EPA-600/2-77-178
Revised June 1978

Environmental Protection Technology Series

ELECTRON MICROSCOPE MEASUREMENT OF AIRBORNE ASBESTOS CONCENTRATION

A Provisional Methodology Manual

EPA Report Collection
Regional Center for Environmental Information
U.S. EPA Region III
Philadelphia, PA 19103

Environmental Sciences Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triang[illegible]rth Carolina 27711

ALL-STATE LEGAL®
DEFENDANT'S EXHIBIT
195

HWBUI0008813

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
ENVIRONMENTAL SCIENCES RESEARCH LABORATORY
RESEARCH TRIANGLE PARK
NORTH CAROLINA 27711

NOTICE OF REVISED PROVISIONAL METHODOLOGY MANUAL

The enclosed copy of the EPA publication "Electron Microscope Measurement of Airborne Asbestos Concentrations - A Provisional Methodology Manual" represents a revised edition of document EPA-600/2-77-178 which was first published in August 1977.

The changes are mostly minor and have been made primarily for the sake of clarity. The most important changes are in the section (2.5.2.4) on Fiber Classification Rules, wherein the procedure is described in more explicit terms and should be easier to follow.

Please discard any copies you may have of the earlier edition.

HWBUI0008814

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into five series. These five broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The five series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies

This report has been assigned to the ENVIRONMENTAL PROTECTION TECHNOLOGY series. This series describes research performed to develop and demonstrate instrumentation, equipment, and methodology to repair or prevent environmental degradation from point and non-point sources of pollution. This work provides the new or improved technology required for the control and treatment of pollution sources to meet environmental quality standards.

EPA REVIEW NOTICE

This report has been reviewed by the U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policy of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

EPA-600/2-77-178
Revised June 1978

ELECTRON MICROSCOPE MEASUREMENT OF AIRBORNE ASBESTOS CONCENTRATIONS

A Provisional Methodology Manual

by

Anant V. Samudra
Colin F. Harwood
John D. Stockham
IIT Research Institute
Chicago, Illinois 60616

Contract No. 68-02-2251

Project Officer

Jack Wagman
Director, Emissions Measurement and Characterization Division
Environmental Sciences Research Laboratory
Research Traingle Park, North Carolina 27711

ENVIRONMENTAL SCIENCES RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, N.C. 27711

HWBUI0008816

DISCLAIMER

This manual has been reviewed by the Environmental Sciences Research Laboratory, U. S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U. S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

HWBUI0008817

FOREWARD

Asbestos or asbestiform minerals include several types or groups of fibrous crystalline substances with special thermal and electrical properties that have long encouraged their use in the manufacture of such products as roofing, insulation, brake linings, fireproof curtains, etc. Their occurrence as pollutants in the ambient air and in supplies of food and drinking water has caused considerable concern because occupational exposures to asbestos have been found to induce mesothelioma of the pleura and peritoneum, as well as cancer of the lung, esophagus, and stomach, after latent periods of about 20 to 40 years.

Electron microscopy is currently the principal technique used to identify and characterize asbestos fibers in ambient air and water samples. Because of the poor sensitivity and specificity of conventional bulk analytical methods, electron microscopy is also being used for routine measurement of airborne or waterborne asbestos concentrations. The several laboratories that perform such analyses generally have reasonable internal self consistency. However, interlaboratory comparisons have shown that the results obtained by the separate laboratories are often widely different.

This manual describes a provisional optimum electron microscope procedure for measuring the concentration of asbestos in air samples. It results from a study, carried out under EPA Contract No. 68-02-2251, to evaluate the various methods currently in use in the various laboratories. Statistical analysis was used to evaluate the effects of the many interacting sub-procedures and arrive at an optimum composite procedure.

This manual does not provide the vast amount of data that supports the provisional methodology. These data are included in the final report on EPA Contract No. 68-02-2251.

Jack Wagman
Project Officer

HWBUI0008818

ABSTRACT

This manual describes a provisional optimum electron microscope (EM) procedure for measuring the concentration of asbestos* fibers** in air samples. The main features of the method include depositing an air sample on a polycarbonate membrane filter, examining an EM grid specimen in a transmission electron microscope (TEM), and verifying fiber identity by selected area electron diffraction (SAED).

This provisional manual results from a study to develop an optimum EM procedure for airborne asbestos determination. The analytical data supporting the provisional methodology are included in a separate final report.

---

* Asbestos is used as a collective term for the six minerals: chrysotile, amosite, crocidolite, and the asbestiform varieties of anthophyllite, actinolite and tremolite.

** The term fiber is used for a particle with an aspect ratio 3:1 or greater and with substantially parallel sides.

HWBUI0008819

TABLE OF CONTENTS

1. PROVISIONAL METHODOLOGY - SUMMARY 1
2. METHODOLOGY 2
2.1 Air Sampling 3
2.1.1 Air Sampling Parameters 3
2.1.2 Sample Time Periods 5
2.2 Sample Storage and Transport 6
2.3 Carbon Coating the Filter. 6
2.4 Transfer of the Sample to the EM Grid. 9
2.5 Examination of the Grid by Transmission Electron Microscopy 11
2.5.1 Low Magnification 11
2.5.2 High Magnification 12
2.5.2.1 Calibration Magnification at Fluorescent Screens 12
2.5.2.2 Loading Levels 12
2.5.2.3 Fiber Counting Rules 12
2.5.2.4 Fiber Classification Rule 14
2.5.2.5 Counting at Low Loading Level . . 16
2.5.2.6 Counting at Medium Loading Level . 16
2.5.2.7 Counting at High Loading Level . . 17
2.6 Recording of Data 17
2.6.1 Recording Formal 17
2.6.2 Computer Coding Forms 19
2.7 EM Data Analysis 19
2.7.1 Checking Data on Key Punch Cards 19
2.7.2 Separating Very Large Sized Bundles 20
2.7.3 Fortran Program for Obtaining Characterizing Parameters 20
2.7.4 Printout of Results on each TEM Grid . . . 20
2.7.5 Summary of Results for a Typical Air Sample 20
2.7.6 Precision of TEM Estimates 21
2.7.7 Analyzing Data on Very Large Bundles of Fibers 21

HWBUI0008820

2.8 Ashing, Sonification, and Reconstitution 22
2.9 Limits of Detection 23
3. PREPARATION OF BLANKS 24

References 27
Appendix A - Instrumentation and Supplies 28
Appendix B - Magnification Calibration 34
Appendix C - Listing for the Fortran Program CONLAB 37
Appendix D - Illustrative Tables 41

HWBUI0008821

LIST OF TABLES AND FIGURES

Table		Page
1	Suggested Sampling Times for Determining Airborne Asbestos Concentrations	5
2	Data Recording Sheet	17
3	Minimum Detection Limit Using High-Volume Air Sampler	23

Figures

1	Modified Jaffe Washer Method	9
2	Two Methods of Examining a Grid	12

HWBUI0008822

ELECTRON MICROSCOPE MEASUREMENT OF AIRBORNE ASBESTOS CONCENTRATIONS

A Provisional Methodology Manual

1. PROVISIONAL METHODOLOGY - SUMMARY

(1) Take an air sample on a polycarbonate membrane filter, 0.4 μm, using a high-volume or personal sampler.

(2) Coat the filter with a 40 nm thick film of carbon using a vacuum evaporator.

(3) Transfer the deposit from the polycarbonate filter to an electron microscope grid using a modified Jaffe washer. The Jaffe washer is prepared as follows. A 60 or 100 mesh stainless steel mesh is placed on top of a paper filter stack or foam sponge contained in a petri dish. Chloroform is carefully poured into the petri dish until the level is just touching the stainless steel mesh. A 1 mm x 2 mm portion of carbon coated polycarbonate filter is placed particle side down on a 200 mesh carbon coated copper electron microscope (EM) grid and this pair is placed on the steel mesh. The 1 mm x 2 mm portion is wetted with a 5 μℓ drop of chloroform. The polycarbonate filter will dissolve in about 24 to 48 hours.

(4) Examine the EM grid under low magnification in the TEM to determine its suitability for high-magnification examination. Ascertain that the loading is suitable and is uniform, that a high number of grid openings have their carbon film intact, and that the sample is not contaminated.

(5) Systematically scan the EM grid at a magnification of about 20,000X (screen magnification 16,000X) for chrysotile and possibly a lower magnification for cases where predominant amphibole fibers are present. Record the length and breadth of all fibers that have an aspect ratio of greater than 3:1 and have substantially parallel sides. Observe the morphology of each fiber through the 10X binocular and note whether a

HWBUI0008823

tubular structure characteristic of chrysotile asbestos is present. Switch into SAED mode and observe the diffraction pattern. Note whether the pattern is typical of chrysotile or amphibole, or whether it is ambiguous or neither chrysotile nor amphibole.

(6) Count 100 fibers in several grid openings, or alternatively, count all fibers in at least 10 grid openings. If more than 300 fibers are observed in one grid opening, then a more lightly loaded filter sample should be used. If no other filter sample can be obtained, the available sample should be transferred onto a 400 mesh grid. Processing of the sample using ashing and sonification techniques should be avoided wherever possible.

(7) Fiber number concentration is calculated from the following equations

$$\text{Fibers/m}^3 = \frac{\text{Total No. of Fibers}}{\text{No. of EM Fields}} \cdot \frac{\text{Total Effective Filter Area, cm}^2}{\text{Area of an EM Field, cm}^2} \cdot \frac{1}{\text{Volume of Air Sampled, m}^3}$$

Fiber mass for each type of asbestos in the sample is calculated by assuming that the breadth measurement is a diameter; thus, the mass can be calculated from

$$\text{Mass } (\mu g) = \frac{\pi}{4} \cdot (\text{length, } \mu m) \cdot (\text{diameter, } \mu m)^2 \cdot (\text{density, g/cm}^3) \cdot 10^{-6}$$

The density of chrysotile is assumed to be 2.6 g/cm^3, and of amphibole 3.0 g/cm^3. The mass concentration for each type of asbestos is then calculated from

HWBUI0008824

$$\text{Mass Concentration } (\mu g/m^3) \text{ of a Particular Type} = \frac{\text{Total Mass of all Fibers of that Type } (\mu g)}{\text{Volume of Air Sampled } (m^3)}$$

(8) Other characterizing parameters of the asbestos fibers are:

(a) Length and width distributions of chrysotile fibers

(b) Volume distribution of chrysotile fibers

(c) Fiber concentration of other asbestos minerals

(d) Relative proportion of chrysotile fibers with respect to total number of fibers.

2. METHODOLOGY

2.1 Air Sampling

Collect the sample of airborne asbestos on 0.4 μm pore size polycarbonate filters using the shiny smooth side as the particle capture surface. In cases where polycarbonate filters cannot be used, the air sample may be collected on a high efficiency membrane filter, e.g. cellulose acetate, which can then be prepared by using an ashing procedure described in Section 2.8. Use the high-volume air sampler [1]*, or in certain instances, the personal dust sampler.[2] When 110V, 60 cycle power supply is available, a variety of other combinations of pumps and filters and other techniques can be used for air sample collection.[3-5]

2.1.1 Air Sampling Parameters

Sampling rates vary with the type and model of sampler and with the type and pore size of filter used to collect an air sample. Typically, a high-volume air sampler fitted with a 20 cm x 25 cm, 0.4 μm pore size, polycarbonate filter will have a flow rate of about 700 ℓ/min (25 cfm) at a pressure drop of 145 cm of water across the filter. By comparison, a

---

* Numbers in brackets denote the literature references.

HWBUI0008825

personal dust sampler, operated with a 37 mm diameter, 0.4 μm pore size, polycarbonate filter, is set, by a flow controller, to sample at a flow rate of 2 ℓ/min. The pressure drop across the filter is 20.9 cm of water.

