the rule as if there were a clear and sharp distinction between values above and below 5,000 s/cm^2. Rather, there is a gradient of concern as a function of increasing dust contamination, and values of 4,000 s/cm^2 (which would not trigger EPA intervention) are only slightly less of concern than values of 6,000 s/cm^2 (which would trigger EPA intervention).

In addition, it is important to recognize that if a Type II error occurs, the exposure that results is not expected to be long-lasting, but to diminish over time (because all known primary sources will have been removed or encapsulated). In this regard, EPA will provide HEPA vacuums to all residents in Libby, so routine cleaning by residents will, over time, have nearly the same effect on dust as active remediation by EPA. Finally, if a Type I error occurs, the funds and resources expended are not actually "wasted", since the presence of asbestos in dust does pose a hazard regardless of level, and removal of dust at locations that are actually somewhat lower than 5,000 s/cm^2 will only decrease risk and hasten the achievement of overall goals.

As seen by inspection of Figure 1, these target error rates can be achieved if the sensitivity of each dust analysis is 1,000 s/cm^2 or less. This sensitivity can be achieved most easily by adjusting the number of grid openings counted, or by using ashing to reduce the amount of non-asbestos debris in the sample (and hence reducing the dilution factor that must be used to avoid overloading).

Step 7. Optimize the Design for Obtaining Results

The SAP for dust may be revised and refined as additional information is collected during the project.

B. MEASUREMENT/DATA ACQUISITION

B1. SAMPLING PROCESS DESIGN

Indoor dust samples will be collected and analyzed in accord with this SAP under several different but inter-related programs at the site, including the following:

1. CSS. The Contaminant Screening Study (CSS) is an interim step in the performance of a full Remedial Investigation (RI) as the site. The CSS was initiated in May 2002 with the goal of assigning every property in Libby into one of three categories: remediation required, no remediation required, or additional information required. The CSS investigation uses a combination of visual inspections, verbal interviews, and soil sampling to screen each property in the study area for the presence or absence of potential sources of LA.

2. Pre-Design Investigations. Pre-design investigations will be performed at all homes that have been identified during the CSS as requiring one or more types of remediation. Pre-design inspection teams will collect any additional samples (potentially including indoor dust samples) required to determine specific remediation needs.

3. RI sampling. For homes that went through the CSS and were identified as requiring further evaluation (i.e., there was no clear trigger for cleanup, but there was some

indicator that dust may be contaminated), EPA will revisit the property, primarily to take dust samples.

As noted above, because indoor dust is a secondary source, sample collection and analysis is not required at all locations, but only when there is an indication that dust contamination might be of concern. The triggers for collection of a dust sample under each program vary, and are described in the project plan for each program (CDM 2002, 2003a, 2003b). Some possible examples of triggers for dust collection include the following:

- The property is located near a known historic or current source of LA or vermiculite release
- LA and/or vermiculite were detected above levels of concern in soil samples from the property
- The building previously had interior vermiculite-containing insulation (VCI), currently has non-exposed vermiculite, or the presence of VCI (past and/or present) cannot be determined
- The property is or was occupied by a worker at the mine
- The property is or was occupied by a person with asbestos-related lung disease

Specific triggers for dust collection and analysis may be revised as data collection proceeds and new information becomes available.

When a dust sample is collected, the indoor sampling locations at each such property will be selected in the field based on where contaminated dust is most likely to be found. All dust samples will be a composite, generally from three sub-locations, each 100 square centimeter (cm^2) in area. The number of samples and sample locations are determined as follows:

- Two dust samples (each a composite of three 100-cm^2 templates) will be collected on each level of the living space (i.e., finished basement, ground floor, 2nd floor). One composite sample from each floor will be collected from accessible horizontal surfaces (i.e., windowsill, shelving, cabinets, etc.). The second composite sample on each floor will be collected from high-traffic walkways (including solid surfaces and rugs or carpets), focusing on the most probable walkway for tracking contamination into that level of the building.

- Unfinished attics, basements, and attached garages will not be considered part of the living space and will be characterized separately. One three-template composite sample will be collected from high-traffic walkways and horizontal surfaces inside attics, unfinished basements, and attached garages.

- Outbuildings (i.e., shed, detached garages) will be sampled only if soil is observed to be contaminated, if the outbuilding contains, or is known to have contained, VCI, or if there are other indications of secondary sources (residents worked at the mine or have

asbestos-related disease, etc.). One three-template composite sample will be collected from the outbuilding entryway and horizontal surfaces.

Note that whenever exposed VCI is visible at a sampling location (i.e., a specific level of a specific building), a dust sample will not be collected from that location. This is because the presence of visible exposed VCI is taken as *prima fascia* evidence that active remediation of the VCI and any associated dust will be required. Therefore, collection and analysis of a dust sample is not needed.

B2. SAMPLING METHODS REQUIREMENTS

Dust sampling is performed in basic accord with the American Society for Testing Materials (ASTM) Method D5755-95 (ASTM 1995), except as noted below. In brief, dust is collected using a microvacuum that consists of a battery-operated low volume sampling pump connected to a 25-millimeter (mm) vacuum dust sampler cassette equipped with a mixed cellulose ester (MCE) filter with a pore size of 0.45 um.

Typically, three sub-areas, each 100 cm^2 in area, will be sampled. Each sub-area will be delineated using a template. At each sub-area, dust will be drawn into the cassette by activating the pump and passing the nozzle along the surface for 2 minutes in a manner sufficient to vacuum up the settled dust, as detailed in ASTM D5755-95. After dust from the last sub-area is collected, the cassette will be capped, sealed, and labeled as described in Section B3 (below).

Pump calibration will be performed by using a primary standard calibration device (e.g. Dry-Cal) with a 25 mm diameter 0.45 micron MCE filtered cassette inline before sample collection. The flow-rate of the low-volume sampling pump will be 2 liters per minute (approximate air velocity of 100 (± 10) cm/sec based on the flow rate and the 6.35 mm tubing diameter) throughout the sample period.

