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Research Method for Sampling and Analysis of Fibrous Amphibole in Vermiculite Attic Insulation

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A technical panel was convened for this effort consisting of Eric Chatfield, Chatfield Technical Consulting, Ltd., Greg Meeker, United States Geological Survey, USGS, James Millette, MVA Inc., and Jeanne Orr, Reservoirs Environmental, Inc.

In addition to the invited panel, a number of representatives from U.S. EPA and other government agencies participated in the two day meeting. These included: Lauren Drees, U.S. EPA - ORD; Joe Fernback, NIOSH - CDC; Peggy J. Forney, U.S. EPA - NEIC; Mary Goldade, U.S. EPA - Region 8; Kathleen Meier, U.S. EPA - ORD; Aubrey Miller, U.S. EPA - Region 8; Paul Peronard, U.S. EPA - Region 8; Gigi Salama, U.S. EPA - ORD; Greg Sayles, U.S. EPA - ORD; Glenn Shaul, U.S. EPA - ORD; Chon Shoaf, U.S. EPA - ORD; Subhas Sikdar, U.S. EPA - ORD; John H. Smith, U.S. EPA - OPPT; Richard Troast, U.S. EPA - OERR; Jim Webber, NY State Dept. Of Health; Chris Weis, U.S. EPA - NEIC; John Wheeler, CDC - ATSDR; and Roger Wilmoth, U.S. EPA - ORD. Meeting support staff consisted of Cat Cole - Court Reporter, Marilyn Joos, U.S. EPA - ORD and Phyllis McKenna, U.S. EPA - ORD.

Cover photo by Glenn M. Shaul, U.S. EPA. All other photos by Eric J. Chatfield, Chatfield Technical Consulting, Inc.

Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory (NRMRL) is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threaten human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.

Lee A. Mulkey, Acting Director
National Risk Management Research Laboratory

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1 INTRODUCTION AND BACKGROUND

1.1 General

The purpose of this procedure is to provide the U. S. Environmental Protection Agency's (EPA) Office of Research and Development (ORD) with a method for the characterization of the fibrous amphibole content of vermiculite attic insulation (VAI). This procedure is to be used in an ORD research project dealing with determining the airborne levels of fibrous amphiboles in residences where VAI is used. This procedure was developed from input received from fibrous amphibole monitoring experts at an interagency meeting on "Analytical Method for Bulk Analysis of Vermiculite," held in Greater Cincinnati, Ohio on July 17-18, 2003. This procedure was developed from a method prepared by Eric J. Chatfield, Ph.D., Chatfield Technical Consulting, Inc., Mississauga, Ontario, Canada, for Mr. Wayne Toland, U.S. Environmental Protection Agency, EPA Region 1, Boston, MA, 02114. The current method has streamlined the original method to provide a means for analytical laboratories to determine the presence of fibrous amphiboles in VAI. For analysis of vermiculite in other materials, the analyst is referred to the original method by Chatfield, [Chatfield (2000)].

This method provides an approach to determine the percentage of fibrous amphibole present in VAI. EPA is determining this percentage for purposes of selecting residences to sample during the research project. EPA does not correlate the percentage of fibrous amphibole, as determined by this method, with risk or remediation. While the principles of this procedure may be applied to the analysis of other vermiculite materials, it may be necessary for the user to address any unique characteristics of these alternate materials with appropriate modifications to this procedure.

Vermiculite is a naturally occurring mineral that has the unusual property of expanding into "books" or worm-like accordion shaped pieces when heated. The expanded vermiculite is a light-weight, fire-resistant, absorbent, and odorless material. These properties allow vermiculite to be used in numerous applications, including attic insulation. Sizes of vermiculite products range from very fine particles to large (coarse) pieces nearly an inch in dimension. Vermiculite attic insulation (VAI) is a pour-in product, fragments of which are generally approximately 5 mm to 1cm in dimension, and is usually light-brown or gold in color. An example of VAI is shown as Figure 1 as well as on the cover of this document. The object highlighted by the black box in the cover photo is a large fragment of fibrous amphibole which is grey in color.

As is the case for most minerals, deposits of vermiculite usually contain other mineral phases, many of which are removed during processing. The process by which vermiculite is concentrated from the crude ore is referred to as beneficiation. During beneficiation of crude vermiculite ore, the vermiculite is also segregated into different size fractions for different applications. Larger sizes of vermiculite flakes command a higher price.

Vermiculite from Vermiculite Mountain (also called Zonolite Mountain) near Libby, MT is likely to contain fibrous amphibole. This fibrous amphibole displays a continuum of

Figure 1. Example of Exfoliated Vermiculite Attic Insulation
(Photo by E. J. Chatfield)

morphologies from acicular to asbestiform. Vermiculite from other sources may or may not contain fibrous amphibole. During the beneficiation the fibrous amphibole may, to a large extent, be removed from the vermiculite. However, some of the fibrous amphibole, if present, may pass through the beneficiation process and appear in the final vermiculite product.

Assuming that amphibole fragments are present in the beneficiated vermiculite, the amount of amphibole present in the final exfoliated product depends on the practices of the exfoliation facility. During exfoliation, the vermiculite expands to 5 - 15 times its original volume, and these very light fragments are separated by air entrainment. The other minerals present in the original beneficiated vermiculite are not useful, and represent material (usually referred to as "rock") that must be disposed of by the exfoliation facility. Some facilities return the "rock" to the vermiculite after the exfoliation process, and it is therefore incorporated into the final product. Other facilities dispose of the "rock" as a waste material. The importance of this to the analyst is that non-vermiculite fragments may be common in some samples but relatively rare in others.

1.2 Required Characteristics for an Analytical Method for Determination of Fibrous Amphibole in Vermiculite Attic Insulation

This method assumes that Vermiculite Attic Insulation (VAI) is normally used as purchased, and is not ground or pulverized to a powder.

This analytical method incorporates a procedure by which fibrous amphibole can be separated from the bulk material, without generating additional fine fibers by crushing or grinding of the material. Neither scanning electron microscopy (SEM) nor transmission electron microscopy (TEM) is an appropriate method for determination of the weight percent fibrous amphibole in vermiculite, because the size range of fiber bundles of fibrous amphibole that may be present in vermiculite extends up to approximately the dimensions of the vermiculite flakes, and the majority of the weight of fibrous amphibole is represented by these larger fiber bundles that are very much larger than can be examined by SEM/TEM. Any attempt to measure the weight concentration by SEM/TEM will usually yield a value that significantly under-estimates the actual concentration. However, SEM/TEM is an appropriate method for determination of the numerical concentration of fine fibers. This analytical method incorporates a procedure by which fine fibers can be separated from the vermiculite, without generating additional fine fibers by crushing or grinding of the material.