The two types of samplers can be compared by dividing the volumetric flow rate by the effective filtration area of the filters. The high-volume sampler, with an effective filtration area of 406.5 cm^2, operates at a rate of 28.7 $cm^3/cm^2/sec$ while the personal dust sampler, with an effective filtration area[ǂ] of 6.7 cm^2, operates at a rate of 5.0 $cm^3/cm^2/sec$. Thus, the filtering rate of the high-volume sampler is about five times higher than that of the personal sampler. Some research investigators contend that the higher face velocity of the high-volume sampler results in a lower fiber retention efficiency. These investigators expect the fibers to align perpendicular to the collection filter, and hence, better able to penetrate through the pores in the filter. They recommend collecting air samples at as low a face velocity as feasible and proportionately extending the sampling time. The optimization study, upon which this provisional methodology is based, tends to support the contention but the reason remains obscure.

Personal dust samplers are used frequently to assess respirable dust levels. When used in this mode, they are preceeded with a nylon cyclone that collects fibers and other particles with aerodynamic diameters in excess of 10 μm. To be comparable with the results of the high-volume sampler, it is recommended that the personal sampler be operated without the cyclone.

It is recommended that a cellulose acetate membrane filter with a pore size of 5 μm be used to support the polycarbonate filter in the samplers. It should be placed between

---

ǂ The effective filtration area varies with the style or manufacturer and hence should be measured.

HWBUI0008826

the polycarbonate filter and the wire mesh filter support of the high-volume sampler, or the glass frit filter support of the personal sampler. The cellulose acetate membrane acts as a diffusion plate and aids in obtaining a uniform deposit on the polycarbonate filter. It also decreases the possibility of contaminating the filter with particles from the sampler frame.

It is recommended that flow rate meters be checked periodically during sampling to ascertain constancy of flow rate.

2.1.2 Sample Time Periods

As a guide, the following time periods are suggested for the sampling of airborne asbestos. It is recommended that samples be collected at all three of the suggested time periods until experience dictates otherwise. Sampling at the three time periods increases the probability that one of the samples will be suitably loaded with asbestos to permit quantification of the asbestos by the direct transfer technique.

Table 1

SUGGESTED SAMPLING TIMES FOR DETERMINING AIRBORNE ASBESTOS CONCENTRATIONS

Proximity to Source	Sampler Type	Suggested Sampling Times, min
Point Source 90 m	High-volume	15, 30, 60
	Personal	75, 150, 300
Near Source 90-180 m	High-volume	30, 120, 480
	Personal	150, 600
Distant Source 0.8-1.6 km	High-volume	240, 480, 1440
	Personal	not recommended

HWBUI0008827

2.2 Sample Storage and Transport

After acquiring the sample, every precaution must be taken to assure its integrity and prevent contamination and loss of fibers until the sample is examined under the electron microscope. The polycarbonate filter should be removed immediately from the filter holder with great care and tacked, with cellophane tape, to the bottom of a clean plastic petri dish. The dish cover should then be secured and all necessary sample identifying marks and symbols applied to the cover. With the 20 cm x 25 cm high-volume filters, it may be necessary to cut the filter into 5 cm x 5 cm segments and store each segment in separate petri dishes. A consistent notation must be used so that the location and orientation of each segment with respect to the original filter is not lost. It is recommended that the petri dishes containing the filters be maintained in a horizontal position at all times during storage and transportation to the analyzing laboratory. At the present time, there are no reliable estimates on the loss of fibers from polycarbonate filters prior to carbon coating the filters in the laboratory.

If other membrane filters, such as cellulose acetate, are used for collecting airborne particles, it is generally believed that the storage and transport do not affect these filters because of their high retention efficiency. However, there are no reliable data for comparing retention efficiency of polycarbonate filters and other membrane filters.

Suitable blank and standard filters should be introduced at this stage in the analytical process and carried through the remaining procedures along with the samples.

2.3 Carbon Coating the Filter

The polycarbonate filter with the sample deposit and suitable blanks and standards should be coated with carbon as soon after sampling is completed as possible. The carbon

HWBUI0008828

coating forms an almost continuous film over the filter and bonds the collected particles to the filter surface. Losses are thus reduced during subsequent handling of the filter, and during the transfer process to the electron microscope grid. A carbon film of about 40 nm thickness is most suitable. All experimental equipment and supplies are listed in Appendix A.

It is highly recommended that the handling and processing of the filters after their receipt by the analyzing laboratory be conducted in a clean room or clean bench to reduce the possibility of contamination. Tweezers should be used for handling the filters; static charge eliminators will facilitate handling of the polycarbonate filters by neutralizing the surface electrostatic charge.

Because a thin, uniform, carbon film is desired, the coating of the filter deposit with carbon should be carried out in a vacuum evaporator. Carbon sputtering devices should be avoided because they produce a film of uneven thickness. Too thick a film can lead to problems during the subsequent steps in the procedure, particularly filter dissolution, fiber sizing, and fiber identification. Electron diffraction patterns tend to be faint when operating the TEM at less than 100 KV.

Typically, vacuum evaporators accept samples as large as 10 cm in diameter. Thus, if the personal sampler was used for sample collection, the entire filter may be carbon coated at one time. It is convenient to use the petri dish in which the polycarbonate filter is being stored. After inspecting the filter to be sure it is securely tacked to the bottom of the petri dish, remove the cover and place the bottom of the dish containing the filter in the vacuum evaporator for coating. If the airborne asbestos was collected on the 20 cm x 25 cm polycarbonate filter using the high-volume sampler, the entire filter cannot be coated at once. Portions, about 2.5 cm x 2.5 cm,

HWBUI0008829

should be cut from the central region of the filter using scissors or scalpel. The portions should be tacked with cellophane tape to a clean glass microscope slide and placed in the vacuum evaporator for coating.

Any high-vacuum, carbon evaporator may be used to carbon coat the filters (caution again: carbon sputtering devices should not be used). Typically, the electrodes are adjusted to a height of 8-10 cm from the level of the turn-table upon which the filters are placed. A spectrographically pure carbon electrode sharpened to a 0.1 cm neck is used as the evaporating electrode. The sharpened electrode is placed in its spring-loaded holder so that the neck rests against the flat surface of a second graphite electrode. The samples, in either a petri dish bottom or on a glass slide, are attached to the turn-table with double-sided cellophane tape.

The manufacturer's instructions should be followed to obtain a vacuum of about 1×10^{-5} torr in the bell jar of the evaporator. With the turn-table in motion, the carbon neck is evaporated by increasing the electrode current to about 15 amperes in 10 seconds, followed by 25-30 seconds at 20-25 amperes. If the turn-table is not used during carbon evaporation, the particulate matter is not coated from all sides and there is a shadowing effect which is not desirable. The evaporation should proceed in a series of short bursts until the neck of the electrode is consumed. Continuous prolonged evaporation is not recommended since overheating and consequent polymerization of the polycarbonate filter may easily occur and impede the subsequent step of dissolving the filter. The evaporation process may be observed by viewing the arc through welders goggles. (CAUTION: never look at the arc without appropriate eye protection.) A rough calculation shows that a graphite neck of 5 mm^3 volume, when evaporated over a spherical surface of 10 cm radius, will yield a carbon layer 40 nm thick.

HWBUI0008830

After carbon coating, the vacuum chamber is slowly returned to atmospheric pressure, the filters are removed and placed in clean, marked petri dishes, and stored in a clean bench.

2.4 Transfer of the Sample to the EM Grid

The transfer of the collected airborne asbestos from the coated polycarbonate filter to an electron microscope grid is accomplished in a clean room or bench using a Jaffe washer technique [6] with some modification.

Transfer is made in a clean glass petri dish about 10 cm diameter and 1.5 cm high. A stack of 40 clean, 5½ cm diameter paper filter circles is placed in the dish; alternatively, a 3 cm x 3 cm x 0.6 cm piece of polyurethane foam (like those used as packing in Polaroid film boxes) may be used. Spectroscopic grade chloroform is poured into the petri dish until it is level with the top surface of the paper filter stack or the foam. On top of the stack or foam a piece of (about 0.6 cm x 0.6 cm) 60-mesh stainless steel screen is placed. Several transfers may be completed at one time and a separate piece of mesh is used for each grid. Details of the modified Jaffe washer and the washing process are illustrated in Figure 1.

Sections of the carbon-coated polycarbonate filter on which the sample is deposited are obtained either by using a punch to punch out 2.3 mm discs or sharp scissors to cut out approximately 1 mm x 2 mm rectangles. A section is laid carbon side down on a 200-mesh carbon-coated electron microscope (TEM) grid. (Alternatively, one may use formvar-coated grids or uncoated TEM grids. Here, the carbon coat on the polycarbonate filter forms the grid substrate.) Minor overlap or underlap of the grid by the filter section can be tolerated since only the central 2-mm portion of the grid is scanned in the microscope. This pair (TEM grid and filter section) is picked up with tweezers and carefully placed on the moist

HWBUI0008831

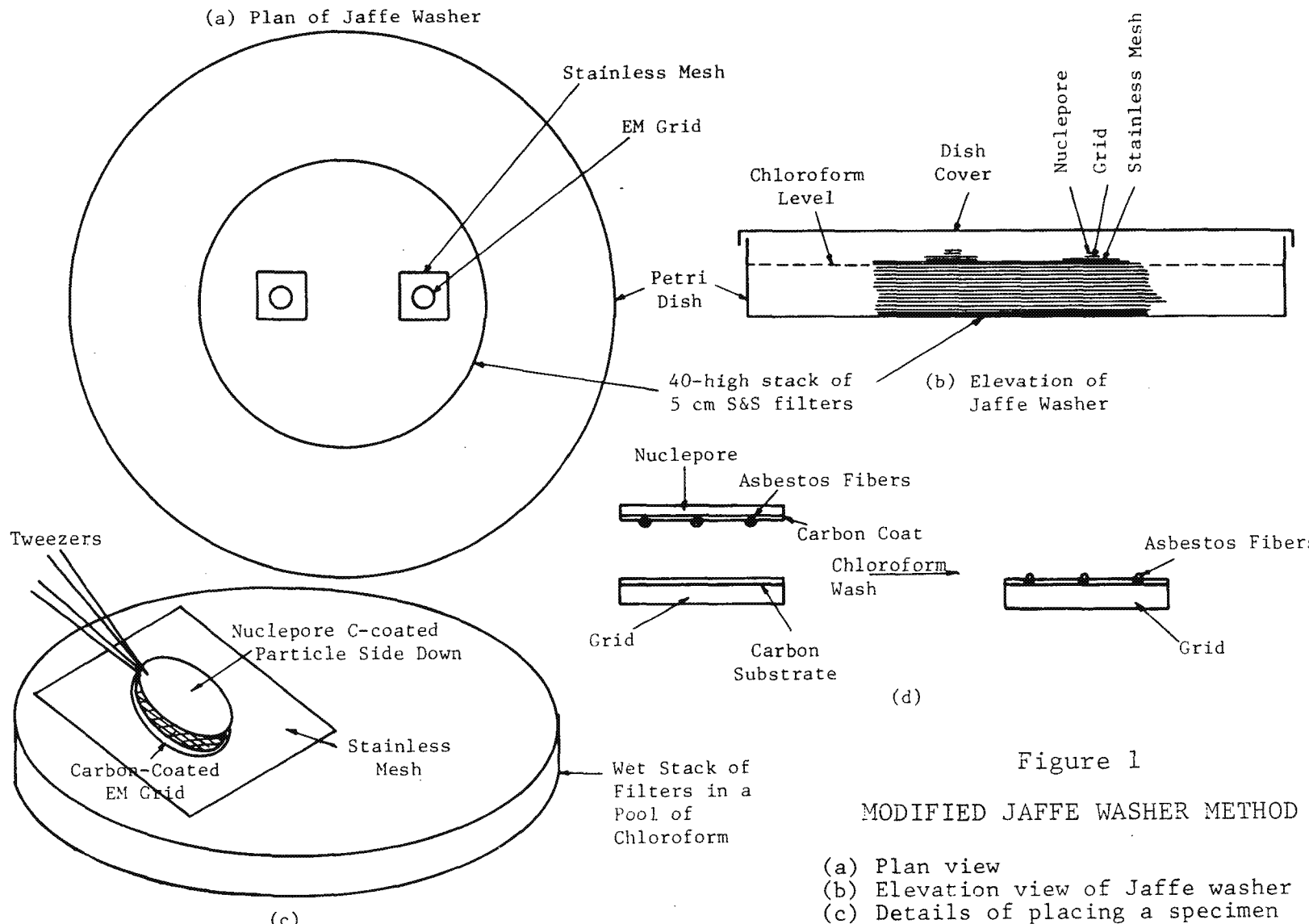


Figure 1

MODIFIED JAFFE WASHER METHOD

(a) Plan view
(b) Elevation view of Jaffe washer
(c) Details of placing a specimen for washing
(d) Principle of the Jaffe method

HWBU0008832

stainless steel mesh of the Jaffe washer. The 1 mm x 2 mm section is wetted immediately by a 5 µℓ drop of chloroform.