B3. SAMPLE DOCUMENTATION, HANDLING AND CUSTODY REQUIREMENT

Field Log Book

Each sampling team will maintain a field log book in accord with CDM SOP 4-1 (Field Logbook Content and Control). Typically the following information is recorded for each location visited:

- Names of team members
- Location (address) of sample collection site
- Date and time of sample collection
- Number and location of samples collected
- Location(s) of any visible VCI (dust samples will not be collected at these locations)
- Any deviations from standard procedures
- Any special circumstances that influenced sample collection

Field Data Sheets

Detailed information on each dust sample collected will be entered onto a field data sheet in accord with SOP CDM-Libby-03. Field data sheets to be used for dust are presented in Appendix A.

Sample Numbering System

All dust samples collected during the CSS and RI will be assigned a sample number in accord with the numbering system defined in the project-specific plan. These sample numbers may be assigned in any order that is convenient for field supervisors and sampling crews. In order to minimize the chance of error in number assignment, pre-printed sheets of adhesive labels with sequential sample numbers will be prepared and provided to field crews. Each sheet will have four identical labels for each sample number. Once a sample is collected, one adhesive label will be attached to the sample cassette, one to the zip-lock bag used to contain the cassette, one to the appropriate field data sheet, and one in placed in the field log book.

Sample Handling and Custody Requirements

Once the dust sample has been collected and sealed as described in ASTM D5755-95 (with modifications described above), a custody seal will be affixed to the cassette. The cassette will be placed into a separate zip-lock baggie. Custody of all indoor dust samples will be relinquished to the sample coordinator for hand-delivery to the onsite laboratory or shipment to an offsite laboratory. If dust samples are to be shipped, they will be packaged and shipped in accordance with CDM SOP 2-1, Packaging and Shipping of Environmental Samples (CDM 2002).

The chain-of-custody for all samples will be prepared in accordance with CDM SOP 1-2. All required paper work, including sample container labels, chain-of-custody forms, custody seals and shipping forms will be fully completed in ink (or printed from a computer) prior to shipping of the samples to the laboratory. All corrections to the chain-of-custody record will be initialed and dated by the person making the corrections. Each chain-of-custody form will include signatures of the appropriate individuals indicated on the form. The originals will accompany the samples to the laboratory, and copies documenting each custody change will be recorded and kept on file.

Upon receipt at the laboratory, the samples will be given to the laboratory sample custodian. The shipping container(s) will be opened and the contents inspected. Chain-of custody forms will be reviewed for completeness, and samples will be logged and assigned a unique laboratory sample number. Any discrepancies or abnormalities in samples will be noted and the sample coordinator for the project will be promptly notified.

All sample cassettes must be maintained by the laboratory under chain-of-custody until otherwise instructed by the laboratory coordinator.

Record Keeping

The Volpe technical lead will maintain all original hard copy and electronic data generated during the project, including log books, field data sheets, analytical data packages, and other relevant reports. One copy of each chain-of-custody will be kept by the sample coordinator. A complete copy of all relevant records will be provided to EPA.

B4. ANALYTICAL METHODS REQUIREMENTS

All dust samples will be analyzed for asbestos content using transmission electron microscopy (TEM) coupled with energy dispersive spectroscopy (EDS). Samples will be prepared for TEM analysis using an indirect preparation, as described in ASTM D5755-95, with project-specific modification as described in SOP SRC-LIBBY-05.

B4.1 Sample Preparation

Basic Procedure

The sample cassette is opened and the contents are rinsed out and resuspended in 100 mL of a particle free-mixture of water and ethanol (50:50 by volume). A fraction of this resuspension fluid (up to a maximum of 50 mL) is then applied to a secondary MCE filter such that the total dust loading on the secondary filter does not exceed approximately 20-25%. A fraction of this secondary filter is then used for preparation of two or more TEM grids, as detailed in ASTM D5755-95.

Check of Sensitivity

The sensitivity of an analysis is given by the following equation:

$$\text{Sensitivity (s/cm}^2\text{)} = \text{EFA} / (\text{GO} \cdot \text{AGO} \cdot \text{AREA} \cdot \text{F})$$

where:

EFA = effective area (mm^2) of the secondary filter
GO = Number of grid openings counted
AGO = Area of a grid opening (mm^2)
AREA = Area from which the dust sample was collected (cm^2)
F = Fraction of the original sample applied to the secondary filter

As discussed in the DQO section, a minimum sensitivity of 1,000 s/cm^2 is required in order to achieve the specified decision error tolerances. It is expected that for most dust samples, this

sensitivity can be achieved by analysis of 10 grid openings. For example, given typical analytical parameters (EFA = 1295 mm^2, GO= 10, AGO = 0.01 mm^2, AREA = 300 cm^2, F = 0.1), the sensitivity will be 432 s/cm^2. If the dust content of the sample is so high that a higher dilution is required (e.g., F < 0.05), then a sensitivity of 1,000 s/cm^2 cannot be obtained using 10 grid openings, but can be achieved by increasing the number of grid openings to some number between 11 and 20. In this case, the sample should be analyzed by counting the number of grid openings needed to achieve the target sensitivity. If a sensitivity of 1,000 s/cm^2 cannot be achieved by counting up to 20 grid openings, an ashing step is required in order to reduce debris loading (thereby allowing a larger value of F), as described below.

If the necessary sensitivity cannot be achieved even after ashing, then the laboratory should complete a laboratory modification form to summarize the issues associated with that sample.

Ashing (If Needed)

Based on the results of the first analysis, the laboratory should estimate the volume of resuspension fluid needed to achieve the detection limit required and apply that volume to a new secondary filter. The entire filter should be ashed and the residue re-suspended and filtered as described in EPA 540/2-90/005a (USEPA 1990), as modified in SOP SRC-LIBBY-02 (Revision 1).

B4.2 Sample Analysis

All dust samples will be analyzed by TEM as described in ASTM D5755-95, except for project-specific modifications described in SOP SRC-LIBBY-05.