It is most important to recognize that reliable and reproducible results cannot be obtained by analysis of small quantities of samples. Any amphibole particles present in vermiculite are usually much fewer in number than the flakes of vermiculite, and if only a small sample size is analyzed the number of amphibole particles included in the sample will be small and often unrepresentative.

1.3 Analytical Considerations Specific to Vermiculite from Libby, Montana

Prior to 1990, a large proportion of the U.S. consumption of vermiculite originated from the mine at Libby, Montana. Depending on the date of production, beneficiated vermiculite from Libby may have contained several percent of fibrous amphibole, down to a fraction of a percent shortly before the mine was closed in 1990.

From an analytical perspective, it is important to recognize that, with relatively simple, but appropriate, analytical procedures specified in this method, the fibrous amphibole in vermiculite from the Libby mine can be readily recognized and the weight percent of fibrous amphibole can be estimated in the range of less than approximately 0.01% to several percent by weight. This measurement can be made using conventional chemical laboratory equipment, a stereo-binocular microscope and a polarized light microscope. Samples of vermiculite attic insulation that originated from the Libby mine will generally yield sufficient fibrous amphibole to determine the approximate weight concentration by weighing.

1.4 Analytical Considerations for Vermiculite Sources Other Than Libby

Analysis of vermiculite attic insulation from sources other than Libby may be a matter of establishing a sufficiently low limit of detection, and discriminating between fibrous and non-fibrous amphibole.

1.5 Safety Precautions

This method does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use. All materials associated with this analysis should be used and disposed of with due consideration for any potential hazard.

2 PRINCIPLE OF METHOD

2.1 Types of Measurement

Two types of measurement are specified in this method; each of the two measurements examines a different fraction of the VAI sample which is processed/separated into three fractions by soaking and swirling in water. Larger fragments of fibrous amphibole are separated from VAI by washing the sample in water. In general , the larger pieces of amphibole will sink to the bottom of the container while the vermiculite will float on the surface, effecting a separation. The material which floats on the water is referred to in this procedure as "floats fraction." The material which sinks in the water is referred to as "sinks fraction." Even though larger pieces of amphibole should fall out in the water wash, there may also be some amphibole suspended in the water and/or entrained in the floating vermiculite, regardless of whether larger pieces fall out. The water used for the washing is referred to in this method as the "suspended particles fraction."

Each of the two fractions separated from the VAI sample are further prepared and analyzed differently and in the specified sequence. The "sinks" are analyzed by optical microscopy for mineral fragments which may be fibrous amphibole. If fibrous amphiboles are detected in the examination of the sinks, the analysis may be terminated. If no fibrous amphiboles are detected, the suspended particles fraction is analyzed next for amphibole fibers which may have remained in the suspended particles fraction. If fibrous amphiboles are detected in the suspended particles fraction, the analysis may be terminated. If fibrous amphiboles are not detected in the suspended particles fraction, the analysis is terminated. The analytical process is described in Figure 2. The procedures are described in detail in the following sections.

(a) Rapid Screening Procedure for Sinks Fraction ("Sinks")

The weight percent fibrous amphibole is determined by separating fibrous amphibole from VAI and weighing it. A known weight of the VAI is first suspended in water. Most of the vermiculite floats to the top of the suspension, and this vermiculite is removed and saved for possible further examination. After allowing time for most of the suspended material to settle, the water is decanted and saved, and the sediment is dried and weighed. The dried sediment is examined under a stereo-binocular microscope. If there is more than approximately 0.01% of fibrous amphibole in the original sample, the fiber bundles are readily recognized during the stereo-microscope examination, and it is possible to hand-pick these fiber bundles from the sediment and weigh them. Representative fibrous amphibole particles are identified by PLM, SEM-EDS or TEM-EDS.

(b) **Procedure for "Suspended Particles Fraction."**

If no fibrous amphibole is detected in the sediment, the suspended particles fraction saved from the wash above, is analyzed. ***This suspension should be filtered within 24 hours of the washing in order to minimize bacterial growth in the sample.*** Aliquots of the suspension are filtered through membrane filters, and TEM specimens are prepared from the filters. The TEM specimens are examined, and fibers are identified and their dimensions are recorded. Alternately, the sample may be prepared and analyzed by SEM. The balance of the suspension is filtered on to a pre-weighed membrane filter. The filter is dried and weighed to obtain the total weight of suspended particles.

