

X. APPENDIX II

ANALYTICAL METHOD - FIBER COUNT

Principle of the Method

(a) Environmental dust samples are collected by drawing air through a membrane filter by means of a battery-powered personal sampling pump.

(b) The filter is transformed from an opaque solid membrane to a transparent, optically homogeneous gel.

(c) The fibers are sized and counted by phase-contrast microscopy at 400-450X magnification.

Range and Sensitivity

(a) This method has been successfully applied at concentrations of 10,000 to 20,000,000 fibers/cu m (0.01 to 20 fibers/cc) for fibers longer than 5 μm . Large deviations from the specified conditions of the method may result in filters with either too few or too many fibers. Too few fibers will yield air concentration estimates of low statistical precision.

(b) A sensitivity of 10,000 fibers/cu m (0.01 fiber/cc) has been reported [JM Dement, written communication, 1975] based on a 4-hour sample at 2 liters/minute air flow.

Interferences

All particulates, such as asbestos or mineral wool, with a length-to-width ratio of 3 to 1 or greater, and length greater than 10 μm should, in

the absence of other information, be considered as glass fibers and counted as such. Asbestos interference can be eliminated using phase contrast, polarized light microscopy.

Advantages of the Method

(a) The fiber count method allows for repeated counts, and storage for counting at a later time. The method consumes only part of the filter, thereby allowing for at least one replicate sample analysis at a later time.

(b) Fiber counts are assumed to be more toxicologically significant than fiber weight for fibers less than 3.5 μm in diameter.

(c) Fiber size determinations may be performed.

Disadvantages of the Method

(a) The fiber count method is slow and tedious.

(b) Variation in counts may be significant between different observers.

(c) The sensitivity of the method is dependent on the sampling time and flowrate. The sensitivity and useful range of this method has not been determined specifically for fibrous glass but is based on the method recommended for asbestos.

Apparatus

(a) Optical Equipment

(1) Microscope body with binocular head, 10X Huygenian

eyepieces, and Koehler illumination.

(2) Porton reticle.

(3) Mechanical stage, and stage micrometer with 0.01-mm subdivisions.

(4) Abbe or Zernike condenser fitted with phase ring with a numerical aperture equal to or greater than the numerical aperture of the objective.

(5) A phase-ring centering telescope or Bertrand lens and a green filter if recommended by the microscope manufacturer.

(6) Fiber mounting equipment

(A) Microscope slides, and cover slips, usually 0.17 mm thick.

(B) Scalpel, tweezers, lens tissues, and glass rod or spatula for mounting procedures.

(b) Wheaton Balsam Bottle.

Reagents

(a) Dimethyl phthalate.

(b) Diethyl oxalate.

Analysis of Samples

(a) Calibration and Standardization

(1) Porton Reticle and the Counting Field

The fiber count procedure consists of comparing fiber length with calibrated circles, and counting all fibers $> 10 \mu\text{m}$ in length within a

given counting field. A Porton reticle is used for this purpose. The Porton reticle is a glass plate inscribed with a series of circles and rectangles. The square on the left, divided into six rectangles, is defined as the counting field.

(2) Placement in Eyepiece

Place the Porton reticle inside one Huygenian eyepiece, resting it on the field-limiting diaphragm. Keep the reticle clean, since dirt on the reticle will be in focus and will complicate the counting and sizing process.

(3) Stage Micrometer

The Porton reticle cannot be used for counting until it has been properly calibrated with a stage micrometer. Most stage micrometer scales are approximately 2 mm long, divided into units of 10 μm .

(4) Microscope Adjustment

When adjusting the microscope, follow the manufacturer's instructions while observing the following guidelines.

(A) The light source image must be in focus and centered on the condenser iris or annular diaphragm.

(B) The object for examination must be in focus.

(C) The illuminator field iris must be in focus, centered on the sample, and opened only to the point where the field of view is illuminated.

(D) The phase rings (annular diaphragm and phase-shifting elements) must be concentric.

