EPA-600/4-83-043
September 1983

# ANALYTICAL METHOD FOR DETERMINATION OF ASBESTOS FIBERS IN WATER

by

Eric J. Chatfield and M. Jane Dillon
Electron Optical Laboratory
Department of Applied Physics
Ontario Research Foundation
Sheridan Park Research Community
Mississauga, Ontario, Canada L5K 1B3

Contract 68-03-2717

Project Officer

J. MacArthur Long
Analytical Chemistry Branch
Environmental Research Laboratory
Athens, Georgia 30613

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
ATHENS, GEORGIA 30613

This Method has been assigned the EPA method number of 100.1

NTIS Number PB 83 - 260471

DISCLAIMER

The information in this document has been funded wholly or in part by the United States Environmental Protection Agency under Contract No. 68-03-2717 to Ontario Research Foundation. It has been subject to the Agency's peer and administrative review, and it has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

# 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 raiseth 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$^{R}$ 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

## FIGURES



## TABLES

# 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 an 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 s lender 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: $A_{2-3}B_5(Si,Al)O_{22}(OH)_2$, 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° and 124°.

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 treater 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: $(Mg,Fe)_3Si_2O_5(OH)_4$.

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 $\mu m^2$ 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:

$$A = \frac{\pi}{4}\left(\frac{D}{M} + 2000\, C_s \theta^3\right)^2$$

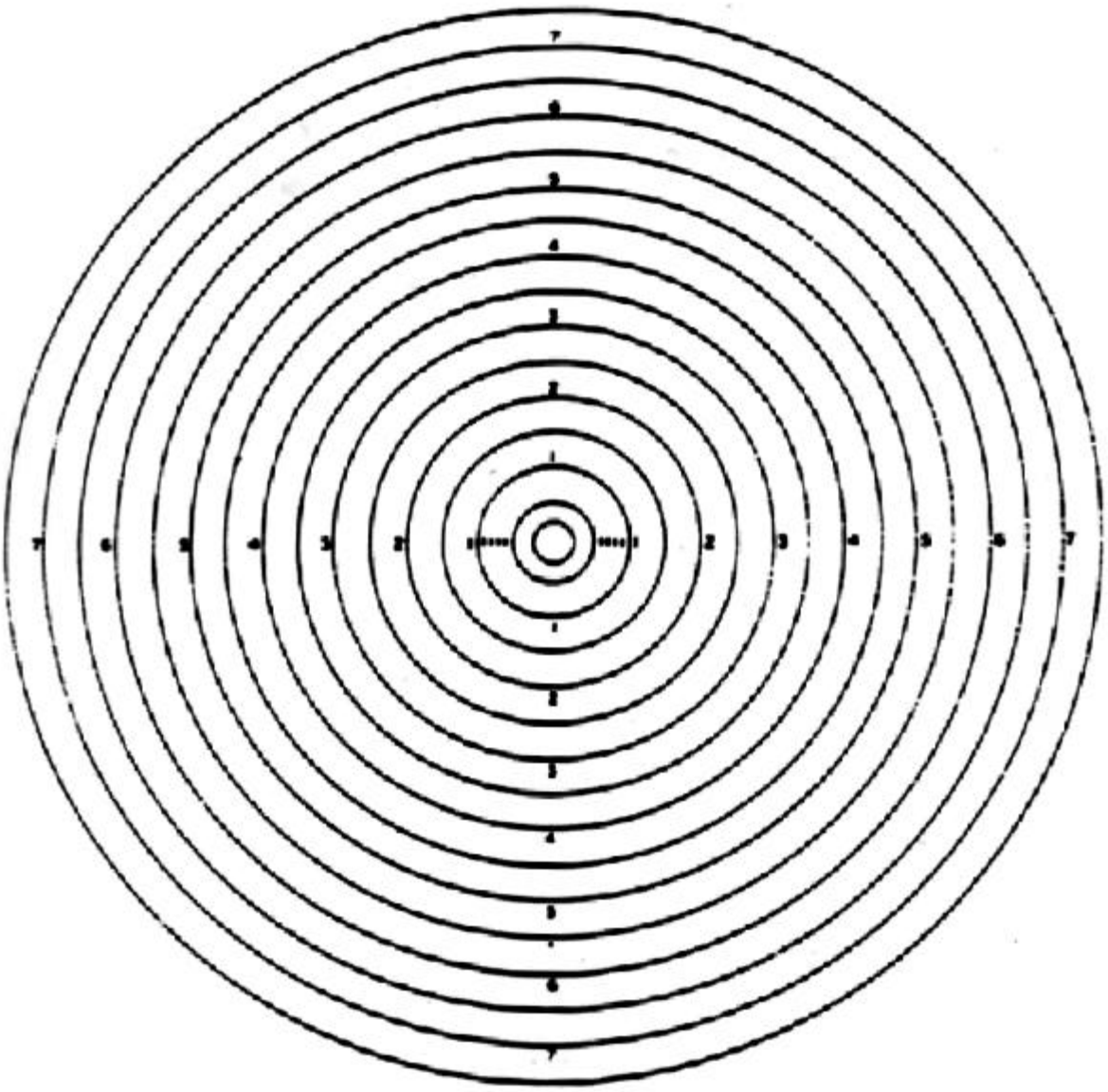


Figure 1. Calibration Markings on TEM Viewing Screen.

where:

| | | |
|---|---|---|
| A | = | Effective SAED area in $\mu m^2$ |
| D | = | Diameter of SAED aperture in µm |
| M | = | Magnification of objective lens |
| $C_s$ | = | Objective lens spherical aberration coefficient in mm |
| $\theta$ | = | 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 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° rotation combined with tilting through at least +30° to -30°, or tilting through at least +30° to -30° 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 12 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. XX4,0 025 00) or equivalent with a 0.2 µm 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 µm 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 chrysatile 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 mm 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 acct labware are required. The general supplies include a detergent for cleaning apparatus, marking pens for labeling glass and plastic apparatus, glass microscopy slides, lens paper (for preparation of Jaffe Washer and lining of TEM arid 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 dropping 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 p re-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 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.