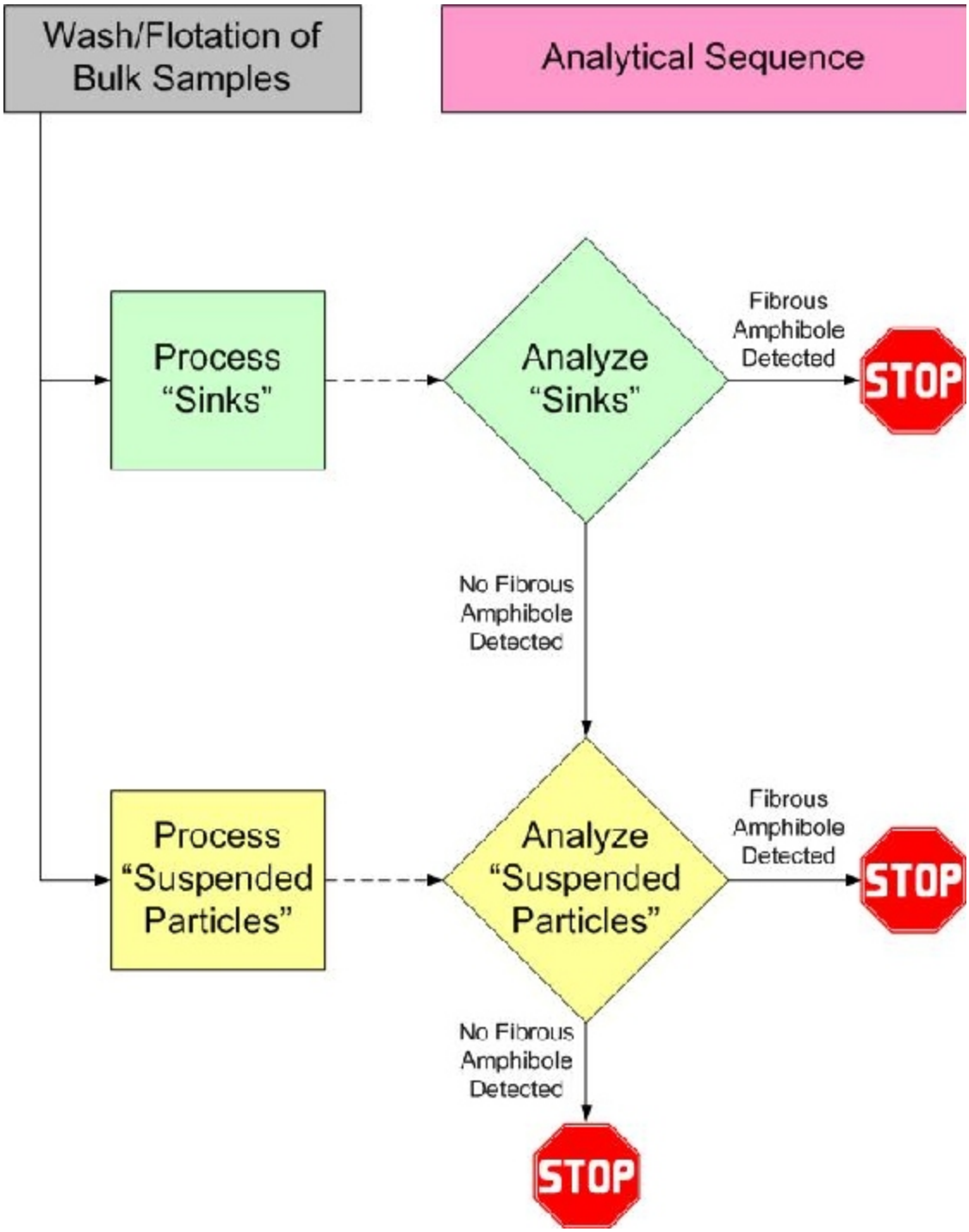

Figure 2. Analytical Sequence Flow Chart

3 SCOPE AND FIELD OF APPLICATION

3.1 Substance determined

3.1.1 Weight Percent Fibrous Amphibole

The rapid screening method specifies a procedure to determine the weight percent of fibrous amphibole.

3.1.2 Numerical Concentration of Suspended Amphibole Fibers

The method specifies a TEM or SEM procedure to determine the concentration of suspended fibrous amphiboles in VAI. The concentration of suspended fibrous amphiboles is expressed as the numerical concentration per gram of sample. The lengths, widths and aspect ratios of the fibers and bundles are measured. The method allows determination of the type(s) of fibers present. As for all routine TEM/SEM analytical methods, this method cannot always discriminate between an individual fiber of the fibrous and non-fibrous analogues of the same amphibole mineral.

3.2 Type of Sample

The method is defined for samples of vermiculite attic insulation.

3.3 Range

The range of fibrous amphibole weight concentration that can be measured is estimated to be approximately 0.01% to 100%.

The minimum suspended particle concentration that can be measured is dependent on the volume of the suspension that can be filtered while still yielding filters that are appropriately-loaded for preparation of TEM/SEM specimens. The minimum for the suspended particle concentration can be lowered by examination of a larger area of the TEM/SEM specimens. There is no maximum, since the analytical parameters can always be adjusted to accommodate high fiber concentrations.

3.4 Limit of Detection

For the rapid screening method, the limit of detection for fibrous amphibole is estimated to be less than approximately 0.01% by weight.

Theoretically, for determination of the concentration of suspended particles, the limit of detection can be lowered indefinitely by increasing the volume of liquid filtered during specimen preparation, and by increasing the area of the TEM/SEM specimens examined in the electron

microscope. In practice, for a particular area of TEM/SEM specimens examined, the lowest achievable limit of detection is controlled by the total amount of particulate material in the suspended particle size range. There is an upper limit to the volume of the final suspension that can be filtered, if TEM/SEM specimens of appropriate particulate loading are to be obtained. Lower limits of detection can be achieved by increasing the area of the TEM/SEM specimens that is examined. In order to achieve lower limits of detection for fibers and bundles longer than 5 µm, and for PCM equivalent fibers (fibers detected under TEM/SEM that would be expected to also be seen by Phase Contrast Microscopy techniques: usually fibers greater than 5 um in length, and greater than 0.25 um in width.), lower magnifications are specified which permit more rapid examination of larger areas of the TEM/SEM specimens when the examination is limited to these dimensions of fiber.

4 DEFINITIONS

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

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

where the most common constituents are:

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.

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°.

Analytical filter: A filter through which an aqueous suspension of particles is passed, and from which TEM/SEM specimen grids are prepared.

Asbestiform: Aggregates of long, thin, flexible mineral particles resembling organic fibers that occur in significant quantity and quality to be economically useful.

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

Beneficiation: The process in which vermiculite is concentrated from the crude ore and separated into different size fractions.

Filter Blank: A structure count made on TEM/SEM specimens prepared from an unused filter, to determine the background measurement.

Cleavage: The breaking of a mineral along specific crystallographic directions.

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

Cluster: A structure in which two or more fibers, or fiber bundles, are randomly oriented in a connected grouping.

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

Exfoliation: A process in which vermiculite flakes are expanded by sudden heating or by chemical action.

Fibril: The smallest structural unit of a fiber bundle.

Fibrous: The tendency of certain minerals to crystallize in needle-like grains or fibers, including the asbestiform habit.

Fiber (countable): Fiber (countable): For this method a countable fiber is defined as an elongate particle with a minimum aspect ratio of 3:1.

Fiber (mineral): An elongate particle or parallel group of elongate particles. On average in a population of fibers, the lengths of fibers are much greater than their widths (over ten times). Note that for different fiber count methods, the minimum aspect ratio (the relationship between the length and width) used to define a fiber may vary. Fiber morphologies can include acicular (needle-like) and asbestiform.

Fiber bundle: A structure composed of parallel, smaller diameter fibers attached along their lengths. A fiber bundle may exhibit diverging fibers at one or both ends.