Mineral Type Classification

Each countable asbestos structure shall be assigned to one of three broad classes of asbestos mineral class as follows:

Mineral Class	Description
Libby Amphibole (LA)	Any amphibole asbestos similar to that observed in ores obtained from the mine in Libby. This includes mainly actinolite, tremolite, richterite, and winchite, as well as magnesio-arfedsonite and ferro-edenite.
Other Amphibole (OA)	Other types of amphibole asbestos, including amosite, anthophyllite, and crocidolite. These forms of asbestos are not thought to be related to the mine in Libby.
Chrysotile (C)	Serpentine asbestos. This form of asbestos is the most common type in building materials, and is not thought to be related to the mine in Libby.

This assignment shall be based on a combination of particle morphology, electron diffraction pattern, and EDS spectrum. A discussion of the EDS spectrum associated with LA fibers is presented in USGS (2002).

Stopping Rules

All samples shall be analyzed by examination of a minimum of 4 grid openings. After a minimum of 4 grid openings, the count may be stopped when one of the following conditions is satisfied:

- A sensitivity of less than 1,000 s/cm^2 has been achieved
- A total of 20 grid openings have been examined
- A total of 50 countable LA structures have been recorded

All data shall be recorded in an electronic data sheet as provided in Appendix B. The sensitivity may be calculated using the electronic spreadsheet provided in Appendix C.

B5. QUALITY CONTROL

Quality Control (QC) is a component of the SAP, and consists of the collection of data that allow a quantitative evaluation of the accuracy and precision of the field data collected during the project. QC samples that will be collected during this project include the following types of samples.

Lot Blanks

Whenever a new lot of filter cassettes are received from the supplier, one cassette from the lot will be selected at random and submitted for analysis to ensure that no significant asbestos contamination exists on the filters from that lot. These will be supplied to the laboratory with a request for analysis using a DIRECT PREPARATION, since this increases the sensitivity and provides maximum likelihood of detecting any contamination on the filters. Results for lot blanks shall be reported only as fiber loading (s/mm^2), since there is no applicable value for the area vacuumed.

No samples shall be collected in cassettes that have not been subject to a lot blank check. All filters in the lot will be rejected if one or more asbestos structures (any type) are observed.

To date, no asbestos fiber has been observed on any lot blank.

Field Blanks

Field blanks will be collected at a frequency of 1 per 50 field samples (2 percent). This frequency is used in place of the more customary rate of 5% because of experience gained at the

site which indicates that the occurrence rate of LA fibers on field blanks is very low, as shown below:

Parameter	ISO	AHERA
Total number	1738 (100%)	1534 (100%)
Number with reported asbestos	45 (2.6%)	2 (0.13%)
Number with reported LA	7 (0.4%)	1 (0.07%)

Based on database download on July 31, 2003.

Field blanks are prepared by opening the end of a microvacuum cartridge at a sampling location and exposing to air for 5-30 seconds. The end is then closed and the sample submitted for analysis. Dust field blanks are used to determine if airborne asbestos is causing detection on dust samples and/or if cross-contamination is occurring during sampling procedures.

Like lot blanks, field blanks will be supplied to the laboratory with a request for analysis using a DIRECT PREPARATION, since this increases the sensitivity and provides maximum likelihood of detecting any contamination on the filters. Results for lot blanks shall be reported only as fiber loading (s/mm^2), since there is no applicable value for the area vacuumed.

If one or more LA structures is detected on a field blank, that result should be promptly communicated to the EPA regional chemist or her delegate. These individuals will evaluate the data, confer with project managers as needed, and decide what corrective actions may be required. Specific decisions may vary, depending on the nature of the samples in the same analytical group and the intended use of the data.

Laboratory-Based QC Samples

Each laboratory will prepare and analyze six different types of QC sample in accord with the frequency specified in Modification LB-00029. Responses for samples that are outside expected limits are also specified in Modification LB-00029.

B6. INSTRUMENT CALIBRATION AND FREQUENCY

All instruments shall be calibrated at a frequency in accord with NVLAP requirements. A log shall be maintained for each instrument that documents all calibration and maintenance activities.

B7. DATA MANAGEMENT

Data generated during this project will be managed using the basic methods and procedures established for this project. In brief, all data are entered into a project-specific database by

appropriately trained data entry staff. The data entered into the database includes all relevant field information regarding each environmental samples collected, as recorded on the Field data Sheets and in the field log book, as well as the analytical results provided by the laboratory. All data entries are reviewed and evaluated for accuracy by the data entry manager or his/her delegate. All original data records (both hard copy and electronic) will be cataloged and stored in their original form by the Volpe Technical Lead until otherwise directed by the EPA RPM.

C. ASSESSMENT AND OVERSIGHT

C1. ASSESSMENTS AND RESPONSE ACTIONS

Quality Assurance assessments performed during this project will include the following:

1) Oversight of field sampling activities
2) Oversight of sample handling and chain of custody procedures
3) Laboratory inspections

The following individuals or their delegates are authorized to perform any of the assessments above:

- EPA RPM
- EPA Regional Chemist
- Volpe Project Manager

Assessment of field sampling and other project activities may occur at any time and without prior notification. Each of the individuals above has authority to specify any appropriate response action that may be deemed necessary to resolve problems detected during the assessments. This could range from a simple review of approved SOPs with field staff to address minor problems, to requiring submission of corrective action reports and follow-up audits, up to a temporary stop work order to provide time for senior project managers to address more significant issues.

C2. REPORTS TO MANAGEMENT

Reports to management will be prepared and provided as described in each project-specific project plan. The Volpe Project Manager will provide the EPA RPM and the EPA technical leads and/or their delegates with regular verbal reports on project status.

D. DATA VALIDATION AND USABILITY

Data generated in accord with the guidance set forth in this SAP will be evaluated and/or reviewed for level of quality and usability as described in this section.