(5) Porton Reticle Calibration Procedure

Each eyepiece-objective-reticle combination on the microscope must be calibrated. Should any of the three be changed (disassembly, replacement, zoom adjustment, etc) the combination must be recalibrated. Calibration may change if the interpupillary distance is changed. For proper calibration, the following procedure should be followed closely.

Using a 10X objective, place the stage micrometer on the mechanical stage and focus and center the image. Change to the 40-45X objective and adjust the first scale division to coincide with the left boundary of the Porton rectangle. Count the number of divisions between the left and right boundaries of the long horizontal dimension of the largest rectangle, estimating any portion of the final division. This measurement represents 200 L units and the measurement is then divided by 200 to find "L." The large rectangle is 100 L units long on the short vertical dimension. The calculated "L" is inserted into the formula $D = L(2N)^{1/2}$ where "N" is the circle number (indicated on the reticle) and "D" is the circle diameter. Since the circle diameters vary logarithmically, every other circle doubles in diameter. For example, number three is twice the diameter of number one; number four is twice the counting field area consisting of the left six smaller rectangles can be calculated from the relation 10,000 L. The reticle calibration is now completed for this specific objective-eyepiece-reticle combination.

(b) Preparation of Mounting Solution

An important part of the sample evaluation is the mounting process which involves a special mounting medium of prescribed viscosity. The proper viscosity is important to expedite filter clearing and to minimize particle migration. Once the sample has been mounted, an elapsed time of

approximately 15 minutes is needed before the sample is ready for evaluation.

Combine the dimethyl phthalate and diethyl oxalate in a 1 to 1 ratio by volume and pour the solution into a Wheaton balsam bottle. Add 0.05 gram of new membrane filter/ml of solution to reach the necessary viscosity. The mixture must be stirred periodically until the filter material is dissolved and a homogeneous mixture is formed. The normal shelf life of the mounting solution is about 6 months. Approximately 300 samples can be prepared from 20 ml of mounting solution.

(c) Sample Mounting

Cleanliness is important. The working area must be kept clean to prevent sample contamination and erroneous counts. The following steps should be followed when mounting a sample.

(1) Clean the slides and cover slips with lens tissue. Lay the slide down on a clean surface with the frosted end up. It is good practice to rest one edge of the cover slip on the slide and the other edge on the working surface. By doing this, you keep from becoming contaminated.

(2) Wipe all the mounting tools clean with lens tissue and place them on a clean surface (such as lens tissue). When mounting a series of filters, wipe the scalpel clean before cutting a sector of each sample [see (5) below].

(3) Apply a small drop of mounting solution onto the center of the slide with a glass rod. It may be necessary to adjust the quantity of solution used or the size of the wedge. The correct amount will result in the solution extending only slightly beyond the filter boundary. If the

quantity is greater than this, adverse particle migration may occur.

(4) With a spatula or a supplemental glass rod, spread the mounting media into a triangular shape. The size of this triangle should coincide with the dimension of the filter wedge.

(5) Separate the middle and bottom sections of the field monitor case to expose the fragile filter. Cut a triangular wedge from the center to the edge of the filter using a scalpel. The size of the wedge should approximate one-eighth of the filter surface. The filter should be handled gently so that no material will be lost.

Grasp the filter wedge with tweezers on the outer area of the filter which was clamped between the monitor case sections. Do not touch the filter with fingers. Place the wedge, fiber-bearing side up, upon the mounting medium.

(7) Lift the cover slip with the tweezers and carefully place it on the filter wedge. Once this contact has been made, do not reposition the cover slip.

(8) Label the slide with the sample number and current date before proceeding to the next filter.

(9) The sample should become transparent after about 15 minutes. If the filter appears cloudy, it may be necessary to press very lightly on the cover slip. This is rarely necessary, however.

(10) Examine the slide within 3 days. The sample mount should be discarded after 3 days if it has not been counted because crystals which appear similar to glass fibers may begin to grow at the mounting media/air interfaces; they seldom present any problems if the slide is examined within 3 days. In any case, do not perform counting or

sizing around the edges of the filter.