Fibrous structure: A fiber, or connected grouping of fibers, with or without other particles.

Fine fiber: A fiber of aspect ratio greater than or equal to 3:1, longer than 5 µm.

Funnel blank: A structure count made on SEM/TEM specimens prepared by the direct-transfer method from a filter used for filtration of a sample of distilled water.

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

Limit of detection (weight percent): The fiber structure concentration in amphibole fibers in g/g or mg/mg, equivalent to the limit of detection of the balance used for weighing the sample and

the sinks fraction.

Limit of detection (suspended particles): The calculated fibrous amphibole structure concentration in structures/g, equivalent to counting of 2.99 fibrous amphibole structures in the analysis.

Matrix: A structure in which one or more fibers, or fiber bundles, touch, are attached to, or partially concealed by, a single particle or connected group of non-fibrous particles.

"Suspended Particles Fraction:" The water remaining following the washing of a sample of vermiculite attic insulation. This portion may contain suspended fibrous amphibole.

"Sinks Fraction:" The portion of a sample of vermiculite attic insulation which sinks when the material is " washed" in a container of water.

Structure: A single fiber, fiber bundle, cluster or matrix

5 ABBREVIATIONS

ED - Electron diffraction

EDS - Energy dispersive X-ray spectroscopy

MCE - Mixed cellulose ester

PC - Polycarbonate

PCM - Phase contrast optical microscopy

PLM - Polarized light microscopy

SEM - Scanning electron microscope

TEM - Transmission electron microscope

VAI - Vermiculite Attic Insulation

6 EQUIPMENT AND APPARATUS

6.1 General

General laboratory equipment, such as glass beakers, disposable pipets, disposable plastic beakers and measuring cylinders, is required, with the addition of the specific items listed below. Some analyses do not require all of the equipment listed.

Note: *Additional reagents and equipment are listed in the referenced TEM and SEM preparation methods.*

6.2 Sampling

6.2.1 Scoop, metal (approximately 12 by 5 cm) with a flat edge

6.2.2 One gallon plastic resealable bags

6.2.3 Sample labels and markers

6.2.4 Chain-of-custody forms

6.3 Sample preparation

6.3.1 Laboratory balance, sensitivity 0.0001 gram

6.3.2 Laboratory magnetic stirrer

6.3.3 Teflon coated magnetic stirrer bars

6.4 Rapid Screening Method for "Sinks Fraction" by Stereomicroscopy/PLM

6.4.1 Water aspirator

6.4.2 Stereo-binocular microscope, 10x to 40x magnification

6.4.3 Polarized light microscope

6.4.3 Drying oven, capable of drying samples at 100° C

6.4.4 Desiccator, cabinet type for drying filters

6.5 Measurement of "Sinks Fraction" and "Suspended Particles" by SEM/TEM

6.5.1 Peristaltic pump capable of pumping 15-25 mL/minute

6.5.2 Glass filtration system, 25 mm diameter

6.5.3 Transmission electron microscope, as specified in ISO 13794

6.5.4 Energy dispersive x-ray analysis system, as specified in ISO 13794

6.5.5 Scanning electron microscope, with energy dispersive x-ray analysis system as specified in EPA SRC-LIBBY-02 (Rev. 1).

6.5.6 Ultrasonic bath, calibrated by Appendix B in ISO 13794, with capacity for 1 liter beaker

7 REAGENTS

7.1 Ethanol, reagent grade

7.2 Reagent water, either freshly-distilled or deionized water, filtered through an MCE filter of maximum porosity 0.22 µm, and meeting the requirements of ASTM D 1193 for reagent water.

Note: For analyses incorporating TEM specimen preparation, it is important that the reagent water be freshly produced and filtered, in order to minimize bacterial interferences on TEM specimens.

8 SELECTION AND PRE-TREATMENT OF SAMPLE FOR ANALYSIS

8.1 Types of Sample

This method addresses specifically the analysis of vermiculite attic insulation. Other vermiculite containing materials may be analyzed using the principles of this method but appropriate modifications may be required to meet specific needs of the sample. The user should refer to the method by Chatfield for other applications, US EPA (2000).

8.2 Obtaining a Representative Sample of VAI

A 1-gallon resealable plastic bag full of VAI is collected for each sample. Care must be given to collecting a representative sample of the material. A metal scoop (approximately 12 cm by 5 cm) with a flat edge is recommended for collecting randomly spaced aliquots of VAI to make up the 1-gallon sample total. The scoop must be thrust into the VAI until it reaches the substrate, moved along the bottom, then raised through the remaining material and deposited in the sample bag. Multiple scoops of material are collected to make up the 1 gallon sample. This procedure is intended to insure that any heavy materials, such as fibrous amphiboles which may have settled in the VAI, will be sampled. A minimum of three 1-gallon samples are recommended

for each sampling site.

Vermiculite attic insulation may have a substantial water content, and so all samples shall be dried at 100° C for 2 hours and until the sample reaches constant weight before analysis. The sample shall be weighed before and after drying to obtain the weight of water, so that the final results can be expressed in terms of the original weight or dry weight of the sample.

8.3 Obtaining a Representative Sub-Sample for Analysis

If amphibole is present in VAI, the size range of the fragments of amphibole is usually approximately the same as that of the vermiculite flakes, because during the beneficiation process the material is segregated into several different size categories. The fragments of amphibole are distributed randomly throughout the VAI, and the number of these fragments is generally much lower than the numbers of vermiculite flakes. Accordingly, if a reproducible analysis is to be obtained, it is necessary to select a sub-sample of VAI sufficiently large that a statistically-valid number of the amphibole fragments are included. The weight of sub-sample required for analysis is dependent on the size grade of the vermiculite. Table 1 gives recommended approximate weights **of vermiculite** that should be used for the initial sub-sample. For products such as VAI, the material is likely to be primarily vermiculite and the weights given in Table 1 will generally apply. If additional materials have been added to or have contaminated the VAI, a visual estimate of the proportion of vermiculite in the product should be made and the starting weights in Table 1 should be proportionately increased.

Table 1. Recommended Sub-Sample Weights of Vermiculite for Analysis

Size of Vermiculite Flakes, mm	Recommended Minimum Sub-sample Weight for Analysis, grams
<2	5
>2 - <5	10
>5	50

The sub-sample shall be obtained from the original sample by the cone and quarter method. On a clean surface, such as a sheet of aluminum foil, form the sample into a cone. Using a thin flat sheet of metal or rigid plastic, divide the cone into two parts, vertically from the apex. Form either of the two fractions into a cone, and repeat the procedure until one of the separate fractions is of a suitable weight for analysis.

