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Environmental Asbestos Assessment Manual

Superfund Method for the Determination of Asbestos in Ambient Air

Part 1: Method

INTERIM VERSION

DISCLAIMER

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1. INTRODUCTION

This is a sampling and analysis method for the determination of asbestos in air. Samples are analyzed by transmission electron microscopy (TEM). Although a small subset of samples are to be prepared using a direct procedure, the majority of samples analyzed using this method will be prepared using an indirect technique. The method allows for the determination of the mineralogical type(s) of asbestos present and for distinguishing asbestos from non-asbestos minerals. In the method, asbestos structures are characterized as fibers, bundles, clusters, or matrices and the length and width of each asbestos structure are measured. Although the method is designed specifically to provide results suitable for supporting risk assessments at Superfund sites, it is applicable to a wide range of ambient air situations.

To support a risk assessment, this method addresses two objectives:

(a) to provide increased precision at the low concentrations of asbestos typically found in the environment;

(b) to provide measurements that can be compared with risk factors derived from existing epidemiology studies.

An additional consideration addressed in this method is the need to control sampling and analysis costs.

The method focuses on sampling requirements for individual sampling stations and the analysis of sample filters collected at such stations. During a site investigation, sampling stations would be arranged in an array designed to distinguish spatial trends in airborne asbestos concentrations. Sampling schedules would be fashioned to establish temporal trends. Thus proper design of a comprehensive sampling strategy, detailing the design of the array of sampling locations and the schedule for sample collection, is also critical to the success of an investigation. However, design of a sampling strategy is necessarily site specific and site-specific considerations are beyond the scope of this document.

Satisfying the two method objectives listed above requires innovations that tax the limits of available technology. Consequently, several variations were considered during development and this method represents a workable compromise among several technical constraints. Although the method has not been validated as a whole and the feasibility of a few procedures needs to be better documented, many of the component procedures of this method have been performed in the laboratory. Thus, the principle features of the method are well enough established that the method can be profitably employed in current field investigations. There is no better way to acquire the necessary experience and data for completing method validation. At the same time, until a validation study and appropriate pilot studies are completed, this should be considered an interim method.

Details of the considerations addressed during the development of this method are provided in a companion Technical Background Document, Part 2 of this report, under separate cover. A summary is presented in Chapter 2 (Background) of this document.

NOTE

This document is intended to serve several audiences including site project managers, field sampling teams, data reviewers, and laboratory analysts. The document may be separated into segments so that individuals may focus on the sections of most interest to their particular roles in a project.

2. BACKGROUND

During the development of this method, existing sampling and analysis technologies were considered to determine an appropriate approach for achieving defined method objectives while remaining within the known constraints associated with the measurement of airborne asbestos. The method is designed to provide the analytical sensitivity and precision necessary to distinguish background from asbestos concentrations typical of environmental contamination. The method is also designed to provide results recorded in a format that allows comparison with published risk factors or other proposed representations of the biological activity of asbestos.

Recent developments toward understanding the relationship between asbestos exposure and risk indicate that, to assess risks, modern field measurements should be tailored to quantify asbestos characteristics that best relate to biological activity. This is true despite the fact that existing risk factors are derived from epidemiology studies in which asbestos concentrations were derived primarily by phase contrast microscopy (PCM). A broader range of asbestos structures potentially contribute to biological activity than can be detected by PCM.

To provide the needed capabilities for distinguishing the broadest range of asbestos characteristics, transmission electron microscopy (TEM) was selected as the analytical technique employed in this method. Although considered, both PCM and scanning electron microscopy (SEM) were rejected for use in this method due to their inherent limitations.

The method is designed to provide the best description possible of the nature, numerical concentration, and sizes of asbestos-containing particles found in an air sample. The method of data recording specified in the method is designed to allow re-evaluation of the structure characterization and counting data without the necessity for re-examination of the specimens.

Currently-recognized methods require collection of airborne particulate on a membrane filter, followed by preparation of TEM specimens from the filter. Airborne asbestos is collected either on mixed cellulose ester (MCE) filters or polycarbonate filters. TEM specimens can be prepared from such membrane filters either by a direct-transfer technique or by an indirect method.

Direct-transfer TEM specimen preparation methods have the following advantages:

(a) the particulate and fiber size distributions are undisturbed during specimen preparation;

(b) the limited amount of specimen manipulation reduces the possibility of fiber loss or introduction of extraneous contamination.

However, the direct-transfer TEM preparation methods also have some significant disadvantages:

(a) the achievable detection limit is restricted by the particulate density on the filter, which in turn is controlled by the sampled air volume and the total suspended particulate concentration in the atmosphere being sampled;

(b) the precision of the result is dependent on the uniformity of the deposit of asbestos structures on the sample collection filter;

(c) air samples must be collected that have particulate and fiber loadings within narrow ranges. If too high a particulate loading occurs on the filter, it is not possible to prepare satisfactory TEM specimens by a direct-transfer method. If too high a fiber loading occurs on the filter, even if satisfactory TEM specimens can be prepared, accurate fiber counting will not be possible.

Indirect TEM specimen preparation techniques permit some of the disadvantages of direct-preparation to be overcome:

(a) air samples can be collected without regard to the amount of deposit on the filter surface. The filter loading can be adjusted in the laboratory to provide satisfactory TEM specimens;

(b) interfering particulate material can be completely or partially removed through a combination of dissolution and oxidation (ashing);

(c) the uniformity of distribution of asbestos structures on the filters to be analyzed is improved.

Indirect TEM specimen preparation methods have the following disadvantages:

(a) the size distribution of asbestos structures is modified;

(b) there is increased opportunity for fiber loss or introduction of extraneous contamination;

(c) when sample collection filters are ashed, any fiber contamination in the filter medium is concentrated on the TEM specimen grid.

The question as to whether direct or indirect specimen preparation yields the "correct" result (in terms of representing biological activity) is currently unresolved. It can be argued that the direct methods yield an under-estimate of the asbestos structure concentration because many of the asbestos fibers present are concealed by other particulate material with which they are associated. Conversely, the indirect methods can be considered to yield an over-estimate because some types of complex asbestos structures disintegrate during the preparation, resulting in an increase in the numbers of structures counted.

2.1. SENSITIVITY

To provide the required sensitivity at asbestos levels typically found in the environment, it is necessary either to selectively concentrate asbestos, or to count structures over a much greater area of a TEM specimen grid than has traditionally been required. To the extent that it can be employed, selective concentration is the least costly of the alternatives for increasing sensitivity. The optimal asbestos concentration on a filter requires filter loadings of total particulate matter in excess of what can generally be tolerated for analysis using direct-transfer methods for TEM specimen preparation. Consequently, an indirect technique for TEM specimen preparation is employed in this method, which incorporates steps allowing for the selective concentration of asbestos.

In most ambient environments, a proportion of the suspended particulate is organic, consisting of soot, spores, pollens and other debris from vegetation. Organic materials such as these can be removed from the analysis by low-temperature ashing.

It is common to find substantial numbers of calcium sulfate fibers (gypsum) in airborne particulate collected in urban environments. These can arise from various sources, and they can also be generated either in the atmosphere or on the sample collection filter by reaction of airborne calcite or dolomite particles with atmospheric sulfur dioxide. Gypsum can be removed by dissolution in water or dilute acids.

Carbonates are another major component of most exterior atmospheres. Calcite and dolomite are commonly found in urban atmospheres; these originate from various sources, including erosion of concrete and cement, local geology, and in some cases from industrial operations. Such carbonates can be removed by extraction with hydrochloric acid. If acid extraction procedures are carried out correctly, no chemical or crystallographic degradation of asbestos can be detected by routine methods of TEM analysis.

Removal of a large proportion of the suspended particulate by use of these techniques permits the asbestos present in the sample to be concentrated on to a smaller area of the TEM specimen. Consequently, the area of the TEM specimen that must be examined to achieve a particular analytical sensitivity is proportionately reduced. Also, many of the non-asbestos fibrous structures normally found in a directly-prepared TEM specimen, each of which must be identified and rejected from the asbestos structure count, do not appear on the indirectly-prepared TEM specimen.

For many environmental samples, a selective concentration of the asbestos structures, incorporating low temperature ashing, re-suspension in water, and acidification with HCl is capable of removing substantial amounts of interfering particulate from the analysis, and, for a

particular analytical sensitivity, reducing the area of the TEM specimens that must be examined.

The concentration steps included in this method, together with the requirement to measure very low airborne asbestos concentrations, make control of asbestos contamination more critical than for specimens prepared by direct-transfer techniques. Accordingly, the procedures in this method have been designed to minimize the effects of contamination.

2.2. SAMPLED AIR VOLUMES

This method incorporates an indirect preparation procedure to provide flexibility in the amount of deposit that can be tolerated on the sample filter and to allow for the selective concentration of asbestos prior to analysis. To minimize contributions to background contamination from asbestos present in the plastic matrices (polycarbonate or mixed cellulose ester) of membrane filters while allowing for sufficient quantities of asbestos to be collected, this method also requires the collection of a larger volume of air per unit area of filter than has traditionally been collected for asbestos analysis.

Due to the need to collect large volumes of air, higher sampling flow rates are recommended in this method than have generally been employed for asbestos sampling in the past. As an alternative, samples may be collected over longer time intervals but this restricts the flexibility required to allow samples to be collected while uniform meteorological conditions prevail. Higher flow rates through a 25 mm filter result in increased face velocities at the filter surface. Potential problems associated with the higher flow rates and increased face velocities include:

(a) destruction of the filter due to failure of its physical support under force from the increased pressure drop;

(b) leakage of air around the filter mount so that the filter is bypassed;

(c) damage to the asbestos structures due to increased impact velocities.

The recommended flow rates and face velocities have been employed in air sampling using membrane filters in the past. Based on such studies, (c) is not likely to be a problem. Using the filters and flow-rates specified, the first two concerns (a and b) are also unlikely to impose limitations. However, documented experience at the increased flow rates with the types of filter cassettes to be used in this method is very limited. Consequently, a pilot study is recommended to confirm that the structural integrity of a 25 mm filter cassette is not altered at the increased flow rates recommended. There is also some question

whether available pumps can maintain such flow rates throughout the collection of large volumes of air while the filter loading and, correspondingly, the resistance to flow steadily increase.

2.3. FILTER SELECTION

Selection of the type of filter to be used in this method was determined by considering the performance of the filter during sample collection, sample handling, and TEM specimen preparation (by an indirect technique). Concerns include the degree of asbestos loss that may occur during sample transport or handling, the efficiency with which asbestos is transferred from the filter to the TEM specimen, and the level of asbestos contamination potentially introduced to the TEM specimen from the filter material itself. Both mixed cellulose ester (MCE) and polycarbonate filters were evaluated.

It is generally accepted that deposited particles may move on the surface of polycarbonate filters when these filters are heavily loaded. Such movement may occur either during sample transport or handling in the laboratory. Such movement may potentially lead to asbestos losses between the time samples are collected and the time they are prepared for analysis.

Unlike the smooth surface of a polycarbonate filter, MCE filters exhibit sponge-like surfaces that trap filters within layers of porous material. The potential for movement of deposits on the surface of this type of filter is generally considered minimal. Correspondingly, the potential for loss of asbestos during transport or handling is also reduced. Thus, MCE filters are preferred over polycarbonate filters in terms of ease of handling.

Two methods are available for removal of sampled particulate from the sample collection filter in an indirect preparation: complete ashing of the filter and the particulate deposit, or washing of the particulate deposit from the filter surface followed by ashing of the wash-suspended deposit only. A number of problems have been experienced with the ashing of filters during indirect-transfer procedures. The efficiency with which deposited asbestos is transferred by washing has not been thoroughly investigated.

Complete ashing of a filter causes any asbestos contamination that may be present within the polymer matrix of the filter to be transferred to the final TEM preparation. Direct-transfer preparations from polycarbonate filters are known to yield a measurable background of asbestos contamination and current knowledge indicates that ashing of polycarbonate filters will yield an even higher value. Direct-transfer preparations of MCE filters generally yield low background asbestos contamination. However, ashing of either filter may yield higher levels of background asbestos contamination due to sources of contamination associated with the preparation itself.

Problems that have been experienced in association with low temperature ashing include both potential sources of additional contamination and filter behavior potentially leading to asbestos losses. During filter ashing, background contamination can arise from within the asher chamber, the containers in which the filters are ashed, the distilled water supply used for re-dispersing the ash, the pipettes used for transfer of dispersion for filtration, and the filtration funnel.

During ashing, both MCE and polycarbonate filters have occasionally been observed to curl up and give the appearance of being completely ashed, yet asbestos is trapped in a few very large, incompletely ashed particles that do not disperse. Consequently, such asbestos is lost and does not appear on the final TEM preparation. Procedures for avoiding this problem need to be refined.

Due to the problems associated with ashing the filters, washing of the particulate from the filter surface into ethanol is considered a superior approach. It is considered that washing of particulate from the surface of a polycarbonate filter can be accomplished with high removal efficiency. However, contamination of polycarbonate filters by asbestos has been measured by various laboratories, and currently appears to be within the range of 50-200 asbestos structures/sq.mm. <u>on the active filter surface</u>.[1]

During the washing of the filter surface, asbestos contamination on the surface of a polycarbonate filter (observed on preparations from direct-transfer procedures) may be detached along with the collected particulate and contribute to the background against which the concentrations of any collected asbestos must be measured. If the filter is removed from the cassette and the washing of the filter is performed ultrasonically in a beaker, contamination from <u>both sides</u> of the filter may be detached, leading to higher background counts. Therefore, potential but unquantified problems may be associated with the washing of polycarbonate filters.

Currently, it is not known whether collected particulate can be efficiently removed by washing the surfaces of 0.45 μm or 0.8 μm pore size MCE filters. The MCE filter has a sponge-like texture, and many of the fibers collected are embedded or entrapped within this structure. It has been shown that any attempt to use ultrasonic techniques results in disintegration of the filter surface and release of fragments of

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1 Certain laboratories have consistently reported lower values for polycarbonate filter contamination than other laboratories. Although this is well known and the matter has been addressed by numerous investigators, the source of the apparent discrepancy has not been elucidated.

filter material. It is possible that this disintegration of the filter surface may also release most of the collected particulate, but this has not been demonstrated. On the basis of current knowledge, it is unlikely that the small amount of polymer removed from the surface of the filter would contribute a significant level of contamination to the analysis, because contamination levels on the surface of MCE filters have been demonstrated to be very low.

Based on the above considerations, MCE filters were selected for use in this method. Although available data suggest that the optimum combination of filter and preparation technique would be for samples to be collected on MCE filters and to re-disperse the particulate in water by washing of the filters, little is known concerning the efficiency with which asbestos can be removed from the surface of an MCE filter by washing. It is known that some of the filter polymer is detached from the filter surface during washing and particles of this polymer can interfere with the analysis. Thus, ashing of the wash suspension would be necessary in order to remove the filter polymer fragments. Because the efficacy of the filter washing procedure has not been adequately substantiated, the preparation procedure incorporated into this method is ashing of the filter followed by re-dispersion of the ash in water.

2.4. PRECISION

To maximize precision, potential sources of variation within the method must be controlled. To minimize systematic variation during sampling and analysis, the method specifies detailed procedures. Random variation is minimized by maximizing the number of structures to be identified and counted, which includes increasing the probability of encountering asbestos structures during analysis, as discussed in Section 2.1. In addition, detailed and unambiguous counting rules and rules for identification are specified in the method to minimize variation due to subjective interpretation.

Airborne asbestos is often found, not as single fibers, but as very complex structures that may or may not be aggregated with equant (non-asbestos) particles. The mineralogy of structures found suspended in an ambient atmosphere must be identified to confirm a structure as asbestos. Such structures can often be identified unequivocally, if sufficient measurement effort is expended. However, if each structure were to be identified in this way, the analysis becomes prohibitively expensive. Because of instrumental deficiencies or because of the nature of the particulate, some structures cannot be positively identified as asbestos, even though the measurements all indicate that they could be asbestos. Subjective factors therefore contribute to this measurement, and consequently a very precise procedure for identification and enumeration of asbestos is incorporated in this method.

Despite the imposition of systematic counting and identification criteria, there are large potential errors in characterizing asbestos in ambient atmospheres, associated with the variability between filter samples and the performance of individual microscopists. For this reason, a replicate sampling and analysis scheme is also incorporated in order to determine the accuracy and precision of the method.

2.5. ASBESTOS CHARACTERISTICS

To analyze asbestos samples so that results can be related to potential risks, it is necessary to quantify the characteristics of asbestos that relate to biological activity. This involves enumeration of individual structures within certain size categories with particular emphasis on the longest and thinnest structures. Although the range of dimensions over which asbestos structures contribute to biological activity has yet to be precisely defined, this method is designed to provide a detailed characterization of structures encompassing the entire range of potential importance.

When evaluating detailed asbestos size characterizations, it is important to consider the effects of sample preparation. The appearance of the size distribution representing a sample of asbestos may vary depending on whether the sample was prepared by a direct or an indirect technique. Existing risk factors are based largely on studies incorporating direct transfer techniques.[2] Because indirect preparation is preferred to achieve the desired analytical sensitivity for environmental analysis (see Section 2.1), a procedure for evaluating the relationship between structure counts on samples prepared by an indirect technique and by a direct transfer technique is included in the method.

2.6. COST CONSIDERATIONS

In environmental samples, the amount of airborne asbestos is usually low relative to the total suspended particulate concentration. Therefore, when using a direct-transfer preparation, large areas of the TEM specimen grids must be examined to obtain a statistically valid measurement for asbestos. Consequently, the analysis is expensive. It is therefore not feasible to perform this type of analysis on all of the filters collected as part of a site investigation.

A cost-effective compromise has been incorporated into this method. A two phase sampling and analysis scheme has been adopted. In Phase 1, the majority of samples collected at a site will be prepared by

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[2] PCM analyses are performed directly on sample filters where the filter material has been rendered transparent by a suitable solvent. Since the fibers are observed as originally deposited, this corresponds closely to a direct transfer technique for TEM analysis.

the indirect-transfer technique (to maximize precision) and analysis will be performed using an efficient evaluation procedure. Air volumes collected on Phase 1 samples may be maximized subject to the limitations of sampling equipment.

In Phase 2 a small subset of samples (5 to 10%) will be collected and analyzed using an extended TEM examination to derive a relationship between counts from samples prepared by a direct technique and counts from samples prepared by an indirect technique for each of the various structure size fractions of interest that are observed in Phase 1 and Phase 2 samples. Filters from Phase 2 samples will be split so that half may be prepared by the direct-transfer procedure and half by the indirect-transfer procedure. Because half of each filter to be analyzed under Phase 2 will be prepared by the direct-transfer technique, the volume of air collected for each sample is limited by the loading that can be tolerated during analysis of a specimen prepared using a direct-transfer technique. Phase 1 and 2 samples shall be collected simultaneously.

3. OVERVIEW OF METHOD

Samples are collected and prepared for TEM examination by one of two techniques depending on the purpose for the sample. The majority of air samples (denoted Phase 1 samples) will be analyzed using an indirect procedure for preparation of TEM specimens, optimized to provide low detection limits and high precision. A small number of samples (denoted Phase 2 samples) will be collected in such a way that they can be analyzed using both indirect and direct procedures for preparation of TEM specimens, to allow comparisons to be made between the results from the two specimen preparation procedures. TEM examination procedures used for the two sets of samples also differ.

3.1. SAMPLE COLLECTION

A sample of airborne particulate is collected by drawing a measured volume of air through a 25 mm diameter, 0.45 μm pore size MCE membrane filter by means of a pump. Air volumes collected on Phase 1 samples will be maximized. Air volumes collected on Phase 2 samples will be limited to provide optimum loadings for filters to be prepared by a direct-transfer procedure.

3.2. SAMPLE PREPARATION

TEM grids will be prepared according to either 3.2.1 or 3.2.2 below.

3.2.1. Indirect TEM Specimen Preparation

This preparation will be applied to all Phase 1 and Phase 2 samples. The filter is split and half of the filter is stored for future use. The remaining half of the filter is ashed in a low-temperature plasma asher. The residual ash is ultrasonically dispersed in freshly-distilled water. The suspension is acidified using hydrochloric acid, and immediately filtered through a 25 mm diameter, 0.1 μm pore size MCE filter. The filter is dried and the filter structure is collapsed using a mixture of dimethyl formamide, acetic acid and water. A thin film of carbon is evaporated onto the collapsed filter surface and small areas are cut from the filter. These areas of filter are supported on TEM specimen grids and the filter medium is dissolved away by a solvent extraction procedure.

NOTE

An alternate procedure for indirect preparation in which filter deposits are washed off of MCE filters followed by the ashing of the wash-suspended deposit may be substituted into this method upon completion of validation in a pilot study.

3.2.2. Direct TEM Specimen Preparation

The remaining one-half of the filters from all Phase 2 samples will be prepared by this procedure. One quarter of the filter is collapsed using a mixture of dimethyl formamide, acetic acid and water. The collapsed filter is etched for a short time in a low temperature plasma asher to remove the surface layer of filter polymer which may have encapsulated asbestos structures during the collapsing procedure. A thin film of carbon is evaporated onto the collapsed filter surface and small areas are cut from the filter. These areas of filter are supported on TEM specimen grids and the filter medium is dissolved away by a solvent extraction procedure.