When all the samples are in place in the washer, more chloroform is carefully added to increase the level back to where it just touches the top of the paper filter stack. Raising the chloroform level any higher may float the TEM grid off the mesh or displace the polycarbonate filter section; neither is desirable. The cover is placed on the washer and weighted to improve and seal and reduce the evaporation of the chloroform.

More chloroform should be added periodically to maintain the level within the washer. After a minimum of 24 hours, the polycarbonate filter should be completely dissolved. The TEM grid is removed by picking up the stainless steel mesh with tweezers and placing it on a clean filter. When all traces of chloroform have evaporated, the grid may be lifted from the mesh and examined in the electron microscope or stored for future examination.

2.5 Examination of the Grid by Transmission Electron Microscopy

2.5.1 Low Magnification

The grid is observed in the transmission electron microscope at a magnification of 500X to determine its suitability for detailed study at high magnification. The grid is rejected if:

(a) The carbon film over a majority of the grid opening is damaged and not intact. If so, the transfer step 2.4 must be repeated to obtain a new grid.

(b) The fibers give poor images and poor diffraction patterns due to organic contamination or interference due to non-fibrous particles. If so, the filter may be ashed, redispersed, and refiltered (see Section 2.8). Ashing step allows elimination of organic matter and facilitates diluting to minimize the interference from other particles.

HWBUI0008833

2.5.2 High Magnification

2.5.2.1 Calibrating Magnification at Fluorescent Screen

It is important to know the exact value of magnification at the fluorescent screen for the most common settings of the electron microscope. The method for calibrating magnification is illustrated in Appendix B.

2.5.2.2 Loading Levels

The method for examining the grid for fiber counting is a function of the fiber loading on the filter. Three generalized loading levels may be encountered.

(a) Low Loading -- less than 50 fibers in a full grid opening (80 μm x 80 μm).

(b) Medium Loading -- 50 to 300 fibers in a full grid opening.

(c) High Loading -- more than 300 fibers per full grid opening.

2.5.2.3 Fiber Counting Rules

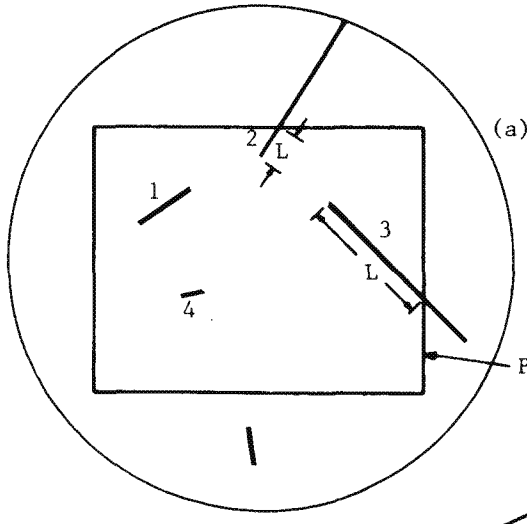
In making a fiber count, the following rules are to be observed:

(a) A field of view is defined. In some microscopes, it is convenient to use the central rectangular portion of the fluorescent screen which is lifted for photographic purposes [see Figure 2(a)]. On other microscopes, a scribed circle or the entire circular screen may be used as the field of view. The area of the field of view must be accurately measurable.

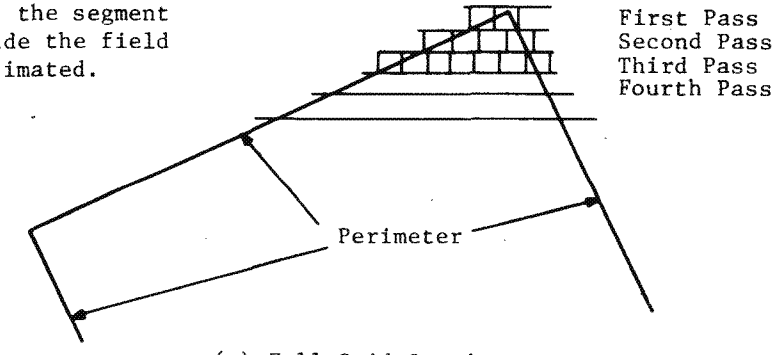
(b) All fibers within the field of view are counted and their length and width estimated and noted.

(c) Fibers which extend beyond the perimeter of the field of view are counted. The width of these fibers is measured but their length is measured as only that portion which lies within the field of view. Such fibers are noted by the letter "L" as the length information is recorded, indicating that it is a limit case [see Figure 2(a)]. In the final analysis, such fibers are treated as half-fibers (half-counts).

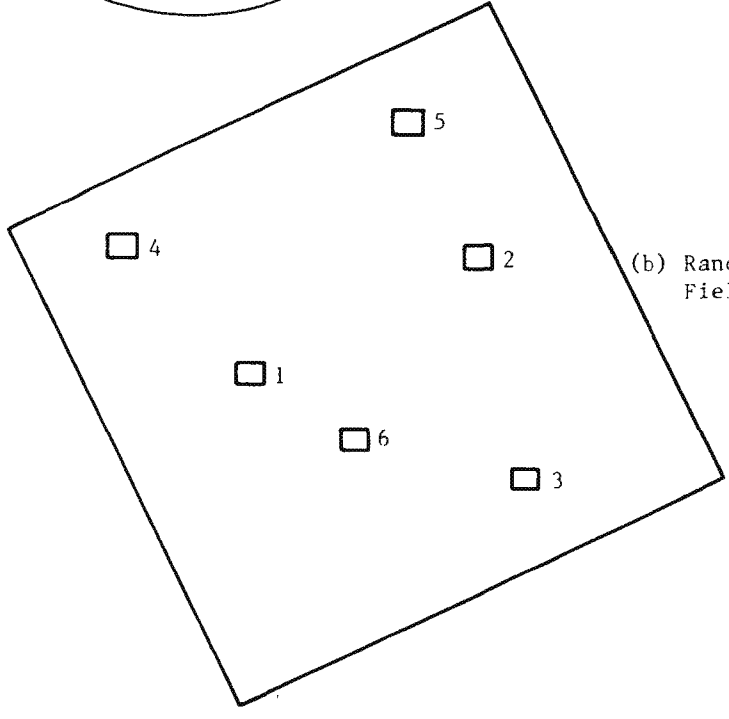
HWBUI0008834



(a) Field of View:
Fibers 2 & 3 are crossing the perimeter; the segment of length inside the field of view is estimated.



(c) Full Grid Opening:
Method of scanning.



(b) Random Choice of Field of View

Figure 2

TWO METHODS OF EXAMINING A GRID

HWBUI0008835

(d) Tightly bound bundles of fibers are counted as a single fiber and an estimate made of their average length and width. Fibers which touch or cross are counted separately. Some subjective judgement is required but fortunately, borderline cases are rare. Notation is also made in recording the data that the fiber was a bundle.

(e) Selection of the grid opening and the selection of a field of view within a grid opening should be done on a random basis [see Figure 2(b)]. This is important for avoiding biases and to ensure the statistical validity of the results.

(f) Morphological comparison with standard specimens is used as a basis for rejecting non-asbestos particles such as plant parts and diatoms. Where doubt exists, the electron diffraction pattern of the particles should be examined.

2.5.2.4 Fiber Classification Rules

Fibers are classified by observation of their morphology and electron diffraction patterns. It is recommended that both morphological and diffraction pattern study be done at zero degree tilt angle.

The following rules should be followed when classifying a fiber:

(a) Observe a fiber at a TEM screen magnification of about 16,000X through a 10X binocular. At such high magnifications, the tubular structure of chrysotile is usually apparent (compare with standard specimens). Fibers showing the tubular structure may be classified tentatively as chrysotile. A few other minerals, e.g. halloysite, show tubular structure, but can be recognized from chrysotile by electron diffraction. Amorphous matter can get inside the tube or cover the chrysotile fiber, and thus obscure the tubular structure. Therefore, non-tubular structure does not rule out chrysotile.

Amphibole asbestos fibers usually have a lath or plate-like shape, are more electron dense, and show thickness contours and other diffraction contrasts which change due to beam heating. Morphological features alone may not be a sufficient basis for distinguishing chrysotile from amphiboles and other fibrous minerals.

HWBUI0008836

(b) Electron diffraction patterns from single fibers of asbestos minerals fall into distinct groups. The chrysotile asbestos pattern has characteristic streaks on layer lines other than the central line and some streaking also on the central line. There are spots of normal sharpness on the central layer line and on alternate lines (2nd, 4th, etc.). The repeat distance between layer lines is about 0.53 nm.

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.

(c) Transmission electron micrographs and selected area electron diffraction patterns obtained with standard samples should be used as guides to fiber identification.[7-9]

From the examination of the electron diffraction patterns, fibers are classified as belonging to one of the following categories:

- Chrysotile
- Amphibole group (includes amosite, crocidolite, anthophyllite, tremolite and actinolite)
- Ambiguous (incomplete spot patterns)
- Non-asbestos (minerals other than the six asbestos minerals)
- Unknown (no spot pattern)

It should be noted that other particles with fibrous morphology also give layer patterns; for example, pyroxenes. The complete quantitative indexing and deriving interplanar d-spacings from diffraction patterns is a time consuming and complex undertaking and is not feasible for routine analysis.

It is not possible to inspect electron diffraction patterns for some fibers. There are several reasons for the absence of a recognizable diffraction pattern. These include contamination of the fiber, interference from nearby particles, too small a fiber, too thick a fiber, and non-suitable orientation of the fiber. Some chrysotile fibers are destroyed in the electron beam resulting in patterns that fade away within seconds of

HWBUI0008837

being formed. Some patterns are very faint and can be seen only under the binocular microscope. In general, the shortest available camera length must be used and the objective lens current may need to be adjusted to give optimum pattern visibility for correct identification. Use of a 20 cm camera length and a 10X binocular to inspect the SAED pattern on the tilted screen is recommended.

2.5.2.5. Counting at Low Loading Level

When fewer than 50 fibers per grid opening are encountered, the preferred counting method is to scan the entire grid opening and defining the full grid opening as one field. With the microscope magnification at 20,000X, a series of parallel scans across the grid square are made starting with the top corner of the square and ending at the bottom [see Figure 2(c)]. (With the tilting section of the fluorescent screen used as a single field of view, approximately 300-400 fields will be observed if the entire grid opening is scanned.) Fibers noted in each full grid opening (or single field) are classified in accordance with the procedure described above.

Additional grid openings are selected, scanned, and counted until the total number of fibers counted exceeds 100, or a minimum of 10 grid openings have been scanned, whichever occurs first.

2.5.2.6 Counting at Medium Loading Level

When the loading on the filter is in the range of 50 to 300 fibers per grid opening, counting is done on randomly selected fields of view. At a nominal magnification of 20,000X, fields are randomly selected within a grid opening until a total of 20 fibers have been counted, sized, and classified. (Generally 20-40 fields of view are observed per grid opening.) After about 20 fibers have been counted, another grid opening is selected and an additional 20 fibers (approx.) are counted. This procedure is repeated for 5 grid openings until a minimum

HWBUI0008838

of 100 fibers are counted. (When estimating fibers of a particular type of asbestos, counting is continued until 50-100 fibers of that type are counted.)

2.5.2.7 Counting at High Loading Level

When the fiber loading exceeds 300 fibers per grid opening, the filter should ideally be rejected in favor of a filter sample taken for a shorter time period.

If no other filter sample is possible and the number of fibers above 300 is not too great (up to 400), then a filter section should be transferred to a 400 mesh grid and the procedure repeated as for medium filter loading levels. The 400 mesh grid opening is much smaller in area than a 200 mesh grid opening, and hence can be scanned much faster with less fatigue for the operator.