D1. DATA REVIEW, VERIFICATION, VALIDATION, and EVALUATION

Data Verification

Data verification is a consistent and systematic process that determines whether the data have been collected in accordance to the specifications as listed in the approved SAP, its appendices/attachments or approved modifications. This process is independent of data validation, and is conducted at various levels both internal and external to the data generator (laboratory).

Data Validation/Evaluation

Data validation is generally defined as an evaluation of the technical usability of the verified data with respect to planned objectives. Data validation is performed external to the data generator (laboratory) by applying a defined set of performance criteria to the body of data in the evaluation process. This may include checks of some or all of the calculations in the data set, and reconstruction of some or all final reported data from initial laboratory data (e.g., photographs, EDS spectra). It is in the data validation process that data qualifiers for each verified datum are evaluated and assigned. It extends beyond the analytical method or contractual compliance to protocols to address the overall technical usability of the generated data. EPA does not currently offer guidance on the validation of asbestos sample analysis. Therefore, project-specific review criteria have been developed and continue to be refined. These project-specific review criteria are referred as "Data Evaluation" to avoid confusion if data validation procedures are defined by EPA in the future. Further, data evaluation is performed as a component to the overall Data Quality Assessment (DQA) process which will be employed as a project-specific review on overall precision and accuracy of site samples.

D2. VERIFICATION AND EVALUATION METHODS

Data Verification

Data verification will include a review of the findings of all QA assessment activities (see Section C), including assessments of field collection procedures, sample labeling methods, chain-of-custody procedures, and all assessments of analytical data collection, recording and reporting. If any deviations are identified, the potential impact of those deviations on the reliability of the data will be assessed, and that information will be provided to the EPA RPM, the EPA technical leads, and the Volpe project manager.

Data verification for field samples will be performed by ensuring that each sample is accompanied by a complete chain of custody record, a complete field data sheet, a properly completed laboratory data sheet, and electronic data are properly imported into the project database. Any sample that lacks one or more of the required sets of documentation will be excluded unless the missing sample identification and documentation can reliably be obtained. In addition, the comments sections of the field data sheet and electronic laboratory data

deliverable for each sample will be reviewed to determine if there are any notations which indicate that the sample may not be reliable. Any sample with a notation which indicates that the result may not be reliable will be considered unreliable unless a subsequent review determines that the datum is reliable. Data points determined to be unreliable or invalid will be permanently flagged with an "R" qualifier in the project database and excluded from subsequent data analyses, summaries, and reports. Documentation for the justification for the "R" qualifier as well as any other qualification assignments will be placed in the permanent project file.

Data Evaluation

The data evaluation process or Data Quality Assessment consists of two parts. First, a subset of data packages are reviewed against a data package checklist to determine if the raw data package is complete. The first 3-5 data packages prepared by each analytical laboratory will be reviewed. Subsequent data package reviews will be performed on a tri-monthly basis where data packages are randomly selected for each analytical laboratory.

The second part is a Data Quality Assessment (DQA) review. The DQA review process employs as a project-specific evaluation of overall precision and accuracy of site quality control (QC) samples. This DQA will be performed on a regular intervals to determine is QC samples such as blanks, duplicates, and reference samples/performance evaluation samples are within project-specific limits for precision and accuracy. These quantitative or qualitative limits of acceptability defined for precision and accuracy are discussed below.

> Precision: Precision is defined as the agreement between a set of replicate measurements without assumption or knowledge of the true value. Agreement is often expressed as the relative percent difference (RPD) for duplicate measurements or standard deviation for larger numbers of replicates. Data on precision are obtained by analyzing duplicate or replicate samples.
>
> Accuracy: Accuracy is a measure of the closeness of a sample analysis result to the "true" value. The accuracy of an analytical method is generally assessed by inserting a series of blind "performance evaluation" (PE) samples into the laboratory sample stream, where the "true" concentration of analyte in each PE sample is known. However, PE samples are not available for asbestos in dust, so accuracy will be evaluated primarily by an evaluation of agreement between repeat analyses, both within a laboratory and between laboratories.

Data quality assignment recommendations resulting from the DQA review will be documented in a technical memorandum and qualifications applied to electronic data (database) accordingly.

D3. RECONCILIATION WITH DQOs

The Data Quality Assessment reports will be reviewed by the EPA Regional Chemist and the Volpe Project manager to determine its usability in accordance with EPA guidance on assessing

data useability for risk assessment (USEPA 1992), and to determine whether the data are adequate to meet the project objectives.

E. REFERENCES

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CDM. 2003b. Pre-Design Sampling and Analysis Plan. *Document in preparation.*

NIST. 1994. Airborne Asbestos Method: Standard Test Method for Verified Analysis of Asbestos by Transmission Electron Microscopy - Version 2.0. SOP prepared by S. Turner and E.B. Steel, U.S. Department of Commerce, National Institute of Standards and Technology. NIST IR 5351. March 1994.