3.3. ANALYSIS

The TEM specimen grids are examined at both low and high magnifications to check that they are suitable for analysis before carrying out a quantitative examination on randomly-selected grid openings. In addition to isolated fibers, ambient air samples often contain more complex aggregates of fibers, with or without equant particles. Some particles are composites of asbestos fibers with other materials. Individual fibers and these more complex structures are referred to as "asbestos structures". A coding system is used to record the type of fibrous structure, and to provide the optimum description of each of these complex structures.

The method requires that separate examinations be made for asbestos structures of all sizes (incorporating asbestos fibers with lengths greater than 0.5 μm) and for long asbestos structures (incorporating structures longer than 5 μm). In both cases, asbestos structures are defined as those exhibiting mean aspect ratios equal to or greater than 5:1. This TEM examination procedure allows for specification of a lower analytical sensitivity for the measurement of the concentration of asbestos structures longer than 5 μm.

In the TEM analysis, electron diffraction (ED) is used to examine the crystal structure of a fiber, or fibrous components of complex structures, and the elemental composition is determined by energy dispersive X-ray analysis (EDXA). For a number of reasons, it is not possible to identify (determine the mineralogy of) each structure unequivocally, and structures are classified according to the techniques that have been used to identify them. A simple code is used to record the manner in which each structure is classified.

The classification procedure is based on successive inspection of the morphology, the electron diffraction pattern, and the energy dispersive X-ray spectrum. Confirmation of the identification of chrysotile is only by quantitative ED, and confirmation of amphibole is only by quantitative EDXA and quantitative zone-axis ED.

Several levels of analysis to confirm mineralogy are specified, the higher levels providing a more rigorous approach to the identification of structures. The procedure permits a minimum required asbestos identification procedure to be defined on the basis of previous knowledge, or lack of it, about the particular sample. Attempts are then made to achieve this defined minimum procedure for each asbestos structure, and the degree of success is recorded for each. The two codes remove from the microscopist the requirement to interpret observations made during the TEM examination, and allow this evaluation to be made later without the requirement for re-examination of the TEM specimens.

The lengths and widths of all classified asbestos structures are recorded. The number of asbestos structures found on a known area of the TEM specimen grids, together with the equivalent volume of air filtered through this area, are used to calculate the airborne concentration in asbestos structures/liter of air.

This method requires that the desired analytical sensitivities for the measurements of:

(a) asbestos structures of all sizes (incorporating structures longer than 0.5 μm); and,

(b) asbestos structures longer than 5 μm,

shall be specified before air samples are collected. In both cases, structures to be counted are defined as those exhibiting aspect ratios equal to or greater than 5:1. It will not always be possible to achieve these analytical sensitivities, because the volume of air that can be sampled is dictated by the nature and concentration of the suspended particulate in the atmosphere being sampled. To some degree, this limitation can be overcome by selective concentration of asbestos structures during the specimen preparation procedures and by examination of a larger area of the TEM specimens. However, the ease and cost of achieving a specific value for the analytical sensitivity will vary from sample to sample.

4. SCOPE AND FIELD OF APPLICATION

This method defines procedures for analyzing of airborne asbestos collected by drawing a known volume of air through mixed cellulose ester (MCE) filters. The method is suitable for the determination of asbestos in ambient air.

4.1. SUBSTANCE DETERMINED

The method defined uses transmission electron microscopy for determination of the concentration of asbestos in ambient atmospheres. This method includes measurement of the lengths and widths of asbestos structures and a calculation of their aspect ratios. In the method, asbestos structures are characterized as fibers, bundles, clusters, or matrices. The method allows for the determination of the mineralogical type(s) of asbestos present. The method, however, cannot discriminate between individual fibers of the asbestos and non-asbestos analogues of the same amphibole mineral.

4.2. RANGE

The range of concentrations that can be determined on a TEM specimen grid is approximately 50 to 7000 asbestos structures/mm^2. The lower limit is generally defined by the requirement that the concentration of asbestos collected from the air be distinguishable from background contamination contributed by the method and the filter. The upper limit is defined by the concentration at which asbestos structures will significantly overlap and interfere with proper detection.

4.2.1. Upper Limit of Range

The upper limit of the range for detecting asbestos on a filter may be limited both by excessive overlap of asbestos structures and by the presence of other interfering particles. In practice, if obscuration of asbestos structures is not to be a significant factor in a method's precision, the final TEM specimen grid should not exhibit more than approximately a 10% coverage by particulate. For the indirect TEM specimen preparation method, this maximum permissible particulate loading is determined by the concentration of that portion of the total suspended particulate concentration that cannot be removed by procedures such as oxidation or chemical dissolution.

Using the direct-transfer preparation method, it has been found that if the total particulate loading of the sample collection filter exceeds approximately 10 $\mu g/cm^2$ of filter surface, corresponding to approximately 10% coverage of the collection filter by particulate, the TEM specimens are loaded to a point that obscuration of asbestos structures by particulate becomes a significant source of error. If the particulate loading

exceeds approximately 25 $\mu g/cm^2$ of filter surface, the specimens are seriously overloaded and it is often not possible to prepare TEM specimens that have grid openings with intact carbon film.

The degree that non-asbestos particulates may interfere with asbestos analysis is a function of the concentration of total suspended particulate (TSP) present in the atmosphere in addition to the asbestos being sampled. Concentrations of the TSP vary over several orders of magnitude depending on location, time of day, and weather. Urban and agricultural sites tend to have significantly higher concentrations of the TSP than rural locations. Consequently, higher volumes of air may be collected at rural locations before interference by the TSP becomes a limiting factor.

In addition to variation in overall concentration, the composition of the TSP varies significantly as a function of location. At urban sites and specific rural locations, the TSP tends to be composed principally of organic matter that can be ashed or inorganic substances that are soluble in acidified media. Agricultural locations and other rural locations frequently exhibit higher concentrations of refractory silicate particles.

Due to the wide spatial and temporal variation in the TSP concentrations, a general rule for estimating levels can not be provided. However, a review of available environmental studies indicates that air volumes of 1 m^3 per cm^2 of filter area may reasonably be collected for direct preparation samples in most urban and rural environments. Subject to the types of the TSP present, samples collected for indirect preparation can be loaded to levels up to an order of magnitude higher. Data on the range of local levels of TSP should be obtained and evaluated prior to finalizing a sampling plan for a site.

4.2.2. Lower Limit of Detection

The detection limit theoretically can be lowered indefinitely by:

(a) filtration of progressively larger volumes of air;

(b) use of intermediate specimen preparation procedures to concentrate the collected particulate on to smaller areas of the TEM specimen grids; and,

(c) examination of greater areas of the TEM specimens in the electron microscope.

Although it might appear that the detection limit can be lowered indefinitely by examination of a greater area of the TEM

specimens, this is not actually the case if unused filters (blanks), or the analytical procedure itself, contribute background asbestos. Although it is considered that the background asbestos structure counts of TEM specimens prepared by the collapsed MCE filter technique (direct preparation) are very low, there are few data currently available concerning the asbestos content of the MCE filter polymers themselves, which might contribute to background during an indirect preparation.

In a technique that incorporates complete ashing of MCE filter membranes, any asbestos contained in the filter polymer will be concentrated on the final TEM specimens. Accordingly, this effect should be minimized by collection of the maximum volumes of air possible, and a continuous program of blank measurements must form part of the quality assurance procedures.

4.3. ANALYTICAL SENSITIVITIES

Based on a review of data from past environmental asbestos investigations and published studies of rural and urban background, the lower end of the ranges of concentrations typical of these types of studies center on 5 structures/liter (s/L) for asbestos structures of all sizes and 0.2 s/L for structures longer than 5 μm. The significance of the asbestos structures longer than 5 μm is discussed in Section 2.5. To distinguish among such concentrations at acceptable levels of statistical significance, it is assumed that a minimum of 10 asbestos structures should be identified and counted (see Section 4.2 of the Technical Background Document, Part 2 of this report). Thus, analytical sensitivities (defined as the concentration represented by the detection of 1 structure) required by this method are 0.5 s/L and 0.02 s/L for asbestos structures of all sizes and asbestos structures longer than 5μm, respectively.

4.4. DIMENSIONAL DETECTION LIMITS

Microscopists vary in their ability to detect very small asbestos structures. Therefore, a minimum length of 0.5 μm has been defined as the shortest fiber to be incorporated in the reported results.

The minimum detectable width for asbestos structures counted in this method is determined by the ability of an operator to detect them in a routine examination at the specified magnification of the method. The minimum magnification specified in this method (10,000) is more than sufficient to ensure visibility of the thinnest chrysotile asbestos fibrils.

5. DEFINITIONS

Acicular: The shape shown by an extremely slender crystal with cross-sectional dimensions which are small relative to its length.

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

$$A_{0-1}B_2C_5T_8O_{22}(OH,F,Cl)_2$$

where:

A = K, Na;

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

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

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

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

Amphibole asbestos: Amphibole in an asbestiform habit.

Analytical sensitivity: The calculated airborne concentration, in asbestos structures/liter, equivalent to counting of one asbestos structure in the analysis.

Asbestiform: A specific type of mineral fibrosity in which the fibers and fibrils possess high tensile strength and flexibility.

Asbestos: A term applied to a group of fibrous silicate minerals that readily separate into thin, strong fibers that are flexible, heat resistant and chemically inert.

Asbestos component: A term applied to any individually identifiable asbestos sub-structure that is part of a larger asbestos structure.

Asbestos structure: A term applied to any contiguous grouping of asbestos fibers, with or without equant particles.

Aspect ratio: The ratio of length to width of a particle.

Blank: A fiber count made on TEM specimens prepared from an unused filter, to determine the background measurement. Blanks consist of filter blanks, field blanks and laboratory blanks.

Bragg Angle: The angle between a crystal surface and an incident ray of incoming x-radiation.

Bundle: A fiber composed of parallel, smaller diameter fibers attached along their lengths.

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

Chrysotile: A mineral of the serpentine group which has the nominal composition:

$$Mg_3Si_2O_5(OH)_4$$

In some varieties of chrysotile, the silicon may be partially substituted by Al or less commonly by Fe. The magnesium may be partially substituted by Fe, Ni, Mn or Co. Some varieties contain Na, Cl or both. Chrysotile is a highly fibrous, silky variety, and constitutes the most prevalent type of asbestos.

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

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

Cluster: An assembly of randomly oriented fibers.

Component count: For any sample, a tally that includes the individually identified components of complex asbestos structures and each single asbestos structure with no identifiable components.

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

Detection limit: The calculated airborne fiber concentration in fibers/liter, equivalent to counting of 3.69 fibers in the analysis.

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

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

Energy dispersive X-ray analysis: Measurement of the energies and intensities of X-rays by use of a solid state detector and multi-channel analyzer system.

Eucentric: The condition when an object is placed with its center on a rotation or tilting axis.

Fiber: An elongated particle which has parallel or stepped sides. In this method, a fiber is defined to have an aspect ratio equal to or greater than 5:1.

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

Fibrous structure: A contiguous grouping of fibers, with or without equant particles.

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

Filter Blank: An unused filter which is analyzed to determine the background asbestos structure count.

Habit: The characteristic crystal form or combination of forms of a mineral, including characteristic irregularities.

Identify: During analysis, the use of a sequential set of procedures to determine and confirm the mineralogy of a structure.

Laboratory Blank: An unused filter which is analyzed along with sample filters to determine the background asbestos structure count.

Matrix: A connected assembly of asbestos fibers with particles of another species (non-asbestos).

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

PCM equivalent structure: A structure of aspect ratio greater than or equal to 3:1, longer than 5 μm, and which has a mean diameter between 0.2 μm and 3.0 μm for a part of its length greater than 5 μm. In this method, PCME structures also must contain at least one asbestos component.

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

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

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

$$Mg_3Si_2O_5(OH)_4$$

Structure Count: For any sample, a tally of each individually identified asbestos structure regardless of whether the structure contains identifiable components. This is equivalent to a count of the total number of separate asbestos entities encountered on the sample.

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

Unopened fiber: A large diameter asbestos fiber bundle which has not been separated into its constituent fibrils or fibers.

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

6. SYMBOLS AND ABBREVIATIONS

6.1. SYMBOLS

Å	-	Ångström unit (10^{-10} meter)
eV	-	electron volt
kV	-	kilovolt
L/min	-	liters per minute
μg	-	microgram (10^{-6} grams)
μm	-	micrometer (10^{-6} meter)
nm	-	nanometer (10^{-9} meter)
Pa	-	Pascal
s/L	-	structures per liter
W	-	watt

6.2. ABBREVIATIONS

DMF	-	Dimethyl formamide
ED	-	Electron diffraction
EDXA	-	Energy dispersive X-ray analysis
FWHM	-	Full width, half maximum
HEPA	-	High efficiency particle absolute
MCE	-	Mixed cellulose ester
PCM	-	Phase contrast optical microscopy
PCME	-	Phase contrast microscopy equivalent
SAED	-	Selected area electron diffraction
SEM	-	Scanning electron microscope

STEM	-	Scanning transmission electron microscope
TEM	-	Transmission electron microscope
TSP	-	Total suspended particulate
UICC	-	Union Internationale Contre le Cancer

7. EQUIPMENT AND APPARATUS

7.1. AIR SAMPLING - EQUIPMENT AND CONSUMABLE SUPPLIES

7.1.1. Filter Cassette

Commercially available field monitors, comprising 25 mm diameter three-piece cassettes, with conductive extension cowls shall be used for sample collection. The cassette must be new and not previously used. The cassette shall be loaded with an MCE filter of pore size 0.45 μm, and supplied from a lot number which has been qualified as low background for asbestos determination. The filter shall be backed by a 5 μm pore size MCE filter, and supported adequately so that distortion of the filter by the differential pressure across it does not occur during sampling. The cassettes shall be purchased with the required filters in position, or shall be assembled in a laminar flow hood or clean area such that the filter blank determinations meet the criteria of Section 10.6. When the filters are in position, a shrink cellulose band or adhesive tape should be applied to cassette joints to prevent air leakage. Suitable precautions shall be taken to ensure that the filters are tightly clamped in the assembly so that significant air leakage around the filter cannot occur.

7.1.2. Sampling Pump

The sampling pump shall be capable of a flow-rate and pumping time sufficient to achieve the desired volume of air sampled. The face velocity through the filter shall be between 4.0 cm/s and 65.0 cm/s. The sampling pump used shall provide a non-fluctuating air-flow through the filter, and shall maintain the initial volume flow-rate to within ±10% throughout the sampling period. A constant flow or critical orifice controlled pump meets these requirements. Flexible tubing shall be used to connect the filter cassette to the sampling pump. A means for calibration of the flow-rate of each pump is also required.

7.1.3. Stand

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

7.1.4. Rotameter

A rotameter or other flow measuring device calibrated such that the operator can measure flow rates to ± 5% accuracy at the expected sampling flow rate.

7.2. SPECIMEN PREPARATION LABORATORY

Asbestos, particularly chrysotile, is present in varying quantities in many laboratory reagents. Many building materials also contain significant amounts of asbestos or other mineral fibers that may interfere with the analysis if they are inadvertently introduced during preparation of specimens. It is most important to ensure that during preparation, contamination of TEM specimens by any extraneous asbestos fibers is minimized. All specimen preparation steps shall therefore be performed in an environment where contamination of the sample is minimized. The primary requirement of the sample preparation laboratory is that a blank determination shall yield a result which will meet the specifications in 10.6. A minimum facility considered suitable for preparation of TEM specimens is a laminar flow hood. However, it has been established that work practices in specimen preparation appear to be more important than the type of clean handling facilities in use. Preparation of samples shall be carried out only after acceptable blank values have been demonstrated.

NOTE

Activities involving manipulation of bulk asbestos shall not be performed in the same area as TEM specimen preparation, because of the possibilities of contaminating the TEM specimens.

7.3. LABORATORY EQUIPMENT

7.3.1. Transmission Electron Microscope

A TEM operating at an accelerating potential of 80-120 kV, with a resolution better than 1.0 nm, and a magnification range of approximately 300 to 100,000 shall be used. The ability to obtain a direct screen magnification of about 100,000 is necessary for inspection of fiber morphology; this magnification may be obtained by supplementary optical enlargement of the screen image by use of a binocular if it cannot be obtained directly. It is also required that the viewing screen be calibrated (as shown in Figure 7.1) with concentric circles and a millimeter scale such that the lengths and widths of fiber images down to 1 mm width can be measured in increments of 1 mm.

For Bragg angles less than 0.01 radians, the TEM shall be capable of performing ED from an area of 0.6 μm^2 or less, selected from an in-focus image at a screen magnification of 20,000. This performance requirement defines the minimum separation between particles at which independent ED patterns can be obtained from

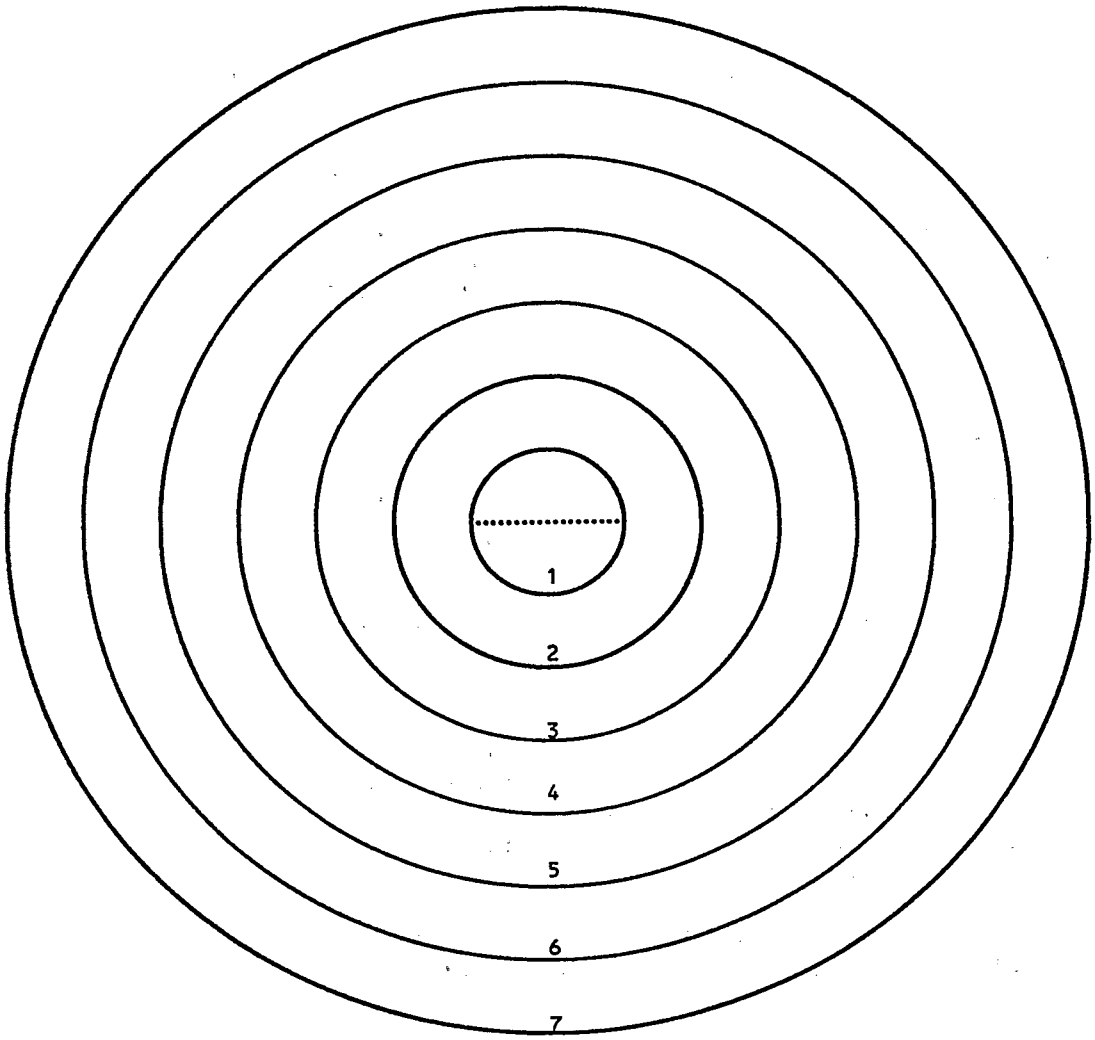

Figure 7.1: Calibration Markings on the TEM Viewing Screen

each particle. If SAED is used, the performance of a particular instrument may normally be calculated using the following relationship:

$$A = 0.7854 \times (D/M + 2000 \times C_s\theta^3)^2$$

Where:
- A = the effective SAED area in μm^2
- D = the diameter of the SAED aperture in μm
- M = the magnification of the objective lens
- C_s = the spherical aberration coefficient of the objective lens in mm
- θ = maximum required Bragg angle in radians

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

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

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

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

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

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

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

7.3.2. Energy Dispersive X-ray Analyzer

The TEM shall be equipped with an energy dispersive X-ray analyzer capable of achieving a resolution better than 175 eV (FWHM) on the MnK_α peak. Since the performance of individual combinations of TEM and EDXA equipment is dependent on a number of

geometrical factors, the required performance of the combination of the TEM and X-ray analyzer is specified in terms of the measured X-ray intensity obtained from a fibre of small diameter, using a known electron beam diameter. Solid state X-ray detectors are least sensitive in the low energy region, and so measurement of sodium in crocidolite shall be the performance criterion. The combination of electron microscope and X-ray analyzer shall yield, under routine analytical conditions, a background-subtracted NaK_{α} integrated peak count rate of more than 1 count per second (cps) from a 50 nm diameter fiber of UICC crocidolite irradiated by a 250 nm diameter electron probe at an accelerating potential of 80 kV. The peak/background ratio for this performance test shall exceed 1.0.