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 containers. It is not easy to indicate when oxidation is complete, but this treatment as described has been found to be adequate for all

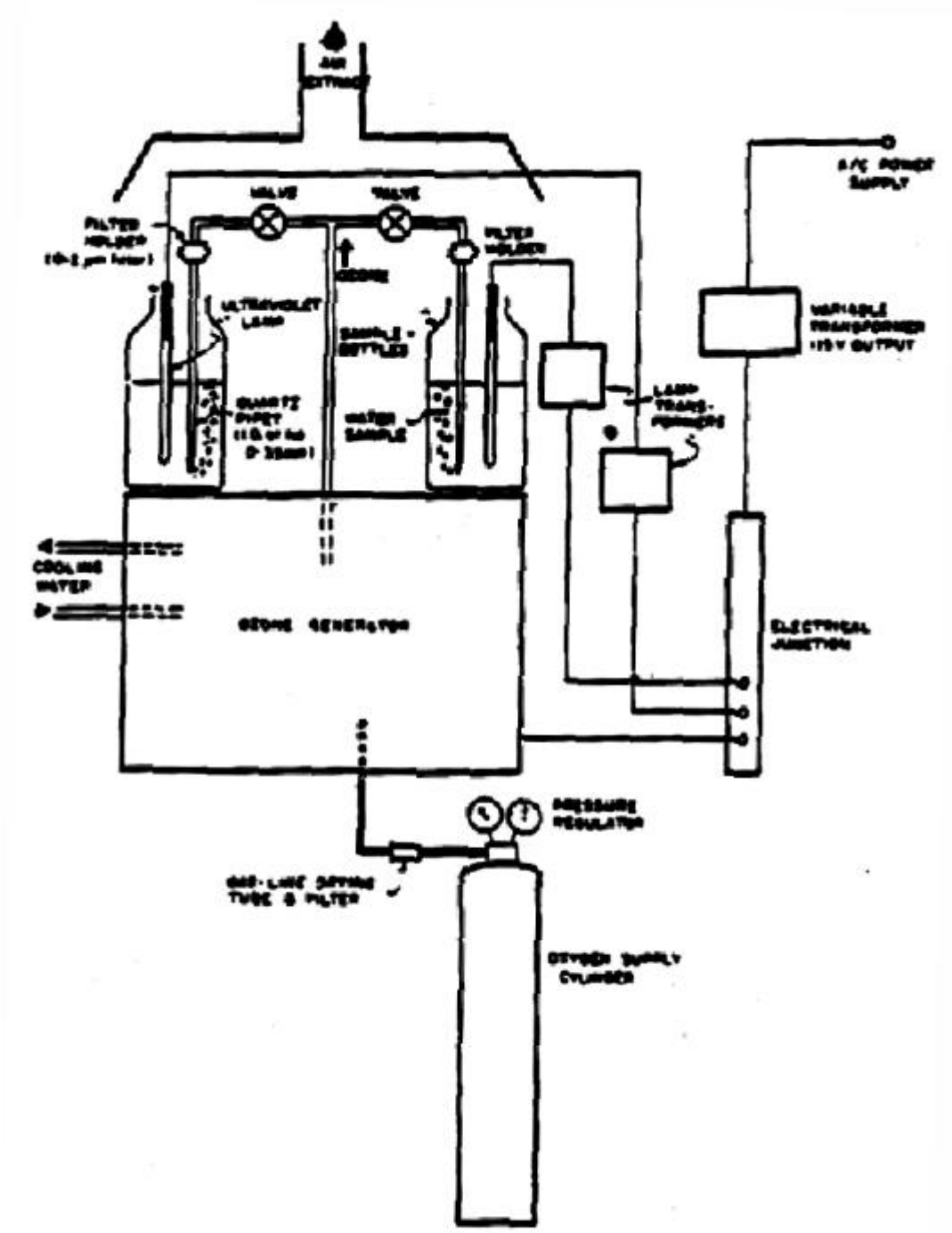



Figure 2. Diagram of Ozone-UV Equipment.

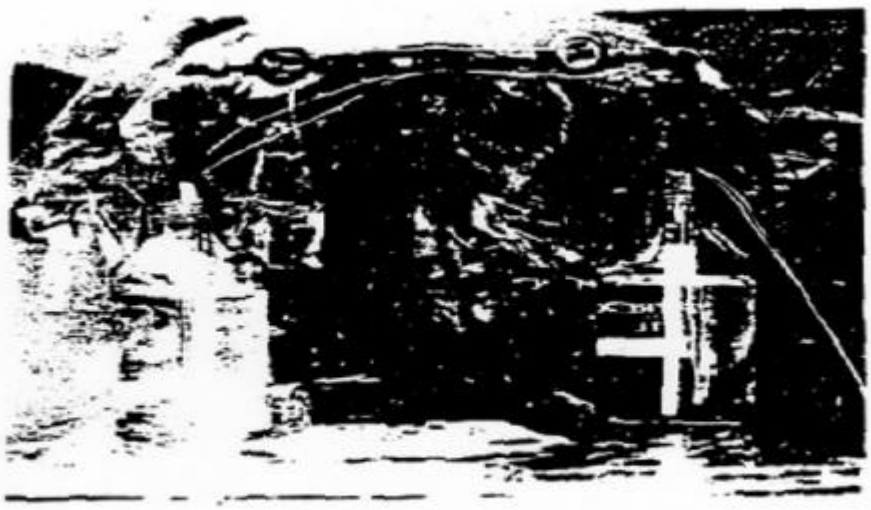


Figure 3. Ozone-UV Oxidation of Water Samples in Glass Bottles. The ozone supply line has been split into two lines to permit simultaneous oxidation of two samples. A valve and a filter holder are incorporated in each of the supply lines to the samples.

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 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.

**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 | 06 | $1.5 \times 10^7$ |
| 0.5 | 2.8 | $3.0 \times 10^6$ |
| 1.0 | 5.7 | $1.5 \times 10^6$ |
| 2.0 | 11 | $0.8 \times 10^6$ |
| 5.0 | 28 | $3.0 \times 10^5$ |
| 10 | 57 | $1.5 \times 10^5$ |
| 25 | 142 | $6.0 \times 10^4$ |
| 50 | 285 | $3.0 \times 10^4$ |
| 100 | 570 | $1.5 \times 10^4$ |

[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 $cm^2$

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 μm 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 μm 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 cleaned, 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 labeled, 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.