8.4 Pre-Treatment of Sub-Samples

This method is designed specifically for the analysis of VAI. In cases where VAI may have been mixed with or contaminated by other materials, some pretreatment of samples may be needed. For such samples, the user is referred to the original method by Chatfield, USEPA (2000).

9 PROCEDURE FOR ANALYSIS

9.1 General

A sub-sample of the vermiculite attic insulation is weighed and prepared for analysis by "washing" a sample of material in water. The expanded vermiculite will float in the water suspension and large fragments of minerals (including amphibole) will separate and sink in the suspension. The "sinks" are collected, dried, weighed, and analyzed. If no fibrous materials are found in the "sinks", the suspended particles fraction is analyzed for suspended particles. If neither the "sinks" or the "suspended particles fraction" contain fibrous amphibole material, the analysis is terminated. The rapid screening analysis using PLM as described in 9.2 should be used. The percent weight of fibrous amphibole is determined from the weight of the fibrous amphibole and the weight of the original sub-sample.

If no fibrous amphibole is found in the "sinks", it will be necessary to determine if any suspended fibrous amphibole particles are present in the "suspended particles fraction" in the wash used to separate the fibrous amphiboles from the VAI. The number of suspended fibrous amphibole particles is determined by counting and sizing each fiber by SEM/TEM. The number of suspended fibrous amphibole particles in the sample is determined from the number of suspended fibrous amphibole particles counted and the weight of the original sub-sample, as described in 9.3.

9.2 Rapid Screening Analysis to Determine the Weight Percent of Fibrous Amphibole in VAI

9.2.1 General

The rapid screening analysis is designed to determine the minimum weight percent of fibrous amphibole in a VAI sample.

9.2.2 Separation of Vermiculite from other Components by Flotation on Water

Place 800 mL of reagent water into a 1000 mL glass beaker. Using a spoon, place a portion of the VAI sub-sample into the beaker, and immerse the vermiculite several times by pushing it under the surface using the spoon. Remove the floating vermiculite (an open tea strainer works well for this application) and save it for possible additional analysis. Continue to

wash portions of the vermiculite in this manner until all of the sub-sample (weighed according to Table 1) has been treated. Carefully remove all fragments of vermiculite from the surface of the water, and allow the suspension to settle for 60 minutes. After this period of time, any amphibole fibers thicker than approximately 3 µm will have settled to the bottom of the beaker. Using a pump or syphon, transfer the supernatant liquid to a second beaker. Using ethanol, wash the sediment from the first beaker into a glass petri-dish and dry the sediment by placing the petri-dish on a slide warmer at a temperature of approximately 60°C. Use of an oven for drying the sediment is not recommended, because of the hazards associated with evaporation of ethanol in a closed environment. Transfer the sediment to a pre-weighed dish, and weigh the dish to obtain the weight of the sediment. Figure 4 shows an example of sediment after the water sedimentation procedure.

9.2.3 Optional Preparation of SEM/TEM Specimens From the Suspended Particles Fraction

If fibrous amphibole is detected visually in the sediment from the wash liquid, it can be assumed that suspended amphibole fibers are present in the suspended particles fraction. If fibrous amphibole is not detected visually in the wash liquid, there is still a possibility that suspended particles of amphibole fibers, too small for detection visually or by the stereo binocular microscope and PLM, could be present. This possibility can be confirmed or discounted by examination of particles in the aqueous suspension by SEM/TEM. Prepare analytical filters by the procedure described in 9.3.3. It is beyond the scope of this document to describe the preparation of TEM specimens from membrane filters; these procedures are fully described in ISO 13794.

9.2.4 Stereo-Binocular Microscope Examination of the "Sinks"

The "sinks" will contain particles large enough to detect with a stereo-binocular microscope. The "sinks" will include any large amphibole particles present in the original sub-sample. There are three possible outcomes which define the extent to which further analytical work on these "sinks" is necessary. The procedure shall be either (a), (b) or (c).

(a) If the sample originated from Libby, Montana, the "sinks" will likely contain a major proportion of large fiber bundles that are gray-green in color, and are easily visible under the stereo-binocular microscope at magnifications up to 40x. If a sub-sample of sufficient size was used, numerous fiber bundles should be present in the "sinks", as shown in Figure 3. The analyst will generally have no difficulty recognizing these fiber bundles. There are two options for determining the weight of fiber bundles in the "sinks," use forceps to: (1) move the fiber bundles into another previously weighed empty vessel and weigh them; or (2) remove non-fiber bundles from the sinks and weigh the remaining "fiber-bundle sinks." The analyst must determine which will be the more efficient approach. The fiber bundles picked from such "sinks" are shown in Figure 4. After the fiber bundles have been weighed, representative bundles shall be selected for identification by either PLM, SEM or TEM. The morphology, color and optical properties of the fibrous amphibole in vermiculite originating from

Figure 3. Example of Sediment or "Sinks" After Flotation of VAI
(Photo by E. J. Chatfield)

Libby are characteristic (Bandli and Gunter, 2001) (Wylie and Verkouteren, 2000), and with experience, the analyst need go no further than mounting representative fiber bundles in a high dispersion liquid of refractive index 1.630, in which the very fine fibers exhibit dispersion staining colors of magenta to gold (parallel) and blue (perpendicular). Representative fiber bundles may be examined by SEM or TEM, and the EDS spectra obtained may be used as the basis for identification. Examples of EDS spectra for Libby amphiboles are shown in Appendix A.

(b) If the sample originated from a mine other than Libby, Montana, few fibrous amphibole bundles, if any, may be observed in the "sinks" during the stereo-binocular microscope examination. However, the "sinks" may contain a large proportion of non-fibrous amphibole fragments. An example of these fragments is shown in Figure 5. Non- fibrous amphibole fragments are prismatic and may have crystal faces intersecting at angles of approximately 56° and 124°. In well-crystallized material, these angles can be recognized by examination of the ends of elongated fragments, such as shown in Figure 5. The total amount of non-fibrous amphibole may be estimated by hand-picking of fragments and weighing, using the same

Figure 4. Fibrous Amphibole Bundles Hand-Picked from Sinks after Flotation of VAI which Originated from Libby, MT.
(Scale divisions = 1 mm, Photo by E. J. Chatfield)

Figure 5. Example of Non-Fibrous Tremolite Detected in a Vermiculite Sample
(Photo by E. J. Chatfield)

procedure as defined in (a). If required, identification and quantification of the individual non-fibrous amphiboles present are best performed by SEM, since there is an overlap in the optical properties of amphiboles such as actinolite and mixtures of amphibole types may be present.