When the loading level is so high that fibers touch and overlap and no other sample is available, then the filter should be ashed, dispersed, and refiltered to yield a lower concentration level. Details for this procedure are given in Section 2.8.

2.6 Recording of Data

It is advantageous to record the TEM data in a systematic form so that it can be transferred to computer data cards for statistical analysis.

2.6.1 Recording Format

A suggested data sheet format is shown in Table 2. The entries at the top describe the sample (identification, the storage box, and storage location), the sampling parameters (volume of air sampled, total effective area of the filter), and the TEM parameters (screen magnification, area of one field of view in cm^2, etc.).

Column 1 -- EM grid opening identification number

Column 2 -- Identification number for the field of view

HWBUI0008839

Table 2

DATA RECORDING SHEET

Sample: _____ Vol. of Air Sampled: 9.2 m^3

Storage Box No.: _____ Effective Area of Membrane: 406.5 cm^2

Location in Box: _____

Magnification: 17,000

Area of One Field: 0.182 x 10^{-6} cm^2

Grid Opening I.D.	Field of View Number	Fiber Number	Cumulative Fiber Count	Fiber Width, mm	Fiber Length, mm	Fiber Identification by Morphology and Electron Diffraction
1	1	1	1	1.0	20	Chrysotile
		2	2	0.25	7	Ambiguous
	2	1	3	0.5	17	Ambiguous
		2	4	1.0	10	Chrysotile
		3	5	1.0	18	Chrysotile
		4	6	0.75	22	Chrysotile
		5	7	1.0	12	Chrysotile
		6	8	0.5	10	No Pattern
	3	1	9	0.25	24	Chrysotile
		2	10	0.75	18	Ambiguous
		3	11	1.0	10	Chrysotile
		4	12	1.0	8	Chrysotile
		5	13	0.25	10	No Pattern
	4	1	14	0.25	6	Chrysotile
		2	15	0.25	20	Ambiguous
		3	16	0.5	5	No Pattern
	5	1	17	2.0	65	Chrysotile
		2	18	1.0	10	Ambiguous
		3	19	0.5	4	No Pattern

and so on.

HWBU0008840

Column 3 -- Fiber sequence number within a given field of view

Column 4 -- Cumulative number of fibers counted

Column 5 -- Fiber width in mm

Column 6 -- Fiber length in mm

Column 7 -- Fibers extending beyond the perimeter of the field, marked with L (limiting case)

Column 8 -- Fiber identification, chrysotile, amphibole, ambiguous, or no pattern or non-asbestos

2.6.2 Computer Coding Forms

A Fortran program has been developed (see Appendix C) to analyze the data obtained from the electron microscope study. In order to use this Fortran program, it is recommended that data from the notes be transferred to IBM computer coding sheets to facilitate key punching. The coding scheme is given in Table D-1 and an illustration is presented in Table D-2 of Appendix D. The scheme is sufficiently broad to keep all relevant information, such as sample code number, laboratory code number, operator code number, TEM grid number, etc. Ashing factor refers to the dilution or concentration resulting from the ashing and reconstitution step. It is defined as the ratio of the redeposition filter area to the area of the filter segment ashed. For example, if a segment of 5 cm^2 area was ashed and the ash suspension deposited on 25 mm diameter final filter (effective area 2 cm^2), the ashing factor is 0.4. The area of the field of view when multiplied by the ashing factor gives the corrected area of the field.

2.7 EM Data Analysis

2.7.1 Checking Data on Key Punch Cards

Key punch cards are checked by obtaining a printout of all cards as illustrated in Table D-3 of Appendix D. This printout helps in detecting key-punching errors by comparison with the coding forms.

HWBUI0008841

2.7.2 Separating Very Large Sized Bundles

At present, separating bundles of fibers from the data is done by inspection of printout of the input data. The computer program can be modified to exclude the very large sized fibers from the analysis.

2.7.3 Fortran Program for Obtaining Characterizing Parameters

Each analyzing laboratory can develop its own computer program to facilitate statistical analysis and to obtain the necessary characterizing parameters. One Fortran program called CONLAB was specially developed at IITRI for obtaining several important characterizing parameters. The listing for this program is given in Appendix C. The program gives characterizing parameters for each TEM grid used.

2.7.4 Printout of Results on each TEM Grid

A typical printout of results on each TEM grid (for the data in Table D-3) is given in Tables D-4 and D-5. The parameters shown are:

Fiber counts for each category

Fiber concentration per cm^2 of filter
Fiber concentration per m^3 of air

Mass concentration per cm^2 of filter
Mass concentration per m^3 of air

Length (μm) Mean
Std. Deviation

Diameter (μm) Mean
Std. Deviation

Volume $(\mu m)^3$ Mean
Std. Deviation

2.7.5 Summary of Results for a Typical Air Sample

Summaries of the results are obtained using relevant quantities from the printouts in Tables D-4 and D-5. Shown

HWBUI0008842

in Table D-6 are the characterizing parameters for all fibers and for chrysotile fibers.

2.7.6 Precision of TEM Estimates

When more than one TEM grid is used, it is possible to obtain the mean values and 95% confidence levels on the means. This is done for each important parameter. The method consists of obtaining the mean, $\bar{x}$, the standard error of the mean, SEm, and t-value [10] (0.025, n - 1) for n - 1 degrees of freedom, where n = number of TEM grids examined and hence n replicates available. The 95% confidence limits are given by $\bar{x} \pm t \cdot (SEm)$.

In the illustrative case, Table D-6, the following four parameters are given:

(1) Fiber number concentration of all fibers, $10^6/m^3$ of air

(2) Volume concentration of all fibers, 10^{-9} cm^3/m^3 of air

(3) Fiber number concentration of chrysotile, $10^6/m^3$ of air

(4) Mass concentration of chrysotile fibers, $\mu g/m^3$ of air

The t-value decreases sharply with greater replication. For example, t = 12.7 for n = 2 and decreases to 4.3 for n = 3 and to 2.77 for n = 5 and so on. The standard error of the mean also decreases with greater replication. Hence, to increase the precision of the TEM estimates, 3 or 4 replicates per sample should be analyzed.

2.7.7 Analyzing Data on Very Large Bundles of Fibers

Fiber bundles should be reported separately as the number concentration of large bundles or fiber aggregates (greater than 1 μm^3 each) per m^3 of air. In general, these are few and these computations can easily be done using a desk calculator.

No attempt is made to compute either the volume or the mass of bundles because of the large uncertainty in assigning dimensions to aggregates.

HWBUI0008843

2.8 Ashing, Sonification, and Reconstitution

Some air samples (especially samples collected over several hours) may contain high levels of organic contaminant. This organic matter obscures the fibrous particles, and interferes with the proper counting, sizing, and identification. Such samples should be ashed and reconstituted. In cases where a flat, polycarbonate filter cannot be used for initial sample collection, and a depth filter (cellulose acetate) with high retention efficiency must be used, the ashing step may be used to reconstitute the particles on a flat filter for TEM analysis. The procedure for ashing is as follows:

A section of known area (e.g. 1 cm x 1 cm) is cut from the polycarbonate or cellulose acetate filter used to collect the air sample and is placed in a clean glass vial (30 mm diameter x 80 mm high). The membrane is positioned such that the particle collection side (shiny side) faces the glass wall. The vial is placed in an upright position in a low-temperature asher. Using manufacturer's instructions, vacuum is obtained and the filter is ashed at 40 watts power in oxygen plasma. Oxygen is admitted at 2 psi pressure. Though the membrane vanishes in about a half-hour, the ashing is continued for about 3-4 hours to ensure complete ashing. The ashing chamber is allowed to slowly reach atmospheric pressure. The vial is removed and 10 ml of filtered distilled water containing 0.1 percent filtered Aerosol OT is added. The vial is placed in a 100 ml beaker containing 50 ml of water, and this beaker is placed in a low-energy, ultrasonic bath. Ultrasonic energy is applied for 15 minutes to disperse all of the ash.

A 25 mm diameter filtering apparatus is assembled with a 25 mm diameter, 0.1 μm pore size polycarbonate filter with 5 μm pore size cellulose ester filter backing on the glass frit. Suction is applied and the filters are recentered if necessary. The filter funnel is mounted, the vacuum is turned off and suction is allowed to cease. Two ml of distilled water is added to the funnel followed by the careful addition of the water containing

HWBUI0008844

the dispersed ash. Suction is applied to filter the sample. The vial should be rinsed with 10 ml of 0.1 percent Aerosol OT at least twice and the contents carefully transferred to the filtration funnel before the funnel goes dry. At the end of filtration, the suction is stopped. The filter is then dried in still air and stored in a disposable petri dish. After drying, the filter is ready for carbon-coating (see Section 2.3) and transfer of the sample to an EM grid (see Section 2.4).

The effective area of the redispersion filter and the area of the section cut for ashing from the original membrane must be taken into account when computing the fiber concentration, etc., in the TEM data analysis.

2.9 Limits of Detection

The minimum detection limit of the electron microscope method for the counting of airborne asbestos fibers is variable and depends upon the amount of total extraneous particulate matter in the sample and the contamination level in the laboratory environment. This limit also depends on the air sampling parameters, loading level, and the electron microscope parameters used.

In this provisional method, 100 fields, each with an area 0.18×10^{-6} cm^2 are scanned. Assuming that a fiber count has an accuracy of ± 1 fiber, then the detection limit is

$$\text{Detection Limit} = \frac{1}{100} \cdot \frac{\text{Area of Filter } (cm^2)}{0.18 \times 10^{6} \ (cm^2)} \cdot \frac{1}{\text{Vol. of Air } (m^3)}$$

In some cases (for very lightly loaded samples) when four, full grid openings are scanned, each grid opening with an area of 0.72×10^{-4} cm^2, the detection limit is

HWBUI0008845

$$\text{Detection Limit} = \frac{1}{4} \cdot \frac{\text{Area of Filter } (cm^2)}{0.72 \times 10^{-4} \ (cm^2)} \cdot \frac{1}{\text{Vol. of Air } (m^3)}$$

Table 3 gives an indication of the magnitude of the detection limit, calculated for the high-volume sampler method. It is seen that the minimum detection limit is lower for very dilute samples. Examining full grid openings leads to a lower value of minimum detection limit because of the large area scanned, as compared with the field of view method. With a given sample, the detection limit can be lowered considerably but the experimental effort required also increases. The guidelines of using 100 fields of view or four full grid openings represent a judicious compromise, between a reasonable experimental effort and a fairly low value of the detection limit. Also, using two or more TEM grids will reduce the detection limit further and also improve the precision of the estimates.

3. PREPARATION OF BLANKS

Even after taking utmost precautions to avoid asbestos contamination, one cannot rule out the possiblity of some contamination. It is a good practice to check contamination periodically by running blank samples.

A blank sample may consist of a clean filter, subjected to all the processing conducted with an actual air sample. These may include ashing, resuspension, redeposition, carbon coating, transferring to TEM grid, and TEM examination.

When analyses of blank samples show significant background levels of asbestos, these should be subtracted from the values obtained for field samples. Also, the minimum detection limit may be calculated as twice or three times the standard deviation of the blank or background value.

HWBUI0008846

Table 3

MINIMUM DETECTION LIMIT USING HIGH-VOLUME AIR SAMPLER

	Sampling Duration	Vol. of Air Sampled m^3	Field of View Method* (1 fiber in 100 fields) million fibers/m^3	Full Grid Opening** (1 fiber in 4 grid openings) million fibers/m^3
Point Source	½ hr	21	1.07	0.07
Near Source	2 hr	84	0.27	0.02
Distant Source	8 hr	336	0.067	0.005

* $$\text{Detection Limit} = \frac{1 \text{ Fiber}}{100 \text{ fields}} \cdot \frac{406 \text{ cm}^2}{0.18 \times 10^{-6} \text{ cm}^2/\text{field}} \cdot \frac{1}{\text{Vol. of Air Sampled, m}^3}$$

** $$\text{Detection Limit} = \frac{1 \text{ Fiber}}{4 \text{ Grids}} \cdot \frac{406 \text{ cm}^2}{0.72 \times 10^{-4} \text{ cm}^2/\text{grid}} \cdot \frac{1}{\text{Vol. of Air Sampled, m}^3}$$

HWBUI0008847

REFERENCES

1. Tentative Method of Analysis for Suspended Particulate Matter in the Atmosphere (High-Volume Method) 11101-01-70T, Methods of Air Sampling and Analysis; Intersociety Committee, American Public Health Association, 1015 18th Street, N.W., Washington, DC, 1972.