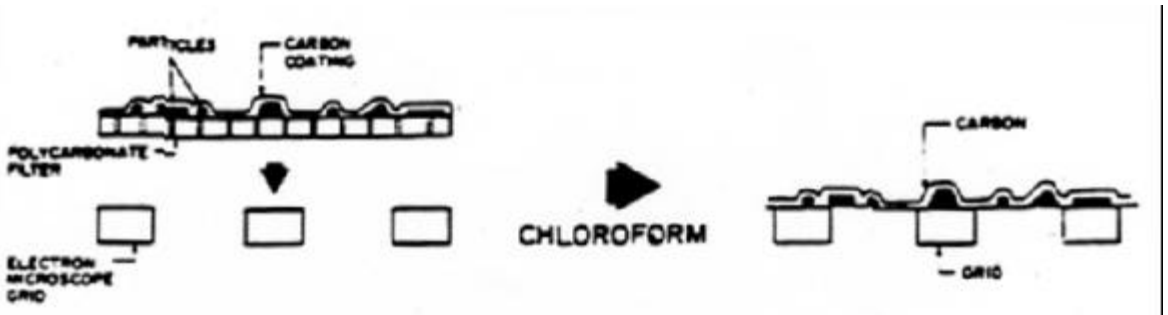
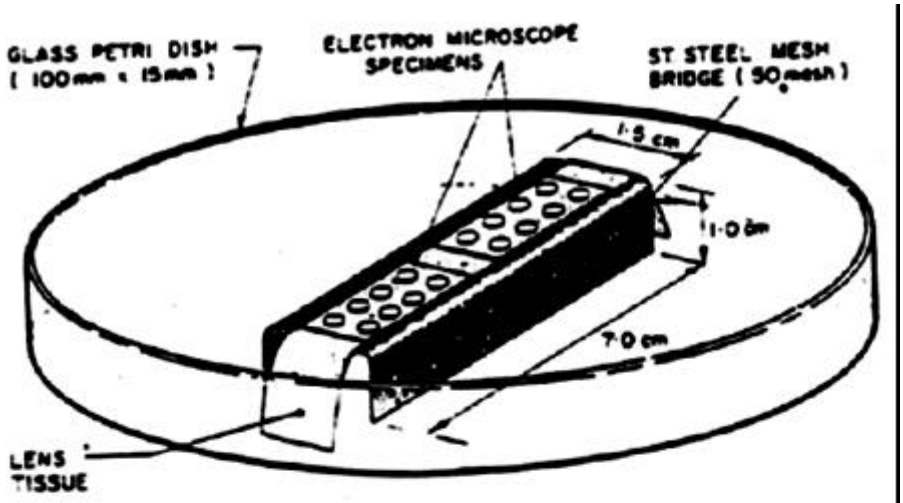



Figure 4. Nuclepore Dissolution Technique

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 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 aloma 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 mm to 50 mm 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 avacuum 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 class 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.

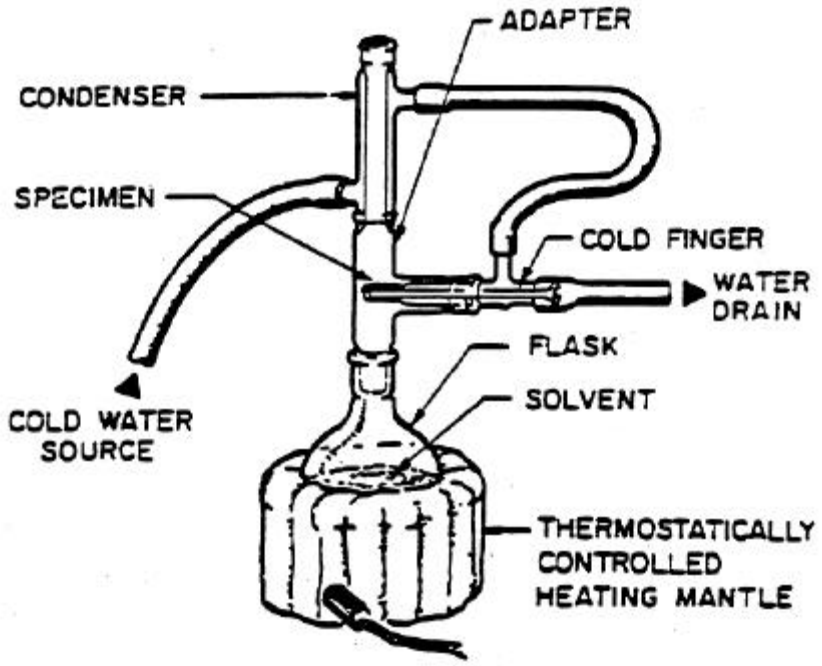



Figure 6. Condensation Washer.

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 an 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 µm 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 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

**ASBESTOS ANALYSIS - WATER SAMPLE DATA**

| | | |
|---|---|---|
| SEQ: | SAMPLE: | CODE |
| JOB: | | |

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 line of 60 characters)

_____

_____

_____

_____

_____

FIBER CLASSIFICATIONS:

COUNT: NAM TM CM CD CQ CMQ CDQ UF AD AX ADX AQ ADQ AZQ

PROCESS: AZZ

| 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 | | Fiber Classification | | | | | | | | | | | | | | | Fiber Size | | EDXA Peak Areas | Comments |
| No. | Dimensions (mm) | NAM | TM | CM | CD | CQ | CMQ | CDQ | UF | AD | AX | ADX | AQ | ADQ | AZQ | AZZ | Length (mm) | Width (mm) | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |
| | x | | | | | | | | | | | | | | | | | | | |

Continued on Page

Figure 8. Sheet for Recording Fiber Classification and Measurement Data.

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 mats 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.