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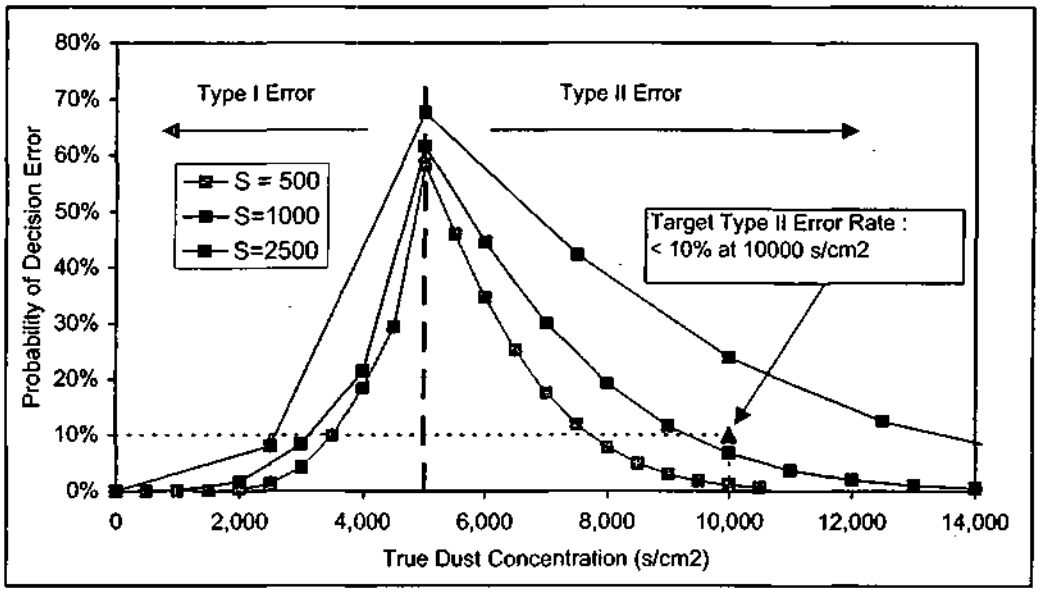
USEPA. 1992. Guidance for Data Useability for Risk Assessment (Part A). U.S. Environmental Protection Agency, Office of Emergency and Remedial Response. Publication 9285.7-09A. April, 1992.

USEPA. 2003. Screening Level Estimates of Exposure and Risk from Libby Amphibole in Air, Dust, and Soil. Report prepared by USEPA Region 8 with technical assistance from Syracuse Research Corporation.

USGS. 2002. Guide to Analysis of Soil Samples from Libby, Montana for Asbestos Content by Scanning Electron Microscope and Energy Dispersive Spectroscopy. U.S. Geological Survey Administrative Report prepared by Amy Bern, Greg Meeker, and Isabelle Brownfield. October 17, 2002.

FIGURE 1. PROBABILITY OF DECISION ERRORS



EXPLANATION. The estimated concentration of asbestos in a dust sample (s/cm2) is calculated as N*S, where N = number of structures observed and S = sensitivity. The sensitivity (s/cm2) is a function of the area of the secondary filter, the area of a grid opening, the number of grid openings counted, the area vacuumed, and any dilution factors used to prepare the filter. Because of statistical variation, there is uncertainty in any observed value of N. This uncertainty is described by the Poisson distribution, which predicts the chances of observing a count of N if the true concentration is equal to some specified value (e.g., 5000 s/cm2). The three curves above are derived using the Poisson distribution to calculate the statistical chances of observing a count that will result in an decision error, assuming a decision rule of 5,000 s/cm2.

APPENDIX A

FIELD DATA SHEETS FOR DUST

Sheet No: D-___

LIBBY MONTANA SITE INVESTIGATION
FIELD SAMPLE DATA SHEET FOR
DUST

Address or Location ID:_____
GPS (if no address available): Northing_____ Easting_____
Owner:_____
Land Use Category: Residential School Commercial Mining Other (_____)

Date:_____
Sampling Team:_____

Data Item	Cassette 1	Cassette 2	Cassette 3
Field ID Number			
Index ID			
Category (circle)	FS Blank	FS Blank	FS Blank
Location Description			
Sample area (cm^2)			
Flow Meter Type			
Flow Meter ID Number			
Pump ID Number			
Start-Time			
Start-Flow (L/min)			
Stop-Time			
Stop-Flow (L/min)			
Pump fault?	No Yes	No Yes	No Yes
Field Comments			

APPENDIX B

ELECTRONIC DATA SHEET FOR AIR AND DUST

Check with Volpe or SRC for latest spreadsheet version number

APPENDIX C

ELECTRONIC SPREADSHEET FOR CALCULATING SENSITIVITY

"Sensitivity Calc.xls"

DUST SENSITIVITY

Input values in yellow.

S = EFA / (GO * Ago * Area * F)

Constants

EFA (secondary filter)	1295	mm2
Ago	0.01	mm2
Area	300	cm2
F	0.100	(use calcualtion on right if needed)

SENSITIVITY (s/cm2)

	Number of GO Counted			
	4	10	15	20
Sensitivity	1079	432	288	216

Calculation of F (no ashing)

Total resuspension volume	100	mL
Volume applied to secondary filter	10	mL
Value of F	0.100	

Calculation of F (with ashing)

Total resuspension volume (pre-ashing)	100	mL
Volume applied to filter for ashing	20	mL
Volume used to resuspend ashed residue	50	mL
Volume applied to secondary filter	25	mL
Value of F	0.100	

Tabbed Page: 25

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GUIDE TO ANALYSIS OF SOIL SAMPLES FROM LIBBY, MONTANA FOR ASBESTOS CONTENT BY SCANNING ELECTRON MICROSCOPE AND ENERGY DISPERSIVE SPECTROSCOPY

by

Amy Bern
Greg Meeker
Isabelle Brownfield

U.S. Geological Survey Administrative Report
For the U.S. Environmental Protection Agency

October 17, 2002

This report is preliminary and has not been reviewed for conformity with U.S. Geological Survey editorial standards. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Fibrous materials can not be reliably identified as amphibole by morphology alone. Other materials, such as calcite, pyroxene, or biotite, can also have cleavage that produces long thin particles or scrolls. Libby, Montana soils contain many of these minerals. To accurately identify amphibole fibers by SEM analysis, EDS spectra must be obtained to verify that mineral composition is consistent with an amphibole.

Two EDS spectra of amphiboles are illustrated in Figure 1a. The first is an example of an amphibole from Libby Montana. The second is an International Union Against Cancer (UICC) standard tremolite. Libby amphiboles contain the elements Na, Mg, Al, Si, K, Ca, Ti, Mn, and Fe in varying amounts, but similar to those shown in Figure 1a. F and Cl also occur, but usually not in quantities great enough to show on EDS spectra. Some diagnostic spectra of other minerals are presented for comparison.