2. Recommended Procedures for Sampling and Counting Asbestos Fibers; Joint AIHA-ACGIH Aerosol Hazards Evaluation Committee, American Industrial Hygiene Assoc. Journal, 36(2):83-90, 1975.

3. Standards of Performance for New Stationary Sources; Federal Register, 36(247):24876 ff, December 23, 1971.

4. Standards of Performance for New Stationary Sources; Federal Register, 42(160):41754 ff, August 18, 1977.

5. Ranade, M.B., Sampling Interface for Quantitative Transport of Aerosols; EPA-650/2-74-016, 1973.

6. Jaffe, M.A., Handling and Washing Fragile Replicas; Proc. Electron Microscope Society of America, Toronto, Sept. 1948, J. Appl. Phys., 19(12):1189, 1948.

7. Clark, R.L. and C.O. Ruud, Transmission Electron Microscopy Standards for Asbestos; Micron, 5:83-88, Pergamon Press, 1974.

8. Ruud, C.O., C.S. Barrett, P.A. Russell and R.L. Clark, Selected Area Electron Diffraction and Energy Dispersive X-ray Analysis for the Identification of Asbestos Fibers, A Comparison; Micron, 7(2):115-132, 1976.

9. Mueller, P.K., A.E. Alcocer, R.Y. Stanley and G.R. Smith, Asbestos Fiber Atlas; U.S. Environmental Protection Agency Publication No. EPA-650/2-75-036, April 1975. National Technical Information Service, Springfield, Va.

10. Fisher, R.A. and F. Yates, Statistical Tables for Biological, Agricultural and Medical Research Workers; Table IV, 6th Ed., Stechert-Hafner, Inc., New York, 1964.

HWBUI0008848

Appendix A

INSTRUMENTATION AND SUPPLIES

HWBUI0008849

Appendix A

INSTRUMENTATION AND SUPPLIES

A. INSTRUMENTATION

1. Transmission Electron Microscope

A transmission electron microscope should be capable of 100 kv of accelerating voltage, 1 nm resolution, and a magnification range of 300 to 100,000X. The instrument should be capable of selected area electron diffraction analysis on areas 300 nm diameter. The fluorescent screen should have either a millimeter scale, concentric circles of 1, 2, 3, and 4 cm radii, or other devices to estimate the length and width of fibrous particles. All modern transmission electron microscopes meet these requirements.

2. Vacuum Evaporation

A vacuum evaporator is required for depositing a layer of carbon on the polycarbonate filters and for preparing carbon-coated EM grids. The evaporator should have a turntable for rotating the specimen during coating.

3. Low-Temperature Plasma Asher

A low-temperature plasma asher is required when the quantities of organic matter in the air sample are very high and interfere with the detection and identification of asbestos. Oxygen should be used for plasma ashing. The sample chamber should be at least 10 cm diameter, so that glass vials can be positioned vertically (e.g., Plasmod, Tegal Corporation, Richmond, CA or equivalent).

B. SUPPLIES

1. Jaffe Washer: For dissolving polycarbonate filters. This item is not available commercially. The assembly is described in Section 2.4 and illustrated in Figure 1.

HWBUI0008850

2. Filtering Apparatus: 47 mm filtering funnel (e.g., Cat. No. XX1504700, Millipore Corp. Order Service Dept., Bedford, MA 01730). 25 mm filtering funnel (Cat. No. XX1002500, Millipore Corp. Order Service Dept., Bedford, MA 01730). These are used to filter dispersed ash samples.

3. Vacuum Pump: A vacuum pump is needed to filter ash suspensions. It should provide up to 20 in. of mercury. Such vacuum pumps are available from any general laboratory supply house.

4. EM Grids: 200-mesh copper or nickel grids with carbon substrate are needed. These grids may be purchased from manufacturers of electron microscopic supplies (e.g., Cat. No. 1125, E.F. Fullam, Schenectady, NY) or prepared by standard electron microscopic grid preparation procedures. Finder grids may be substituted and are useful if the re-examination of a specific grid opening is desired (e.g., Cat. No. 1458, H-2 London 200 Finder grids, E.F. Fullam, Schenectady, NY or Cat. No. 17420 200 mesh carbon-coated nickel grids, Ladd Research Industries, P.O. Box 901, Burlington, VT 05401).

5. Membrane Filters: Polycarbonate

   (a) 47 mm diameter, 0.4 μm pore size Nuclepore® membranes or equivalent.

   (b) 37 mm diameter Nuclepore® membranes for use with the personal dust samplers.

   (c) 25 mm diameter, 0.4 μm pore size Nuclepore® membranes or equivalent to filter dispersed ash suspension.

   (d) 20 cm x 25 cm, 0.4 μm pore size Nuclepore® membranes or equivalent for collecting air samples using the high-volume sampler.

6. Membrane Filters: Cellulose acetate (to be used as backing filters)

   (a) 47 mm diameter, 5.0 μm pore size Millipore® or equivalent.

HWBUI0008851

(b) 37 mm diameter, 5 μm pore size Millipore® filters or equivalent for use with personal dust samplers.

(c) 25 mm diameter, 5 μm pore size Millipore® filters or equivalent.

(d) 20 cm x 25 cm, 5 μm pore size Millipore® filters or equivalent for use with the high-volume sampler.

7. Air Samplers:

(a) High-volume sampler, see reference 1 (e.g., Sierra Instruments, Model 305, 3756 N. Dunlap St., St. Paul, Mn. 55112 or equivalent).

(b) Personal dust sampler, see reference 2 (e.g., MSA Gravimetric Dust Sampling Kit, MSA Co., Pittsburgh, Pa. 15208 or equivalent).

8. Glass Vials: 30 mm diameter x 80 mm long; for holding filter during ashing. 50 ml beakers can be used instead of vials.

9. Glass Slides: 5.1 cm x 7.5 cm; for support of filters during carbon evaporation.

10. Scalpels: With disposable blades and scissors.

11. Tweezers: Several pairs for the many handling operations.

12. Doublestick Cellophane Tape: To hold filter section flat on glass slide while carbon coating.

13. Disposable Petri Dishes: 50 mm diameter and 100 mm diameter for storing membrane filters.

14. Static Eliminator: 500 microcuries PO-210. (Nuclepore Cat. No. V090POL00101) or equivalent. To eliminate static charges from membrane filters.

15. Carbon Rods: Spectrochemically pure, 3.0 mm diameter, 4.6 mm long with 1.0 mm neck. For carbon coating

HWBUI0008852

(Cat. No. 42350, Ladd Research Industries, P.O. Box 901, Burlington, VT 05401 or equivalent).

16. Ultrasonic Bath: (50 watts, 55 KHz). For dispersing ashed sample and for general cleaning.

17. Graduated Cylinder: 500 ml

18. 10 µl Microsyringe: For administering drop of solvent to filter section during sample preparation.

19. Carbon Grating Replica: 2160 lines/mm. For calibration of EM magnification (e.g., Cat. No. 1002, E.F. Fullam, Schenectady, NY or equivalent).

20. Specimen Grid Punch: For punching 3 mm diameter sections from membranes (e.g., Cat. No. 1178, E.F. Fullam, P.O. Box 444, Schenectady, NY 12301 or Cat. No. 16250, Ladd Research Industries, P.O. Box 901, Burlington, VT 05401).

21. Screen Supports: Copper or stainless steel; 6 mm x 6 mm, 60-100 mesh. To support specimen grid in Jaffe washer.

22. Filter Paper: S&S #589 Black Ribbon or equivalent (5½ cm circles). For preparing Jaffe washer.

23. Chloroform: Spectro grade, doubly distilled. For dissolving polycarbonate filters.

24. Acetone: Reagent grade or better. For cleaning the various tools.

25. Asbestos: Chrysotile (Canadian), crocidolite, amosite. UICC (Union International Contre le Cancer) standards. Reference asbestos samples available commercially (e.g., Duke Standards Company, 455 Sherman Avenue, Palo Alto, CA 94306 or Particle Information Service, 600 South Springer Road, Los Altos, CA 94022 or equivalent).

26. Petri Dish: Glass (100 mm diameter x 15 mm high). For modified Jaffe washer.

HWBUI0008853

27. Cleanser: Alconox, Inc., New York, NY 10003 or equivalent. For cleaning glassware. Add 7.5 g Alconox to a liter of distilled water.

28. Aerosol OT: 0.1% solution (Cat. No. So-A-292, Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219). Used as a dispersion medium for ashed filters. Prepare a 0.1% solution by diluting 1 ml of the 10% solution to 100 ml with distilled water. Filter through 0.1 µm pore size polycarbonate filter before using.

29. Parafilm: American Can Company, Neenah, WI Used as protective covering for clean glassware.

30. Pipettes: Disposable, 5 ml and 50 ml.

31. Distilled or Deionized Water: Filter through 0.1 µm pore size polycarbonate filter. Used for making all reagents and for final rinsing of glassware, and for preparing blanks.

31. Storage Box for TEM Grids: Cat. No. E-0174 Grid Holders, JEOL U.S.A., Inc., 477 Riverside Avenue, Medford, Mass. 02155 or equivalent.

33. Squeeze Bottles: For keeping double-filtered distilled water and 0.1 percent Aerosol OT solution.

34. Welders Protective Goggles

HWBUI0008854

Appendix B

MAGNIFICATION CALIBRATION

HWBUI0008855

Appendix B

MAGNIFICATION CALIBRATION

(1) Align the electron microscope using the instruction manual provided by the manufacturer.

(2) Insert mag-calibration grating replica (with 54864 lines per inch, or 2160 lines per mm, e.g., Cat. No. 1002, E.F. Fullam, Schenectady, NY) in the specimen holder.

(3) Switch on the beam, obtain the image of the replica grating at 20,000X magnification (or the magnification at which the asbestos samples will be analyzed) and focus.

(4) If the fluorescent screen has scribed circles of known diameters, proceed as follows. Using stage control, align one line tangentially to circumference of one circle. Count the number of lines in a diameter perpendicular to the lines. In most cases, the other end of the diameter will be in-between the Nth and $N + 1^{th}$ line. You can estimate the fractional spacing by eye. Alternatively, one can estimate the separation between lines using the scribed circles.

(5) If X line spacings span Y mm on the fluorescent screen using this grating replica, the true magnification is given by

$$M = \frac{Y \times 2160}{X}$$

The readings should be repeated at different locations of the replica and the average of about 6 readings should be taken as the representative or true magnification for that setting of the electron microscope.

HWBUI0008856

Line Spacings X	mm on Screen Y	Magnification M
9.5	83	18871
9.3	80	18580
7.0	60	18514
8.8	80	19636
9.0	80	19200
9.0	80	19200
		Average 19000

On most electron microscopes with large (18 cm dia.) fluorescent screens, the magnification is substantially constant only within the central 8-10 cm diameter region. Hence, calibration measurements should be made within this small region and not over the entire 18 cm diameter.