(c) One situation that sometimes occurs is that, during the stereo-microscope examination, only a few amphibole fiber bundles may be visible in the "sinks", along with fragments of non-fibrous amphiboles and other minerals. In this case, it is unlikely that random sampling of particles for either SEM particle counting or PLM examination would include any of these fiber bundles, and a false-negative result would be reported. If the aggregate of the amphibole fiber bundles is within the range of the laboratory balance, the best approach is to pick them from the "sinks" and weigh them. The statistical validity of the calculated concentration may be limited by the low number of fiber bundles.

If it is found that the aggregate weight of the fiber bundles is below the sensitivity of the balance, it is necessary to approximate their weight concentration by other methods. A more sensitive micro-balance may be used when available. But when a micro-balance is not available, two approaches to determining an estimate of the fibrous amphibole concentration are available, as described in (1) and (2).

(1) an estimate of the upper limit of the fibrous amphibole concentration may be made by assuming the sensitivity of the balance as the weight of fibrous amphibole. In many cases, this may be sufficient for the purpose;

(2) An approximation of the number of particles in the "sinks" may be made by estimation of the average particle size and assuming that they all have a density of ~ 3.1. The weight percentage of any observed fibrous amphibole fiber bundles may then be approximated by a simple ratio of the number of fibrous amphibole bundles to the calculated number of particles in the "sinks". This approach yields only an approximation of the concentration.

In the event that a low concentration of fibrous amphibole is reported, representative fiber bundles shall be identified either by PLM, SEM, or TEM. In the majority of cases, general identification of fibrous amphibole (not individual amphibole species) can be identified satisfactorily by PLM alone.

9.3 Determination of Concentration of Suspended Fibrous Amphibole Particles by Electron Microscopy

9.3.1 Introduction

In this procedure, the suspended particles fraction saved from Section 9.2.2 is analyzed. SEM/TEM specimens are prepared from aliquots taken from the suspension, and the balance of the suspension is filtered through a pre-weighed filter. After drying, the filter is weighed to determine the total weight of suspended particles. Readily-available laboratory apparatus is used to perform this measurement.

9.3.2 Separation of Suspended Particles

After all of the floating vermiculite has been removed and the suspended particles fraction decanted from the "sinks," make the suspension up to a volume of 1 liter using reagent water. Place the beaker into a calibrated ultrasonic bath for 2 minutes. Remove the beaker from the ultrasonic bath, and mix the contents by air bubbling using filtered air.

9.3.3 Preparation of SEM/TEM Specimens From "Suspended Particles Fraction"

Filtration of the aqueous suspension is a very critical procedure because it is important to obtain uniform deposits of particulate on the analytical filters. The following procedure shall be used.

(a) Set up the filtration system and connect to a vacuum source;

(b) Add freshly distilled water to the filtration unit base component until there is a raised meniscus;

c) Place a 5 µm pore size cellulose ester filter on to the water meniscus. The filter will centralize. Apply the vacuum very briefly in order to bring the filter into contact with the base component;

(d) Add freshly distilled water to the top of the cellulose ester filter, and place the analytical filter (either a 0.2 µm maximum pore size capillary-pore polycarbonate filter or a 0.22 µm maximum pore size cellulose ester filter) on to the water surface. Apply the vacuum very briefly again in order to bring both filters into contact with the base component;

(e) Install the filtration reservoir and clamp the assembly together.

(f) Before filtering the aqueous suspensions, prepare a funnel blank by filtration of 40 mL of freshly-distilled water. This sample is a control to ensure that the filtration equipment is clean and the reagent water is not contaminated by fibers.

(g) The volume of the aqueous suspension to be filtered depends on either the particulate concentration or the amphibole fiber concentration. The volume of the aqueous suspension required to produce an analytical filter with a suitable particulate or fiber loading for analysis often cannot be predicted, and it is usually necessary to prepare and examine several analytical filters corresponding to filtration of different aliquots. The number of grid openings on the TEM specimens that require examination in order to achieve a particular analytical sensitivity are shown in Table 2.

(h) The aqueous suspensions are generally not stable; it is therefore necessary to prepare all analytical filters immediately. Uniform deposits of particulate on the analytical filters cannot be assured if liquid volumes smaller than 5 mL are filtered using filtration systems of 199 mm^2 active area; accordingly, where it is required to filter volumes smaller than 5 mL, the aliquot shall be diluted with freshly-distilled and filtered water to a volume exceeding 5 mL.

(I) Pour the aliquot of the suspension into the filtration reservoir, and apply the vacuum. If the volume of the aliquot is larger than the capacity of the filtration reservoir, do not allow the level of liquid in the reservoir to fall below 5 cm depth before the remaining volume is added. Failure to observe this precaution may result in disturbance of the filtered particulate and non-uniform deposition.

(j) With the vacuum still applied, unclamp the filtration assembly and remove the filtration reservoir. Using clean tweezers, remove the analytical filter and transfer it to a petri-dish. Allow the filter to air dry before placing the cover on the petri-dish.

(k) For the beaker blank, prepare only one analytical filter by filtration of the entire 40 mL suspension.

Table 2. Examples of the minimum number of grid openings of TEM specimens required to be examined to achieve a particular analytical sensitivity and limit of detection.

Analytical sensitivity (10^6 Fibers/g)	Limit of detection (10^6 Fibers/g)	Volume of Suspension Filtered (mL)				
		0.01	**0.03**	**0.1**	**0.3**	**1**
0.1	0.3	551	184	56	19	6
0.2	0.6	276	92	28	10	4
0.3	0.9	184	62	19	7	4
0.4	1.2	138	46	14	5	4
0.5	1.5	111	37	12	4	4
0.7	2.1	79	27	8	4	4
1	3	56	19	6	4	4
2	6	28	10	4	4	4
3	9	19	7	4	4	4
4	12	14	5	4	4	4
5	15	12	4	4	4	4
7	21	8	4	4	4	4
10	30	6	4	4	4	4

NOTES

In Table 2, it is assumed that the initial sample weight was 50 grams, the volume of water used to disperse the sample is 1 liter, the active area of the analytical filter is 199 mm^2, and the TEM grid openings are square with a linear dimension of 85 µm. The limit of detection is defined as the upper 95% confidence limit of the Poisson distribution for a count of zero structures. In the absence of background, this is equal to 2.99 times the analytical sensitivity. Non-zero backgrounds observed during analysis of blank filters will degrade the limit of detection.

