



Formulae: Various MW: Various CAS: Various RTECS: Various

METHOD: 7402, Issue 3 EVALUATION: PARTIAL Issue 1: 15 May 1989
Issue 3: 1 August 2022

OSHA: 0.1 f/cc asbestos fibers (>5 µm) [1]
MSHA: 1 f/cc/30 min excursion; carcinogen; 0.1 asbestos fibers/cc, 8-hr TWA (>5 µm) [2]
NIOSH: 1 f/cc/30 min excursion; carcinogen; 0.1 f/cc (>5 µm) [3]
OTHER OELS: [4,5]

PROPERTIES: solid, fibrous, elongate mineral particles (EMP), crystalline, anisotropic

SYNONYMS [CAS #]: actinolite [77536-66-4] or ferroactinolite [15669-07-5]; amosite [12172-73-5]; anthophyllite [77536-67-5]; serpentines: (chrysotile [12001-29-5]; antigorite [12168-92-2]; lizardite [12161-84-1]); crocidolite [12001-28-4]; tremolite [77536-68-6]; amphibole asbestos [1332-21-4].

SAMPLING		MEASUREMENT	
SAMPLER:	FILTER, 25-mm diameter cellulose ester membrane, 0.45- to 1.2-µm pore size, conductive cassette	TECHNIQUE:	MICROSCOPY, TRANSMISSION ELECTRON (TEM)
FLOW RATE:	0.5 to 16 L/min	ANALYTE:	Asbestos fibers
VOL-MIN:	400 L @ 0.1 fiber/cc (Step 4, sampling)	SAMPLE PREPARATION:	Modified Jaffe wick
-MAX*:	*Adjust for 100 to 1300 fibers/mm ²	EQUIPMENT:	Transmission electron microscope; energy dispersive X-ray system (EDX) analyzer
SHIPMENT:	Routine, pack to reduce shock. NOTE: Do not use untreated polystyrene foam or other static charge-inducing packing materials.	CALIBRATION:	Qualitative electron diffraction; calibration of TEM magnification and EDX system
SAMPLE STABILITY:	Stable	RANGE:	100 to 1300 fibers/mm ² filter area [6]
BLANKS:	2 to 10 field blanks minimum per set	ESTIMATED LOD:	1 confirmed asbestos fiber above 95% of expected mean blank value
ACCURACY		PRECISION (\bar{S}_r):	0.28 when 65% of fibers are asbestos; 0.20 when adjusted fiber count is applied to phase contrast microscopy (PCM) count [7]
RANGE STUDIED:	80 to 100 fibers counted		
BIAS:	Not determined		
OVERALL PRECISION (\hat{S}_{RT}):	See EVALUATION OF METHOD		
ACCURACY:	Not determined		

APPLICABILITY: The working range is 0.04 to 0.5 fiber/cc for a 1000-L air sample. The LOD depends upon sample volume and quantity of interfering dust and is <0.01 fiber/cc for atmospheres free of interferences. This method is not designed for quantification of the air concentration of asbestos fibers, alone; it determines the fraction of fibers that are asbestos from a filter prepared and analyzed following NIOSH 7400, Asbestos and other fibers by PCM [8]. The method is not meant as a confirmatory method, but rather an investigative or modifiable research method.

INTERFERENCES: Other amphibole particles that have aspect ratios greater than 3:1 and elemental compositions similar to the asbestos minerals may interfere in the TEM analysis. Some non-regulated amphibole minerals may give electron diffraction patterns similar to regulated amphiboles. High concentrations of background dust interfere with fiber identification.

OTHER METHODS: This method is designed for use with NIOSH 7400, Asbestos and other fibers by PCM [8]. If a confirmatory method is needed, it is recommended to use an EPA level III [9] analysis or a current equivalent method for asbestos identification. NOTE: Though the intent and scope here is for regulated asbestos, project specific or individual investigators may want to further define (exclusive) or expand (inclusive) to include other target elongate particles of interest.

REAGENTS:

1. Acetone, reagent grade*.
NOTE: Dimethylformamide (DMF) 35%, reagent grade / Glacial acetic acid 15% / distilled water / 50% solution can be used as an alternative clearing agent.
2. Asbestos standard bulk reference materials: NIST Standard Reference Material (SRM) 1866b, 1867, 1876a or suitable substitutes when not available.