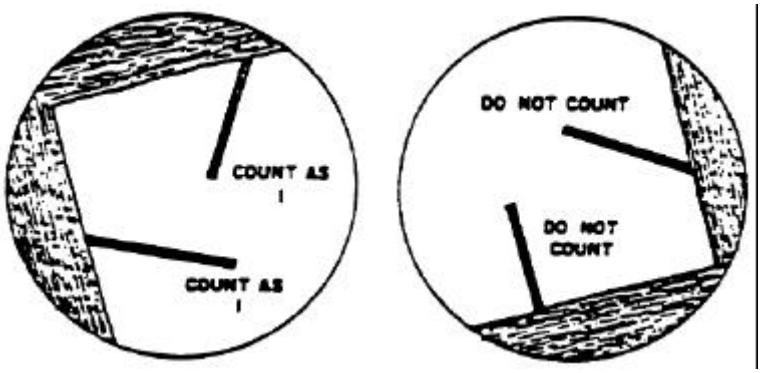



**Figure 9. Counting of Fibers Which Overlap Grid Bars.**

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 continues.

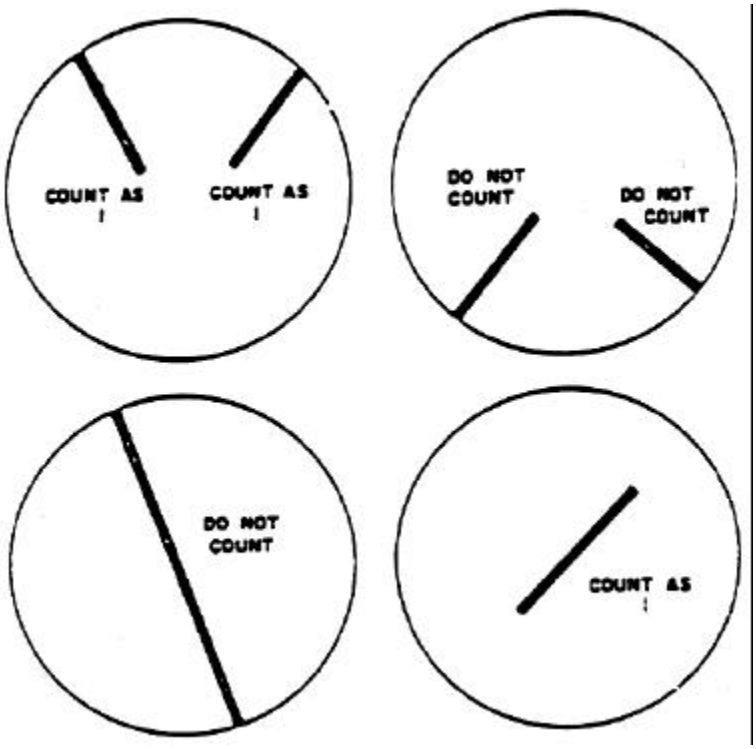



**Figure 10. Counting of Fibers Which Extend Outside the Field of View.**

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.

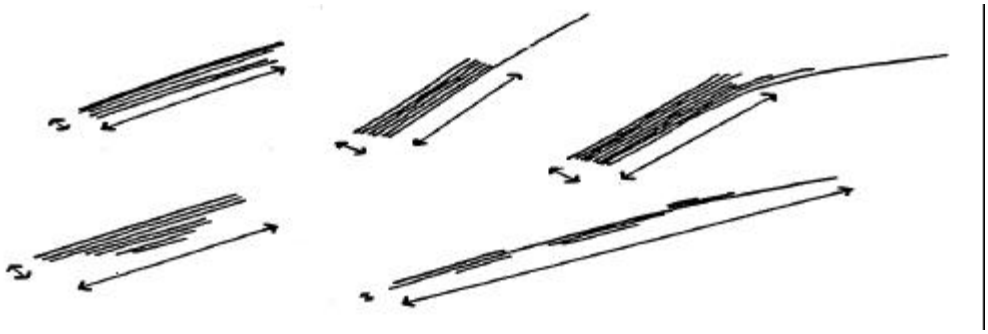


**Figure 11. Counting and Measurement of Fiber Bundles. Each bundle to be counted as one fiber with dimensions as indicated by arrows.**

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 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.


**Figure 12. Counting of Fiber Aggregates.**

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 procedures 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.

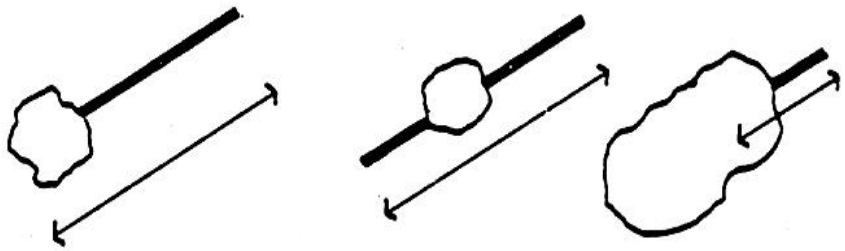


**Figure 13. Counting and Measurement of Fibers Attached to Non-Fibrous Debris.**

## 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, 6 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 how 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.

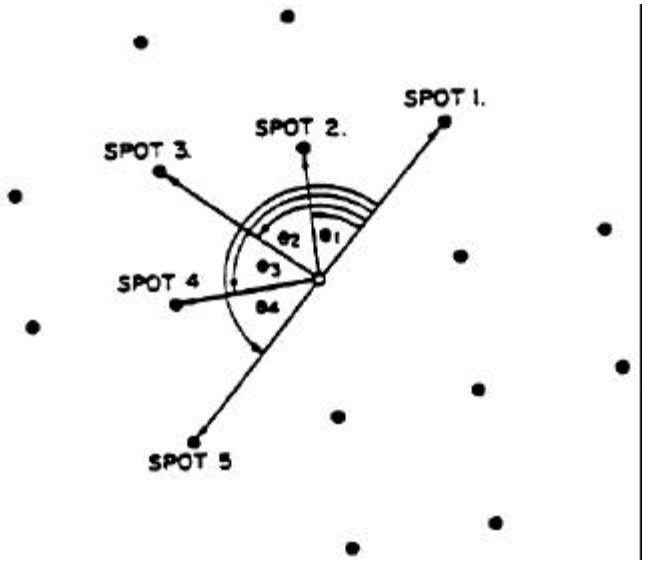



**Figure 14. Measurement of Zone Axis SAID Patterns.**

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 or the calibration pattern (111 and 200) must also be measured with the same precision.