Biotites contain many of the same elements as Libby amphiboles. The Al and Mg content is used to distinguish biotite from amphibole. Biotites contain much more aluminum than Libby amphiboles. EDS spectra of Libby micas are provided in Figure 1b for comparison.

Pyroxene and Libby amphibole can be difficult to tell apart, but usually can be distinguished by the absence of K and higher Al in pyroxene. An EDS spectrum for a typical pyroxene is given in Figure 1c.

The best way to determine if the chemical composition is consistent with an amphibole is to perform a "quantitative" analysis.[1] In most cases a "standardless" analysis will provide sufficient accuracy to give confidence that the material is compatible with an amphibole. For this analysis, oxygen should be determined by stoichiometry and cation ratios for Na, Mg, Al, Si, K, Ca, Ti, Mn, and Fe should be calculated based on 23 oxygens. The cation total should equal between 15 and 16. Examples of EDS analyses for Libby Amphibole weight percentages and cations are given in Table 1.

Table 1. EDS Analysis of Libby Amphibole Fibers.

Weight Percent									
	Na2O	MgO	Al2O3	SiO2	K2O	CaO	TiO2	MnO	Fe2O3
1	4.7	21	0.43	57	1.3	7.5	0.33	0.17	7.7
2	5.5	21	0	60	2.1	4.4	0	0.03	8.0
3	1.6	23	0.14	59	0.37	12	0.12	0.01	3.1

Cation Totals (all Fe is Fe3+)										
	Na	Mg	Al	Si	K	Ca	Ti	Mn	Fe3	Total
1	1.22	4.27	0.07	7.77	0.22	1.09	0.03	0.02	0.81	15.50
2	1.44	4.11	0.00	8.07	0.35	0.63	0.00	0.00	0.84	15.43
3	0.42	4.64	0.02	7.90	0.06	1.78	0.01	0.00	0.32	15.16

	T Site Total	C Site Total	B Site Total	A Site Total	Cation Total
1	7.87	5.00	2.00	0.63	15.50
2	8.00	5.02	2.00	0.42	15.43
3	7.93	4.96	2.00	0.26	15.16

T site-Si ≅ 8 C site-Al + Fe + Mg + Ti + Mn ≅ 5 A+B sites-Ca + Na + K >= 2

1. Quantitative analysis requires matrix corrections to correct for atomic number, absorption, and fluorescence effects. Matrix correction routines (e.g. ZAF or Phi Rho Z) assume that the sample is homogeneous in the volume analyzed, and has a flat polished surface. If these conditions are not met the results can have errors that are larger than what is normally expected. The magnitude of this error will depend on the size, shape and composition of the sample or particle and also on the analytical conditions, particularly accelerating voltage (Small and Armstrong, 2000). Our experience with the analysis of single structures of Libby amphibole using the analytical conditions specified in this procedure is that the errors for all elements are typically within +/-10 % relative concentration of the expected value, and often much better.

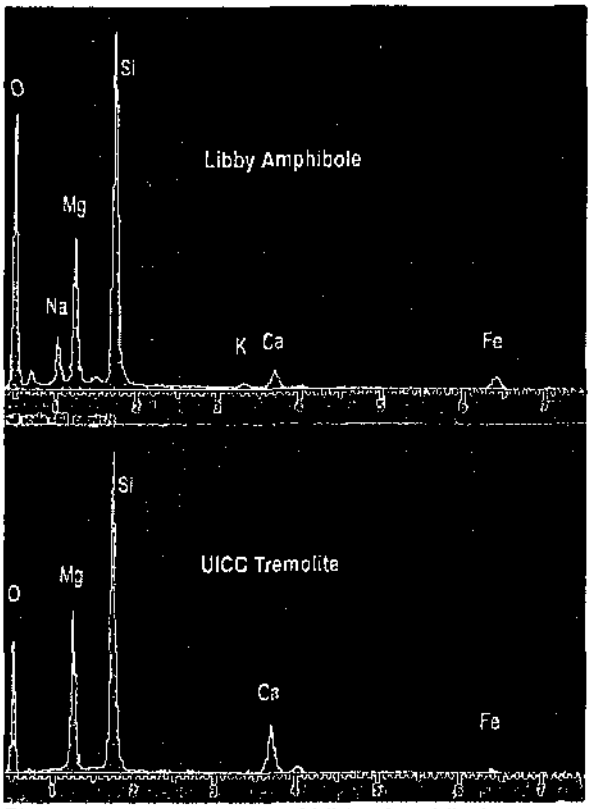

Figure 1a. EDS Spectra of Libby Amphibole and Tremolite.

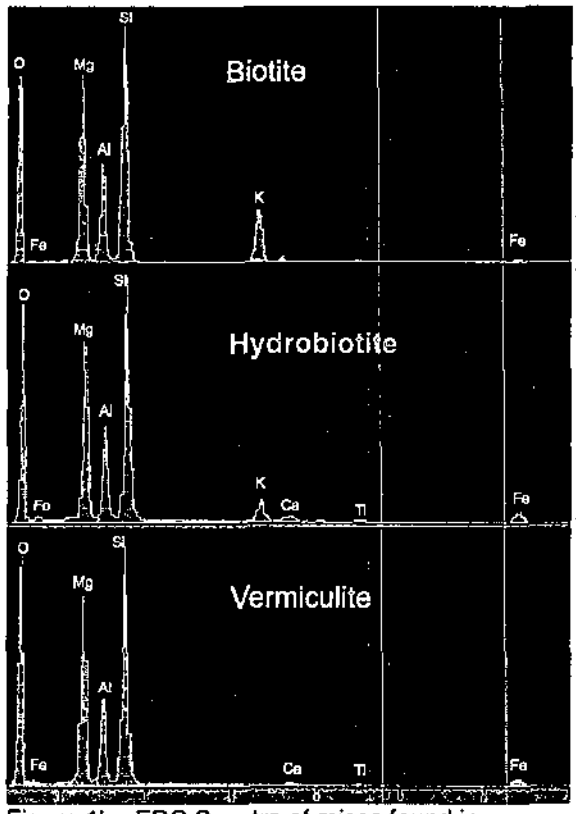

Figure 1b. EDS Spectra of micas found in Libby Samples.