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

7.3.3. Computer

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

7.3.4. Plasma Asher

For ashing of filters or particulate deposits washed from filters, a plasma asher, with a radio frequency power rating of 100 W or higher, shall be used. The asher shall be supplied with a controlled oxygen flow, and shall be modified, if necessary, to provide a valve to control the speed of air admission so that rapid air admission does not disturb the ashed particulate. It is recommended that filters be fitted to the oxygen supply and the air admission line.

7.3.5. Filtration Apparatus

After re-dispersal of the ashed particulate in water, the particulate suspension is filtered through a membrane filter of 25 mm diameter. A glass frit support is required in order to obtain a uniform deposit on the filter. The reservoir must be easily cleaned in order to prevent sample cross-contamination. A 25 mm analytical filter holder (Millipore Corporation, Cat. No. XX10 025 00) or equivalent has been found to be suitable.

7.3.6. Filtration Manifold

When a number of samples are to be filtered, several

filtration units can be operated simultaneously from a single vacuum source by using a multiple port filtration manifold (Millipore Corporation, Cat. No. XX26 047 35 or equivalent has been found to be suitable). The manifold should include valves to permit each port to be opened or closed independently.

7.3.7. Vacuum Pump

A pump is required to provide a vacuum of approximately 20 kPa for the filtration of water samples. A water jet pump (Edwards High Vacuum Inc., Grand Island, NY 14072, Cat. No. 01-C046-01-000-female connection or 01-C039-01-000-male connection or equivalent) has been found to provide sufficient vacuum for a 3-port filtration mani-fold and also incorporates a non-return valve to prevent back-streaming.

7.3.8. Vacuum Coating Unit

A vacuum coating unit capable of producing a vacuum better than 0.013 Pa shall be used for vacuum deposition of carbon on the membrane filters. A sample holder is required which will allow a glass microscope slide to be continuously rotated during the coating procedure. A mechanism which allows the rotating slide also to be tilted through an angle of approximately 45° during the coating procedure is recommended. A liquid nitrogen cold trap above the diffusion pump may be used to minimize the possibility of contamination of the filter surfaces by oil from the pumping system. The vacuum coating unit may also be used for deposition of the thin film of gold, or other calibration material, when it is required on TEM specimens as an internal calibration of ED patterns.

7.3.9. Sputter Coater

A sputter coater with a gold target may be used for deposition of gold on to TEM specimens as an internal calibration of ED patterns. Experience has shown that a sputter coater allows better control of the thickness of the calibration material.

7.3.10. Solvent Washer (Jaffe Washer)

The purpose of the Jaffe washer is to allow dissolution of the filter polymer while leaving an intact evaporated carbon film supporting the fibers and other particles from the filter surface. One design of a washer that has been found satisfactory for various solvents and filter media is shown in Figure 7.2. Several acceptable variations have been described in the scientific literature. When specimens are not being inserted or removed, the petri dish lid should be in place. The washer should be cleaned before it is used for each batch of specimens.

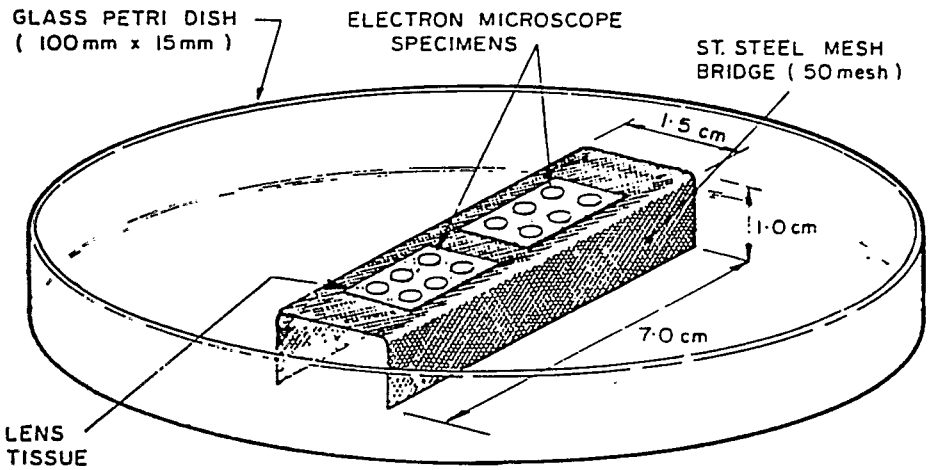

Figure 7.2: Design of a Solvent Washer (Jaffe Washer).
Solvent is added until the meniscus contacts the underside of the stainless steel mesh

7.3.11. Condensation Washer

For more rapid dissolution of the filter polymer, a condensation washer, consisting of a flask, condenser and cold finger assembly, with a heating mantle and means for controlling the temperature may be used. A suitable assembly is shown in Figure 7.3, and acetone should be used as the solvent for MCE filters.

7.3.12. Slide Warmer or Oven

Either a slide warmer or an oven shall be used for heating slides during the preparation of TEM specimens. It is required to maintain a temperature of 65-70°C.

7.3.13. Ultrasonic Bath

An ultrasonic bath is required for cleaning of apparatus used for TEM specimen preparation, and for re-dispersal of ashed residues from MCE filters.

7.3.14. Carbon Grating Replica

A carbon grating replica with about 2000 parallel lines per mm shall be used to calibrate the magnification of the TEM.

7.3.15. Calibration Grids for EDXA

For calibration of the efficiency of the EDXA system, reference silicate standard material must be used. The National Institute for Standards and Technology (NIST) Standard Reference Material (SRM) 2063 is a sputtered thin film containing known concentrations of magnesium, silicon, calcium and iron. Other suitable calibration materials include riebeckite, chrysotile, halloysite, phlogopite, wollastonite and bustamite. The mineral used for calibration of the EDXA system for sodium must be prepared using a gold TEM grid.

7.3.16. Carbon Rod Sharpener

The use of necked carbon rods, or equivalent, allows the carbon to be evaporated on to the filters without creating sufficient heat to damage the filters.

7.3.17. Disposable-Tip Micropipettes

A disposable tip micropipette, capable of transferring a volume of approximately 30 μL, is required for the preparation of TEM specimen grids.

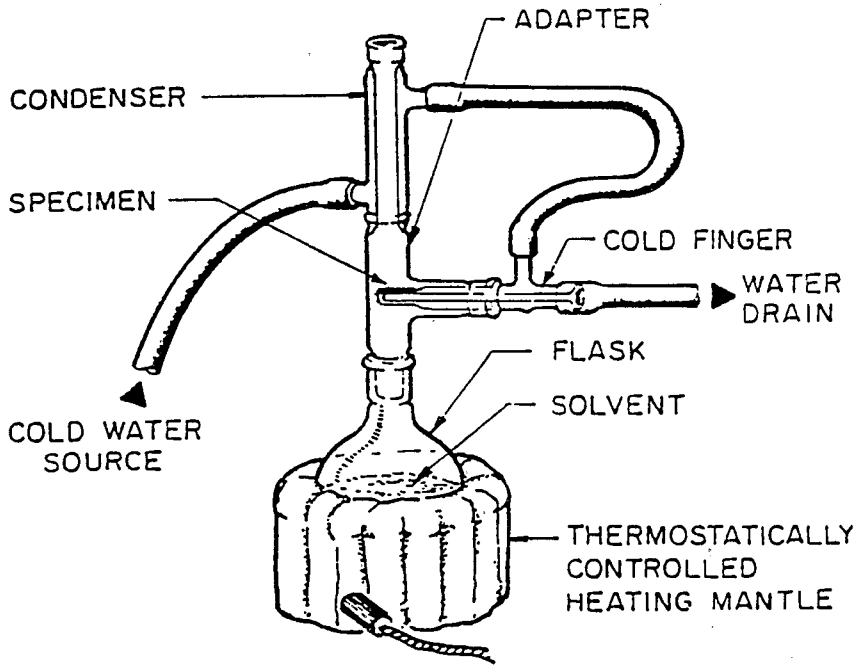

Figure 7.3: Design of a Condensation Washer

7.4. CONSUMABLE LABORATORY SUPPLIES

7.4.1. Glass Beakers

Borosilicate glass beakers of 100 mL capacity are required for ashing of filters and re-dispersal of ashed residues.

7.4.2. Membrane Filters

For filtration of the particulate suspensions, two types of filter are required:

(a) MCE filters of 0.1 μm pore size (Millipore Corporation VCWP 025 00 or equivalent) are used to collect the particles from the liquid suspension;

(b) MCE filters of 5 μm pore size (Millipore Corporation SMWP 025 00 or equivalent). One of these filters is placed between the glass frit of the filtration apparatus and the 0.1 μm pore size filter, in order to ensure a uniform particulate deposit.

7.4.3. Copper Electron Microscope Grids

Two-hundred mesh TEM grids are required. Grids which have grid openings of uniform size such that they meet the requirement of 10.4.2 should be chosen.

7.4.4. Gold Electron Microscope Grids

Two-hundred mesh gold TEM grids are required to mount TEM specimens when sodium measurements are required in the fiber identification procedure. Grids that have grid openings of uniform size such that they meet the requirement of 10.4.2 should be chosen.

7.4.5. Carbon Rod Electrodes

Spectrochemically pure carbon rods are required for use in the vacuum evaporator during carbon coating of filters.

7.4.6. Disposable Tips for Micropipette

Disposable tips for a micropipette are required for measurement of 30 μL volumes of the filter collapsing solution.

7.4.7. Routine Electron Microscopy Tools and Supplies

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

7.4.8. Reference Asbestos Samples

Asbestos samples are required for preparation of reference TEM specimens of the primary asbestos minerals. The NIST SRM 1866 contains well-characterized samples of chryostile, crocidolite, and amosite, although compositional data are not available for these standards. The UICC set of minerals may be used as additional reference materials.

8. REAGENTS

8.1. FRESHLY-DISTILLED WATER

A supply of freshly-distilled water must be available. The water must be produced using an all-glass still. Any water used for re-dispersal of ash, or for final rinsing of glassware must have been distilled no longer than 24 hours prior to use.

8.2. DIMETHYL FORMAMIDE, ANALYTICAL GRADE

8.3. GLACIAL ACETIC ACID, ANALYTICAL GRADE

8.4. ACETONE, ANALYTICAL GRADE

8.5. HYDROCHLORIC ACID, ANALYTICAL GRADE

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

9. AIR SAMPLE COLLECTION

Sampling procedures for this method were developed in consideration of and designed to be compatible with the analytical requirements of this method including, primarily, the need to achieve the desired analytical sensitivity.

Analytical sensitivity is a function of the volume of air sampled, the active area of the collection filter, the dilution or concentration factor introduced during specimen preparation, and the area of the TEM specimen over which asbestos structures are counted. If total airborne dust levels are high, it may be necessary to terminate sampling before the required volume has been sampled. If this happens, the analytical sensitivity required can be achieved only by counting structures over a greater area of the TEM specimen, or by use of a specimen preparation technique that incorporates some selective concentration of asbestos structures relative to other particulate species. For example, depending on the nature of the suspended particulate, a concentration factor up to about 2 may be possible for 25 mm filters, if the entire filter is used in the analysis. Larger concentration factors are possible for larger sample filters. In either case, the number of grid openings to be examined will be proportionately reduced.

The number of grid openings that must be examined in order to achieve a particular analytical sensitivity, without any selective concentration of the asbestos structures, is shown in Table 9.1. An average grid opening size of 0.0081 mm^2 is assumed in this table. However, grid opening sizes vary depending on the manufacturer of the grid. When using this table to derive the number of grid openings to be counted to achieve a defined analytical sensitivity, the number of grid openings to be counted must be adjusted to preserve the total areas represented. For example, if a volume of 5000 liters of air is collected, the table indicates that 96 grid openings must be counted to achieve an analytical sensitivity of 0.1 s/L. This corresponds to an area of 0.78 mm^2 (96 x 0.0081). If the average grid opening size on another grid is 80 μm square (resulting in an average grid opening area of 0.0064 mm^2), than a total of 122 grid openings will have to be counted. to cover the same area and achieve the same analytical sensitivity. Under no circumstances should fewer than 4 grid openings be counted.

The volume in liters required to achieve a specified analytical sensitivity is calculated from the formula:

$$V = A_f/(N \times A_g \times S \times F)$$

where:

A_f = Active area of sample collection filter in mm^2

N = Number of grid openings to be examined. N shall be rounded upwards to the next highest integer

A_g = Mean area of grid openings in mm^2

S = Analytical sensitivity in structures/liter

F = Concentration factor.

Table 9.1 - **Examples of the Minimum Number of Grid Openings Required to Achieve a Particular Analytical Sensitivity**

Analytical sensitivity	Volume of air sampled (liters)					
s/L	2000	5000	7000	10000	15000	20000
0.01	2377	951	680	476	317	238
0.02	1189	476	340	238	159	119
0.03	793	317	227	159	106	80
0.04	595	238	170	119	80	60
0.05	476	191	136	96	64	48
0.07	340	136	98	68	46	34
0.1	238	96	68	48	32	24
0.2	119	48	34	24	16	12
0.3	80	32	23	16	11	8
0.4	60	24	17	12	8	6
0.5	48	20	14	10	7	5
0.7	34	14	10	7	5	4
1.0	24	10	7	5	4	4
2.0	12	5	4	4	4	4
3.0	8	4	4	4	4	4
4.0	6	4	4	4	4	4
5.0	5	4	4	4	4	4
7.0	4	4	4	4	4	4
10.0	4	4	4	4	4	4

NOTES

In this table, it is assumed that the collection filter area is 385 mm^2, there is no concentration or dilution introduced during specimen preparation, and the TEM grid openings are 90 μm square.

For grid openings with dimensions other than 90μm square, the minimum number of openings to be counted should be adjusted by the ratios of the squares of the grid opening dimensions, such that the area of the TEM specimen examined remains the same.

The concentration factor F arises from the fact that, when using an indirect specimen preparation technique, the fibers on a particular area of the TEM specimen have usually been transferred from a different area of the original sample collection filter. The concentration factor may correspond to a concentration or a dilution of the original fiber density on the collection filter. It is calculated from the ratio between the areas of the original and analytical filters, the volume of water used to re-disperse the ashed particulate, and the volume of the final dispersion used to prepare the analytical filter. For the purposes of calculating the required volume of air to be sampled in order to achieve the specified analytical sensitivity when a direct transfer preparation is to be used, the concentration factor (F) is assumed to be 1.

9.1. REQUIRED SENSITIVITY

The required analytical sensitivities for characterizing environmental samples is established in section 4.3 as 0.5 s/L and 0.02 s/L for asbestos structures of all sizes and asbestos structures longer than 5 μm, respectively. For other applications of this method, different analytical sensitivities should be established prior to sample collection. Air volumes to be collected in this method are selected to provide the desired analytical sensitivity when coupled with the counting of a manageable number of grid openings.

9.2. AIR VOLUME

The sampling rate and the period of sampling shall be selected to yield as high a sampled volume as possible, which will minimize the influence of filter contamination. Wherever possible, a volume of 15 cubic meters shall be sampled for those samples intended for analysis only by the indirect TEM preparation method (Phase 1 samples). For those samples to be prepared by both the indirect and the direct specimen preparation methods (Phase 2 samples), the volumes must be adjusted so as to provide a suitably-loaded filter for the direct TEM preparation method (See Section 4.2.1). It has been found that the volume cannot normally exceed 5 cubic meters in an urban or agricultural area, and 10 cubic meters in a rural area for samples collected on a 25 mm filter and prepared by a direct transfer technique.

Prior to planning a sampling program, air volumes to be collected should be adjusted based on available information characterizing typical local TSP concentrations (see Section 4.2.1). It has not proven possible to judge the loading of filters in real time during sampling so that filters may easily become overloaded to the point were analysis by the direct method becomes impossible. To minimize the potential that filters will have to be rejected for analysis due to overloading, conservative estimates of the loading rate based on typical TSP concentrations have been developed so that an "optimum" volume of air per unit area of filter may be collected. However, because such estimates tend to be conservative to avoid overloading, a fair percentage of the filters analyzed from such

a sampling effort tend to be underloaded forcing a corresponding increase in analysis costs.

NOTE

One option is to collect filters at several loadings to bracket the estimated optimum loading for a particular site. Such filters can be screened in the laboratory so that only those filters closest to optimal loading are analyzed.

9.3. FLOW RATE

To remain within known constraints on filter face velocities as reported in Section 2.2, an upper limit to the range of acceptable flow rates for this method is 15 L/min. In practice, available pumps that can be used for environmental sampling at remote locations operate under load at a maximum of approximately 12 L/min. Consequently, the recommended 15 m^3 of air for Phase 1 samples requires approximately 20 hours to collect. Such pumps typically draw 6 amps at full power so that 2 lead/acid batteries should provide sufficient power to collect a full sample. The lower volumes required for Phase 2 samples can be collected in shorter periods with a corresponding reduction in stored energy requirements. The use of line voltage, where available, eliminates the difficulties associated with transporting stored electrical energy.

At many locations, wind patterns exhibit strong diurnal variations. Therefore, intermittent sampling (sampling over a fixed time interval repeated over several days) may be necessary to accumulate 20 hours of sampling time over constant wind conditions. Other sampling objectives also may necessitate intermittent sampling. The objective is to design a sampling schedule so that samples are collected under uniform conditions throughout the sampling interval. This method provides for such options.

9.4. SAMPLING PROCEDURES

Before air samples are collected, unused filters shall be analyzed as described in Section 10.6 to determine the mean background asbestos structure count for the analytical procedure.

Air samples shall be collected using cassettes as described in 7.1.1. The cassettes used for sampling shall be new and unused. Sampling shall be conducted with the cassette open-face. During sampling, the filter cassette shall be supported on a stand so that it is isolated from the vibrations of the pump. The cassette shall be held facing vertically downwards at a height of approximately 1.5-2.0 m above ground/floor level and connected to the pump with a flexible tube. In many cases it will likely be sufficient to collect samples with a short cassette. If conditions dictate the need for additional protection, however, extension cowls may be affixed to the front of the cassette. Such cowls must be

constructed of electrically conducting material to minimize electrostatic effects.

The sampling flow-rate should be measured at the cassette at the beginning and at the end of the sampling period. Sampling equipment shall be selected such that either the sampling flow rate can be maintained to within ± 10%, using a flow controller or critical orifice, or flow rates shall be measured at least every 4 hours. If at any time the measurement indicates that the flow-rate has decreased by more than 30%, the sampling shall be terminated. The mean value of these flow-rate measurements shall be used to calculate the total air volume sampled. After sampling, a cap shall be placed over the open end of the cassette, and the cassette packed in a clean plastic bag for return to the laboratory. Field blank filters shall also be included, as described in Section 10.6, and processed through the remaining analytical procedures along with the samples.

Should intermittent sampling be required, sampling cassettes must be covered between active periods of sampling. To cover the sampler: turn the cassette to face upward, attach a rotameter to measure the flow rate, turn off the sampling pump, and place the cap. To resume sampling: remove the cap, turn on the sampling pump, attach a rotameter to measure the flow rate, and invert the cassette to face downward.

10. PROCEDURE FOR ANALYSIS

10.1. INTRODUCTION

Filters representing Phase 1 samples will be prepared by the indirect method. Filters representing Phase 2 samples will be split. Half of the Phase 2 filters will be prepared by the indirect method and a quarter sector of the filter will be prepared by the direct method.

10.1.1. Indirect TEM Specimen Preparation Method

The indirect method requires collection of particulate from a large volume of air on a 0.45 μm pore size MCE filter. In this procedure, half of the filter is ashed by treatment in a low temperature plasma asher, and the ashed residue is re-dispersed in distilled water. Immediately before filtration, the suspension of particles is acidified using hydrochloric acid to remove any acid-soluble species. The suspension is filtered through a 0.1 μm pore size MCE filter. The filter is collapsed using a mixture of dimethyl formamide, acetic acid and water. A sector of the collapsed filter is carbon coated, and TEM specimen grids are prepared from the carbon coated filter by the Jaffe washer technique, using dimethyl formamide as the solvent.

Use of the 0.1 μm pore size membrane filter eliminates the requirement for the plasma etching step incorporated into both NIOSH Method 7402 and the method of Burdett and Rood. In previous work by Chatfield, Dillon and Stott, no statistically-significant fiber losses could be detected when TEM grids were prepared from 0.1 μm pore size MCE filters without including the etching step.