The camera constant ($\lambda L$) required for the computer program is given by:

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

where:

| | | |
|---|---|---|
| $\lambda$ | = | Wavelength of the incident electrons |
| L | = | Effective camera length in mm |
| a | = | Unit cell dimension in Angstroms |
| D | = | Diameter of the (h, k, 1) diffraction rings in millimeters |
| h, k, l | = | Miller indices of the scattering plane of the crystal. |

Using gold, the camera constant is given by:

| | | |
|---|---|---|
| $\lambda L$ | = | 2.3548 D (first ring) |
| $\lambda L$ | = | 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 K$\alpha$ 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 foe 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 CD 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.

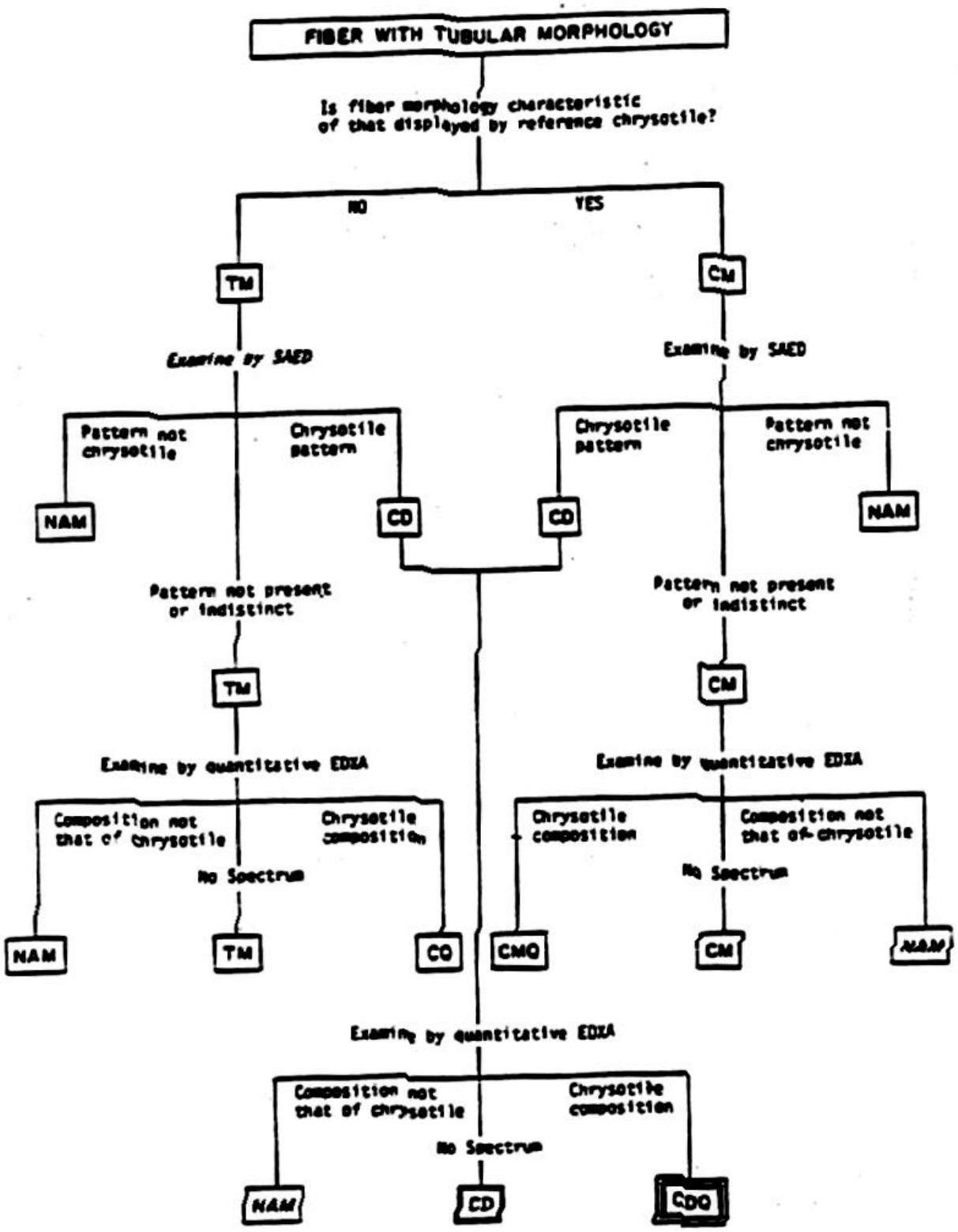



Figure 15. Classification Chart for Fiber With Tubular Morphology.

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 |

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.

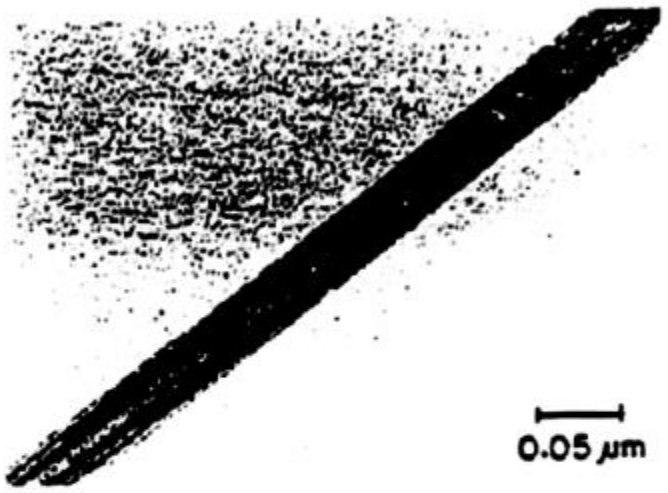



Figure 16A. TEM Micrograph of Chrysotile Fibril, showing Morphology.