*See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Sampler: field monitor, 25-mm, three-piece cassette with ca. 50-mm electrically-conductive extension cowl, cellulose ester membrane filter, 0.45- to 1.2- μ m pore size, and backup pad.
NOTE 1: Analyze representative filters for fiber background before use. Discard the filter lot if mean count is >5 fibers/100 fields.
NOTE 2: Use an electrically-conductive extension cowl to reduce electrostatic effects on fiber sampling and during sample shipment. Ground the cowl when possible during sampling.
NOTE 3: Pore size choice depends on sampling time requirements, air flow rate, pump loading, amount of dust in the air, battery capacity, etc.
2. Personal sampling pump, 0.5 to 16 L/min, with flexible connecting tubing
3. Microscope, transmission electron, operated at ca. 100 kV, with electron diffraction, energy-dispersive X-ray capabilities, and having a fluorescent screen with inscribed or overlaid calibrated scale (Step 15).
NOTE 1: The scale is most efficient if it consists of a series of lines inscribed on the screen or partial circles every 2 cm distant from the center.
NOTE 2: A digital camera in conjunction with image analysis software may be used for asbestos fiber measurement and recording.
NOTE 3: Measurement may be further facilitated with built in electronic measuring Heads Up Devices (HUD), artificial intelligence (AI), etc. when available. All such innovations should be calibrated, and operation checked before use.
4. Diffraction grating replica with known number of lines/mm.
5. Slides, glass, pre-cleaned, 25- x 75-mm.
6. Knife, surgical steel, curved blade.
7. Tweezers.
8. Flash vaporization system for clearing

- filters on glass slides using acetone. Use a drying oven or warming plate located in fume cabinet if using DMF/glacial acetic acid.
9. Indexed grids, 200-mesh TEM copper, (optional: carbon-coated).
 10. Petri dishes, 15-mm depth. The top and bottom of the petri dish must fit snugly together. To ensure a tight fit, grind the top and bottom pieces together with an abrasive such as carborundum to produce a ground-glass contact surface (if using acetone).
 11. Foam, clean polyurethane, spongy, 12-mm thick, 100 mesh stainless steel screen.
NOTE: These components depend on your Jaffe wick washer design.
 12. Filters, Whatman No. 1 qualitative paper or equivalent, or lens paper.
 13. High carbon vacuum evaporator (HVCE) with rotating stage.
NOTE: A carbon thread evaporator may also be used that has a vacuum better than 10^{-5} torr and rotating stage.
 14. Cork borer, about 8-mm, or scalpel and blades.
 15. Pen, waterproof, marking.
 16. Reinforcement, page, gummed.
 17. Carbon rods, sharpened to 1-mm x 8-mm.
NOTE: Carbon thread can be used.
 18. Microscope, light, phase contract (PCM), with Walton-Beckett graticule (see NIOSH 7400).
 19. Grounding wire, 22- gauge, multi-strand.
 20. Tape, shrink, or adhesive.

SPECIAL PRECAUTIONS: Acetone is extremely flammable (flash point = 0 °F). Take precautions not to ignite it. Heating of acetone must be done in a fume hood using a flameless, spark-free heat source. Asbestos is a confirmed human carcinogen, handle only in a well-ventilated fume, HEPA, or class I laminar flow hood (none of which should recirculate the air), with lab coat, goggles, and chemical resistant gloves.

SAMPLING, SAMPLE TRANSPORT, AND STORAGE:

Refer to the NIOSH 582 course or modern equivalent that is being offered by outside organizations for more details about sampling.