Indirect preparation of TEM specimens for determination of asbestos is particularly sensitive to problems of contamination by extraneous asbestos. This method requires that very low detection limits be achieved so that control of extraneous asbestos contamination becomes even more important. The ability to meet the blank sample criteria is critically dependent on the cleanliness of equipment and supplies. All supplies such as microscope slides and glassware should be considered as potential sources of asbestos contamination. All solvents used should be filtered through a 0.2 μm pore size filter or they should be distilled in a glass still. It is necessary to wash all glassware before it is used and the final washing of glassware should be performed using freshly-distilled water. Any tools or glassware that come into contact with the air sampling filters or TEM specimen preparations should be washed both before use and between handling of individual samples. Where possible, disposable supplies should be used.

10.1.2. Direct TEM Specimen Preparation Method

The direct TEM specimen preparation method requires that the air volume sampled must not result in a filter that is over-loaded by particulate. Particulate is collected on a 0.45 μm pore size MCE filter. A quarter sector of the MCE filter is collapsed using a mixture of dimethyl formamide, acetic acid and water. The collapsed filter is etched for a short time in a low temperature plasma asher. The collapsed and etched filter is carbon coated, and TEM specimen grids are prepared from the carbon coated filter by the Jaffe washer technique, using dimethyl formamide as the solvent.

10.2. PREPARATION OF TEM SPECIMEN GRIDS BY THE INDIRECT METHOD

10.2.1. Cleaning of Sample Cassettes

Asbestos can adhere to the exterior surfaces of air sampling cassettes and this asbestos can be inadvertently transferred to the sample during handling. To prevent this possibility of contamination, and after ensuring that the cassette is tightly sealed, wipe the exterior surfaces of each sampling cassette with a clean, wet paper towel before it is taken into the clean facility or laminar flow hood.

10.2.2. Ashing of MCE Filter

Open the filter cassette and remove the MCE filter. Place the filter on a clean microscope slide and cut it in half, using a curved scalpel blade. Return one half of the filter to the cassette. Place the other half filter in the bottom of a 100 mL glass beaker and cover the top of the beaker with a piece of aluminum foil. Make approximately 8 1-2 mm perforations in the aluminum foil. Completely ash the half filter in the low temperature plasma asher, using operating conditions as defined in Appendix A.

10.2.3. Re-dispersal of Ashed Residues

After the filter has been completely ashed, remove the beaker from the asher, add 40 mL of distilled water, and place the beaker in the ultrasonic bath for a period of 15 minutes. Using a disposable plastic pipette, add 0.2 mL of concentrated hydrochloric acid and stir the suspension. After addition of the acid, place the beaker in the ultrasonic bath for a period of 5 minutes. Filtration of the suspension must be performed immediately after the ultrasonic treatment. Power in the ultrasonic bath should be maintained below 0.1 watt/mL.

10.2.4. Filtration of the Aqueous Suspension

The separation of suspended particulate by filtration of the aqueous suspension through a membrane filter is a critical step in the analytical procedure. The objective is to produce an analytical filter on which the suspended solids from the sample are distributed uniformly, and at an acceptable loading for the microscopical examination.

The volume to be filtered generally depends on either the total suspended solids content or the asbestos structure concentration of the sample, and the intention is to prepare a filter that has a loading not exceeding about 50 asbestos structures per grid opening and with a particulate coverage not exceeding 10% of the filter area. Some judgment is required to achieve the optimum loading and, if the asbestos concentration is very low, it will be found that the suspended solids concentration will limit the volume that can be filtered. The maximum particulate loading on the filter that can be tolerated is about 10 $\mu g/cm^2$.

In practice, the best procedure for optimizing the loading on the final filter is to prepare several filters using different volumes of the suspension. Any fraction of the 40 mL suspension (prepared as per Section 10.2.3) may be selected as a unit for filtration. For example, aliquots containing 1 mL, 4 mL, 8 mL, and 20 mL fractions from the original suspension may be used to prepare a series of increasingly loaded filters. However, if the concentration of suspended solids dictates that a very small volume of the suspension be filtered, small volume aliquots need to be further diluted prior to filtering. **Do not attempt to filter a volume of less than 10 mL.**

If aliquots smaller than 10 mL from the original suspension are to be filtered, it is difficult to ensure that a uniform deposit of particulate is obtained on the filter unless the aliquot is first diluted. Samples of high solids content, or of high asbestos concentration, may require filtration of volumes less than 10 mL. Such aliquots shall be diluted with freshly-distilled water so that the filtered volumes exceed the minimum of 10 mL. These dilutions shall be made by transferring a known volume of the sample (such as an aliquot from the original suspension) to a disposable plastic beaker and making the volume up to a known volume with freshly-distilled water. The mixture shall be stirred vigorously before sub-samples are taken for filtration.

The following instructions for filtration must be followed precisely:

(a) Assemble the filtration base and turn on the vacuum. The upper surface of the filtration base (both the glass

frit and the ground mating surface) must be dry before the membrane filters are installed. Place a 5 μm pore size MCE filter on the glass frit. If the filter appears to become wet by capillary action on residual water in the glass frit it must be discarded and replaced by another filter. Place a 0.1 μm pore size MCE filter, smooth side facing up, on top of the 5 μm filter. The mating surface of the reservoir component of the filtration apparatus should be dried by shaking off any surplus water and draining on paper towel or tissue. Position the reservoir on the filters and firmly clamp it, taking care not to disturb the filters. The vacuum should not be released until the filtration has been completed.

It is necessary to comment on the use of filtration equipment that is still wet after washing, since improper procedures at this point can seriously compromise the results. If the glass frit is wet when the first filter is applied to it, capillary action will result in some areas of the filter structure being filled by water. When the second, smaller pore size filter is added and the vacuum is applied, the differential pressure across the 5 μm pore size filter will be insufficient to overcome the surface tension of the water in the filled areas. Thus no filtration will take place through the corresponding areas of the 0.1 μm pore size filter, and a grossly non-uniform deposit of particulate will be obtained.

(b) Add the required volume of sample to the filtration funnel. Disposable plastic beakers and pipettes provide a means of measuring the sample volumes without introducing problems of sample cross-contamination. The reservoir may not be sufficiently large to accommodate the total volume of liquid to be filtered. In this case, more of the sample may be added during the filtration, but this should be done carefully and only when the reservoir is more than half full. In this way the addition of liquid will not disturb or affect the uniformity of particulate already deposited on the filter. Do not rinse the sides of the reservoir, and avoid other manipulations which may disturb the particulate deposit on the filter.

(c) Disassemble the filtration apparatus, and transfer the 0.1 μm pore size filter to a labelled, clean plastic petri dish. Place the cover loosely over the top of the dish to limit any deposition of material on the filter, and allow the filter to dry. Discard the 5 μm pore size filter.

10.2.5. Selection of Area of Filter for Preparation

Using clean tweezers, and a clean, curved scalpel blade, cut out a 90 degree sector of the filter while it is still in the plastic petri dish.

10.2.6. Preparation of Solution for Collapsing MCE Filters

Mix 35 mL of dimethyl formamide, 15 mL of glacial acetic acid with 50 mL of freshly-distilled water. Store this mixture in a clean bottle.

10.2.7. Filter Collapsing Procedure

Using a micropipette with a disposable tip, place approximately 30 μL of the collapsing solution on a cleaned microscope slide, and using the end of the pipette tip spread the liquid over the area to be occupied by the filter sector. Place the filter sector, active surface upwards, on top of the solution, lowering the edge of the filter at an angle of about 20 degrees so that air bubbles are not created. Remove any solution not absorbed by the filter by allowing a paper tissue to contact the liquid at the edge of the filter. More than one filter sector may be placed on one slide, and a laboratory blank filter sector shall also be prepared on each slide. Place the slide either on a thermostatically-controlled slide warmer at a temperature of approximately 65-70°C, or in an oven at the same temperature, for about 10 minutes. The filter collapses slowly to about 15% of its original thickness. The procedure leaves a thin, transparent plastic film adhering to the microscope slide, with particles and fibers embedded in the upper surface.

10.2.8. Carbon Coating of Filter Sectors

Place the glass slide holding the filter sectors on the rotation-tilting device, approximately 10-12 cm from the evaporation source, and evacuate the evaporator chamber to a vacuum better than 0.013 Pa. The evaporation of carbon shall be performed in very short bursts, separated by some seconds to allow the electrodes to cool. If evaporation of carbon is too rapid, the surface of the filter may be damaged. The thickness of carbon required is dependent on the size of particles on the filter, and approximately 30-50 nm has been found to be satisfactory. If the carbon film is too thin, large particles will break out of the film during the later stages of preparation, and there will be few complete and undamaged grid openings on the specimen. Too thick a carbon film will lead to a TEM image which is lacking in contrast, and the ability to obtain SAED patterns will be compromised. The carbon film thickness should be the minimum possible, while retaining most of the grid openings of the TEM specimen intact.

10.2.9. Preparation of the Jaffe Washer

Place several pieces of lens tissue on the stainless steel bridge, and fill the washer with dimethyl formamide to a level where the meniscus contacts the underside of the mesh, resulting in saturation of the lens tissue. If it is intended to complete the washing of the specimens in the condensation washer, the pieces of lens tissue should be of such a size that they will fit on to the stainless steel mesh of the condensation washer cold finger. Three TEM specimen grids shall be prepared from each of the coated analytical filters, and for each of the analytical filters, three new specimen grids should be placed on to one of the pieces of lens tissue.

10.2.10. Placing of Specimens Into the Jaffe Washer

Using a curved scalpel blade, cut along the radius of the coated filter sector, approximately 1 mm from the edge. Discard the thin strip of coated filter, which usually exhibits signs of edge effects introduced during the collapsing procedure. Starting from the freshly-cut edge, cut three approximately 3 mm square pieces of the coated filter. Select three squares to represent the center and the outer periphery of the active surface of the filter. Place each square of filter, carbon side up, on one of the TEM specimen grids already on the lens tissue in the Jaffe washer. Cover the Jaffe washer with the lid, and allow the washer to stand. Specimens are normally cleared after approximately 4 hours.

10.2.11. Rapid Preparation of TEM Specimens From MCE Filters

An alternative washing procedure may be used to prepare TEM specimens from MCE filters more rapidly than can be achieved by the Jaffe washing procedure. After the specimens have been washed in a Jaffe washer for approximately 1 hour, transfer the piece of lens tissue supporting the specimens to the cold finger of a condensation washer operating with acetone as the solvent. Operate the condensation washer for approximately 30 minutes. This treatment removes all remaining filter polymer.

10.3. PREPARATION OF TEM SPECIMEN GRIDS BY THE DIRECT METHOD

10.3.1. Cleaning of Sample Cassettes

After ensuring that the cassette is tightly sealed, wipe the exterior surfaces of each sampling cassette with a clean, wet paper towel as described in 10.2.1 before it is taken into the clean facility or laminar flow hood.

10.3.2. Selection of Area of Filter for Preparation

Using clean tweezers, remove the MCE filter from the filter cassette, and cut a sector as described in 10.2.5.

10.3.3. Filter Collapsing Procedure

Collapse the filter sector as described in 10.2.7.

10.3.4. Plasma Etching of the Filter Surface

The conditions required in a particular plasma asher shall first be established using the procedure defined in Appendix A. Place the microscope slide holding the collapsed filter sectors in the plasma asher, and etch for the time and under the conditions determined. Care should be taken to ensure that the correct conditions are used. After etching, admit air slowly to the chamber and remove the microscope slide.

10.3.5. Carbon Coating of Filters

Carbon coat the microscope slide holding the collapsed filter portions as described in 10.2.8.

10.3.6. Preparation of the Jaffe Washer

Prepare the Jaffe washer as described in 10.2.9.

10.3.7. Placing of Specimens into the Jaffe Washer

Place specimens in the Jaffe washer as described in 10.2.10. Specimens are normally cleared after approximately 4 hours.

10.3.8. Rapid Preparation of TEM Specimens from MCE Filters

The alternative rapid washing procedure, described in 10.2.11, may be used to prepare TEM specimens from MCE filters more rapidly than can be achieved by the Jaffe washing procedure.

10.4. CRITERIA FOR ACCEPTABLE TEM SPECIMEN GRIDS

Examine the TEM specimen grid in the TEM at a magnification sufficiently low (300-1000) so that complete grid openings can be inspected. Reject the grid if:

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

(b) the sample is over-loaded with particulate. If the specimen grid exhibits more than approximately 10% obscuration on the majority of the grid openings, the specimen will be designated as over-loaded. This filter cannot be analyzed satisfactorily because the grid is too heavily loaded with debris to allow separate examination of individual particles by ED and EDXA and obscuration of structures by other particulate would lead to under-estimation of the structure count;

(c) the particulate deposits on the specimen are not uniformly distributed from one grid opening to the next. If the particulate deposits on the specimen are obviously not uniform from one grid opening to the next, the specimen will be designated as non-uniform. This condition is caused either by use of an unsatisfactory procedure for the water filtration or it is a consequence of the fundamental nature of the airborne particulate. Such a filter cannot be analyzed satisfactorily;

(d) the TEM grid is too heavily loaded with asbestos structures to make an accurate count. Accurate counts cannot be made if the grid has more than approximately 7000 asbestos structures/mm^2; or,

(e) less than approximately 75% of the grid openings have unbroken carbon film over the whole grid opening. Since the breakage of carbon film is usually more frequent in areas of heavy deposit, counting of the intact openings can lead to an underestimate of the structure count. An additional carbon coating may be applied to the carbon coated filter and new specimen grids prepared. The larger particles can often be supported by using a thicker carbon film. If this action does not produce acceptable specimen grids, this filter cannot be analyzed and a filter prepared with a lighter loading of particulate should be selected.

If any one or more of the conditions described in (b), (c), (d) or (e) exists, the specimen grids cannot be analyzed.

10.5. PROCEDURE FOR STRUCTURE COUNTING BY TEM

10.5.1. Introduction

The TEM examination consists of a count of asbestos structures, which are present on a specified number of grid openings. Asbestos structures are classified into groups on the basis of morphological observations, ED patterns and EDXA spectra. The total number of asbestos structures to be counted depends on the statistical precision desired. In the absence of asbestos structures, the area of the TEM specimen grids which must be examined depends on the analytical sensitivity required. The

precision of the asbestos structure count depends not only on the total number of asbestos structures counted, but also on their uniformity from one grid opening to the next. Additional asbestos structure counting will be necessary if greater precision is required.

In order that the estimate of the asbestos structure density on the sampling filter shall not be based on the asbestos structure deposits found within the small area represented by one specimen grid, grid openings shall be examined on at least two of the three specimen grids prepared. The results shall then be combined in the calculation of the asbestos structure density. Structure counts shall be made at a magnification of approximately 20000, and will be terminated at the number of asbestos structures as defined below, except that the count shall be continued until a minimum of 4 grid openings have been examined. Otherwise, the asbestos structure count shall continue to that number of grid openings at which the specified analytical sensitivity has been achieved.

10.5.2. Measurement of Mean Grid Opening Area

The grid opening area shall be measured for the type of TEM specimen grids in use. This may be performed either on an optical microscope at a calibrated magnification of about 400, or at a calibrated magnification in the TEM. A mean value for the grid opening dimensions may be used if this value can be shown to be sufficiently precise. If a mean value is used, the standard deviation of the mean of 10 openings selected randomly from each of 10 grids shall be less than 5%. Alternately, the dimensions of one grid opening on each of the grids examined shall be measured and the mean of these used in the calculations for the particular analysis.

Values for the number of grid openings to be counted to achieve a desired level of sensitivity (Table 9.1) will be adjusted based on the average grid opening size derived in this section. The procedure for performing the conversion is presented in a note at the bottom of Table 9.1.

10.5.3. TEM Alignment and Calibration Procedures

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

10.5.4. Determination of Stopping Point

Before structure counting is commenced, the area of specimen to be examined in order to achieve the required analytical

sensitivity shall be calculated. The maximum number of grid openings to be examined shall be calculated from the formula:

$$N = A_f/(A_g \times V \times S \times F)$$

where:

N = Number of grid openings to be examined. N shall be rounded upwards to the next highest integer

A_f = Area of collection filter in mm^2
A_g = Area of TEM specimen grid opening in mm^2

V = Volume of air sampled in liters

S = Required analytical sensitivity in asbestos structures/liter

F = Concentration factor.

The concentration factor F arises from the fact that the asbestos structures on a particular area of the TEM specimen have usually been transferred from a different area of the original sample collection filter. The concentration factor may correspond to a concentration or a dilution of the original asbestos structure density on the collection filter. It is calculated from the ratio between the areas of the original and analytical filters, the volume of water used to re-disperse the ash, and the volume of the final dispersion used to prepare the analytical filter. F is calculated from the following formula:

$$F = A_a \times V_f/(A_f \times V_r)$$

where:

A_a = Area of filter ashed
A_f = Area of analytical filter
V_r = Volume of water used to re-disperse ashed particulate
V_f = Volume of dispersion filtered through analytical filter

10.5.5. General Procedure for Structure Counting and Size Analysis

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

Use a form similar to that shown in Figure 10.1 to record the structure counting data. Insert the first specimen grid into the TEM. Select a typical grid opening and set the screen magnification to the calibrated value (approximately 20,000). Adjust the sample

TEM ASBESTOS COUNT

Page of

Preparation Date: / / By:

Analysis Date: / / By:

y m d

Report Number:

Filename:

Sample Description:

Sample Number:

Sample Filter Area: sq.mm.

Area of Filter Taken: sq.mm.

Analytical Filter Area: sq.mm.

Redispersal Volume: mL

Liquid Volume Filtered: mL

Magnification:

Air Volume: Liters

Grid Opening Dimension: um

Grid Opening	Structure Number	Class	Structure Type	Length (mm)	Width (mm)	Comments

Figure 10.1: Counting Form

height until the features in the center of the TEM viewing screen are at the eucentric point. Set the goniometer tilt angle to zero.

In column 1 of the structure counting form, record the sequential number of the grid opening. Position the specimen so that the grid opening is positioned with one corner visible on the screen. Move the image by adjustment of only one translation control, carefully examining the sample for asbestos structures, until the opposite side of the grid opening is encountered. Move the image by a pre-determined distance less than one screen diameter, using the other translation control, and scan the image in the reverse direction. Continue the procedure in this manner until the entire grid opening has been inspected in a pattern similar to that shown in Figure 10.2.

When a fibrous structure is detected, assign a sequential number in column 2, perform the identification procedures required as detailed in Appendix D, and enter the appropriate compositional classification on the fiber counting form in column 3. Assign a morphological classification to the structure according to the procedures in Appendix C, and record this in column 4. Measure on the TEM viewing screen the length and width of the image of each of the components of the fibrous structure in mm and record these measurements in columns 5 and 6 of the structure counting form.

If fibrous structures are present that are of obvious biological origin or that are determined to be non-asbestos, record data for a minimum of the first 10 such structures; further recording of data from non-asbestos structures is optional. After a fibrous structure has been examined and measured, re-locate the original field of view accurately before continuing scanning of the specimen. Failure to do this may cause asbestos structures to be overlooked or counted twice. The point at which the examination is to be terminated depends on the specimen, and is defined by either (a), (b) or (c) below:

(a) for Phase 1 samples, which have been prepared by the indirect TEM specimen preparation method <u>only</u>, complete the examination at the end of the grid opening on which the 50th asbestos structure is counted;

(b) for Phase 2 samples, which have been prepared both by the direct and indirect TEM specimen preparation methods, complete the examination at the end of the grid opening on which the 100th asbestos structure is counted; or

(c) for either of the sample types, until the number of grid openings required to achieve the specified analytical sensitivity for asbestos structures of all lengths, calculated according to 10.5.4, have been inspected, whichever occurs

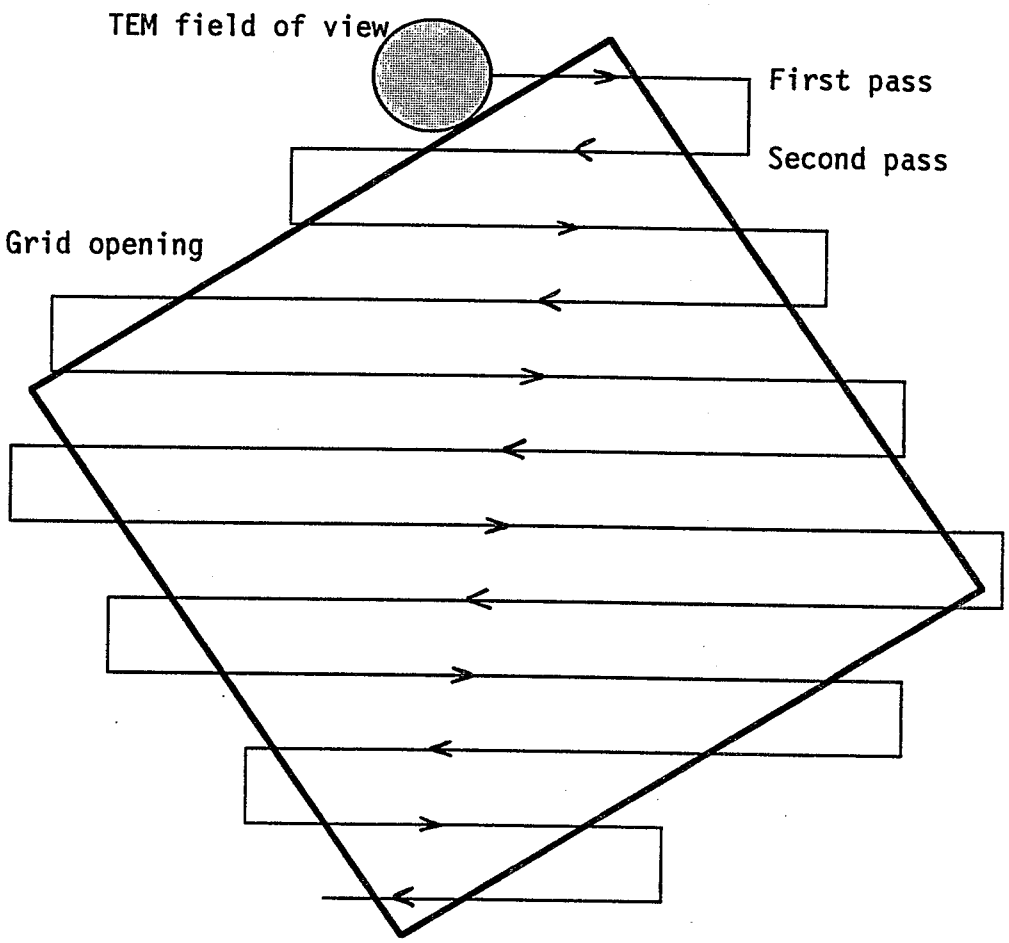

Figure 10.2: Scanning Procedure for TEM Specimen Examination

first. The data shall be drawn approximately equally from the two grids. Regardless of the value calculated according to 10.5.4, asbestos structures shall be counted on a minimum of 4 grid openings. Table 10.1 presents a summary of the counting requirements for Phase 1 and Phase 2 samples.