HWBUI0008857

Appendix C

LISTING FOR THE FORTRAN PROGRAM CONLAB FOR OBTAINING CHARACTERIZING PARAMETERS

HWBUI0008858

Appendix C

LISTING FOR THE FORTRAN PROGRAM CONLAB FOR
OBTAINING CHARACTERIZING PARAMETERS

```
C     PROGRAM CONLAB
C     ANALYZE FIBER AND MASS CONCENTRATIONS
C
      REAL SUMX(7,6),SUMX2(7,6),CONCT(6),CONMAS(6),VOLCT(6),VOLMAS(6)
      REAL FIBCT(6),QTY(7),DEN(2),SDEV(7,6),SLDEV(7,6),GMN(7,6)
      REAL CVAR(7,6),MEAN(7,6),MEAN2(7,6)
      DATA I,ICT,PI/1,0,3.14159/
      DATA DEN/2.6,3.0/
C     QTY(1) = LENGTH
C     QTY(2) = DIAM
C     QTY(3) = MASS OR VOL
C     QTY(4) = LOG LENGTH
C     QTY(5) = LOG DIAM
C     QTY(6) = LOG MASS
C
C     READ LENGTH,DIAM,COMPUTE OTHER DATA, STORE
      READ(5,110,END=190) IGRID,IFLD,IFSEQ,ICSEQ,DIAM,ALEN,IOUT,
     *INFIB,ILAB,IFIL,IPUN,XMAG,AREA,XASH,XVOL,TAREA,ICASE
050   TOTAR=0
      TOTCT=0
      LCT=60
      LCASE=ICASE
      LLAB=ILAB
      LFIL=IFIL
      LPUN=IPUN
      DO 080 I=1,6
      FIBCT(I)=0.0
      VOLCT(I)=0.0
      VOLMAS (I)=0.0
      CONCT(I)=0.0
      CONMAS(I)=0.0
      DO 080 J=1,7
      SDEV(J,I)=0.0
      SLDEV(J,I)=0.0
      GMN(J,I)=0.0
      CVAR(J,I)=0.0
      QTY(J)=0.0
      MEAN(J,I)=0.0
      MEAN2(J,I)=0.0
      SUMX(J,I)=0.0
      SUMX2(J,I)=0.0
080   CONTINUE
      GOTO 1101
100   READ(5,110,END=190) IGRID,IFLD,IFSEQ,ICSEQ,DIAM,ALEN,IOUT,
     *INFIB,ILAB,IFIL,IPUN,XMAG,AREA,XASH,XVOL,TAREA,ICASE
110   FORMAT (I2,2I3,I4,2F6.0,1X,I1,3X,I1,1X,3I1,2X,2F7.1,3F5.1,5X,I2)
      IF  ((ICASE.NE.LCASE).OR.(ILAB.NE.LLAB)
     *  .OR.(IFIL.NE.LFIL).OR.(IPUN.NE.LPUN))
     * GOTO 200
1101  AVOL=XVOL
      FILAR=TAREA
      JLAB=ILAB
      JPUN=IPUN
      JFIL=IFIL
      IF ((IGRID.EQ.LGRID).AND.(IFLD.EQ.LFLD)) GOTO 1110
      TOTAR=TOTAR+AREA*XASH*1.0E-6
      WRITE (6,1102)
1102  FORMAT(132X)
      LCT=LCT+2
      LFLD=IFLD
```


HWBUI0008859

```
      LGRID=IGRID
1110  IF(LCT.LE.50) GOTO 1112
      WRITE (6,1111) ILAB,IFIL,IPUN,LCASE
1111  FORMAT('1',40X,'FIBER AND MASS CONCENTRATION',15X,'LAB ',I1,
     *'  SAMP ',I1,'  GRID ',I2,'  CASE ',I2/
     *50X,'DATA LIST'//
     *1X,'FIB=SEQ FIELD  FLD-SEQ    DIAM',6X,
     *'LENGTH    OUT-COD   FIB-COD   FLD-MAG   ',
     *'FLD=AREA    ASH=MAG    VOL    FILT=AREA'/)
      LCT=0
1112  IF (INFIB.EQ.0) GOTO 1120
      IFIB=INFIB
      IF((INFIB.GE.2).AND.(INFIB.LE.5)) IFIB=INFIB+1
      IF(INFIB.EQ.5) IFIB=2
      IF(INFIB.GE.6) IFIB=5
1120  WRITE(6,1113) ICSEQ,IFLD,IFSEQ,DIAM,ALEN,IOUT,INFIB,XMAG,
     *AREA,XASH,XVOL,FILAR
1113  FORMAT(3X,I4,3X,I3,5X,I3,2(5X,F6.2),2(7X,I1),
     *3(5X,F7.3),2(4X,F5.1))
      LCT=LCT+1
112   IF(IFSEQ.EQ.0) GOTO 100
      IF (IOUT.EQ.2) GOTO 115
      FIBCT(IFIB)=FIBCT(IFIB)+1.0
      GOTO 117
115   FIBCT(IFIB)=FIBCT(IFIB)+0.5
      ALEN=ALEN*2.0
117   ALEN=ALEN/XMAG
      DIAM=DIAM/XMAG
      QTY(1)=ALEN
      QTY(2)=DIAM
      QTY(3)=PI*ALEN*DIAM*DIAM*1.0E-12/4.0
      IF(IFIB.GT.2) GOTO 122
      QTY(7)=QTY(3)*DEN(IFIB)
C     FIND LOG OF EACH QTY, SUM QTY AND LOG
122   DO 149 I=1,3
      IF (QTY(I)) 140,140,145
140   QTY(I+3)=0
      GOTO 149
145   QTY(I+3)=ALOG(QTY(I))
149   CONTINUE
150   DO 159 I=1,7
      SUMX(I,IFIB)=SUMX(I,IFIB)+QTY(I)
      SUMX2(I,IFIB)=SUMX2(I,IFIB)+(QTY(I))*(QTY(I))
159   CONTINUE
      GOTO 100
190   IEND=1
200   DO 205 IFIB=1,5
      TOTCT=TOTCT+FIBCT(IFIB)
      DO 205 I=1,7
      SUMX(I,6)=SUMX(I,6)+SUMX(I,IFIB)
      SUMX2(I,6)=SUMX2(I,6)+SUMX2(I,IFIB)
205   CONTINUE
      FIBCT(6)=TOTCT
      DO 599 IFIB=1,6
      IF (FIBCT(IFIB).EQ.0.0) GOTO 599
      CONCT(IFIB)=FIBCT(IFIB)/TOTAR
      VOLCT(IFIB)=CONCT(IFIB)*FILAR/AVOL
      IF (IFIB-2) 220,220,210
210   CONMAS(IFIB)=FIBCT(IFIB)*100.0/TOTCT
      GOTO 230
220   CONMAS(IFIB)=SUMX(7,IFIB)/TOTAR
      VOLMAS(IFIB)=CONMAS(IFIB)*FILAR/AVOL
230   DO 250 I=1,7
206   MEAN(I,IFIB)=SUMX(I,IFIB)/FIBCT(IFIB)
      MEAN2(I,IFIB)=SUMX2(I,IFIB)/FIBCT(IFIB)
250   CONTINUE
```


HWBUI0008860

```
      DO 270 I=1,3
      SDEV(I,IFIB)=SQRT(ABS(MEAN2(I,IFIB)-
     *      (MEAN(I,IFIB))*(MEAN(I,IFIB))))
      SLDEV(I,IFIB)=SQRT(ABS(MEAN2(I+3,IFIB)-
     *           (MEAN(I+3,IFIB))*(MEAN(I+3,IFIB))))
      CVAR(I,IFIB)=EXP(SLDEV(I,IFIB))-1
      GMN(I,IFIB)=EXP(MEAN(I+3,IFIB))
270   CONTINUE
599   CONTINUE
      WRITE(6,605) JLAB,JFIL,JPUN,LCASE,TOTAR,FILAR,TOTCT,AVOL
605   FORMAT('1',40X,'FIBER AND MASS CONCENTRATION',
     *15X,' LAB ',I1,'  SAMPLE ',I1,'  GRID ',I2,'  CASE ',I2/
     *50X,'SUMMARY'//
     *1X,'TOTAL AREA SCANNED = ',E12.4,'  SQ CM'/
     *1X,'TOTAL AREA FILTER = ',F7.1,8X,'SQ CM'/
     *1X,'TOTAL FIBER COUNT = ',F7.1,8X,'FIBERS'/
     *1X,'TOTAL VOLUME AIR  = ',F7.1,8X,'CUBIC METERS'///
     *30X,'CHRYSOTILE      AMPHIBOLE       AMBIGUOUS        NO PATTERN',
     *'     NON-ASBESTOS       ALL FIBERS'/)
      WRITE(6,607) (FIBCT(IFIB),IFIB=1,6)
607   FORMAT(1X,'FIBER COUNT'/
     *1X,'(FIBERS)',21X,6(F8.1,7X)/)
      WRITE(6,610) (CONCT(IFIB),IFIB=1,6),(VOLCT(IFIB),IFIB=1,6)
610   FORMAT(1X,'FIBER CONCENTRATION'/
     *1X,'(FIBERS PER SQ CM OF FILTER) ',6(E12.4,3X)/
     *1X,'(FIBERS PER CUB METER OF AIR)',6(E12.4,3X)/)
      WRITE(6,615) (CONMAS(IFIB),IFIB=1,5),(VOLMAS(IFIB),IFIB=1,2)
615   FORMAT(1X,'MASS CONCENTRATION',53X,'PERCENT TOTAL FIBERS'/
     *1X,'(GRAMS PER SQ CM OF FILTER)',
     *2X,2(E12.4,3X),3(F6.2,1X,'X',7X)/
     *1X,'(GRAMS PER CUB METER OF AIR)',1X,2(E12.4,3X)/)
      WRITE(6,620) (MEAN(1,IFIB),IFIB=1,6),
     *(SDEV(1,IFIB),IFIB=1,6),
     *(MEAN(4,IFIB),IFIB=1,6),
     *(GMN(1,IFIB),IFIB=1,6),
     *(CVAR(1,IFIB),IFIB=1,6)
620   FORMAT(1X,'LENGTH     MEAN',11X,6(F12.4,3X)/
     *1X,'(MICRONS) STD DEV',8X,6(F12.4,3X)/
     *11X,'MEAN LOG',7X,6(F12.4,3X)/
     *11X,'GEOM MN ',7X,6(F12.4,3X)/
     *11X,'COEF VAR',7X,6(F12.4,3X)/)
      WRITE(6,625) (MEAN(2,IFIB),IFIB=1,6),
     *(SDEV(2,IFIB),IFIB=1,6),
     *(MEAN(5,IFIB),IFIB=1,6),
     *(GMN(2,IFIB),IFIB=1,6),
     *(CVAR(2,IFIB),IFIB=1,6)
625   FORMAT(1X,'DIAMETER   MEAN',11X,6(F12.4,3X)/
     *1X,'(MICRONS) STD DEV',8X,6(F12.4,3X)/
     *11X,'MEAN LOG',7X,6(F12.4,3X)/
     *11X,'GEOM MN ',7X,6(F12.4,3X)/
     *11X,'COEF VAR',7X,6(F12.4,3X)/)
      WRITE(6,630) (MEAN(3,IFIB),IFIB=1,6),
     *(SDEV(3,IFIB),IFIB=1,6),
     *(MEAN(6,IFIB),IFIB=1,6),
     *(GMN(3,IFIB),IFIB=1,6),
     *(CVAR(3,IFIB),IFIB=1,6)
630   FORMAT(1X,'VOLUME     MEAN',15X,6(E12.4,3X)/
     *1X,'(CUB CM)  STD DEV',12X,6(E12.4,3X)/
     *11X,'MEAN LOG',7X,6(F12.4,3X)/
     *11X,'GEOM MN ',11X,6(E12.4,3X)/
     *11X,'COEF VAR',11X,6(E12.4,3X)/)
      IF (IEND.EQ.1) GOTO 9999
      GOTO 050
9999  STOP
      END
```


HWBUI0008861

Appendix D

ILLUSTRATIVE TABLES

HWBUI0008862

Table D-1

ELECTRON MICROSCOPE METHODS FOR ASBESTOS DATA ENTRY FORMAT, PER FIBER

Cols.	Description of Coding-Sheet Field	Permissible Values*
1-2	EM grid opening ID	01 to 99
3-5	EM field ID	001 to 999
6-8	Sequence no. of fiber within field**	0 to 999
9-12	Cumulative sequence no. within sample**	0 to 9999
13-18	Diameter in mm (do code decimal point)	0.0 or greater
19-24	Length in mm (do code decimal point)	0.0 or greater
26	2 if fiber extends beyond perimeter	0, 2
28	1 if a fiber bundle	0, 1
30	Fiber type	1, 2, 3, etc.
32-36	Case identification:	
	Col. 32 - lab	0 to 6
	33 - filter - sample	1, 2, etc.
	34 - punch - grid	1, 2
	35 - instrument type	1, 2, 3
	36 - operator within lab	1, 2
37-43	Magnification in multiples of K = 1000 (do code decimal point)	1.0 to 99.9
44-50	Area of EM field identified in cols. 3-5, in 10^{-6} cm^2 (do code decimal point)	0.001 to 999.99
51-55	Ashing factor	0.1 to 10
56-60	Volume of air sampled in m^3	1.0 to 100.0
61-65	Effective area of the original membrane in cm^2	1.0 to 999.9
71-72	Data set code	1 to 99

* Right-justify numbers in all fields unless a decimal point is entered. A blank is equivalent to a zero.