1. Calibrate each personal sampling pump with a representative sampler in-line [10].
2. For personal sampling, fasten sampler to worker's lapel near worker's mouth. Remove the top cover from cowl extension ("open-face") and orient sampler face down. Wrap joint between extender and monitor body with tape to help hold the cassette together and provide a marking surface to identify the cassette. Where possible, especially at low % relative humidity, attach sampler to electrical ground to reduce electrostatic effects during sampling.
3. Submit at least two field blanks (or 10% of the total samples, whichever is greater) for each set of samples. Remove top covers from the field blank cassettes and store top covers and cassettes in a clean area (e.g., closed bag or box) during sampling. Replace top covers when sampling is completed.
4. Sample at an accurately known flow rate between 0.5 and 16 L/min [11]. Adjust sampling rate, Q (L/min), and time, t (min), to produce fiber density, E, of 100 to 1300 fibers/mm² [3.85×10^4 to 5×10^5 fibers per 25-mm filter with effective collection area ($A_c=385 \text{ mm}^2$)] for optimum accuracy. These

variables are related to the action level (one-half the current standard), L (fiber/cc), of the fibrous aerosol being sampled by:

$$t = \frac{A_c \times E}{Q \times L \times 10^3}, \text{min.}$$

NOTE 1: The purpose of adjusting sampling times is to obtain optimum fiber loading on the filter. A sampling rate of 1 to 4 L/min for 8 h (700 to 2800 L) is appropriate in atmospheres containing ca. 0.1 fiber/cc in the absence of significant amounts of non-asbestos dust. Dusty atmospheres require smaller sample volumes (≤ 400 L) to obtain countable samples. In such cases take short, consecutive samples and average the results over the total collection time. For documenting episodic exposures, use high rates (7 to 16 L/min) over shorter sampling times. In relatively clean atmospheres, where targeted fiber concentrations are much less than 0.1 fiber/cc, use larger sample volumes (3000 to 10000 L) to achieve quantifiable loadings. Take care, however, not to overload the filter with background dust [11].

NOTE 2: For a grid opening to be read, it should be 95% intact and have a loading of no more than 25%. If a grid is less than 50% intact, the support film is doubled or folded, has very uneven loading, or more than 50% of grid openings have incomplete filter dissolution it should be rejected.

5. At the end of sampling, replace top cover and small end caps.
6. Ship samples upright with conductive cowl attached in a rigid container with packing material to prevent jostling and damage.

NOTE: Do not use untreated polystyrene foam in the shipping container because electrostatic forces may cause fiber loss from sample filter.

SAMPLE PREPARATION:

7. Remove circular sections from any of three quadrants of each sample and blank filters using a cork borer [12]. The use of three grid preparations reduces the effect of local variations in dust deposit on the filter.

NOTE 1: An alternative preparation method [8] may be followed:

Cut wedges of about 1/8 to 1/4 for a 25-mm diameter filter, using a curved-blade surgical steel knife with a rocking motion to prevent tearing, while the filter is still in the base of the cassette (preferred method; minimizes potential disturbance of dust on the filter). The entire filter can be used instead of a wedge, but with the understanding that the whole sample is lost if there is a problem in preparation. If the prepared grids are lost or damaged, there can be no QA/QC follow-up. Mounted filters on slides prepared for the NIOSH 7400 PCM method cannot be used or somehow re-prepped to work with this method. Place the wedge or entire filter, dust side up, on a slide using forceps. To prevent cross-contamination of samples, blades should be cleaned between samples.

NOTE 2: Include a blank filter with each batch of filters processed to serve as a lab blank.

8. Affix the circular filter sections (if using a cork borer) to a clean glass slide with a gummed page reinforcement or equivalent. Label the slide with a waterproof marking pen.
NOTE: Up to eight filter sections may be attached to the same slide.
9. Place the slide in a petri dish which contains several paper filters soaked with 2 to 3 mL acetone. Cover the dish. Wait 2 to 4 min for the sample filter(s) to fuse and clear.
NOTE: The "hot block" clearing technique [13] or the DMF clearing technique [14] (35% DMF, 15% Glacial acetic acid, 50% distilled water) of NIOSH 7400 PCM may be used instead of Steps 8 and 9.
10. Transfer the slide to a rotating stage inside the bell jar of a vacuum evaporator or other carbon coating device. Evaporate a 1- x 5-mm section of a graphite rod onto the cleared filter(s). Remove the slide to a clean, dry, covered petri dish [12].
NOTE: A carbon thread evaporator may also be used that has a vacuum better than 10^{-5} torr.
11. Prepare a second petri dish as a Jaffe wick washer, or similar design, with the wicking substrate prepared from filter or lens paper placed on top of a 12-mm thick disk of clean, spongy polyurethane foam [9].
NOTE: Some designs of the Jaffe wick washer use a 100 mesh stainless steel screen as in [9]

Cut a V-notch on the edge of the foam and filter paper. Use the V-notch as a reservoir for adding solvent.