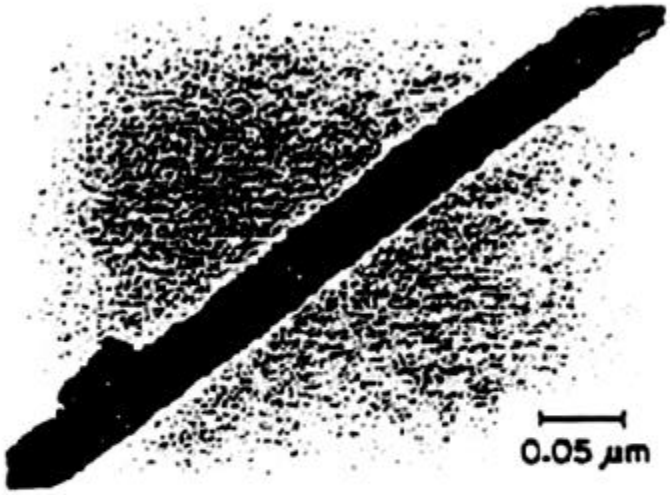



Figure 16B. TEM Micrograph of UICC Canadian Chrysotile Fiber after Thermal Degradation by Electron Beam Irradiation.

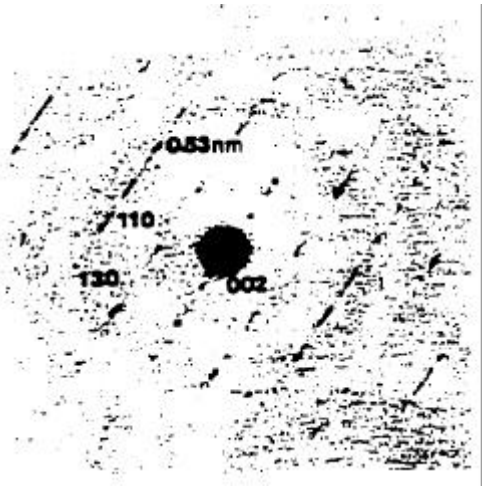



**Figure 17. SAED Pattern of Chrysotile Fiber with Diagnostic Features Labelled. Necessary criteria are the presence of 0.73 nm spacing for the 002 reflections, 0.53 nm spacing for the layer line repeat and characteristic streaking of the 110 and 130 reflections.**

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 CO, 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.

Depending on the particular situation, four levels of analysis can be defined in this analytical procedure, and these are shown in Table 5.

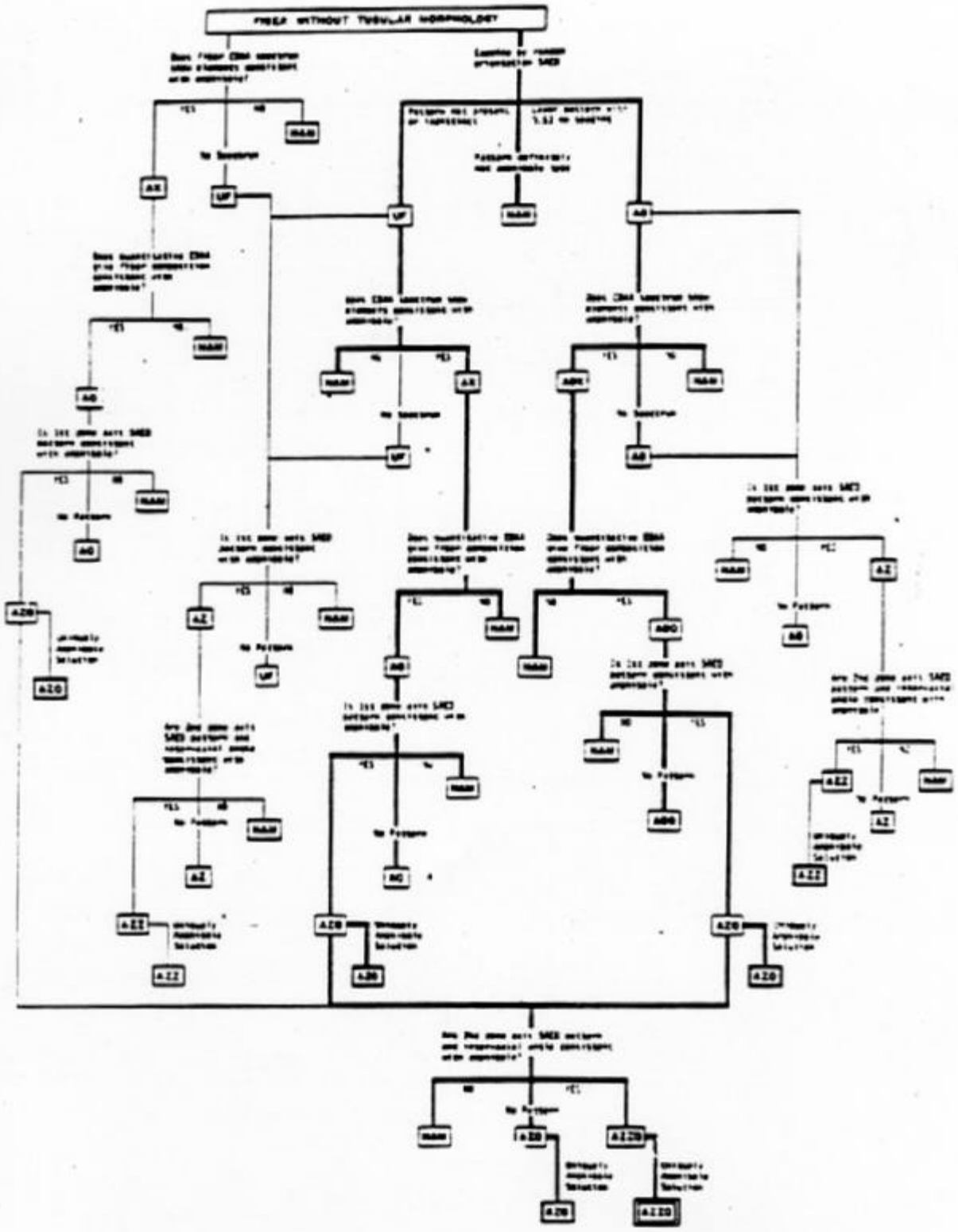


Figure 18. Classification Chart for Fiber Without Tubular Morphology. Bold Lines indicate the Preferred Paths.