** If no fibers are observed in a field, write a one-line record with:

(1) 0 entered for sequence no. of fiber in field and for cumulative sequence no.
(2) diameter and length fields and also columns 26, 28, and 30 blank
(3) grid opening ID, field ID, case ID, magnification, and area, entered as usual.

HWBUI0008863

Table D-3

COMPUTER PRINTOUT OF DATA CARDS

1* Grid Opening	2 ID of Field	3 Seq. No. of Fibers Within a Field	4 Cum. Number of Fibers	5 Fiber Width, μm	6 Fiber Length, μm	7 8 Limit Cases If Bundle	9 Fiber Type Identif.	10 Case ID	11 Screen Mag. x 1000	12 Area of One Field of View x 10^{-6} cm^2	13 Ashing Factor	14 Vol. of Air Sampled, m^3	15 Effective Area of Collection Filter, cm^2	16 Index of Data Set
1	1	1	1	1.	20.		1	31111	17.	.182	1.0	9.2	406.5	3
1	1	2	2	.25	7.		2	31111	17.	.182	1.0	9.2	406.5	3
1	2	1	3	.5	17.		2	31111	17.	.182	1.0	9.2	406.5	3
1	2	2	4	1.	10.		1	31111	17.	.182	1.0	9.2	406.5	3
1	2	3	5	1.	18.		1	31111	17.	.182	1.0	9.2	406.5	3
1	02	4	06	.75	22.		1	31111	17.	.182	1.0	9.2	406.5	3
1	02	5	07	1.	12.		1	31111	17.	.182	1.0	9.2	406.5	3
1	02	6	08	.5	10.		3	31111	17.	.182	1.0	9.2	406.5	3
1	03	1	09	.25	24.		1	31111	17.	.182	1.0	9.2	406.5	3
1	03	2	10	.75	18.		2	31111	17.	.182	1.0	9.2	406.5	3
1	03	3	11	1.	10.		1	31111	17.	.182	1.0	9.2	406.5	3
1	03	4	12	1.	8.		1	31111	17.	.182	1.0	9.2	406.5	3
1	03	5	13	.25	10.		3	31111	17.	.182	1.0	9.2	406.5	3
1	04	1	14	.25	6.		1	31111	17.	.182	1.0	9.2	406.5	3
1	00	2	15	.25	20.		2	31111	17.	.182	1.0	9.2	406.5	3
1	04	3	16	.5	5.		3	31111	17.	.182	1.0	9.2	406.5	3
1	05	1	17	2.	65.	2	1	31111	17.	.182	1.0	9.2	406.5	3
1	05	2	18	1.	10.		2	31111	17.	.182	1.0	9.2	406.5	3
1	05	3	19	.5	4.		2	31111	17.	.182	1.0	9.2	406.5	3
1	06	1	20	1.	30.		1	31111	17.	.182	1.0	9.2	406.5	3
1	06	2	21	2.	32.		1	31111	17.	.182	1.0	9.2	406.5	3
1	07	1	22	1.	25.		1	31111	17.	.182	1.0	9.2	406.5	3
1	08	1	23	.5	5.		2	31111	17.	.182	1.0	9.2	406.5	3
1	09	1	24	.25	6.		2	31111	17.	.182	1.0	9.2	406.5	3
1	9	2	25	1.	30.	2	1	31111	17.	.182	1.0	9.2	406.5	3
1	10	1	26	1.	16.	2	1	31111	17.	.182	1.0	9.2	406.5	3
1	10	2	27	1.	6.	2	1	31111	17.	.182	1.0	9.2	406.5	3
1	10	3	28	1.	12.	2	1	31111	17.	.182	1.0	9.2	406.5	3
1	10	4	29	.25	10.		2	31111	17.	.182	1.0	9.2	406.5	3
1	10	5	30	.25	4.		3	31111	17.	.182	1.0	9.2	406.5	3
2	11	1	31	1.	8.		1	31111	17.	.182	1.0	9.2	406.5	3
2	11	2	32	1.	6.		1	31111	17.	.182	1.0	9.2	406.5	3
2	11	3	33	.5	5.		2	31111	17.	.182	1.0	9.2	406.5	3
2	11	4	34	1.	6.	2	2	31111	17.	.182	1.0	9.2	406.5	3
2	12	1	35	1.	4.		1	31111	17.	.182	1.0	9.2	406.5	3
2	12	2	36	.2	4.		2	31111	17.	.182	1.0	9.2	406.5	3
2	12	3	37	.25	5.		3	31111	17.	.182	1.0	9.2	406.5	3
2	13	1	38	1.	18.		1	31111	17.	.182	1.0	9.2	406.5	3
2	13	2	39	.2	12.		3	31111	17.	.182	1.0	9.2	406.5	3
2	14	1	40	1.	20.		1	31111	17.	.182	1.0	9.2	406.5	3
2	15	1	41	1.	14.		1	31111	17.	.182	1.0	9.2	406.5	3
2	15	2	42	.5	12.		2	31111	17.	.182	1.0	9.2	406.5	3
2	15	3	43	.25	8.		2	31111	17.	.182	1.0	9.2	406.5	3
2	15	4	44	.25	3.		3	31111	17.	.182	1.0	9.2	406.5	3
2	16	1	45	.5	5.		2	31111	17.	.182	1.0	9.2	406.5	3
2	16	2	46	.2	3.		3	31111	17.	.182	1.0	9.2	406.5	3
2	17	1	47	1.	25.	2	1	31111	17.	.182	1.0	9.2	406.5	3
2	17	2	48	1.	20.	2	1	31111	17.	.182	1.0	9.2	406.5	3
2	17	3	49	.5	6.	2	1	31111	17.	.182	1.0	9.2	406.5	3
2	17	4	50	.75	8.		2	31111	17.	.182	1.0	9.2	406.5	3
2	17	5	51	.1	12.		3	31111	17.	.182	1.0	9.2	406.5	3
2	18	1	52	.5	30.		1	31111	17.	.182	1.0	9.2	406.5	3
2	18	2	53	1.5	8.		1	31111	17.	.182	1.0	9.2	406.5	3
2	18	3	54	.2	8.		2	31111	17.	.182	1.0	9.2	406.5	3
2	18	4	55	1.	10.	2	1	31111	17.	.182	1.0	9.2	406.5	3

* See page 44 for detailed explanation of the column headings.

HWBUI0008864

Table D-2

ILLUSTRATION OF FORTRAN CODING FORM SCHEME

1	1	1	1	1.	20.		1	31111	17.	.182	1.0	9.2	406.5		3
1	1	2	2	.25	7.		2								
1	2	1	3	.5	17.		2								
1	2	2	4	1.	10.		1								
1	2	3	5	1.	18.		1								
1	2	4	6	.75	22.		1								
1	2	5	7	1.	12.		1								
1	2	6	8	.5	10.		3								
1	3	1	9	.25	24.		1								
1	3	2	10	.75	18.		2								
1	3	3	11	1.	10.		1								
1	3	4	12	1.	8.		1								
1	3	5	13	.25	10.		3								
1	4	1	14	.25	6.		1								
1	4	2	15	.25	20.		2								
1	4	3	16	.5	5.		3								
1	5	1	17	2.	65.	2	1								
1	5	2	18	1.	10.		2								
1	5	3	19	.5	4.		3								
1	6	1	20	1.	30.		1								
1	6	2	21	2.	32.		1								
1	7	1	22	1.	25.		1								
1	8	1	23	.5	5.		2								
1	9	1	24	.25	6.		2								
1	9	2	25	1.	30.	2	1								

HWBU0008865

Table D-3 (continued)

1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2	19	1	56	.5	13.			1	31111	17.	.182	1.0	9.2	406.5	3
2	19	2	57	.25	6.			4	31111	17.	.182	1.0	9.2	406.5	3
2	19	3	58	.25	12.			3	31111	17.	.182	1.0	9.2	406.5	3
2	20	1	59	.1	8.			2	31111	17.	.182	1.0	9.2	406.5	3
2	20	2	60	1.5	25.			1	31111	17.	.182	1.0	9.2	406.5	3
2	20	3	61	.5	27.	2		2	31111	17.	.182	1.0	9.2	406.5	3
2	20	4	62	.25	10.			2	31111	17.	.182	1.0	9.2	406.5	3
3	21	1	63	.75	15.			1	31211	17.	.182	1.0	9.2	406.5	3
3	21	2	64	.25	6.			2	31211	17.	.182	1.0	9.2	406.5	3
3	21	3	65	.5	15.			2	31211	17.	.182	1.0	9.2	406.5	3
3	21	4	66	.25	3.			3	31211	17.	.182	1.0	9.2	406.5	3
3	22	1	67	.5	6.	2		1	31211	17.	.182	1.0	9.2	406.5	3
3	22	2	68	.5	10.			5	31211	17.	.182	1.0	9.2	406.5	3
3	22	3	69	.25	5.			3	31211	17.	.182	1.0	9.2	406.5	3
3	23	1	70	.5	7.			1	31211	17.	.182	1.0	9.2	406.5	3
3	23	2	71	.25	12.			2	31211	17.	.182	1.0	9.2	406.5	3
3	23	3	72	.2	3.			3	31211	17.	.182	1.0	9.2	406.5	3
3	24	1	73	1.	28.			1	31211	17.	.182	1.0	9.2	406.5	3
3	24	2	74	1.	30.			1	31211	17.	.182	1.0	9.2	406.5	3
3	25	1	75	1.5	6.			1	31211	17.	.182	1.0	9.2	406.5	3
3	25	2	76	.25	8.			3	31211	17.	.182	1.0	9.2	406.5	3
3	26	1	77	.75	13.			1	31211	17.	.182	1.0	9.2	406.5	3
3	26	2	78	.5	10.			2	31211	17.	.182	1.0	9.2	406.5	3
3	26	3	79	.25	5.			3	31211	17.	.182	1.0	9.2	406.5	3
3	27	1	80	.5	26.			1	31211	17.	.182	1.0	9.2	406.5	3
3	28	1	81	.5	18.			2	31211	17.	.182	1.0	9.2	406.5	3
3	29	1	82	2.	17.			1	31211	17.	.182	1.0	9.2	406.5	3
3	29	2	83	.25	3.			3	31211	17.	.182	1.0	9.2	406.5	3
3	30	1	84	1.	3.	2		1	31211	17.	.182	1.0	9.2	406.5	3
3	30	2	85	.5	4.			2	31211	17.	.182	1.0	9.2	406.5	3
3	30	3	86	.25	4.			3	31211	17.	.182	1.0	9.2	406.5	3
4	31	1	87	3.	18.			1	31211	17.	.182	1.0	9.2	406.5	3
4	31	2	88	.5	7.			4	31211	17.	.182	1.0	9.2	406.5	3
4	31	3	89	.5	15.			4	31211	17.	.182	1.0	9.2	406.5	3
4	32	1	90	1.5	25.			1	31211	17.	.182	1.0	9.2	406.5	3
4	32	2	91	1.	15.	2		1	31211	17.	.182	1.0	9.2	406.5	3
4	32	3	92	1.	10.			4	31211	17.	.182	1.0	9.2	406.5	3
4	33	1	93	1.	35.	2		1	31211	17.	.182	1.0	9.2	406.5	3
4	33	2	94	.25	5.			3	31211	17.	.182	1.0	9.2	406.5	3
4	34	1	95	2.	10.	2		1	31211	17.	.182	1.0	9.2	406.5	3
4	34	2	96	1.	15.	2		1	31211	17.	.182	1.0	9.2	406.5	3
4	34	3	97	1.	12.			4	31211	17.	.182	1.0	9.2	406.5	3
4	35	1	98	2.	28.	2		1	31211	17.	.182	1.0	9.2	406.5	3
4	35	2	99	2.	12.			1	31211	17.	.182	1.0	9.2	406.5	3
4	35	3	100	1.	11.			4	31211	17.	.182	1.0	9.2	406.5	3
4	35	4	101	1.	6.			4	31211	17.	.182	1.0	9.2	406.5	3
4	35	5	102	.5	10.			3	31211	17.	.182	1.0	9.2	406.5	3
4	36	1	103	.5	13.	2		1	31211	17.	.182	1.0	9.2	406.5	3
4	36	2	104	.5	4.			4	31211	17.	.182	1.0	9.2	406.5	3
4	36	3	105	.5	10.			3	31211	17.	.182	1.0	9.2	406.5	3
4	37	1	106	.5	9.			1	31211	17.	.182	1.0	9.2	406.5	3
4	37	2	107	.5	5.			4	31211	17.	.182	1.0	9.2	406.5	3
4	37	3	108	.25	6.			3	31211	17.	.182	1.0	9.2	406.5	3
4	38	1	109	1.	45.	2		1	31211	17.	.182	1.0	9.2	406.5	3
4	39	1	110	1.	25.			1	31211	17.	.182	1.0	9.2	406.5	3
4	39	2	111	.25	5.			3	31211	17.	.182	1.0	9.2	406.5	3
4	40	1	112	.5	17.			1	31211	17.	.182	1.0	9.2	406.5	3
4	40	2	113	2.	12.			1	31211	17.	.182	1.0	9.2	406.5	3
4	40	3	114	.75	5.	2		1	31211	17.	.182	1.0	9.2	406.5	3

* See page 44 for detailed explanation of the column headings.