NOTE: The wicking substrate should be thin enough to fit into the petri dish without touching the lid.

12. Place the TEM grid on the filter or lens paper. Label the grids by marking with a pencil on the filter paper or by putting registration marks on the petri dish halves and marking with a waterproof marker on the dish lid. In a well ventilated fume, HEPA, or class I laminar flow hood, fill the dish with acetone until the wicking substrate is saturated.

NOTE 1: The level of acetone should be just high enough to saturate the filter paper without creating puddles.

NOTE 2: DMF usage is preferred instead of acetone.

13. Remove the section of the carbon-coated filter from the glass slide using a razor blade and tweezers. Carefully place the excised filter, carbon side down, on the appropriately labeled grid in the acetone-saturated petri dish. When all filter sections have been transferred, slowly add more solvent to the wedge-shaped trough to raise the acetone level as high as possible without disturbing the sample preparations. Cover the petri dish. Elevate one side of the petri dish by placing a slide under it (allowing drops of condensed acetone to form near the edge rather than in the center where they would drip onto the grid preparation).

NOTE: DMF usage is preferred instead of acetone.

CALIBRATION AND QUALITY CONTROL:

The laboratory should follow one of the TEM asbestos analysis QA/QC checklists published by NIST NVLAP [15] or AIHA LAP [16], or others as is deemed most appropriate by laboratory management.

14. Determine the TEM magnification on the fluorescent screen:
 - a. Define a field of view on the fluorescent screen either by markings or physical boundaries.

NOTE 1: The field of view must be measurable or previously inscribed with a scale or concentric circles (all scales should be metric) [9].

NOTE 2: For instruments where a digital camera is used for measurements, these markings are not necessary.
 - b. Insert a diffraction grating replica into the specimen holder and place into the microscope. Orient the replica so that the grating lines fall perpendicular to the scale on the TEM fluorescent screen. Ensure that goniometer stage tilt is zero.

NOTE: For calibration with a digital camera, the grating orientation is not necessary.
 - c. Adjust microscope magnification to 10,000X. Measure the distance (mm) between the same relative positions (e.g., between left edges) of two widely separated lines on the grating replica. Count the number of spaces between the lines.

NOTE 1: On most microscopes the magnification is constant (1-3%) only within the central 8-cm to 10-cm diameter region of the fluorescent screen. Compare magnification values of your microscope between the central and outer regions of the fluorescent screen and use an area where the variation is less than 3%.

NOTE 2: For a digital camera, use the built-in camera software measuring device or other software.
 - d. Calculate the true magnification (M) on the fluorescent screen: $M = (X \times G)/Y$
 Where: X = total distance (mm) between the two grating lines;
 G = calibration constant of the grating replica (lines/mm);
 Y = number of grating replica spaces counted
 - e. After calibration, note the apparent sizes of 0.25 and 5.0 μm on the fluorescent screen. (These dimensions are the boundary limits for counting asbestos fibers by phase contrast microscopy.)
15. Measure 20 grid openings at random on a 200-mesh finder copper grid by placing a grid on a glass slide and examining it under the PCM. Use the Walton-Beckett graticule to measure the grid opening dimensions. Calculate an average graticule field dimension from the data and use this number to calculate the graticule field area for an average grid opening.

NOTE: A grid opening is considered as one graticule field.
16. Obtain reference selected area electron diffraction (SAED) from standard asbestos materials prepared for TEM analysis.

NOTE: This is a visual reference technique. No quantitative zone-axis analysis is required [9].

- a. Set the specimen holder at zero tilt.
- b. Center a fiber, focus, and center the smallest field-limiting aperture on the fiber. Obtain a diffraction pattern. Record each distinctive pattern and keep the image for comparison to unknowns.

NOTE: Not all fibers will present diffraction patterns. The objective lens current may need adjustment to give optimum pattern visibility. There are many more amphiboles which give diffraction patterns similar to the analytes named on p. 7402-1. Some, but not all, of these can be eliminated by chemical separations. Also, some non-amphiboles (e.g., pyroxenes, some talc fibers) may interfere.