(d) Only those structures that are identified as, or are suspected to be, either chrysotile or one of the amphibole minerals, will be included in either the original or the extended structure count. Other materials, such as gypsum, cellulose fibers, and filter artifacts such as undissolved filter strands, will not be included in the structure count. This restriction is intended to ensure that the best statistical validity is obtained for the materials of interest.

10.5.6. Measurement of Concentration for Asbestos Structures Longer than 5 μm

To increase the statistical validity of the measurement, this method requires that an extended count be made of those asbestos structures with aspect ratios equal to or greater than 5:1 and longer than 5 μm. This separate structure count takes account only of the asbestos structures longer than 5 μm, and scanning of the TEM specimen shall be performed using a magnification of approximately 10,000.

Measurement of the asbestos structure lengths shall be performed at a magnification of 10,000, but asbestos structure diameters shall be measured at a magnification of a minimum of 20,000. For Phase 1 samples, which have been prepared by indirect transfer only, the count is continued until either 50 asbestos structures have been recorded or an area of specimen has been examined that achieves the specified analytical sensitivity for asbestos structures longer than 5 μm. For Phase 2 samples, which have been prepared both by indirect and direct transfer, the count is continued until either 100 asbestos structures have been counted or an area of specimen has been examined that achieves the specified analytical sensitivity for asbestos structures longer than 5 μm. Table 10.1 presents a summary of the counting requirements for Phase 1 and Phase 2 samples.

Only those structures that are identified as, or are suspected to be, either chrysotile or one of the amphibole minerals, will be reported in either the original or the extended structure count. Other materials, such as gypsum, cellulose fibers, and filter artifacts such as undissolved filter strands, will not be included in the structure count. This restriction is intended to ensure that the best statistical validity is obtained for the materials of interest.

TABLE 10.1: SPECIFICATIONS FOR PHASE 1 AND 2 SAMPLING AND ANALYSIS

Setting	Definitions	Phase 1 Samples: High Magnification Scan	Phase 1 Samples: Low Magnification Scan	Phase 2 Samples: High Magnification Scan	Phase 2 Samples: Low Magnification Scan
	Structure sizes to be counted	total	length > 5 μm	total	length > 5 μm
	Number of structures to be counted	50	50	100	100
Ambient:	Volume of air to be collected is 15 m^3				
	Maximum area to be scanned	0.05 mm^2	1.3 mm^2	0.11 mm^2	25 mm^2
	Maximum number of grid openings to be counted	7	160	13	307
	Minimum number of grid openings to be counted	4	4	4	4
Rural:	Volume of air to be collected is 10 m				
	Maximum area to be scanned	0.08 mm^2	2.0 mm^2	0.16 mm^2	3.7 mm^2
	Maximum number of grid openings to be counted	10	240	20	460
	Minimum number of grid openings to be counted	4	4	4	4
Urban and Agricultural:	Volume of air to be collected is 5 m^3				
	Maximum area to be scanned	0.15mm^2	3.9 mm^2	0.32 mm^2	7.5 mm^2
	Maximum number of grid openings to be counted	20	480	40	920
	Minimum number of grid openings to be counted	4	4	4	4
Activity Specific:	Volume of air to be determined in the field				
	Maximum area to be scanned	TBD	TBD	TBD	TBD
	Maximum number of grid openings to be counted	TBD	TBD	TBD	TBD
	Minimum number of grid openings to be counted	4	4	4	4

TABLE 10.1
(continued)

NOTES:

Phase 1 samples will be prepared by the indirect technique. Filters from Phase 2 samples will be split. Half will be prepared by the indirect technique and half will be prepared by the direct technique. Note that the required sensitivity for the Phase 2 samples is higher than that necessary for the Phase 1 samples. Consequently, it is assumed that 20 structures need to be counted for phase 2 samples at a minimum.

The information presented in this table is derived from the equation presented in the Section 10.5.4 assuming that $A_f = 385\ mm^2$, $A_g = 0.0081\ mm^2$, $F = 1$, and that 10 structures will have to be counted for statistical significance. Based on a review of reported background concentrations and typical concentrations encountered in previous studies, it is also assumed that the critical concentration for total structures is 5.0 structures/liter and the critical concentration for structures longer than 5 micrometers is 0.2 structures/liter.

During any site investigation, it will likely be necessary to characterize both ambient air concentrations and concentrations of asbestos associated with specific human activities. Although the method is designed for ambient conditions, it will be possible to adapt it to activity specific sampling by accounting with some adjustment for expected differences in conditions between the two types of samples. The principle difference likely is that a much higher concentration of non-asbestos particulates will likely be contained in air sampled in association with specific, dust-generating activities. As a consequence, the estimated values for maximum tolerable air volumes presented in section 4.2.1 of the text can not be applied to activity specific sampling and some measure of TSP will have to be provided to derive alternate sampling volumes. Thus, "TBD" means: to be determined.

It must be emphasized that the volume of air recommended for collection in each of the environmental settings listed represents a conservative estimate (based on published data) of the maximum that can be collected and still allow analysis without overloading the filter. If additional information regarding the local average concentration of ambient TSP, air volumes to be collected in any of these settings should be optimized accordingly.

To achieve the desired analytical sensitivity, the numbers of grid openings required for counting (as presented in this Table) may either be distributed over a set of specimen grids prepared from a single sample or a larger set of specimen grids prepared from several samples collected in a manner assuring that the samples are appropriately homogeneous (see Section 4.3 of the Technical Background Document, Part 2 of this report).

10.6. BLANK AND QUALITY CONTROL DETERMINATIONS

Before air samples are collected, a minimum of 2 unused 0.45 μm pore size MCE filters from each lot of 100 filters shall be sectioned and analyzed both by the indirect and the direct transfer procedure to determine the mean background asbestos structure concentration. If the mean concentration for all types of asbestos structures, expressed as the concentration per unit area of the sample collection filter, is found to be more than 10 structures/mm^2, the reasons for the high blank values shall be determined and the situation corrected before air samples are collected.

To ensure that contamination by extraneous asbestos structures during specimen preparation is insignificant compared with the results reported on samples, it is necessary to establish a continuous program of blank measurements. The number of field blanks incorporated into the program shall be at least 10% of the total number of samples, and all of these shall be analyzed. In addition, one unused 0.1 μm pore size filter shall be included with every group of samples prepared on one microscope slide.

It is further recommended that laboratory blanks be collected intermittently at all critical phases of the laboratory program. For example, in addition to the filter blanks and field blanks, suspension blanks should be prepared by subjecting a clean ashing tube to the asher, rinsing the tube and completing the standard indirect preparation on the "clean" rinse. Other blanks may be constructed to test for the potential introduction of contamination at other phases of the process. Such blanks need not be analyzed but may be stored to provide a chain for trouble-shooting should a problem with contamination occur.

Initially, and also included at random points in the sampling program, it is necessary to ensure that samples of known asbestos structure concentrations can be analyzed satisfactorily by this procedure. There are many opportunities in the procedure for a low recovery to be obtained, and the recovery must be measured frequently, particularly if more than one laboratory performs the analysis. Reference filters of known concentration will be incorporated, and shall constitute a minimum of 5% of the total analyses. These reference filters shall not be identified to the analytical laboratory prior to the analysis of any group of samples. The results of these reference analyses shall not differ at the 5% significance level from the mean value obtained by a selected group of experienced laboratories.

Since there is a subjective component in the structure counting procedure, it is necessary that re-counts of some specimens be made by different microscopists, in order to minimize the subjective effects. Such recounts provide a means of maintaining comparability between counts made by different microscopists. Variability between and within microscopists and between laboratories shall be characterized. These quality assurance

measurements shall constitute a minimum of approximately 10% of the analyses. Repeat results should not differ at the 5% significance level.

10.7. CALCULATION OF RESULTS

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

11. PERFORMANCE CHARACTERISTICS

11.1. INTERFERENCES AND LIMITATIONS OF STRUCTURE IDENTIFICATION

Unequivocal identification of every chrysotile structure is not possible, due to both instrumental limitations and the nature of some of the component fibers. The requirement for a calibrated ED pattern eliminates the possibility of an incorrect identification of the structure selected. However, there is a possibility of misidentification of other chrysotile structures for which both a morphology and ED pattern are reported on the basis of visual inspection only. The only significant possibilities of misidentification occur with halloysite, vermiculite scrolls or palygorskite, all of which can be discriminated from chrysotile by the use of EDXA and by observation of the 0.73 nm (002) reflection of chrysotile in the ED pattern.

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

11.2. PRECISION AND ACCURACY

11.2.1. Precision

The analytical precision that can be obtained is dependent upon the number of structures counted, and also on the uniformity of the particulate deposit on the original filter. Assuming that the structures are randomly deposited on the filter, if 100 structures are counted and the loading is at least 3.5 fibers/grid opening, computer modeling of the counting procedure shows that a coefficient of variation of about 10% can be expected. As the number of structures counted decreases, the precision will also decrease approximately as $\sqrt{N}$, where N is the number of structures counted. In practice, particulate deposits obtained by filtration are rarely ideally distributed, and it is found that the precision is correspondingly reduced from that predicted by the Poisson distribution. This degradation is a consequence of:

(a) non-uniformity of the filtered particulate deposit;

(b) distortion of the structure distribution by application of the structure counting criteria;

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

(d) variation between microscopists and instruments in their ability to detect and identify structures.

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

11.2.2. Accuracy

There is no independent method available to determine accuracy.

11.3. ANALYTICAL SENSITIVITY

Analytical sensitivity is the concentration corresponding to observation of one asbestos structure in an analysis. The analytical sensitivity of the method can be varied by choice of the area of the collection filter, the volume of air sampled, the dilution or concentration factor used in the specimen preparation and the area of the specimen examined in the TEM. The analytical sensitivity shall be quoted for each sample analysis.

NOTE

In practice, the lowest achievable value of analytical sensitivity is frequently determined by the total suspended particulate concentration in the air sample, since each particle on the TEM specimen must be separated from adjacent ones by a sufficient distance that the particle can be identified without interference. Particulate loadings on filters greater than about 25 $\mu g/cm^2$ usually preclude preparation of TEM specimens.

If the analysis is to be performed with an acceptable expenditure of time, the area of the specimen examined in the TEM must also be limited. The analytical sensitivity that can be achieved for any particular sample depends on the air volume, the area of TEM specimen examined, and the dilution or concentration factor used in the analysis, which in turn is controlled by the amount of particulate that is not removed by the selective concentration procedures.

11.4. LIMIT OF DETECTION

Should asbestos be observed during analysis, it is generally important to distinguish whether such asbestos originated in the sampled medium or if it was introduced as contamination during analysis. Asbestos that can be attributed to the sampled medium is generally considered to have been "detected". Thus, a detection limit is defined as the smallest measurement that is unlikely (probability less than a specified value) to be due entirely to contamination from sources other than the air being sampled. Detection limits are generally quantified by considering the magnitude and frequency of occurrence of the analytical background associated with a particular method. However, detection limits for asbestos methods are difficult to quantify because the distribution of analytical background associated with asbestos analysis tends to be poorly behaved.

To avoid the problems typically associated with defining a detection limit for asbestos analysis, an alternate procedure for distinguishing sampled asbestos from contamination due to analytical background has been incorporated into this method[3]. A statistical test is performed to compare structure concentrations observed on sample filters with structure concentrations observed on appropriate blanks to determine whether differences between the concentrations observed on the two sets of filters are statistically significant.

NOTE

Due to the difficulty in characterizing contributions to observed asbestos on individual samples from contamination introduced as analytical background, tests to distinguish "detected" asbestos from such contamination are better applied to groups of samples. Adjustments to account for analytical background are not recommended for individual samples.

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[3] A detection limit is more appropriate for analytical methods that employ wet-chemical or spectroscopic techniques rather than structure counts (as in the case of asbestos) because the former are invariably associated with a lower limit to the amount (number of molecules) that can be detected. For asbestos, however, analysis by TEM is sufficiently sensitive to detect the smallest, single asbestos structure. Thus the issues involved in setting detection limits for TEM analyses of asbestos structures are quite different than from those involved with detecting populations of molecules such as in gas chromatograph-mass spectrometry (GC-MS) analyses, for example.

12. REPORTING REQUIREMENTS

Reporting requirements for the laboratory include the individual documentation for each sample (sample analysis report) and the cumulative report for any batch of samples from a particular study. The structure counting form to be used by the analyst was presented in Figure 10.1. Required data reduction by the site data reviewer is presented at the end of this chapter, Section 12.3. It is critical that the following documentation requirements be followed exactly to preserve the ability to interpret results without the need to re-examine samples.

12.1. SAMPLE ANALYSIS REPORT

The sample analysis report for each sample shall include at least the following information:

(a) reference to this analytical method;

(b) identification of the air sample and the batch number of the collection filter used;

(c) the dates and times of the air sample collection period;

(d) the volume of air passed through the sample collection filter, the area of filter used for the sample preparation, the volume of water used for re-dispersal, the batch number of the filters used for filtration of the aqueous suspension, and, for the set of TEM specimen grids examined, the volume of the aliquot used in the aqueous suspension filtered;

(e) a complete listing of the structure counting data. The dimensions of the TEM grid openings shall be specified, and for each asbestos structure (or structure component) the following data shall be included: grid opening number, structure number, identification category (morphological classification), structure type (compositional classification), length and width of the structure in μm, and any comments concerning the structure;

(f) a statement of the minimum acceptable identification category and the maximum identification category attempted;

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

(h) separate concentration values for chrysotile and amphibole structures, expressed in asbestos structures/liter[4];

(i) separate concentration values for chrysotile and amphibole structure components, expressed in asbestos structures/liter[4];

(j) the 95% confidence limits for the concentration values, expressed in asbestos structures/liter;

(k) the analytical sensitivity, expressed in asbestos structures/liter;

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

(m) items d - l for the separate count of asbestos structures longer than 5 μm;

An example of a suitable format for the structure counting data is shown in figure 12.1.

NOTE

As improved definitions of the dimensions of asbestos structures critical to the determination of risk become available, the reporting requirements for this method may be modified accordingly. At the same time, sufficient information is preserved in these reporting requirements to allow later re-evaluation of existing analysis results without the need to re-examine the original sample.

12.2. SAMPLE BATCH REPORT

In addition to the sample analysis report for each sample, a summary page should be provided for each batch of samples representing an entire project. The summary sheet should include the following information:

(a) project title;

(b) date samples received and data results reported;

(c) a summary listing of sample results with chrysotile and amphibole reported separately including:

- the sample number;

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[4] If asbestos is not detected during a particular analysis, the resulting mean concentration for that sample should be reported as "NF" for "not found".

- the analytical sensitivity for each size range characterized (structures of all sizes and structures longer than 5 mm)

- the total number of structures counted;

- the concentration of asbestos structures of all sizes in structures per liter with appropriate 95% confidence limits;

- the concentration of asbestos structure components in structures per liter with appropriate 95% confidence limits;

- the concentration of asbestos structures longer than 5 μm in structures per liter with appropriate 95% confidence limits;

- the concentration of asbestos structure components longer than 5 μm in structures per liter with 95% confidence limits;

A sample summary sheet is presented in figure 12.2.

12.3. DATA REVIEW REPORT

Once the Sample Batch Report is received from the laboratory, the following reporting procedure will be performed by the project data reviewer. The data reviewer shall provide a summary sheet reflecting specific steps in data reduction. The summary data sheet shall include the following information:

(a) the sample number;

(b) the type of sample (lab blank, field blank, sampling station identifier);

(c) the analytical sensitivity for each size fraction reported (structures of all sizes and structures longer than 5 μm);

(d) the number of total structures counted;

(e) the concentration of asbestos structures of all sizes in structures per liter with 95% confidence limits;

(f) the concentration of asbestos structure components in structures per liter with 95% confidence limits;

(g) the concentration of asbestos structures longer than 5 μm in structures per liter with 95% confidence limits;

(h) the concentration of asbestos structure components longer than 5 μm in structures per liter with 95% confidence limits;

(i) the ratio of free asbestos fibers to total asbestos structures;

SAMPLE ANALYSIS INFORMATION (Page 1)

Laboratory name | Report number | Date

SAMPLE: 456 Queen Street
San Diego
Downwind sample 1988-09-25
Collected: 8 a.m. to 4 p.m.

ANALYSIS: Name of method
Reference to method

Air volume:	12500.0 liters
Area of collection filter:	385.0 mm^2
Area of filter ashed:	96.3 mm^2
Volume of water used for re-dispersal:	40.0 mL
Volume of suspension filtered:	35.0 mL
Area of analytical filter:	199.0 mm^2
Magnification used for fiber counting:	20500 (high magnification)
	10200 (low magnification)
Mean dimension of grid openings:	90.0 μm

High Magnification Scan

Number of grid openings examined:	38
Analytical Sensitivity:	0.5 s/L
Number of chrysotile structures counted:	63 (including F, M, C and B)
Number of chrysotile structure components counted:	91
Number of amphibole structures counted:	12 (F only)
Number of amphibole structure components counted:	12

Low Magnification Scan

Number of grid openings examined:	357
Analytical sensitivity:	0.02 s/L
Number of long chrysotile structures counted:	3 (B and C)
Number of long chrysotile structure components counted:	14
Number of long amphibole structures counted:	0
Number of long amphibole structure components counted:	0

NOTES: Only structures with components longer than 5μm counted during the low magnification scan.

Figure 12.1A: Format for Reporting of Counting Data, Page 1

SAMPLE ANALYSIS INFORMATION (Page 2)

Laboratory name | Report number | Date

SAMPLE: 456 Queen Street
San Diego
Downwind sample 1988-09-25
Collected: 8 a.m. to 4 p.m.

ANALYSIS: Name of method
Reference to method

	Mean (s/L)	95% Confidence Limits Range (s/L)
Concentration of chrysotile structures:	31.5	24 - 38
Concentration of chrysotile structure components:	45.5	37 - 54
Concentration of long chrysotile structures:	0.06	0 - 0.12
Concentration of long chrysotile structure components:	0.28	0.16 - 0.4
Concentration of amphibole structures:	6	3.1 - 8.9
Concentration of amphibole structure components:	6	3.1 - 8.9
Concentration of long amphibole structures:	NF	0 - 0.07
Concentration of long amphibole structure components:	NF	0 - 0.07

NOTES: NF means Not Found

Long structures and structure components are those greater than 5μm in length.

Figure 12.1B: Format for Reporting of Counting Data, Page 2

TEM ASBESTOS COUNT — RAW DATA (Page 3 and higher)

Grid Opening	Structure Number	Classif-ication*	Structure Type	Length µm	Width µm	Comments
1	1	CD	F	1.7	0.045	
1	2	CD	MS002	5.5	4.3	
1	3	CDQ	MF002	2.6	0.045	
1	4	AD	MM002	2.0	0.53	
2	5	CDQ	B	8.7	0.21	
3	6	CMQ	CS006	8.5	4.3	
3	7	CMQ	CF006	5.41	1.0	
3	8	CMQ	CB006	8.1	1.2	
3	9	CD	DF006	3.6	0.75	
3	10	ADQ	F	9.0	0.045	Crocidolite
3	11	ADQ	F	4.5	0.60	Amosite
4	12	CD	M	3.0	2.0	
5	13	CD	MS013	6.1	1.0	
5	14	CD	MB013	4.1	0.50	
6	15	AQZZ	F	3.2	0.10	Amosite
7			No Fibers			
8	16	CMQ	F	1.3	0.030	
8	17	CD	F	1.1	0.045	

* Classification codes listed in Tables D1 and D2

Figure 12.1C: Format for Reporting Counting Data, Page 3 and Subsequent Pages

Batch #: Sample Medium:

Project: Analysis Type:

Lab: Preparation Technique:

Date:

Sample I.D. Date Received:								
Structures counted: Structure concentration (s/L): Mean Range | Low / High Analytical sensitivity:								
Components counted: Component concentration (s/L): Mean Range | Low / High Analytical sensitivity:								
Long structures counted: Long structure concentration (s/L): Mean Range | Low / High Analytical sensitivity:								
Long components counted: Long component concentration (s/L): Mean Range | Low / High Analytical sensitivity:								

NOTES:

NF = not found

Long structures or components are those longer than 5 μm.