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

| Levels 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 o known and well-characterized sources where discrimination between two or more ampjibole fiber types is requried. | ADQ | Not Applicable |
| 3 | Routine samples from uncharacterized sources in which presence or absence of amphibole is to be confirmed. | ADQ | ASS, 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 ca rbon 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 he 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 carries out. If it is suspected that samples could have been contaminated during the original preparation, the duplicate bottles should be used for the prepreparation 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 $A_l$ 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, pj, is given by $A_j/A$. 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 $A_j$ is $_{npj}$. If the observed number found on that grid opening is $n_j$, then:

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

This value is compared with significance points of the $x^2$ 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 1, 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 31 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:

<u>No fibers detected</u>

The value will be reported as less than 369% of the concentration equivalent to one fiber.

<u>1 to 4 fibers</u>

When 1 to 4 fibers are counted, the result will be reported as less than the corresponding upper 95% confidence limit (Poisson).

<u>5 to 30 fibers</u>

Mean and 95% confidence intervals will be reported on the basis of the Poisson assumption.

<u>More than 30 fibers</u>

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:

| | | |
|---|---|---|
| $n_j$ | = | Number of fibers on the i'th grid opening |
| n | = | Total number of fibers found in k grid openings |
| $p_j$ | = | 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. 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 µm |
| W | = | Width in µm |
| 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 µg/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:

$$C_{(N)k} = \frac{\sum_{i=1}^{i=k} n_i}{\sum_{i=1}^{i=} n_i} \times 100$$

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) Chrysotile 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 I 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 µg/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 $X^2$ 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 ± 6O%. 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 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.

## TABLE 7. INTER-LABORATORY COMPARISON: STANDARD DISPERSIONS

| Laboratory | Sample 1 | | | Sample 2 | | | Sample 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Concentration, MFL | | | Concentration, MFL | | | Concentration, MFL | | |
| | 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 | 300 | 1.58 | 1.17 - 1.99 | 69 |
| 2 | 9.48 | 4.79 - 14.2 | 66 | 32.3 | Not Available | 75 | 2.67 | 1.82 - 3.52 | 56 |
| 3 | 12.1 | 9.20 - 15.0 | 86 | 27.0 | 19.6 - 34.4 | 186 | 1.68 | 1.17 - 2.19 | 55 |
| 4 | 9.26 | 7.12 - 11.4 | 96 | 20.3 | 11.2 - 29.4 | 150 | 1.76 | 0.97 - 2.55 | 32 |
| 5 | 9.66 | 7.37 - 11.9 | 85 | 34.0 | 28.9 - 39.1 | 284 | 1.18 | 0.85 - 1.51 | 42 |
| 6 | 8.99 | 1.22 - 16.7 | 55 | 16.2 | 3.27 - 29.1 | 202 | 1.90 | 1.14 - 2.65 | 63 |
| Mean Concentration | 9.78 | | | 26.3 | | | 1.79 | | |
| Standard Deviation | 0.82 | | | 6.89 | | | 0.49 | | |
| Relative Standard Deviation | 8.4% | | | 26% | | | 27% | | |
| Fiber Species Reported | Chrysotile | | | Chrysotile | | | Amphibole | | |

## TABLE 7. INTER-LABORATORY COMPARISON: STANDARD DISPERSIONS

| Laboratory | Sample 1 | | | Sample 2 | | | Sample 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Concentration, MFL | | | Concentration, MFL | | | Concentration, MFL | | |
| | 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 | 300 | 1.58 | 1.17 - 1.99 | 69 |
| 2 | 9.48 | 4.79 - 14.2 | 66 | 32.3 | Not Available | 75 | 2.67 | 1.82 - 3.52 | 56 |
| 3 | 12.1 | 9.20 - 15.0 | 86 | 27.0 | 19.6 - 34.4 | 186 | 1.68 | 1.17 - 2.19 | 55 |
| 4 | 9.26 | 7.12 - 11.4 | 96 | 20.3 | 11.2 - 29.4 | 150 | 1.76 | 0.97 - 2.55 | 32 |
| 5 | 9.66 | 7.37 - 11.9 | 85 | 34.0 | 28.9 - 39.1 | 284 | 1.18 | 0.85 - 1.51 | 42 |
| 6 | 8.99 | 1.22 - 16.7 | 55 | 16.2 | 3.27 - 29.1 | 202 | 1.90 | 1.14 - 2.65 | 63 |
| Mean Concentration | 9.78 | | | 26.3 | | | 1.79 | | |
| Standard Deviation | 0.82 | | | 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 | | |

## 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 miss concentration for the crocidolite sample was 67.4 $\mu$g/L, which is very close to the known concentration of 50 $\mu$g/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% | |

SELECTED BIBLIOGRAPHY

Anderson, C.M. and J.M. Long (1980). Interim Method for Determining Asbestos in Water, Report EPA-600/4-80-005. U.S. Environmental Protection Agency, Athens, Georgia. Available through National Technical Information Service, Springfield, Virginia 22161.

Asher, I.M. and P.P. McGrath (Editors) (1976). Symposium on Electron Microscopy of Microfibers. Proceedings of the First FDA Office of Science Summer Symposium, Pennsylvania State University. U.S. Government Printing Office, Washington, D.C. 20402.

Bates, R.L. and J.A. Jackson (1980). Glossary of Geology, Second Edition. American Geological Institute, Falls Church, Virginia 22041.

Beaman, D.R. and D.M.File (1976). Quantitative Determination of Asbestos Fiber Concentrations. Anal. Chem. 48(l): 101-110.

Bradley, D.E. (1965). Replica and Shadowing Techniques. In: Techniques for Electron Microscopy. Blackwell Scientific Publications, Alden Press, Oxford, D.H. Kay (ed.) 96-152.

Campbell, W.J., R.L. Blake, L.L. Brown, E.E. Cather, and J.J. Sjoberg (1977). Selected Silicate Minerals and Their Asbestiform Varieties. Bureau of Mines Information Circular 8751. U.S. Bureau of Mines, Avondale Research Center, 4900 LaSalle Road, Avondale, Md 20782.

Chatfield, E.J. (1979). Preparation and Analysis of Particulate Samples by Electron Microscopy, with Special Reference to Asbestos. Scanning Electron Microscopy/1979/I, SEM Inc., AMF O'Hare, Chicago, Illinois 60666. 563-578.