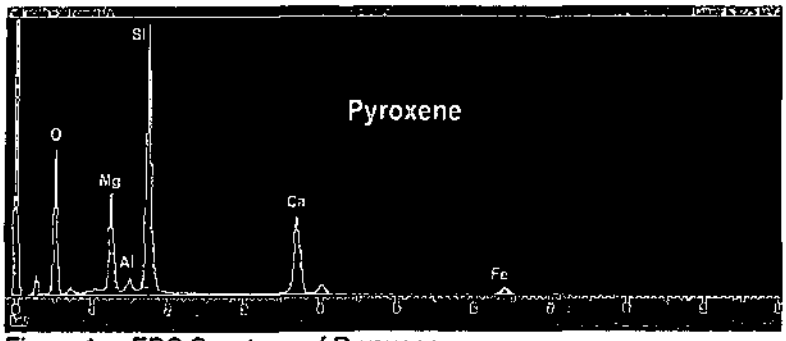

Figure 1c. EDS Spectrum of Pyroxene.

Reference

Small, J.A. and Armstrong, J.T., 2000, Improving the Analytical Accuracy in the Analysis of Particles by Employing Low Voltage Analysis. Microscopy and Microanalysis, vol. 6, supplement 2. Proceedings 2000, p.924-925.

Tabbed Page: 26

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PREPARATION AND ANALYSIS OF SOIL SAMPLES FOR AMPHIBOLE ASBESTOS CONTENT BY SCANNING ELECTRON MICROSCOPY AND ENERGY DISPERSIVE SPECTROMETRY

by

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Introduction

The purpose of this report is to document the procedure for preparing soil samples from Libby, Montana that may contain amphibole asbestos for analysis by scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS).

This method is limited to sample preparation and analysis of soil samples for characterization and analysis of amphibole asbestos particles using SEM/EDS techniques. The asbestos particles of interest are species found at the vermiculite mine site near Libby, Montana, which may include, but may not be limited to various amphiboles as: tremolite, actinolite, winchite, richterite, edenite, magnesioriebeckite, and magnesio-arfvedsonite.

Principle

Careful, consistent sample preparation is essential to obtain results that are reproducible. Glass or mineral standards of known concentration should be run first to calibrate the instrument.

The procedure includes soil drying, homogenization, ashing, crushing, grinding, sieving, and mounting sample. Appropriate care is emphasized to keep contamination and degradation of sample asbestos particles from occurring.

Scope

This method is applicable to the analysis of asbestos-bearing soil samples. Soils of any asbestos concentration can be analyzed by the procedure after preparation as long as calibration is performed.

Apparatus

- HEPA (High-Efficiency Particulate Air filter) negative flow hood
- Forceps
- Kimwipes
- Spatulas
- Stereomicroscope
- Glass petri dishes or aluminum weighing pans
- Heat lamps
- Denver Fire Clay Company No. 1 rock crusher (Model 2P2P1 1121-1) or similar crusher
- Ashing furnace
- Ceramic dishes

- Analytical balance (accuracy to 0.05 g)
- Jones Riffle Splitter (14-0.5 inch chutes, 7x11 inch hopper) or similar
- Mortar and pestle (agate or ceramic)
- 230 µm (60 mesh) sieve with lid and bottom pan
- SEM sample stubs with conductive carbon adhesive tabs
- Polycarbonate filters, 27 mm diameter, 0.4 µm pore size
- Millipore apparatus (small)
- Eppendorf pipette, 20 µL capacity
- Disposable pipette tips
- Tall 800mL beaker
- Razor blade
- Teflon-coated magnetic stirring bar
- Magnetic stirring plate

Reagents

none

Safety precautions

All laboratory personnel must wear safety glasses, a lab coat or apron, and gloves. All procedures involving dry asbestos or potentially asbestos-contaminated soils must be preformed in a HEPA hood. Respirable fibers may exist in the sample. Samples handled outside of the HEPA hood must be covered with aluminum foil or placed in sealed glass jars.

Procedure

Sample Drying--Moisture within the sample will affect measurements performed. If sample is suspected to contain moisture, the following procedure should be used.

Weigh a clean ceramic dish, large enough to hold the sample, to the nearest 0.1 g. Transfer the sample into dish and dry under a heat lamp or in a sample drying oven. For oven drying or heat lamp drying the temperature should not exceed 100°C. Drying time should take less than 24 hours. The sample may need to be stirred to increase the surface area exposed to drying. Drying must be performed under negative flow HEPA hood.

To test for sample dryness, allow the sample to cool and place the sample in the ceramic dish on a weighing pan. Weigh sample to nearest 0.1 g. Remove the sample, wait 10

minutes and weigh it again. If sample weights are the same or more than the previous sample weight, the sample is dry enough. Record sample weight to nearest 0.1 g.

Transfer sample to glass jar(s) with lid(s) for storage and stereoscopic microscope analysis. Plastic jars will increase static buildup, which will attract particles, causing low asbestos quantities to be measured. Therefore, glass jars should be used to minimize this attraction.

Homogenization--Homogenization will ensure that any material analyzed is representative of the entire sample. Through this process, fiber bundles can be separated from organic and other materials. Homogenization will also accelerate the ashing process. The following procedure should be performed if the sample contains particles larger than 3mm in diameter (~6 mesh).

Homogenization is achieved by running the sample through a rock crusher set to crush to 1-3mm. If a rock crusher is not available, one of the following procedures can be used:

1) hand grinding with mortar and pestle
2) milling (Wylie mill, cryomill)

Grinding should be done under negative flow HEPA hood.
After grinding, weigh sample to nearest 0.1 g. This will give an estimate of the weight of sample lost during homogenization. Place the sample into glass containers with lids.