The range presented represents the 95% confidence limits constructed as discussed in Section E.4.

Provide separate sheets for chrysotile and amphiboles or total asbestos, as necessary.

Figure 12.2: Format for the Summary Batch Report

(j) the ratio of free asbestos fibers to total asbestos structures and components;

(k) the ratio of matrices to total asbestos structures;

(l) the ratio of bundles to total asbestos structures;

(m) the ratio of clusters to total asbestos structures;

(n) items i-m for asbestos structures longer than 5 μm;

(o) a complete listing of the structure counting data for each sample (item "e" of section 12.1).

An example of the summary form for data review is provided in Figure 12.3.

ANALYTICAL RESULTS

Case No.:
Site:
Lab:
Reviewer:
Date:

Analysis Type: Air for TEM asbestos
Indirect Preparation

Concentration of Asbestos (Chrysotile unless otherwise noted)

Sample I.D. (number and type)															
Analysis/Preparation															
Parameter	Mean	95% CL Low	95% CL High	Mean	95% CL Low	95% CL High	Mean	95% CL Low	95% CL High	Mean	95% CL Low	95% CL High	Mean	95% CL Low	95% CL High
Number of liters sampled															
HIGH MAGNIFICATION															
Number of grids examined															
Structures/area viewed															
Fibers/area viewed															
Bundles/area viewed															
Clusters/area viewed															
Matrices/area viewed															
Structures/L															
Components/L															
Long structures/L															
Long components/L															
Analytical sensitivity															
LOW MAGNIFICATION															
Number of grids examined															
Structures/area viewed															
Fibers/area viewed															
Bundles/area viewed															
Clusters/area viewed															
Matrices/area viewed															
Long structures/L															
Long components/L															
Analytical sensitivity															

NOTES:

NF = not found

95% CL = 95% confidence limit

The range presented represents the 95% upper or lower confidence limit constructed as discussed in Section E.4.

Figure 12.3: Format for Data Review Summary Report

APPENDIX A - DETERMINATION OF OPERATING CONDITIONS FOR PLASMA ASHER

A.1 INTRODUCTION

It is important to establish standard conditions for operation of the plasma asher, so that:

(a) ashing conditions for MCE filters and particulate are sufficient and remain constant; and,

(b) so that a known amount of etching of MCE filters can be achieved on the surface of filters being prepared by the direct-transfer method.

Therefore, the plasma asher shall be calibrated so that a known material is completely ashed in a constant time. This is carried out by adjusting the radio-frequency power output and the oxygen flow rate, and measuring the time taken to completely oxidize an unused 25 mm diameter 0.45 μm pore size MCE filter.

A.2 PROCEDURE

Place an unused, 25 mm diameter, 0.45 μm pore size MCE filter in the center of a glass microscope slide. Position the slide approximately in the center of the asher chamber. Close the chamber and evacuate to a pressure of approximately 40 Pa, while admitting oxygen to the chamber at a rate of 8-20 cc/min. Adjust the tuning of the system so that the intensity of the plasma is maximized. Measure the time required for complete oxidation of the filter. Determine the power setting that result in complete oxidation of the filter in a period of approximately 15 minutes.

NOTE

Plasma oxidation at high radio-frequency powers will cause the filter to shrink and curl, followed by sudden violent ignition. At lower powers the filter will remain in position, and will become slowly thinner until it becomes nearly transparent. It is recommended that the radio-frequency power be used is selected such that violent ignition does not occur. If powers are used such that the particulate ignites violently, fibers will possibly be disturbed and lost from the analysis.

APPENDIX B - CALIBRATION PROCEDURES

B.1 CALIBRATION OF THE TEM

B.1.1 Calibration of TEM Screen Magnification

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

B.1.2 Calibration of ED Camera Length

Calibrate the camera length of the TEM when used in ED mode. Use a specimen grid supporting a carbon film on which a thin film of gold has been evaporated or sputtered. Form an image of the gold film and select ED conditions. Adjust the objective lens current to optimize the pattern obtained, and measure on the fluorescent viewing screen the diameters of the innermost two rings. Calculate the camera constant, $\lambda \times L$, from the relationship:

$$\lambda \times L = \frac{a \times D}{\sqrt{h^2 + k^2 + l^2}}$$

where:

λ = the wavelength of the incident electrons
$\lambda \times L$ = the effective camera length in mm
a = the unit cell dimensions of gold in Ångström units
D = the diameter of the (hkl) diffraction ring in mm

Using gold as the calibration material, the camera constant is given by:

$\lambda \times L = 2.3548 \times D$ (smallest ring)
$\lambda \times L = 2.0393 \times D$ (second ring)

B.2 CALIBRATION OF THE EDXA SYSTEM

Energy calibration of the EDXA system for a low energy and high energy peak shall be performed regularly. Calibration of the intensity scale of the EDXA system permits quantitative composition data, at an accuracy of about 10% of the elemental concentration, to be obtained from EDXA spectra of silicate minerals involving the elements Na, Mg, Al, Si, K, Ca, Mn, and Fe. SRM 2063 should also be used to calibrate the EDXA system for Mg, Ca, and Fe relative to Si. If quantitative determinations are required for minerals containing other elements, reference standards other than those referred to below will be required. Well-characterized mineral standards permit calibration of any TEM-EDXA combination that meets the instrumental specifications of 6.3.1 and 6.3.2, so that EDXA data from different instruments can be compared. Reference minerals are required for the calibration; the criteria for selection being that they should be silicate minerals with matrices as close as possible to those of the amphiboles or serpentine, and that individual small fragments of the minerals are homogeneous in composition within a few percent.

Determine the compositions of these standards by electron microprobe analysis or chemical methods, and prepare TEM grids from aqueous dispersions of crushed fragments of the same selected mineral specimens. These TEM specimens can then be used to calibrate any TEM-EDXA system so that comparable compositional results can be obtained from different instruments.

NOTE

The microprobe analyses of the mineral standards are made by conventional techniques, which can be found in the bibliography. The mineral is first embedded in a mount of polymethyl methacrylate or epoxy resin. The mount is then ground and polished to achieve a flat, polished surface of the mineral fragment. This surface is then analyzed, using suitable reference standards, preferably oxide standards of the individual elements wherever these are available. It is necessary to take account of the water concentration in the minerals, which in the case of chrysotile amounts to 13% by weight.

Express the results of the electron microprobe analyses as atomic ratios relative to silicon. X-ray peak ratios of the same elements relative to silicon, obtained from the EDXA system, can then be used to calculate the relationship between peak area ratio and atomic ratio. The technique was described by Cliff and Lorimer (See Bibliography).

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

$$\frac{A_i}{A_j} = \frac{k\ C_i}{C_j}$$

where:

A_i = the elemental integrated peak areas for element i
A_j = the elemental integrated peak area for element j
C_i = the concentration or atomic proportion of element i
C_j = the concentration or atomic proportion of element j
k = a constant

To incorporate correction for the particle size effect on peak area ratios (See Small et al. in Bibliography), extend the Cliff and Lorimer technique by obtaining separate values of the constant k for different ranges of fiber diameter. It is recommended that about 20 EDXA measurements be made for each range of fiber diameter. Suitable ranges of fiber diameter are <0.25 μm, 0.25-0.5 μm, 0.5-1.0 μm and >1.0 μm.

Insert the TEM grid into the TEM, obtain an image at the calibrated higher magnification of about 20,000, and adjust the specimen height to the eucentric point. If the x-ray detector is a side-entry variety, tilt the specimen towards the x-ray detector. Select an isolated fiber or particle less than 0.5 μm in width, and accumulate an EDXA spectrum using an electron probe of suitable diameter. When a well-defined spectrum has been obtained, perform a background subtraction and calculate the background-corrected peak areas for each element listed, using energy windows centered on the peaks. Calculate the ratio of the peak area for each specified element relative to the peak area for silicon. All background-subtracted peak areas used for calibration shall exceed 400 counts.

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

APPENDIX C - STRUCTURE COUNTING CRITERIA

C.1 INTRODUCTION

In addition to isolated fibers, other assemblages of particles and fibers frequently occur in air samples. Groupings of asbestos fibers and particles, referred to as "asbestos structures", are defined as fiber bundles, clusters and matrices. The numerical result of a structure count depends strongly on whether the analyst assigns each such assemblage of fibers as a single entity, or as the estimated number of individual fibers which form the assemblage. It is therefore important that a logical system of counting criteria be defined, so that the interpretation of specific structures is the same for all analysts, and so that the numerical result is meaningful. Imposition of specific structure counting criteria generally requires that some interpretation, partially based on uncertain health effects information, be made of each asbestos structure found. It is not the intention of this method to make interpretations based on health effects. Rather, it is intended that a clear separation shall be made between recording of structure counting data and later interpretation of those data. The system of coding specified in this method permits a clear morphological description of the structures to be recorded in a concise manner suitable for later interpretation, if necessary, by a range of different criteria, without the necessity for re-examination of the specimens. The system requires each asbestos structure to be assigned a predominant classification as fiber, bundle, cluster or matrix and in some cases individual components of a structure are separately enumerated. Examples of the various types of morphological structures and the manner in which these shall be recorded are shown in Figure C1.

C.2 STRUCTURE DEFINITIONS AND TREATMENT

C.2.1 Fiber

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

C.2.2 Bundle

A grouping composed of apparently attached parallel fibers shall be counted as a bundle of a width equal to an estimate of the mean bundle width, and a length equal to the maximum length of the structure. The overall aspect ratio of the bundle may be any value, provided that the individual constituent fibers have aspect ratios equal to or greater than 5:1.

C.2.3 Cluster

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

Cluster type A: a disperse and open network, in which both ends of individual fibers and bundles can be separately identified and their dimensions measured. If the cluster consists of up to 5 such fibers or bundles, the individual fibers and bundles comprising the cluster shall be separately counted, measured, and recorded as individual fibers and bundles that are components of the overall cluster. If the cluster consists of more than 5 fibers or bundles it shall be noted as such on the count sheet. In addition to characterizing and measuring individual components, the overall outer dimensions of the cluster will be recorded as prescribed for type B clusters.

Cluster type B: a complex and tightly bound network, in which one or both ends of each individual fiber or bundle are obscured, such that the dimensions of individual fibers cannot be measured. In this case the cluster shall be recorded as a single cluster with no components, and the overall dimensions in the two perpendicular directions defining the maximum and minimum dimensions shall be recorded.

The recording of clusters shall be based on the predominant characteristics of the structures. For example, a cluster consisting predominantly of 4 fibers, but with smaller regions of attached material containing fibers, shall be considered as a type A cluster of 4 fibers to be recorded separately as fibers that are components of the overall cluster.

The procedure for treatment of clusters is illustrated by examples in Figure C2.

C.2.4 Matrix

A fiber, fibers, or bundles, may be attached to, or partially concealed by, a single particle or group of overlapping non-fibrous particles. This structure shall be defined as a matrix. The TEM image does not discriminate between particles that are attached to fibers, and those that, by chance, overlap in the TEM image. It is not known, therefore, whether such a structure is actually a complex particle, or whether it has arisen by a simple overlapping of particles and fibers on the filter.

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

Matrix type A: a disperse grouping of overlapping fibers and/or bundles and associated equant particles or groups of particles in which some

fibers have less than one third of their lengths obscured. If the matrix consists of up to 5 such fibers or bundles, the fibers or bundles shall be recorded as separate fibers or bundles that are components of the overall matrix, and their lengths and widths shall also be recorded. If the matrix consists of more than 5 fibers or bundles, it shall denoted as such on the count sheet. In addition to characterizing and measuring individual components, the overall outer dimensions of the matrix will be recorded as prescribed for type B matrices.

Matrix type B: a structure consisting of an equant particle or linked group of particles, in which the ends of fibers or bundles project from the particles, but the other ends of the fibers or bundles are obscured. Fibers and bundles shall be treated differently, depending on whether the obscured length could not possibly be more than one third of the total length. If the matrix exhibits up to 5 fibers or bundles, those fibers for which the obscured length could not be more than one third of the total length shall be counted as separate fibers or bundles that are components of the overall matrix. The assigned length for each partially-obscured fiber or bundle shall be equal to the visible length plus the maximum possible contribution from the obscured portion. Fibers or bundles which appear to cross the matrix, and for which both ends can be located approximately, shall be included in the maximum of 5 and counted as separate fibers or bundles that are components of the overall matrix. If more than 5 such fibers or bundles can be individually identified, this will be denoted on the count sheet. The residual matrix, if it exhibits additional fiber terminations that cannot be separately counted because the unobscured lengths are too short, shall be recorded as one matrix. All other matrices of type B shall each be recorded as one matrix with no components. The overall dimensions of each matrix in the two perpendicular directions defining the maximum and minimum dimensions shall be recorded.

Matrix type C: a structure in which fibers can be seen and identified in the interior, but which incorporates no fibers which project from the outside edges. This type of matrix can originate as a result of partial dissolution of binders during specimen preparation, when the original particle was a composite material containing asbestos. This type of matrix shall be recorded as a single matrix with no components. The overall dimensions of the matrix in the two perpendicular directions defining the maximum and minimum dimensions shall be recorded.

In practice, structures can occur in which different areas exhibit features of the three types of matrix, and more than one variety of asbestos may be incorporated in the same structure. In this case, the predominant characteristic of the structure should be determined, and then a logical procedure should be followed, in which predominant fibers and bundles are enumerated first up to a maximum of 5 followed by assignment of the remaining asbestos structures according to the counting rules. Complex structures shall be assigned no more than 6 separate components. Examples of the procedure which shall be followed are shown in Figure C3.

C.3 OTHER STRUCTURE COUNTING CRITERIA

C.3.1 Structures that Intersect Grid Bars

Structures that intersect grid bar shall be counted only for two adjacent sides of the grid opening, as illustrated in Figure C4. The length of the structure shall be recorded as twice the unobscured length. Structures intersecting either of the other two sides shall not be included in the count. This procedure ensures that the numerical count will be accurate, and that the best average estimate of length has been made.

C.3.2 Structures That Extend Outside the Field of View

During scanning of a grid opening, systematically count structures that extend outside of the field of view, so as to avoid double-counting. In general, a rule should be established so that structures extending outside the field of view in only two quadrants are counted. Structures without terminations within the field of view shall not be counted. The procedure is illustrated by Figure C5. Measure the length of each such structure by moving the specimen to locate the other end of the structure, and then return to the original field of view before continuing to scan the specimen.

C.4 PROCEDURE FOR DATA RECORDING

When a fibrous structure is detected during the structure count, the components of the structure are first identified according to the procedures in Appendix D. The procedure for recording the morphological description starts by classifying the predominant characteristic of the structure as a fiber, bundle, cluster, or matrix. The code F, B, C or M shall be the first component of the morphological descriptor. Depending on the nature of the individual structure, additional coded descriptions will be added to classify separate components of the structure.

C.4.1 Fibers

On the counting form, isolated fibers as defined in C.2.1 shall be recorded by the simple designation "F". If the fiber is a separately-counted part of a cluster or matrix structure, the sequential structure number shall be attached as a suffix, to identify all such constituent components of the particular cluster or matrix structure. For example, CF004 shall be used to denote a fiber forming part of structure number 004, the overall and predominant characteristic of which is a cluster.

C.4.2 Bundles

On the counting form, isolated bundles as defined in C.2.2 shall be recorded by the designation "B". If the bundle is a

separately-counted part of a cluster or matrix structure, the sequential structure number shall be attached as a suffix, to identify all such constituent components of the cluster or matrix structure. For example, MB004 shall be used to denote a bundle forming part of structure number 004, the overall and predominant characteristic of which is a matrix.

C.4.3 Clusters

On the fiber counting form, isolated clusters, as defined in C.1.4, shall be recorded by the designation "C". The dimensions of the overall structure are recorded. If some components of the cluster have been separately counted, the cluster shall be recorded by the designation CS, followed by the sequential structure number "n". For example, the code CS004 indicates that the primary structure number 004 was a cluster, for which the overall dimensions are specified. Thus if a localized cluster is attached to a group of fibers or bundles, and the fibers and bundles have been counted separately because this procedure was defined by the predominant characteristics of the structure, the sequential structure number shall be attached as a suffix, to identify all constituent components of the structure. For example, CF004 shall be used to denote a fiber forming part of structure number 004, the overall and predominant characteristic of which is a cluster. When more than 5 components can be individually identified, the cluster is still recorded by the designation CS and the overall dimensions of the structure are recorded, but the sequential structure number is excluded from the code and components are not recorded separately.

C.4.4 Matrices

On the fiber counting form, isolated matrices of type B or C, as defined in C.1.5, shall be recorded by the designation "M". The dimensions of the overall structure are recorded. If the type B or C matrix is attached to a group of fibers or bundles, and the fibers and bundles have been counted separately because this procedure was defined by the predominant characteristics of the structure, the sequential structure number shall be attached as a suffix, to identify all constituent components of the structure. For example, the primary matrix structure shall be designated as MS004, and the overall structure dimensions recorded. All separately-counted components of the structure shall carry the suffix 004. Thus the code MB004 shall be used to denote a bundle forming part of structure number 004, the overall and predominant characteristic of which is a matrix. When more than 5 components can be individually identified, the matrix is still recorded by the designation MS and the overall dimensions of the structure are recorded, but the sequential counting number is excluded from the code and components are not recorded separately.

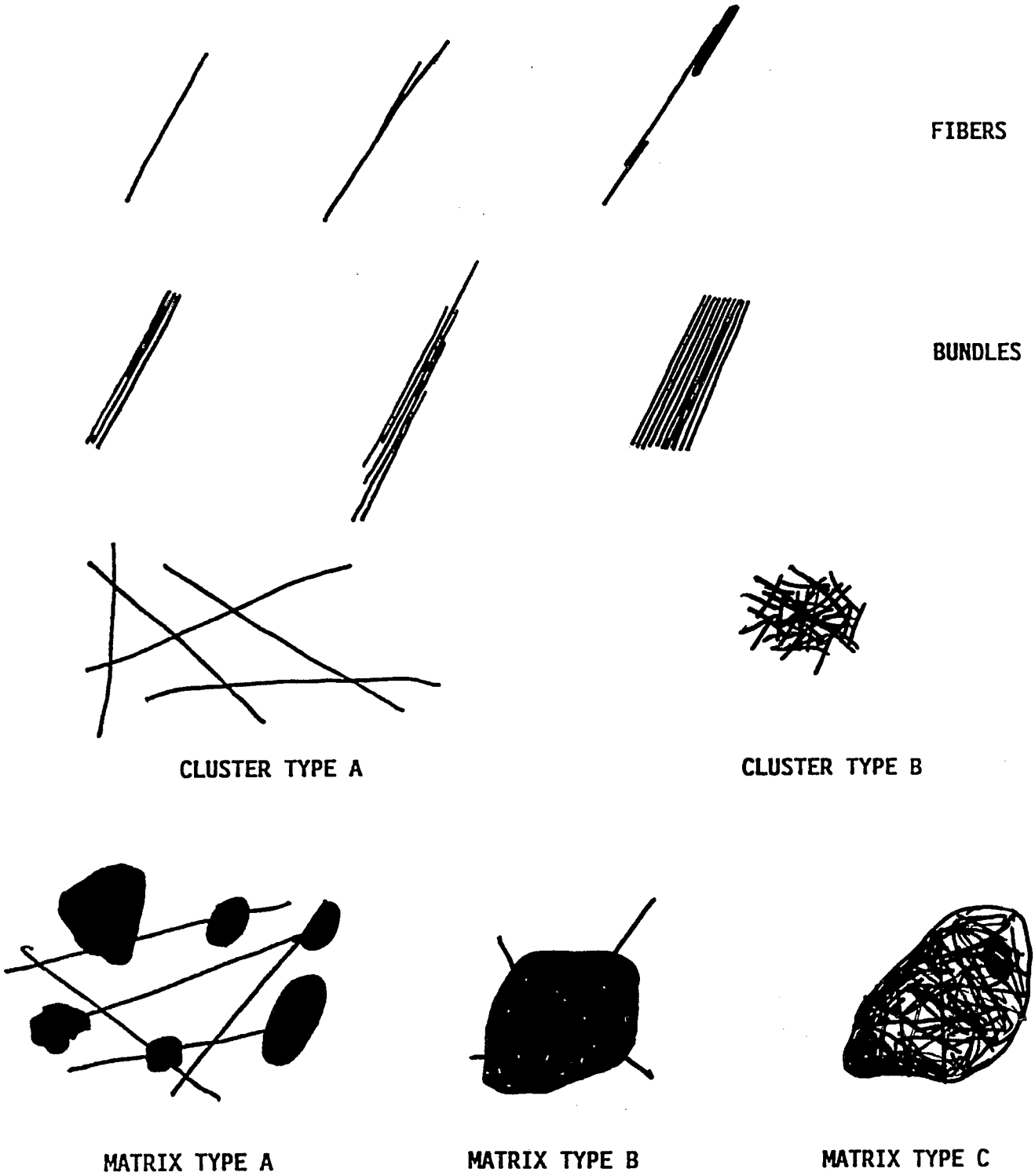
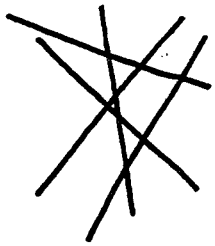
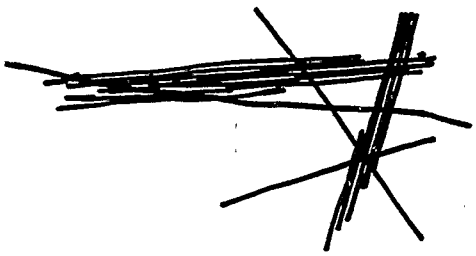


Figure C.1: Fundamental Morphological Structure Types

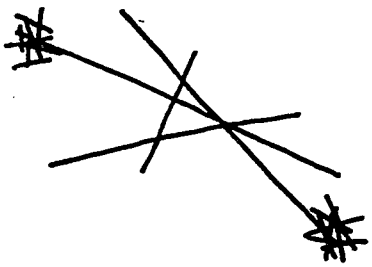
Record primary structure as one cluster designated C.