17. Acquire energy-dispersive X-ray (EDX) spectra on approximately 5 fibers having diameters between 0.25 and 0.5 μm of each asbestos variety obtained from standard reference materials [9].

NOTE 1: The sample may require tilting to obtain adequate signal. Use same tilt angle for all spectra.

NOTE 2: Acquiring a laboratory's own individual reference EDX spectra from asbestos reference materials is particularly important with more efficient EDX detectors coming into use and the differing designs now available.

- a. Prepare TEM grids of all asbestos varieties.
- b. Use acquisition times (at least 100 sec) sufficient to show a silicon peak at least 75% of the monitor screen height at a vertical scale of ≥ 500 counts per channel.
- c. Estimate the elemental peak heights visually as follows:
 - 1) Normalize all peaks to silicon (assigned an arbitrary value of 10).
 - 2) Visually interpret all other peaks present and assign values relative to the silicon peak.
 - 3) Determine an elemental profile for the fiber using the elements Na, Mg, Si, Ca, and Fe. Example: 0-4-10-3-<1 [9].

NOTE: For fibers or elongate particles other than asbestos, determination of other peak heights may also be performed if they are of interest to the laboratory.
- 4) Determine a typical range of profiles for each asbestos variety from reference standards and record the profiles for comparison to unknowns.

MEASUREMENT:

18. Perform a diffraction pattern inspection on all sample fibers counted under the TEM, using the procedures given in Step 17. Assign the diffraction pattern to one of the following structures:
 - a. chrysotile;
 - b. amphibole;
 - c. ambiguous;
 - d. none.

NOTE: There are some crystalline substances which exhibit diffraction patterns similar to those of regulated asbestos fibers. Many of these, (brucite, halloysite, etc.) can be eliminated from consideration by chemistry. There are, however, several minerals (e.g., pyroxenes, massive amphiboles, and talc fibers) which are chemically similar to regulated asbestos and can be considered interferences. The presence of these substances may warrant the use of a level III [9] analysis or modern equivalent before positive identification can be made. If interferences are suspected, morphology can play an important role in making positive identification.

19. Obtain Energy Dispersive Spectra (EDX) in either the TEM or STEM modes from fibers on field samples using procedures in Step 17c. Using the diffraction pattern and EDX spectrum, classify the fiber:
 - a. For a chrysotile structure, obtain EDX spectra on the first five fibers and one out of ten thereafter. Label the range profiles (Na Mg Si Ca Fe) from 0-5-10-0-0 to 0-10-10-0-0 as "chrysotile."
 - b. For an amphibole structure, obtain EDX spectra on the first 10 fibers and one out of ten thereafter. Label element profiles ca. 0-2-10-0-7 as "possible amosite"; profiles ca. 1-1-10-0-6 as "possible crocidolite"; profiles ca. 0-4-10-3-<1 as "possible tremolite"; profiles ca. 0-3-10-0-1 as "possible anthophyllite", and "possible actinolite" ca. 0-4-10-3-1 to 0-1-10-3-3.

NOTE 1: Use laboratory values from reference samples obtained in Step 17 to within ± 1 instead of the above values for analysis. This is due to the use of silicon-drift detectors (and future

detector technologies) coming into common usage, which have increased efficiency in the lower energy range and the variety of detector designs now in use that have differing low energy efficiencies than those EDX detectors used in the 1980's.

NOTE 2: The "possible" designation is used only for EDX analysis. Evaluation of the combined results of SAED, EDX, and morphology determines the designation given in step 21.

- c. For an ambiguous structure, obtain EDX spectra on all fibers. Label profiles similar to the chrysotile profile as "possible chrysotile." Label profiles similar to the various amphiboles as "possible amphiboles." Label all others as "unknown" or "non-asbestos."

20. Counting and Sizing:

- a. Insert the sample grid into the specimen grid holder and scan the grid at zero tilt at low magnification (ca. 300 to 500X). Ensure that the carbon film is intact and unbroken over ca. 75% of the grid openings.
- b. In order to determine how the grids should be sampled, estimate the number of fibers per grid opening during a low-magnification scan (500 to 1000X). This will allow the analyst to cover most of the area of the grids during the fiber count and analysis. Use the following rules when picking grid openings to count [9]:
 - 1) Light loading (<5 fibers per grid opening): count total of 40 grid openings.
 - 2) Moderate loading (5 to 25 fibers per grid opening): count minimum of 40 grid openings or 100 fibers.
 - 3) Heavy loading (>25 fibers per opening): count a minimum of 100 fibers and at least 6 grid openings.