NOTES

It is recommended to prepare several analytical filters from the suspension. If the particulate or fiber concentration is thought to be such that it is required to filter an aliquot of lower volume than 1 mL, use a dilution procedure in which 1 mL of the original suspension is transferred to a clean beaker and diluted with freshly-distilled water to a total volume of 100 mL. After stirring to ensure complete mixing, aliquots of 1 mL, 3 mL, 10 mL and 30 mL from this diluted suspension can then be filtered, corresponding to volumes of 0.01 mL, 0.03 mL, 0.1 mL and 0.3 mL of the original suspension. From the original dispersion, volumes of 1 mL and 3 mL can also be filtered, giving 6 analytical filters with a concentration range of a factor of 300. The requirement for washing of the filtration apparatus is minimized if the aliquots are filtered in order of increasing concentration.

It is beyond the scope of this method to provide detailed instructions for preparation of TEM specimens from membrane filters; these instructions are published in ISO 13794. It is recommended that aliquots of the aqueous suspension of vermiculite be filtered using the method specified in ISO 13794. Blank filters shall be checked from each lot of filters used, or the individual filters if polycarbonate filters are used they may be cleaned to remove the chrysotile,

amosite or crocidolite asbestos contamination reported to be present on this type of filter (Chatfield, 2000, and Webber, 2003). Prepare TEM specimens from the filters using the methods specified in ISO 13794. Prepare SEM specimens from the filters using the methods specified in USEPA SOP No. SRC-LIBBY-02 (Rev.1).

Blank filters shall be checked from each lot of filters used, or if polycarbonate filters are used, individual filters may be cleaned to remove asbestos contamination (Chatfield….Webber..)" Discussion and micrographs of polycarbonate filter contamination can also be found in Millette, J.R., Few, P., and Krewer, J.A., " Asbestos in Water Methods: EPA's 100.1 & 100.2 and AWWA's Standard Method 2570," Advances in Environmental Measurement Methods for Asbestos, ASTM STP 1342, M.E. Beard and H.L. Rook, Eds., American Society for Testing and Materials, 2000.

9.3.4 Examination of TEM Specimens

The number of fibrous amphiboles are counted from 10 grid openings of the TEM grid. Criteria for examination of TEM specimens are specified in ISO 10312 and ISO 13794. For the purpose of VAI analysis, only fibrous amphibole structures longer than 0.5 µm need be considered. The above ISO Standards specify that a magnification of approximately 10,000 is sufficient for determination of the concentration of asbestos structures longer than 5 µm. Classify amphiboles according to the International Mineralogical Association classification (Leake, 1997). A classification may also be obtained using procedures described by Meeker (Meeker, 2003)

9.3.5 Examination of SEM Specimens

Criteria for examination of SEM specimens are specified in USEPA SOP No. SRC-LIBBY-02 (Rev.1).

10 DATA REPORTING

All samples must report the sample identity, the date of analysis, and the analyst.

10.1 Rapid Screening Analysis of "Sinks" to Determine Minimum Weight Percent of Fibrous Amphibole

In the test report, all relevant measurements shall be reported, including:

(a) Initial weigh of the sub-sample;
(b) Weight loss on drying (if applicable);
©) Weight of "sinks" after water separation;
(d) Weight of hand-picked fibrous amphibole;
(e) Assumed sensitivity of the chemical balance;
(f) Identity of the fibrous amphibole in (d) and the method of determination including range of α and γ refractive indices for PLM analyses
(g) Weight percent of fibrous amphibole in the original sub-sample.

10.2 Concentration of Fibrous Amphiboles in the "Suspended Particles Fraction"

In the test report, all relevant measurements shall be reported, including:

(a) Sample identification
(b) Volume of suspension
(c) Volume filtered
(d) Volume filtered for mass determination
(e) Weight of filtered material
(f) Area of filter (effective filtration area)
(g) Area examined (area of field of view and number of fields examined)
(h) Magnification
(i) Number of fibrous amphibole particles counted
(j) Concentration of fibrous amphibole particles in the original sub-sample.

11 ACCURACY AND PRECISION

The accuracy and precision of this method has not been determined. A study will be designed in the future to provide data for determining these values.

11.1 Concentration of "Sinks" to Determine Weight Percent of Fibrous Amphibole

The accuracy of this analysis is limited only by transfer losses during processing, and by the sensitivity of the laboratory balance. The precision is limited by the initial size of the sub-sample, and the statistical effects of large amphibole fiber bundles when there are only small numbers present, or when one or more fiber bundles represent a large proportion of the weight of amphibole detected.

11.2 Concentration of Fibrous Amphiboles in "Suspended Particles Fraction"

There is no independent method to establish the accuracy of measurements of the concentration of suspended particles. The precision of measurements, for measurements based on water suspensions of fibers, is usually limited by the Poisson distribution if filtrations are performed using the specified procedures. Accordingly, the precision can be improved by examination of greater areas of the SEM/TEM specimens in order to collect data on larger numbers of fibers.

12 QUALITY ASSURANCE

Specific quality assurance procedures for measurement of fibrous amphiboles in VAI are under development. Reference materials are needed to allow an assessment of the performance of this method. Until these reference materials are available laboratories should consider using the extensive quality assurance procedures from established programs based upon the principles described in ISO 17025, such as the NIST/NVLAP Asbestos Fiber Analysis Program for PLM

and TEM, the AIHA Bulk Asbestos Proficiency Testing Program, the New York State Laboratory Accreditation Program for Asbestos. Investigators using laboratory services should consider collection of blind replicate samples and analyses by an independent laboratory as a minimum. When reference materials become available, the investigator should consider including these materials as blind samples when submitting samples for analysis.