Record primary structure as 1 cluster designated as CSn. Record 5 fibers as components. Use code CFn for the component fibers.



Record primary structure as 1 cluster designated CSn. Record 3 fibers and 2 bundles as components. Use code CFn for component fibers and code CBn for component bundles.



Record primary structure as 1 cluster designated as CSn. Record 4 fibers and 2 sub-clusters as components. Use code CFn for component fibers and code CCn for component clusters.

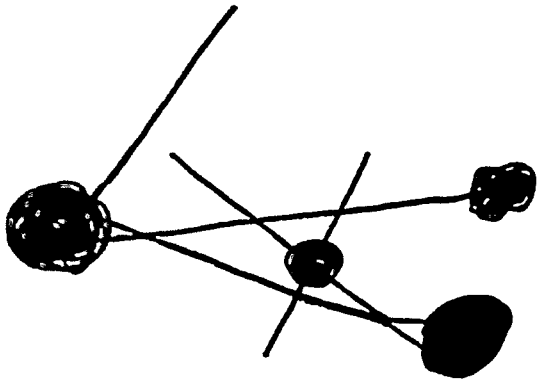
Notes:

"n" is a three digit number identifying the structure number of the primary structure that each component belongs to.

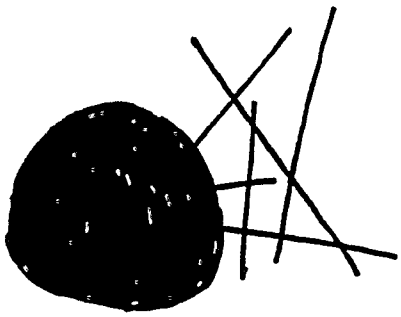
Dimensions assigned to each component represent the best estimate of the maximum length and mean width of that particular component. Dimensions recorded for a primary structure represent the best estimate of the overall outside dimensions of that structure.

Figure C.2: Examples of Recording of Complex Asbestos Clusters

Record primary structure as 1 matrix designated as MSn. Record 1 fiber as a component. Use code MFn for component fiber.



Record primary structure as 1 matrix designated as MSn. Record 5 fibers as components. Use code MFn for component fibers.



Record primary structure as 1 matrix designated as MSn. Record 3 fibers and 1 sub-matrix as components. Use code MFn for component fibers and code MMn for component matrix.

Record as 1 matrix designated as M.

Notes:

"n" is a three digit number identifying the structure number of the primary structure that each component belongs to.

Dimensions assigned to each component represent the best estimate of the maximum length and mean width of that particular component. Dimensions recorded for a primary structure represent the best estimate of the overall outside dimensions of that structure.

Submatrices are only recorded when they incorporate asbestos fibers not accounted for after separation of fiber, bundle, and cluster components from the primary structure.

Figure C.3: Examples of Recording of Complex Asbestos Matrices

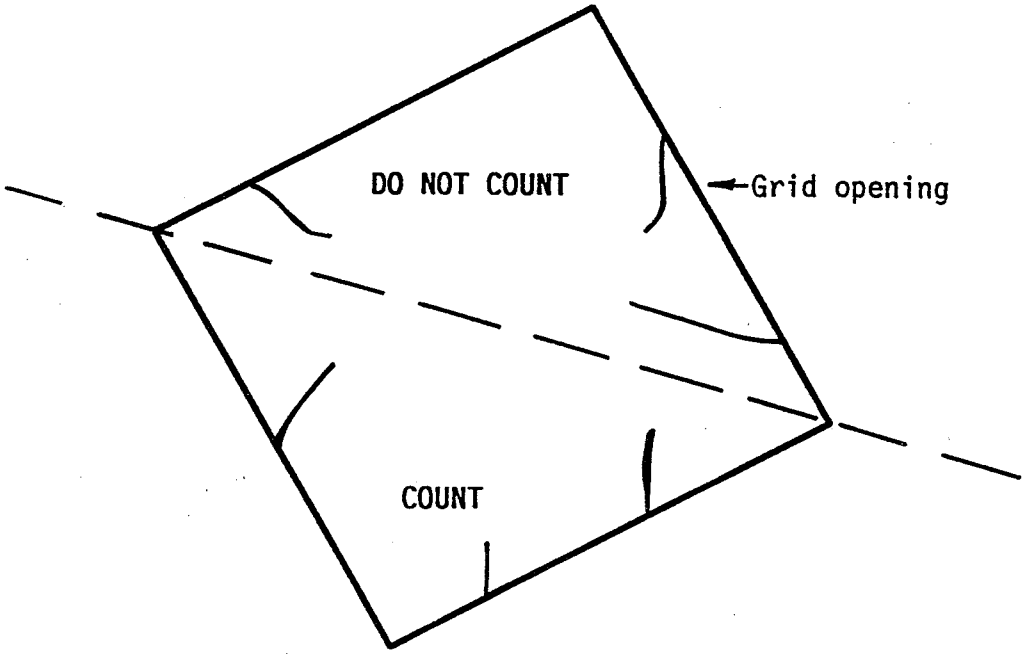


Figure C.4: Counting of Structures That Intersect Grid Bars

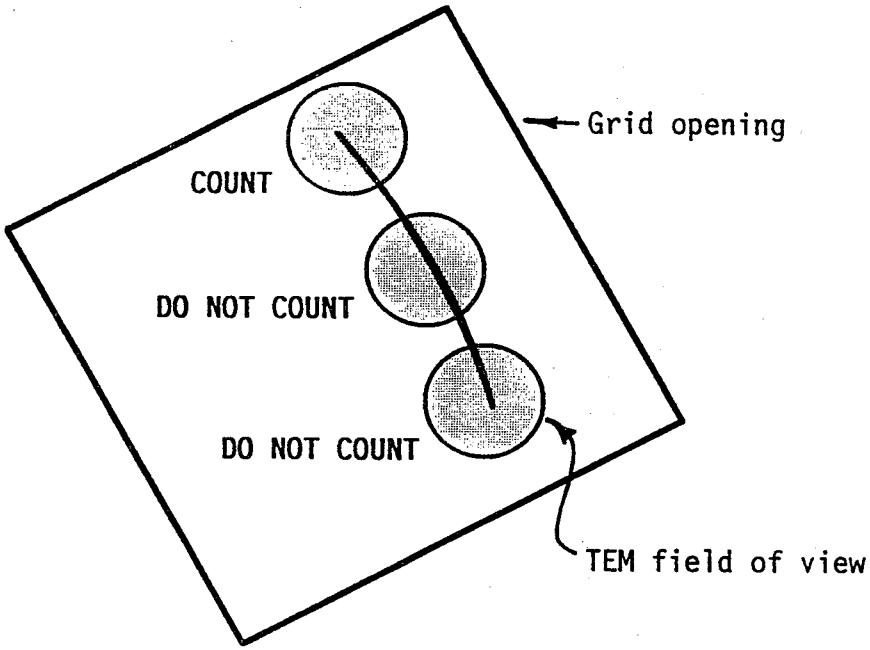


Figure C.5: Counting of Structures That Extend Outside the Field of View

APPENDIX D - FIBER IDENTIFICATION PROCEDURE

D.1 INTRODUCTION

Fibrous structures are classified as asbestos structures by identifying one or more of the constituent fibers or bundles as asbestos. Accordingly, this section refers to identification of fibers rather than structures.

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

D.2 ED AND EDXA TECHNIQUES

D.2.1 General

Initially, classify fibers into two categories on the basis of morphology: those fibers with tubular morphology, and those fibers without tubular morphology. Conduct further analysis of each fiber using ED and EDXA methods. The following procedures should be used when fibers are examined by ED and EDXA.

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

D.2.2 ED Techniques

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

For visual examination of the ED pattern, the camera length of the TEM should be set to a low value of approximately 250 mm and the ED pattern then should be viewed through the binoculars. This procedure minimizes the possible degradation of the fiber by the electron irradiation. However, the pattern is distorted by the tilt angle of the viewing screen. A camera length of at least 2 m should be used when the ED pattern is photographed, if accurate measurement of the pattern is to be possible. It is necessary that, when obtaining an ED pattern to be evaluated visually or to be photographed, the sample height shall be properly adjusted to the eucentric point and the image shall be focussed in the plane of the selected area aperture. If this is not done there may be some components of the ED pattern which do not originate from the selected area. In general, it will be necessary to use the smallest available ED aperture.

For routine sample analysis, calibration films of evaporated gold or other materials shall not be applied to the TEM grids. Such films seriously degrade the visibility of fine chrysotile fibers so that they may not be detected by the EM operator. Moreover, the visibility of ED patterns from chrysotile fibers are also seriously degraded resulting in failure to identify them positively. Both effects lead to significant reduction of the asbestos structure concentrations reported.

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

If a zone-axis ED analysis is to be attempted on the fiber, the sample shall be mounted in the appropriate holder. The most convenient holder allows complete rotation of the specimen grid and tilting of the grid about a single axis. Rotate the sample until the fiber image indicates that the fiber is oriented with its length coincident with the tilt axis of the goniometer, and adjust the sample height until the fiber is at the eucentric position. Tilt the fiber until an ED pattern appears, which is a symmetrical, two dimensional array of spots. The recognition of zone-axis alignment conditions requires some experience on the part of the operator. During tilting of the fiber to obtain zone-axis conditions, the manner in which the intensities of the spots vary should be observed. If weak reflections occur at some points on a matrix of strong reflections, the possibility of multiple diffraction exists, and some caution should be exercised in the selection of diffraction spots for measurement and interpretation. A full discussion of electron diffraction and multiple diffraction can be found in the references by J. A. Gard, P.B. Hirsch et al, and H. R. Wenk, included in the Bibliography. Not all zone-axis patterns that can be obtained are definitive. Only those which have closely-spaced reflections corresponding to low indices in at least one direction should be recorded. Patterns in which all d-spacings are less than about 0.3 nm are not definitive. A useful guideline is that the lowest angle reflections should be within the radius of the first gold diffraction ring (111), and that patterns with smaller distances between reflections are usually the most definitive.

Five spots, closest to the center spot, along two intersecting lines of the zone-axis pattern shall be selected for measurement, as illustrated in Figure D1. The distances of these spots from the center spot and the four angles shown provide the required data for analysis. Since the center spot is usually very over-exposed, it does not provide a well-defined origin for these measurements. The required distances shall therefore be obtained by measuring between pairs of spots symmetrically disposed about the center spot, preferably separated by several repeat distances. The distances must be measured with a precision of better than 0.3 mm, and the angles to a precision of better than 2.5°. The diameter of the first or second ring of the calibration pattern (111 and 200) must also be measured with a precision of better than 0.3 mm.

Using gold as the calibration material, the camera constant is given by:

$$\lambda \times L = 2.3548 \times D \text{ (first ring)}$$
$$\lambda \times L = 2.0393 \times D \text{ (second ring)}$$

D.2.3 EDXA Measurements

Interpretation of the EDXA spectrum may be either qualitative or quantitative. For qualitative interpretation of a spectrum, the elements originating from the fiber are recorded. For quantitative interpretation, the net peak areas, after background subtraction, are obtained for the elements originating from the fiber. This method provides for quantitative interpretation for those minerals which contain silicon. To obtain an EDXA spectrum, move the image of the fiber to the center of the screen and remove the objective aperture. Select an appropriate electron beam diameter and deflect the beam so that it impinges on the fiber. Depending on the instrumentation, it may be necessary to tilt the specimen towards the X-ray detector, and in some instruments, to use Scanning Transmission Electron Microscopy (STEM) mode of operation.

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

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

D.3 INTERPRETATION OF FIBER ANALYSIS DATA

D.3.1 Chrysotile

The morphological structure of chrysotile is characteristic, and with experience, can be recognized readily. However, a few other minerals have similar appearance, and morphological observation by itself is inadequate for most samples. The ED pattern obtained from chrysotile is quite specific for this mineral if the specified characteristics of the

pattern correspond to those from reference chrysotile. However, depending on the past history of the fiber, and on a number of other factors, the crystal structure of a particular fiber may be damaged, and it may not yield an ED pattern. In this case, the EDXA spectrum may be the only data available to supplement the morphological observations.

D.3.2 Amphiboles

Since the fiber identification procedure for asbestos fibers other than chrysotile can be involved and time-consuming, computer program such as developed by Rhoades (see Bibliography) can be used for interpretation of zone-axis ED patterns. The published literature contains composition and crystallographic data for all of the fibrous minerals likely to be encountered in TEM analysis of air samples, and the compositional and structural data from the unknown fiber should be compared with the published data. Demonstration that the measurements are consistent with the data for a particular test mineral does not uniquely identify the unknown, since the possibility exists that data from other minerals may also be consistent. It is, however, unlikely that a mineral of another structural class could yield data consistent with that from an amphibole fiber identified by quantitative EDXA and two zone axis ED patterns.

Suspected amphibole fibers should be classified initially on the basis of chemical composition. Either qualitative or quantitative EDXA information may be used as the basis for this classification. From the published data on mineral compositions, a list of minerals which are consistent in composition with that measured for the unknown fiber should be compiled. To proceed further, it is necessary to obtain the first zone axis ED pattern, according to the instructions in D.2.1.

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

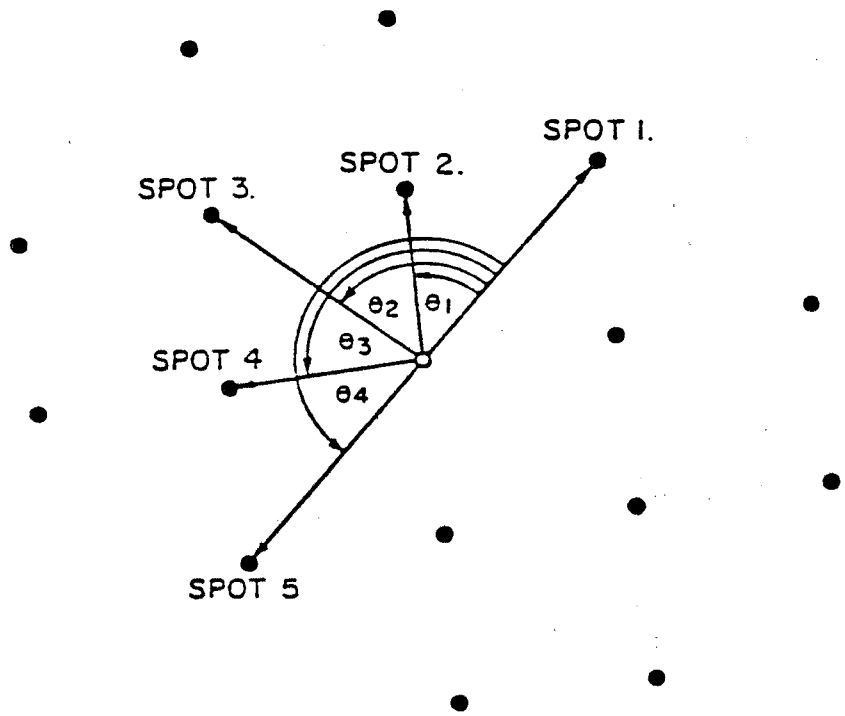


Figure D.1: Measurement of Zone Axis SAED Patterns

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

D.4 FIBER CLASSIFICATION CATEGORIES

It is not always possible to proceed to a definitive identification of a fiber; this may be due to instrumental limitations or to the actual nature of the fiber. In many analyses a definitive identification of each fiber may not actually be necessary if there is other knowledge available about the sample, or if the concentration is below a level of interest. The analytical procedure must therefore take account of both instrumental limitations and varied analytical requirements. Accordingly, a system for fiber classification is used to permit accurate recording of data. The classifications are shown in Tables D1 and D2, and are directed towards identification of chrysotile and amphibole respectively. Fibers shall be reported in these categories.

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

In an unknown sample, chrysotile will be regarded as confirmed only if a recorded, calibrated ED pattern from one fiber in the CD categories is obtained, or if measurements of the ED pattern are recorded at the instrument. Amphibole will be regarded as confirmed only by obtaining recorded data which yields exclusively amphibole solutions for fibers classified in the AQZ, AZZ or AQZZ categories.

D.4.1 Procedure for Classification of Fibers with Tubular Morphology, Suspected to be Chrysotile

Occasionally, fibers are encountered which have tubular morphology similar to that of chrysotile, but which cannot be characterized further either by ED or EDXA. They may be non-crystalline, in which case ED

techniques are not useful, or they may be in a position on the grid which does not permit an EDXA spectrum to be obtained. Alternatively, the fiber may be of organic origin, but not sufficiently definitive that it can be disregarded. Accordingly, there is a requirement to record the fiber, and to specify how confidently each fiber can be identified. Classification of fibers will meet with various degrees of success. Figure D2 shows the classification procedure to be used for fibers which display any tubular morphology. The chart is self explanatory, and every fiber is either rejected as a non-asbestos mineral (NAM), or classified in some way which by some later criterion could still contribute to the chrysotile fiber count.

Morphology is the first consideration, and if this is not similar to that usually seen in chrysotile standard samples, designate the initial classification as TM. Regardless of the doubtful morphology, examine the fiber by ED and EDXA methods according to Figure D2. Where the morphology is more definitive, it may be possible to classify the fiber as having chrysotile morphology (CM).