Note that these grid openings should be selected approximately equally among the three grid preparations and as randomly as possible from each grid.

- c. Count only grid openings that have the carbon film intact. At 400 to 1000X magnification, begin counting at one end of the grid and systematically traverse the grid by rows, reversing direction at row ends.

NOTE: Counting should be done at the same magnification as the original NIOSH 7400 PCM count. Select the number of fields per traverse based on the loading indicated in the initial scan. Count at least 2 field blanks per sample set to document possible contamination of the samples. Count fibers using the following rules:

- 1) Count all particles with diameter greater than 0.25 μm that meet the definition of a fiber (aspect ratio $\geq 3:1$, longer than 5 μm). Use the guideline of counting all fibers that would have been counted under phase contrast light microscopy (NIOSH 7400 PCM). Use higher magnification (10,000X-20,000X) to determine fiber dimensions and countability under the acceptance criteria. Analyze a minimum of 10% of the fibers by EDX and SAED at higher magnification (10,000X-20,000X) until three fibers have been identified as asbestos in order to confirm the presence of asbestos. Afterwards, fibers with the same morphology and EDX spectra under high magnification as the previously identified asbestos can be identified as asbestos without SAED, but every tenth fiber should be analyzed with EDX and SAED for the remainder of the counting. Particles which are of questionable morphology should be analyzed by SAED and EDX to aid in identification.
- 2) Count fibers which are partially obscured by the grid as half fibers.

NOTE: If a fiber is partially obscured by the grid bar at the edge of the field of view, count it as a half fiber only if more than 2.5 μm of fiber is visible.
- 3) Size each fiber as it is counted and record the diameter and length:
 - i. Move the fiber to the center of the screen. Read the length of the fiber directly from the scale on the screen.

NOTE 1: Data can be recorded directly off the screen and later converted to μm by computer.

NOTE 2: For fibers which extend beyond the field of view, the fiber must be moved and superimposed upon the scale until its entire length has been measured.
 - ii. When a fiber has been sized, return to the lower magnification and continue the traverse of the grid area to the next fiber.

- d. Record the following fiber counts:

- 1) f_s, f_b = number of asbestos fibers in the grid openings analyzed on the sample filter and corresponding field blank, respectively.
- 2) F_s, F_b = number of fibers, regardless of identification, in the grid openings analyzed on the sample filter and corresponding field blank, respectively.

CALCULATIONS:

21. Calculate and report the fraction of optically visible asbestos fibers on the filter, $(f_s - f_b)/(F_s - F_b)$. Apply this fraction to fiber counts obtained by PCM on the same filter or on other filters for which the TEM sample is representative. The final result is an asbestos fiber count. The type of asbestos present should also be reported.
22. As an integral part of the report, give the model and manufacturer of the TEM as well as the model and manufacturer of the EDX system.

EVALUATION OF METHOD:

The TEM method, using the direct count of asbestos fibers, has been shown to have a precision of 0.275 (S_r) in an evaluation of mixed amosite and wollastonite fibers. The estimate of the asbestos fraction, however, had a precision of 0.11 (S_r). When this fraction was applied to the PCM count, the overall precision of the combined analysis was 0.20 [7,17].

Issue 3 Revisions:

In this issue, the applicability of the method was refined to state the complementary nature of this method with NIOSH 7400 PCM method [8]. NIOSH 7402 should not be used for quantifying air concentrations of asbestos, since it is designed to mimic the optical magnification range counts of NIOSH 7400 PCM method, but it can be used to determine the fractions of fibers that are asbestos after sample analysis using NIOSH 7400 PCM method [8]. The method evaluation continues to be designated as "partial" due its reliance on historical data [7]. Absent of recent evaluation data, the method is considered partially evaluated. This issue also includes an alternative clearing procedure, an update of the NIST SRM with what is currently available (given availability, NIST standards are preferred), and the use of in column digital cameras.

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