13 REFERENCES

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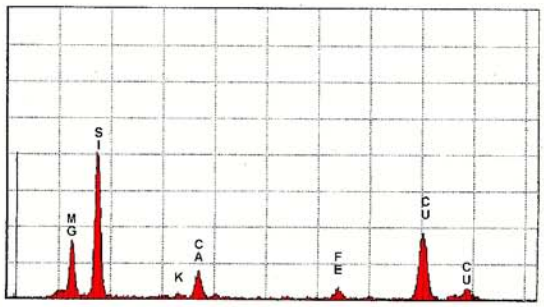
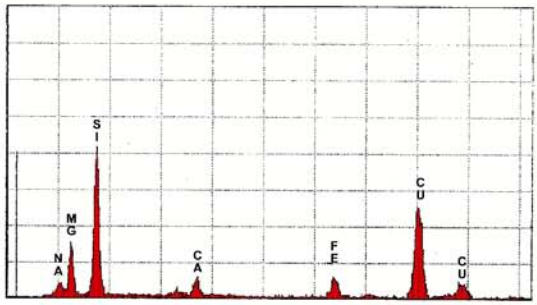
APPENDIX A: **Examples of EDS spectra from Libby amphibole (Spectra by W.B. Hill, MVA, Inc.)**

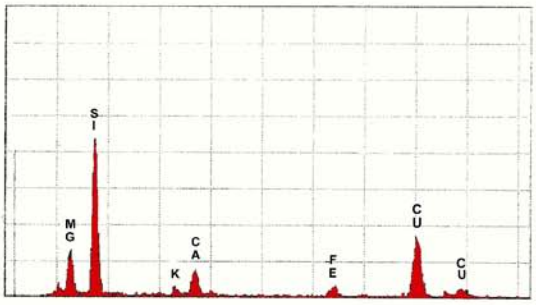
Reference Spectra For Libby Amphiboles



Libby Amphibole - Low Sodium TEM: 100kv MVA, Inc.



Libby Amphibole - High Sodium TEM: 100kv MVA, Inc.



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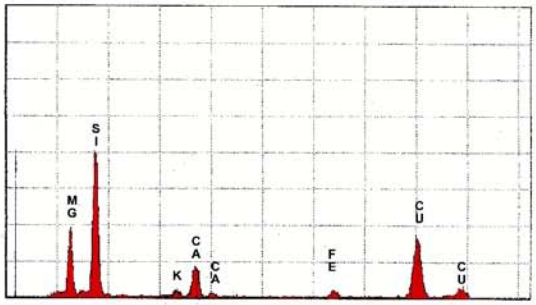

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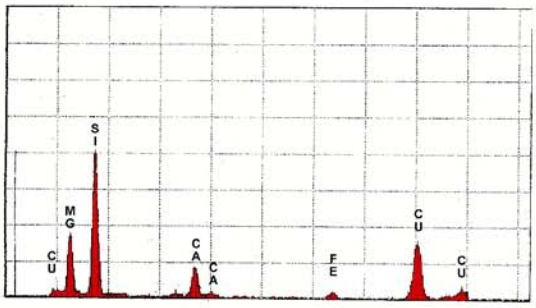
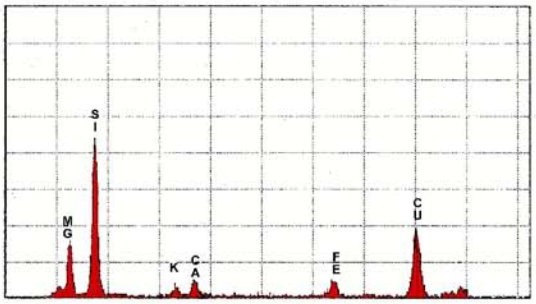
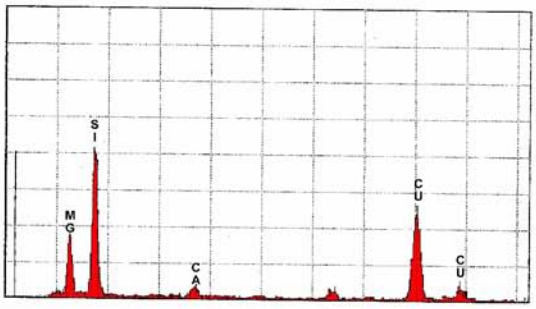


Libby Amphibole - Low Potassium TEM: 100kv MVA, Inc.

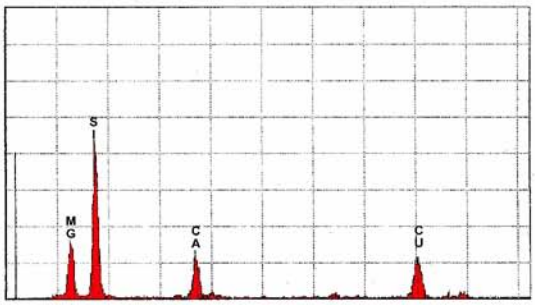


Libby Amphibole - High Potassium TEM: 100kv MVA, Inc.

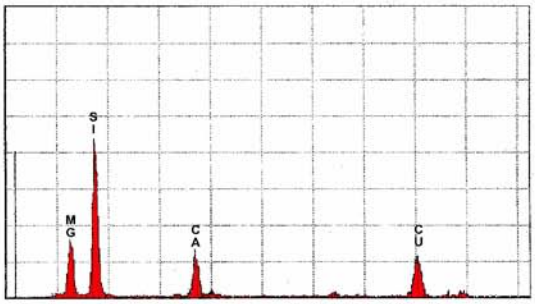
Reference Spectra For Libby Amphiboles



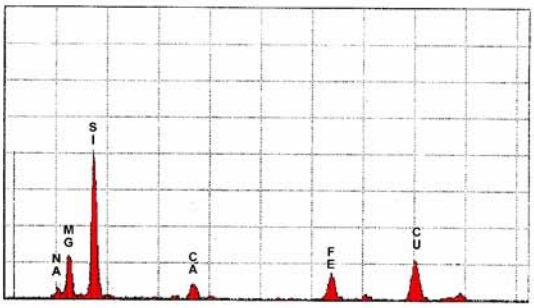
Libby Amphibole - Low Calcium TEM: 100kv MVA, Inc.



Libby Amphibole - High Calcium TEM: 100kv MVA, Inc.



Libby Amphibole - Low Iron TEM:100kv MVA, Inc.



Libby Amphibole - High Iron TEM: 100kv MVA, Inc.