Chatfield, E.J. and M.J. Dillon (1979). A National Survey for Asbestos Fibres in Canadian Drinking Water Supplies. Health and Welfare Canada Report 79-EHD-34. Information Directorate, Department of National Health and Welfare, Brooke Claxton Building, Ottawa, Canada KlA 0K9.

Chatfield, E.J. and R.W. Glass (1976). Improved Methodology for Determination of Asbestos as a Water Pollutant. Ontario Research Foundation Report, Mississauga, Ontario, Canada.

Chatfield, E.J., R.W. Glass and M.J. Dillon (1978). Preparation of Water Samples for Asbestos Fiber Counting by Electron Microscopy, Report EPA-600/4-78-011. U.S. Environmental Protection Agency, Athens, Georgia. Available through the National Technical Information Service, Springfield, Virginia 22161.

Chopra, K.S. (1978). Interlaboratory Measurements of Amphibole and Chrysotile Fiber Concentrations in Water. Journal of Testing and Evaluation, Vol . 6, No. 4, 241-247.

Cook, P.M., I.B. Rubin, C.J. Maggiore, and W.J. Nicholson (1975). X-ray Diffraction and Electron Beam Analysis of Asbestiform Minerals in Lake Superior Waters. Proc. Inter. Conf. on Environ. Sensing and Assessment. Published 1976 IEEE, New York, N.Y., 2(34-1): 1-9.

Deer, W.A., R.A. Howie and J. Zussman (1966). Rock-Forming Minerals, Part 2, Chain Silicates. Longgmans, London, England, 99-192.

Gard, J.A. (Editor) (1971). The Electron-Optical Investigation of Clays. Mineralogical Society, 41 Queen's Gate, London, S.W. 7.

Hirsch, P.B., A. Howie, R.B. Nicholson, D.W. Pashley and M.J. Whelan (1965). Electron Microscopy of Thin Crystals. Butterworths, London, 18-23.

Kay, D.H. (1965). Techniques for Electron Microscopy. Blackwell Scientific Publications, Alden Press, Oxford.

Leake, B.E. (1978). Nomenclature of Amphiboles. The Canadian Mineralogist, 16, 501-520.

Lee, R.J. (1978). Basic Concepts of Electron Diffraction and Asbestos Identification using SAO Part I: Current Methods of Asbestos Identification using SAD. SEM/1978/1 (ed. 0. Johari) AMF O'Hare, Chicago, Illinois, 677-686.

Lee, R.J. (1978). Basic Concepts of Electron Diffraction and Asbestos Identification Using SAD. Part 11: Single Crystal and SAD. Ibid, 687-694.

Lee, R.J., J.S. Lally and R.M. Fisher (1981). Design of a Laboratory for Particulate Analysis, EPA 600/4-81-032. National Technical Information Service, U.S. Dept. of Commerce, Springfield, Va 22161.

Ledoux, R.L. (Editor) (1979). Short Course in Mineralogical Techniques of Asbestos Determination. Mineralogical Association of Canada, c/o Department of Mineralogy, Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario, Canada, M5S 2C6.

Lishka, R.J., J.R. Millette, and E.F. McFarren (1975). Asbestos Analysis by Electron Microscopy. Proc. AWWA Water Quality Tech. Conf. American Water Works Assoc., Denver, Colorado, XIV-1 - XIV-2.

McCrone, W.C. and I.M. Stewart (1974). Asbestos. Amer. Lab. 6(4) 13-18.

Michaels, L. and S.S. Chissick (Editors) (1979). Asbestos (Volume 1) Properties, Applications and Hazards. John Wiley and Sons, New York, N.Y.

Millette, J.R. and E.F. McFarren (1976). EDS of Waterborne Asbestos Fibers In TEM. SEM and STEM. Scanning Electron Microscopy/1976/I (ed. 0. Johari) IIT Research Institute, Chicago, Illinois 60616, 451-460.

National Bureau of Standards Special Publication 506 (1977). Proceedings of Workshop on Asbestos: Definitions and Measurement Methods. Held at NBS, Gaithersburg, MD, (Issued November 1978). U.S. Government Printing Office, Washington, D.C. 20402.

National Bureau of Standards Special Publication No. 619 (1981). Proceedings of the NBS/EPA Workshop on Asbestos Standards: Materials and Analytical Methods. Held at NBS, Gaithersburg, MD, (Issued 1982). U.S. Government Printing Office, Washington, D.C. 20402.

Natrella, M.G. (1966). Experimental Statistics: National Bureau of Standards Handbook 91. U.S. Government Printing Office, Washington, D.C. 20402.

Pearson, E.S. and H.O. Hartley (1958). Biometrika Tables for Statisticians, Volume I. Cambridge University Press, 32 East 57th Street, New York, 22, N.Y.

Russell, P.A. and A.E. Hutchings (1978). Electron Microscopy and X-ray Applications to Environmental and Occupational Health Analysis. Ann Arbor Science Publishers Inc., P.O. Box 1425, Ann Arbor, Michigan 48106.

Samudra, A.V. (1977). Optimum Procedure for Asbestos Fibers Identification from Selected Area Electron Diffraction Patterns in a Modern Analytical Electron Microscope Using Tilted Specimens. Scanning Electron Microscopy, 1977/1 (ed.-O. Johari). IIT Research Institute, Chicago, Illinois 60616, 385-392.

Siegrist, H.G. and A.G. Wylie (1980). Characterizing and Discriminating the Shape of Asbestos Particles. Environmental Research, 23, 348-361.

Smith, J.E. and M.L. Jordan (1964). Mathematical and Graphical Interpretation of the Log-Normal Law for Particle Size Distribution Analysis. J. Colloid Science 19, 549-559.

Spurny, K.R., W. Stober, H. Opiela and G. Weiss (1979). On the Evaluation of Fibrous Particles in Remote Ambient Air. The Science of the Total Environment 11, 1-40.

Steen, D. (1981). Determination du Taux de Contamination de L'Air Par L'Amiante. Institut Universitaire de Medecine du Travail et d'Hygiene Industrielle, Route de-la Clochatte, CH1052, Le Mont-sur-Lausanne, Switzerland.

Wenk, H.R. (Editor) (1976). Electron Microscopy in Mineralogy. Springer-Verlag, New York.