HWBUI0008866

EXPLANATION OF THE COLUMNS IN TABLE D-3

Column 1 -- EM grid opening identification number

Column 2 -- Identification number for the field of view

Column 3 -- Sequence number of a fiber within a given field of view

Column 4 -- Cumulative number of fibers counted

Column 5 -- Fiber width in mm

Column 6 -- Fiber length in mm

Column 7 -- A '2' indicates the fiber crossing the perimeter and, hence, one that is counted as a half-fiber

Column 8 -- A '1' indicates a bundle

Column 9 -- Fiber type identification code
1 → chrysotile
2 → ambiguous
3 → no SAED pattern, etc.

Column 10 -- Case identification. Laboratory code, sample code, TEM grid index, type of TEM instrument code, the operator code, etc.

Column 11 -- Magnification at the TEM fluorescent screen in multiples of 1000

Column 12 -- Area of one field of view in multiples of 10^{-6} cm^2

Column 13 -- Ashing factor, to account for the dilution or concentration resulting in the ashing step. In the procedure without the ashing step, the ashing factor is taken as 1.0.

Column 14 -- Volume of air sampled in m^3

Column 15 -- Effective area of the original air filter in cm^2

Column 16 -- Index for the data set

HWBUI0008867

Table D-4

PRINTOUT FROM PROGRAM CONLAB CHARACTERIZING PARAMETERS PER TEM GRID

FIBER AND MASS CONCENTRATION SUMMARY — LAB 3 SAMPLE 1 GRID 1 CASE 3

TOTAL AREA SCANNED = .4004-05 SQ CM
TOTAL AREA FILTER = 406.5 SQ CM
TOTAL FIBER COUNT = 56.5 FIBERS
TOTAL VOLUME AIR = 9.2 CUBIC METERS

		CHRYSOTILE	AMPHIBOLE	AMBIGUOUS	NO PATTERN	NON-ASBESTOS	ALL FIBERS
FIBER COUNT (FIBERS)		26.5	.0	19.0	10.0	1.0	56.5
FIBER CONCENTRATION (FIBERS PER SQ CM OF FILTER)		.6618+07	.0000	.4745+07	.2498+07	.2498+06	.1411+08
(FIBERS PER CUB METER OF AIR)		.2924+09	.0000	.2097+09	.1104+09	.1104+08	.6235+09
MASS CONCENTRATION				PERCENT TOTAL FIBERS			
(GRAMS PER SQ CM OF FILTER)		.1241-06	.0000	33.63 %	17.70 %	1.77 %	
(GRAMS PER CUB METER OF AIR)		.5482-05	.0000				
LENGTH (MICRONS)	MEAN	1.6582	.0000	.7152	.4471	.3529	1.1036
	STD DEV	1.3292	.0000	.6267	.2188	.0000	1.1182
	MEAN LOG	.0696	.0000	-.6538	-.9470	-1.0415	-.3733
	GEOM MN	1.0720	.0000	.5201	.3879	.3529	.6885
	COEF VAR	1.2066	.0000	.8473	.7397	.0000	1.2524
DIAMETER (MICRONS)	MEAN	.0683	.0000	.0279	.0162	.0147	.0445
	STD DEV	.0099	.0000	.0140	.0071	.0000	.0233
	MEAN LOG	-3.4254	.0000	-3.9964	-4.2171	-4.2195	-3.7716
	GEOM MN	.0325	.0000	.0184	.0147	.0147	.0230
	COEF VAR	2.3513	.0000	.9282	.5463	.0000	1.2885
VOLUME (CUB CM)	MEAN	.7210-14	.0000	.5595-15	.1054-15	.5995-16	.3590-14
	STD DEV	.1554-13	.0000	.6882-15	.1116-15	.0000	.1118-13
	MEAN LOG	-39.3868	.0000	-37.9861	-37.2538	-37.3531	-38.5023
	GEOM MN	.7844-17	.0000	.3183-16	.6620-16	.5995-16	.1900-16
	COEF VAR	.3076+07	.0000	.4293+04	.1586+01	.0000	.7941+05

HWBU0008868

Table D-5

PRINTOUT FROM PROGRAM CONLAB CHARACTERIZING PARAMETERS PER TEM GRID

FIBER AND MASS CONCENTRATION SUMMARY — LAB 3 SAMPLE 1 GRID 2 CASE 3

TOTAL AREA SCANNED = .3640-05 SQ CM
TOTAL AREA FILTER = 406.5 SQ CM
TOTAL FIBER COUNT = 47.0 FIBERS
TOTAL VOLUME AIR = 9.2 CUBIC METERS

		CHRYSOTILE	AMPHIBOLE	AMBIGUOUS	NO PATTERN	NON-ASBESTOS	ALL FIBERS
FIBER COUNT (FIBERS)		20.0	1.0	6.0	12.0	8.0	47.0
FIBER CONCENTRATION (FIBERS PER SQ CM OF FILTER)		.5495+07	.2747+06	.1648+07	.3297+07	.2198+07	.1291+08
(FIBERS PER CUB METER OF AIR)		.2428+09	.1214+08	.7283+08	.1457+09	.9711+08	.5705+09
MASS CONCENTRATION				PERCENT TOTAL FIBERS			
(GRAMS PER SQ CM OF FILTER)		.1204-06	.3294-09	12.77 %	25.53 %	17.02 %	
(GRAMS PER CUB METER OF AIR)		.5320-05	.1455-07				
LENGTH (MICRONS)	MEAN	1.7941	.5882	.6078	.3284	.5147	1.0250
	STD DEV	1.0228	.0000	.2465	.1410	.2116	.9549
	MEAN LOG	.1371	-.5306	-.6029	-1.2002	-.7549	-.4648
	GEOM MN	1.1470	.5882	.5472	.3011	.4701	.6282
	COEF VAR	1.1574	.0000	.6297	.5105	.5447	1.2627
DIAMETER (MICRONS)	MEAN	.0860	.0294	.0245	.0169	.0441	.0522
	STD DEV	.0182	.0000	.0069	.0056	.0147	.0334
	MEAN LOG	-3.5238	-3.5264	-3.7574	-4.1226	-3.1798	-3.6480
	GEOM MN	.0295	.0294	.0233	.0162	.0416	.0260
	COEF VAR	3.3040	.0000	.3865	.3146	.4142	1.3776
VOLUME (CUB CM)	MEAN	.8428-14	.3997-15	.3231-15	.1048-15	.9342-15	.3822-14
	STD DEV	.8552-14	.0000	.2220-15	.1327-15	.8864-15	.6855-14
	MEAN LOG	-41.7513	-35.4559	-35.9903	-37.3179	-34.9870	-38.5986
	GEOM MN	.7373-18	.3997-15	.2342-15	.6209-16	.6387-15	.1725-16
	COEF VAR	.1210+09	.0000	.1378+01	.1471+01	.1582+01	.1319+06

HWBU0008869

Table D-6

SUMMARY OF TEST RESULTS ON ONE AIR SAMPLE (SEE TABLES D-4 AND D-5)

1	2	3	4	5	6	7	8	9	10	11
Data Set Code	Number Conc. of All Fibers, $10^6/m^3$	Size Distribution of All Fibers: Mean Length, μm	Mean Dia., μm	Mean Volume, 10^{-15} cm^3	Volume Conc. of all Fibers, 10^{-15} cm^3/m^3	Number Conc. of Chrysotile, $10^6/m^3$	Size Distribution of Chrysotile: Mean Length, μm	Mean Dia., μm	Mean Volume, 10^{-15} cm^3	Chrysotile Mass Conc. in Air, $\mu g/m^3$
3-1	623.5	1.104	0.044	3.590	2238.4	292.4	1.658	0.068	7.210	5.482
3-2	570.5	1.025	0.052	3.822	2180.5	242.8	1.794	0.086	8.428	5.320
Mean	597.0				2209.4	267.6				5.401
Std. Dev.	37.48				40.94	35.07				0.115
Std. Error (SEm)	26.50				28.95	24.80				0.081
t	12.706									
t • SEm	336.71				367.84	315.11				1.029
95% Conf. Interval										
Upper	933.71				2577.24	582.71				6.430
Lower	260.29				1841.56	(negative)*				4.372

* Negative values are truncated to zero. Such situations are due to limited replication. It is recommended that at least 3 or 4 TEM grids be examined to substantially improve the precision. t-value decreases sharply to 4.30 for n = 3 and to 2.77 for n = 5. Also the standard error decreases with greater replication.

HWBU00008870

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA 600/2-77-178		

4. TITLE AND SUBTITLE	5. REPORT DATE
ELECTRON MICROSCOPE MEASUREMENT OF AIRBORNE ASBESTOS CONCENTRATIONS A Provisional Methodology Manual	August 1977, Revised June 1978 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Anant V. Samudra and Colin F. Harwood	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS IIT Research Institute 10 West 35th Street Chicago, Illinois 60616	10. PROGRAM ELEMENT NO. 1AD712 BA-14 (FY-77) 11. CONTRACT/GRANT NO. 68-02-2251
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Sciences Research Laboratory - RTP, N.C. Office of Research and Development U. S. Environmental Protection Agency Research Triangle Park, N. C. 27711	13. TYPE OF REPORT AND PERIOD COVERED Final 6/75 - 6/77 14. SPONSORING AGENCY CODE EPA/600/09
15. SUPPLEMENTARY NOTES	

16. ABSTRACT

This manual describes a provisional optimum electron microscope (EM) procedure for measuring the concentration of asbestos in air samples. The main features of the method include depositing an air sample on a polycarbonate membrane filter, examining an EM grid specimen in a transmission electron microscope (TEM), and verifying fiber identity by selected area electron diffraction (SAED).

This provisional manual results from a study to develop an optimum EM procedure for airborne asbestos determination. The analytical data supporting the provisional methodology are included in a separate final report.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
*Air pollution *Asbestos *Serpentine *Amphiboles Measurement *Electron microscopy *Electron diffraction	Chrysotile	13B 08G 11E 14B

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	57
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

HWBUI0008871

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Technical Information Staff
Cincinnati, Ohio 45268

OFFICIAL BUSINESS
PENALTY FOR PRIVATE USE, $300
AN EQUAL OPPORTUNITY EMPLOYER

POSTAGE AND FEES PAID
U.S. ENVIRONMENTAL PROTECTION AGENCY
EPA-335

U.S.MAIL

FRANCIS M ALPISER
EPA - REGION III
CURTIS BUILDING 6TH & WALNUT
PHILADELPHIA PA 19106

If your address is incorrect, please change on the above label; tear off; and return to the above address.
If you do not desire to continue receiving this technical report series, CHECK HERE ☐; tear off label, and return it to the above address.

HWBU0008872