Stereoscopic microscope examination-- For major concentrations of coarse-grained asbestos materials in soil samples (estimated 1% by weight or greater), stereoscopic analysis may be sufficient. However, for positive identification of asbestos particles and to accurately determine the quantity and chemical composition of asbestos particles, SEM/EDS analysis is required.

Stereoscopic analysis in multiple fields allows the technician to note homogeneity, texture, color, friability, and fibrous material content.

Stereoscopic analysis should be done in glass petri dishes or aluminum weighing pans. The procedure is performed in a negative flow HEPA hood, or in a glove box. If a HEPA hood is not available, then analysis should be performed while the sample is still in glass jars to protect the analyst from inhaling any amphibole particles in a sample.

Sample Ashing to remove organics—Substantial organic material in the sample can affect or prohibit analysis by SEM. Perform this procedure if the sample appears to contain organic material that could outgas at high vacuum.

Transfer the material to small ceramic dishes under a negative flow HEPA hood.

Use a sample splitter to separate the dry sample into two homogenous splits. Archive one split for further analysis, if needed. Weigh the second split to the nearest 0.1g, then transfer to a ceramic dish using a clean spatula, transferring as much of the dry sample as possible. Smaller sample sizes will ensure the thoroughness of the ashing procedure.

Place the ceramic dishes containing the samples into the furnace. The furnace program should proceed as follows:

1) Increase temperature by 1°C/minute until sample reaches 250°C.
2) Hold temperature at 250°C for 4 hours.
3) Increase temperature by 1°C/minute until sample reaches 480°C.
4) Hold temperature at 480°C for 8 hours.
5) Shut off furnace.
6) Allow sample to cool before removing from furnace.

Furnace and sample temperature should not exceed 500°C. Although amphiboles are stable at higher temperatures, 480°C is sufficient for decomposing organics, therefore, higher temperatures are not required. Quick combustion of the sample is minimized by slowly increasing the temperature to 250°C and then to 480°C. If the temperature of the furnace rises too fast or does not hold at 250°C, the temperature of the sample may rise above 500°C. Quick combustion of organic material may also cause flashing which could cause fine particulates to become airborne and contaminate the furnace and other samples. Slowly heating the sample and holding the sample temperature at 250°C until organic material carbonizes minimizes flashing.

It should be noted that the ashing time is dependant on the furnace temperature, the amount of sample, the amount of organic material in the sample, and the grain size. Ashing time should be adjusted accordingly. Allow the sample to equilibrate to room temperature within the furnace, if possible. If this is not possible, remove the sample from furnace and allow it to reach room temperature under negative flow HEPA hood. When sample has equilibrated, weigh it to the nearest 0.1 g.

Weigh sample and perform stereoscopic analysis.

Split the sample into four equal parts using sample splitter and transfer splits into glass containers with lids to prevent contamination. Plastic containers will attract particles. One

split is for SEM/EDS analysis and others may be used for XRD and/or reflective spectroscopy.

Sieve Sample—For SEM/EDS analysis, further split one of the four sample splits using a Jones Precision Riffle Splitter to obtain a split of approximately 2 grams. Sieve this split through a 230 µm (60 mesh) sieve. Grind the >230 µm fraction and sieve again. While sieving, samples must be under a HEPA hood and a lid should be placed on the sieves. This will prevent contamination and sample loss. Hand grinding is recommended using an agate (or ceramic) mortar and pestle so that the sample just passes through the sieve. Grinding by mechanical means often will over-grind the sample so that most of the grains are too fine. Repeat until the entire sample passes through the 230 µm sieve.

After sieving, place the sample in a glass jar with lid and mix by turning the jar end over end for approximately 10 minutes to ensure homogenization.

Mounting Sample on SEM stub— Prepare an aliquot of the sample for SEM/EDS analysis. Mix with magnetic stirrer and prepare SEM sample stub. The HEPA hood should be freshly cleaned to prevent contamination from previous work.

Set up a Millipore filter apparatus for use with 27 mm polycarbonate filters. Place a few drops of distilled water on the fritted glass surface and place a 27 mm polycarbonate filter (0.4 µm or less pore size) on the water, shiny side up. Attach the top of the apparatus. Add a few milliliters of distilled water on the filter so that no part of the filter is exposed to air. Take a clean Eppendorf pipette tip and with a razor blade, cut enough material off the tip to increase the opening to approximately 1 mm. Transfer 0.5 g of the sample prepared for SEM/EDS analysis to an 800 mL glass beaker containing 125 mL of distilled water and a 2-inch magnetic stirring bar, and place on a magnetic stirring plate. Slowly increase the speed of rotation until all the particles are suspended and a good vortex is achieved without excessive splashing. While the sample is mixing, and the particles are suspended, collect 20 µL of the mixture using the Eppendorf pipette. Transfer this to the polycarbonate filter by dropping the suspension from 3 to 4 cm above the water in the Millipore apparatus. Place two more 20 µL drops of the suspension onto the polycarbonate filter for a total of 60 µL.

Allow the water to filter through the polycarbonate. If a hand-held vacuum pump is available, it may be used to decrease the time it takes for complete filtration. Do not use motorized pumps because sample will become too dry on the filter, increasing the possibility of sample loss. Use forceps to pick up the filter just as the water has passed through but before it is dry and place it on a carbon adhesive tab on a standard SEM mount. The filter needs to be

completely flat on the SEM stub. This is achieved by forming the wet filter into a gentle U-shape. Place the bottom curve of the filter onto the center of the carbon adhesive tab and slowly release the sides so they lay flat. Trim the edges of the polycarbonate filter using a razor blade. After drying, coat the stub with carbon using a carbon evaporator. The stub is then ready to be taken to the SEM in a clean, covered container. Samples must be prepared in a HEPA hood.

The procedure above produces an average area coverage of about 20%.

Background References

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Soil Sample Preparation SOP Revision 1. SOP #IS SI-LIBBY-0 I. Prepared by 13~1 Consulting Group, Inc. for USEPA, Region 8. January 7, 2000.