For classification as CM, the morphological characteristics required are:

(a) the individual fibrils should have high aspect ratios exceeding 10:1, and be about 20 to 40 nm in diameter;

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

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

Examine every fiber having these morphological characteristics by the ED technique, and classify as chrysotile by ED (CD) only those which give diffraction patterns with the precise characteristics shown in Figure D3. The relevant features in this pattern for identification of chrysotile are as follows:

(a) the (002) reflections should be examined to determine that they correspond approximately to a spacing of 0.73 nm;

(b) the layer line repeat distance should correspond to 0.53 nm; and,

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

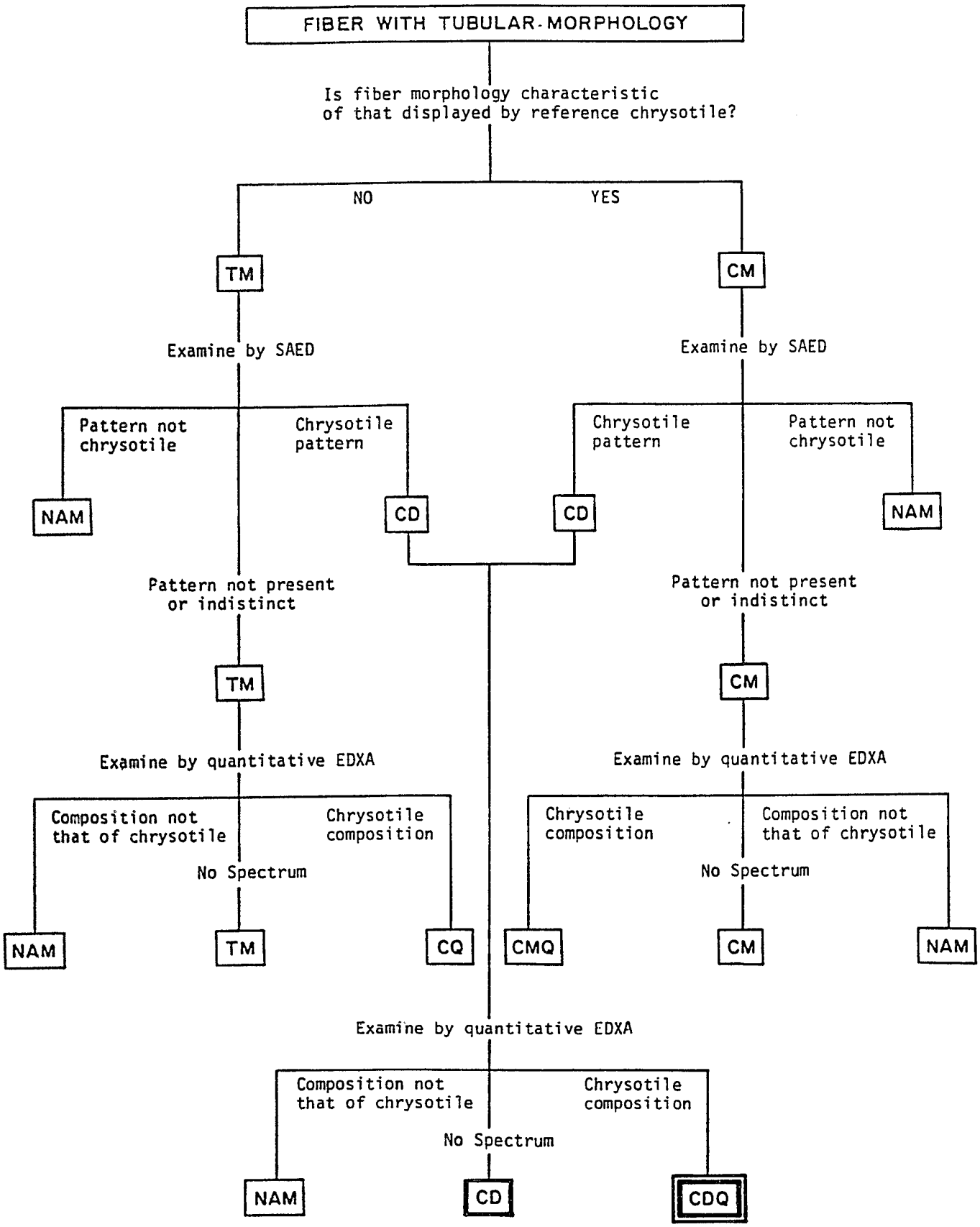


Figure D.2: Classification Chart for Fiber With Tubular Morphology

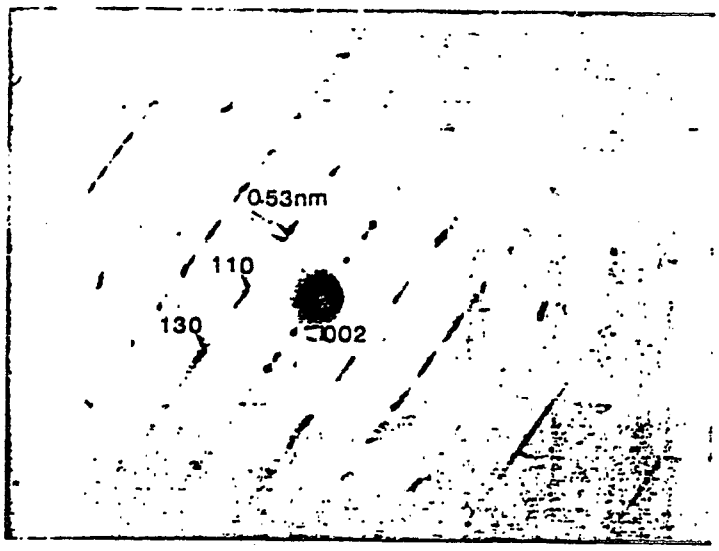


Figure D.3: Chrysotile SAED Pattern

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

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

D.4.2 Procedure for classification of Fibers Without Tubular Morphology, Suspected to be Amphibole

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

The 0.53 nm layer spacing of the random orientation ED pattern is not by itself diagnostic for amphibole. However, the presence of c-axis twinning in many fibers leads to contributions to the layers in the patterns by several individual parallel crystals of different axial orientations. This apparently random positioning of the spots along the layer lines, if also associated with a high fiber aspect ratio, is a characteristic of amphibole asbestos, and thus has some limited diagnostic value. If a pattern of this type is not obtained, the identity of the

fiber is still ambiguous, since the absence of a recognizable pattern may be a consequence of an unsuitable orientation relative to the electron beam, or the fiber may be some other mineral species.

Figure D4 shows the fiber classification chart to be used for suspected amphibole fibers. This chart shows all the classification paths possible in analysis of a suspected amphibole fiber, when examined systematically by ED and EDXA. Two routes are possible, depending on whether an attempt to obtain an EDXA spectrum or a random orientation ED pattern is made first. The normal procedure for analysis of a sample of unknown origin will be to examine the fiber by random orientation ED, qualitative EDXA, quantitative EDXA, and zone-axis ED, in this sequence. The final fiber classification assigned will be defined either by successful analysis at the maximum required level, or by the instrumental limitations. Record the maximum classification achieved for each fiber on the counting sheet in the appropriate column. The various classification categories can then be combined later in any desired way for calculation of the fiber concentration, and a complete record of the results obtained when attempting to identify each fiber is maintained for reassessment of the data if necessary.

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

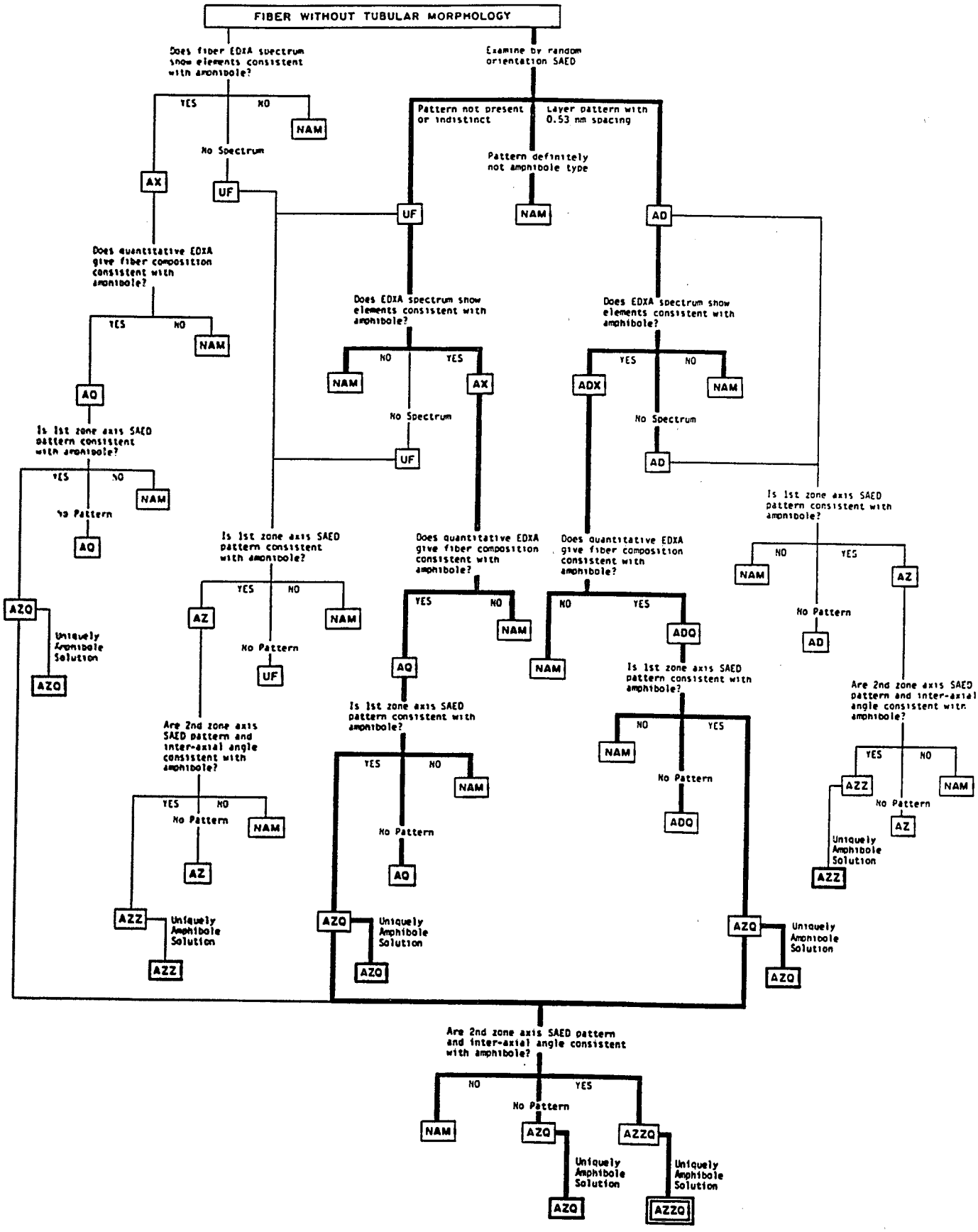


Figure D.4: Classification Chart for Fibers Without Tubular Morphology

Table D1 - **Classification of fibers with tubular morphology**

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

Table D2 - Classification of fibers without tubular morphology

UF	-	Unidentified Fiber
AD	-	Amphibole by random orientation SAED (shows layer pattern of 0.53 nm spacing)
AX	-	Amphibole by qualitative EDXA. Spectrum has elemental components consistent with amphibole
ADX	-	Amphibole by random orientation SAED and qualitative EDXA
AQ	-	Amphibole by quantitative EDXA
AZ	-	Amphibole by one Zone-Axis SAED pattern
ADQ	-	Amphibole by random orientation SAED and Quantitative EDXA
AQZ	-	Amphibole by Quantitative EDXA and one Zone-Axis SAED pattern
AZZ	-	Amphibole by two Zone-Axis SAED patterns with consistent inter-axial angle
AQZZ	-	Amphibole by Quantitative EDXA, two Zone-Axis SAED patterns, and consistent inter-axial angle
NAM	-	Non-asbestos Mineral

APPENDIX E - CALCULATION OF RESULTS

E.1 INTRODUCTION

Calculate the results using the procedures described below. The results can be conveniently calculated using a computer program.

E.2 TEST FOR UNIFORMITY OF FIBER DEPOSITS ON TEM GRIDS

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

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

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

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

This value shall be compared with significance points of the χ^2 distribution, having (k - 1) degrees of freedom. Significance levels lower than 0.1% may be cause for the sample analysis to be rejected, since this corresponds to a very inhomogeneous deposit. If the structure count fails this test, the precision of the result will be uncertain, and new analytical filters should be prepared from the original collection filter.

E.3 CALCULATION OF THE ANALYTICAL SENSITIVITY

Calculate the analytical sensitivity S, in structures/liter, using the formula:

$$S = A_f/(N \times A_g \times V \times F)$$

where:

A_f = Active area of sample collection filter in mm^2
N = Number of grid openings examined
A_g = Mean area of grid openings
V = Volume of air sampled in liters
F = Concentration factor

E.4 CALCULATION OF THE MEAN AND CONFIDENCE LIMITS FOR A REPORTED ASBESTOS STRUCTURE CONCENTRATION

In the asbestos structure count made according to this method, a number of grid openings have been sampled from a population of grid openings and it is required to determine the mean grid opening asbestos structure count for the population on the basis of this small sample. The upper and lower 95% confidence limits are also to be reported with every mean. When such limits are constructed as indicated in Section E.4.2, the interval between the two limits should contain the true mean 90% of the time (95% of the time if the lower confidence limit is zero).

E.4.1 Calculation of the Mean Asbestos Structure Concentration

Calculate the mean concentration from a particular measurement, C, in structures/liter:

$$C = S \times N \times n$$

where:

S = Analytical sensitivity in structures/liter
N = Number of grid openings examined
n = mean number of asbestos structures found per grid opening

E.4.2 Calculation of Upper and Lower 95% Confidence Limits

If the total structures counted from a filter is 30 or less, the Poisson distribution is recommended to be used for constructing confidence limits. Let P(x;m) represent the cumulative probability function from a Poisson distribution with mean m. That is, P(x;m) is the probability of observing x or fewer counts given by

$$P(x;m) = e^{-m}(1 + m + m^2/2! + \ldots + m^x/x!).$$

The upper 95% confidence limit, x_U, on the mean count, based on an observed count of x, is the value that satisfies:

$$P(x,x_U) = 0.05,$$

and the lower 95% confidence limit, x_L, on the mean count, based on an observed count of x, is the value that satisfies

$$1 - P(x-1,x_L) = 0.05$$

(e.g., Miller and Freund 1965)[5]. Tables of P(x;m) for values of m less than 26 may be found in Miller and Freund. A computer program can be easily written for calculating P(x;m) for larger values of m. If such a program is not available, confidence limits may alternately be based on the normal distribution approximation to the Poisson distribution (see Miller and Freund 1965). Corresponding approximations to the upper and lower 95% confidence limits are

$$x_U = x + 1.65*x^{1/2}$$

and

$$x_L = x - 1.65*x^{1/2}.$$

The Poisson distribution is correct whenever structures are randomly distributed in the sampled air and on the filter. However, as a hedge

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[5] Confidence limits derived as described in this section are one-tailed limits meaning that 95% of the distribution lies below the upper 95% confidence limit and, similarly, 95% of the distribution lies above the lower 95% confidence limit. If the two limits are combined to create a confidence interval, the interval between the two limits represents 90% of the distribution (which is different than a 95% confidence interval).

Construction of confidence limits in this manner, rather than the more traditional approach of constructing confidence intervals (where, for example, the 95% confidence interval corresponds to upper and lower 97.5% confidence limits), is recommended to avoid confusion when referring to an asymmetric distribution or when the lower confidence limit falls to zero. When the lower confidence limit falls to zero, for example, the upper 95% confidence limit now corresponds to the upper 95% confidence interval (rather than the 90% confidence interval, as indicated in the last paragraph) because the lower confidence limit (zero) removes nothing from the distribution. Thus, we have chosen to refer to one-tailed confidence limits throughout and avoid emphasis on confidence intervals.

against possible clumping of structures, it is recommended that, whenever the total structure count on a filter is greater than 30, confidence limits also be calculated using the following procedure and the more extreme limits (i.e., larger x_U and smaller x_L) be reported.

Suppose k grid openings are examined and let x_i be the structure count for the ith grid opening. Note that a minimum of four grid openings (k>4) should be examined and they should be of equal size. Then 95% upper and lower confidence limits are calculated according to the formulas

$$x_U = 1.65s$$

$$x_L = 1.65s$$

Where

$$s^2 = [k/(k-1)] \sum_{i=1}^{k} (x_i - x/k)^2,$$

and 1.65 is the cumulative 95th percentage point of the standard normal distribution.

Upper or lower confidence limits for airborne concentrations derived from a particular measurement are computed by multiplying the corresponding upper or lower confidence limits derived as described above for the structure count by the analytical sensitivity of the measurement.

E.4.3 Reporting the Mean and Confidence Limits for a Measured Concentration

Where a result is to be interpreted as a single isolated measurement, the following procedures shall be used for reporting:

No structures detected - the concentration shall be reported as "NF" meaning "not found" and it should be accompanied by the upper and lower 95% confidence limits derived as described in Section E.4.2. Note that the lower 95% confidence limit is zero in this case.

At least 1 structure detected - report the mean concentration derived as described in Section E.4.1 along with the upper and lower 95% confidence limits derived as described in Section E.4.2.

Rules for combining the results of individual measurements as part of a site evaluation are presented in the Technical Background Document, Part 2 of this report.

E.5 DISTINGUISHING DETECTED ASBESTOS FROM ANALYTICAL BACKGROUND

The non-parametric Wilcoxon Test (Hollander and Wolfe 1973) is employed in this method to test for significant differences between asbestos concentrations (in units of s/cm^2) observed on sample filters and concentrations observed on blanks. It must be emphasized, however, that the most useful application of this test is to groups of sample results rather than individual results. This should not be a severe limitation because conclusions based on the totality of data from an entire study tend to be more important than the results from individual analyses.

The Wilcoxon test performs better on multiple samples. A test comparing the concentration from an individual filter to those from a group of blanks can be conducted, but such a test is likely to be of low power (insensitive to small differences). In fact, data from at least 20 blanks are likely required before the test becomes capable of exhibiting a statistically significant difference ($p < -0.05$) between blank concentrations and the concentration observed on a single sample filter. The power of the test will similarly be limited when comparing multiple sample filter analyses to a limited number of blanks. For example, at least seven sample filters likely would have to be combined to find a significant difference from two blanks.

Results of blank analyses will generally be available from two sources: study specific and historical. Within a particular study, two filter blanks are generally analyzed from each lot of filters. Typically, there will also be field blanks. Depending on the length of time that a particular laboratory has been using this method, the laboratory should also be accumulating a historical database of blank analyses. Either set of blank data may be combined with care to provide the needed data to perform the Wilcoxin Test and distinguish sampled asbestos from analytical contamination. However, if different lots of filters exhibit different levels of background contamination or if results from a laboratory change over time (due possibly to changes in methodology or analysts) then it may not be appropriate to include historical blank data in an analysis. On the other hand, if the historical data are representative, use of this data may greatly increase the power of the analysis for detecting airborne concentrations. If historical blank data are used in a statistical test in this method, this is to be noted and results of corresponding tests based only on concurrent blanks shall also be reported.

The following procedure can be used for applying the Wilcoxon test to asbestos analytical results. More detailed discussions of the procedure are available from numerous sources (including Hollander and Wolfe 1973). Suppose M blank and N non-blank samples are available. Let $X_1, \ldots, X_M$ be the measured filter concentrations from the blank samples and $Y_1, \ldots, Y_N$ the measured concentrations from the non-blank samples (in units of structures per mm^2). Combine the M+N concentrations and rank them from smallest to largest; assign sequential integers (beginning with 1) to each concentration arranged from smallest to largest. If there are ties (two or more samples

exhibiting the same concentration) among the M+N observations, assign the average rank to each group of tied observations. (E.g., if the observations are 0, 0, 0, 0, 0.001, 0.002, then the corresponding ranks would be 2.5, 2.5, 2.5, 2.5, 5, 6.) Let W be the sum of the ranks assigned to the concentrations from the non-blank samples. Large values of W are evidence that concentrations from non-blank samples are larger than those from blank samples, and the hypothesis that the blank and non-blank samples have equal concentrations of fibers is rejected if W is large enough.

Tables are available for determining when W is large enough to be statistically significant in cases in which there are no ties and M and N are small (see e.g., Table A.5 in Hollander and Wolfe 1973). As an example of the application of these tables, if N = 4 and M = 5, then W, the sum of the ranks of the concentrations from the non-blank samples, would have to be 28 or larger before it could be concluded at the 0.05 level of significance that the concentrations from the non-blanks are higher than those from the blanks (see Table A.8 in Hollander and Wolfe 1973, page 276).

If ties are present in the data or if the number of samples is so large that tables are not available, a normal approximation to the distribution of W may be used. Define

$$W^* = \frac{W - [N(M+N+1)/2]}{[Var(W)]^{1/2}}$$

where

$$Var(W) = \frac{MN}{12}\left[M+N+1 - \frac{\sum_{j=1}^{g} t_j(t_j^2-1)}{(M+N)(M+N-1)} \right]$$

where g is the number of groups of tied concentrations (included among the combined set of blank and nonblank samples) and t_j is the size of tied group j. The hypothesis test is based on an assumed normal distribution for W^*. Thus one would reject the hypothesis of equal concentrations on blanks and non-blanks at the 0.05 level of significance if $W^* \geq 1.65$. (Note: This normal approximation may not work well if the number of samples is small. Consequently, if there are ties in the data and M or N is less than about 4, consideration should be given to consulting with a statistician regarding a more accurate approach to computing the statistical significance of W.)

E.6 CALCULATION OF ASBESTOS STRUCTURE LENGTH, WIDTH, AND ASPECT RATIO DISTRIBUTIONS

The distributions all approximate to logarithmic-normal, and so the size range intervals for calculation of the distribution shall be spaced logarithmically. The other characteristics required for the choice of size intervals are that they should allow for a sufficient number of size classes, while still retaining a statistically-valid number of asbestos

structures in each class. Interpretation is also facilitated if each size class repeats at decade intervals. A ratio from one class to the next of 1.47 satisfies all of these requirements and this value shall be used. The distributions, being approximately logarithmic-normal, when presented graphically, shall be plotted using a logarithmic ordinate scale and a Gaussian abscissa.

E.6.1 Calculation of Asbestos Structure Length Cumulative Number Distribution

This distribution allows the fraction of the total number of asbestos structures either shorter or longer than a given length to be determined. It is calculated using the relationship:

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

where:

$C(N)_k$ = Cumulative fraction asbestos structures (expressed in percent) which have lengths less than the upper bound of the k'th class

n_i = Number of asbestos structures in the i'th length class

N = Total number of length classes.

E.6.2 Calculation of Asbestos Structure Width Cumulative Number Distribution

This distribution allows the fraction of the total number of asbestos structures either narrower or wider than a given width to be determined. It is calculated in a similar way to that used in E.6.1, but using the asbestos structure widths.

E.6.3 Calculation of Asbestos Structure Aspect Ratio Cumulative Number Distribution

This distribution allows the fraction of the total number of asbestos structures which have aspect ratios either smaller or larger than a given aspect ratio to be determined. It is calculated in a similar way to that used in E.6.1, but using the asbestos structure aspect ratios.

APPENDIX F - BIBLIOGRAPHY

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