(d) Counting and Sizing--Finding and Inspecting Counting Fields

Place the slide on the mechanical stage and position the center of the wedge under the objective lens and focus upon the sample. Nearly all of the particulates (particles and fibers) will be found in the upper 10-15 μm of the filter surface. When counting and sizing, continued use of the fine focus control is required to insure that nothing is missed. Start counting from one end of the wedge and progress along a straight line to the other end (count in either direction from circumference to wedge tip). Haphazard fields are selected without looking into the eyepieces by slightly advancing the slide in one direction with the mechanical stage control.

(e) Achieving Comparable Results

(1) Size only those fibers with a length-to-width ratio equal to or greater than 3:1.

(2) Count only fibers greater than 10 μm in length. (Be as accurate as possible in accepting or rejecting fibers near this length).

(3) Count up to 100 fields if necessary to yield a total count of at least 100 fibers. Count at least 20 fields even if more than 100 fibers are counted.

(4) Select the field of view without looking through the microscope's eyepieces to minimize unconsciously selecting "heavy" or "light" areas.

(5) The fields are selected along the entire length of a radial line running between the outside perimeter and the tip of the wedge.

(6) When an agglomerate (mass of material) covers a significant portion of the field of view (approximately 1/6 or greater), reject the field and select another. (Do not include this field in the number of fields counted.) Record the agglomerated field even though it is not included in the count.

(7) Bundles of fibers are counted as one fiber unless both ends of a fiber crossing another can be clearly resolved.

(8) For fibers that cross either one or two sides of the counting field, the following procedure is used to obtain a representative count. First, arbitrarily select: a) the left and bottom sides, and b) the upper and lower left corners and vertical direction as "decision aids."

Then count any fiber greater than 10 micrometers in length, but only if the fiber:

- a. lies entirely within the counting area, or
- b. crosses the left or bottom sides, or
- c. crosses the upper or lower left corners, or
- d. crosses both the top and bottom sides.

Reject and do not count all other fibers.

Calculations of Airborne Concentrations

Glass fiber airborne concentration may be calculated from the following formula:

$$C = \frac{(F-B)(W)}{(A)(V)}$$

where:

C = Airborne fiber concentrations in fibers >10 μm /cu m.

- F = Average fiber count in fibers $>10 \mu\text{m}/\text{field}$.
- B = Average fiber count of blank(s) or control filter(s) in fibers $>10 \mu\text{m}/\text{field}$. (It is subtracted to eliminate the error or background contamination.)
- W = 855 square mm for 37-mm diameter filters (the portion of the membrane filter which is exposed when mounted in the field monitor case, ie, the effective filter area).
- A = The area of the counting field of a calibrated reticle expressed in square mm/field.
- V = Total air volume collected through filter expressed in milliliters.

XI. APPENDIX III

AIR SAMPLING METHOD - TARED FILTER

Sampling

Breathing zone samples of the total airborne material are collected on a tared 37-mm filter of 0.8- μ m pore size, low ash, polyvinyl chloride, mounted in a filter holder with a 4-mm opening. The sample is collected for a 30-minute period at a sampling rate of 2 liters/minute. A personal sampling technique is employed, with the sampler head fastened to the worker's clothing in the breathing zone. Battery-powered personal sampler pumps, such as those used in the sampling train of the Coal Mine Dust Personal Sampling Units, approved under the provisions of 30 CFR 74 or their equivalent are used to draw air through the filters.

Calibration of Personal Sampler

The accuracy of environmental monitoring can be no greater than the accuracy of the volume of air which is measured. Therefore, the accurate calibration of a sampling device is essential to the correct interpretation of an instrument's indication. The frequency of calibration is dependent on the use, care, and handling of the pump. Pumps should also be recalibrated if they have been misused or if they have just been repaired or received from a manufacturer. If the pump receives hard usage, more frequent calibration may be necessary. Regardless of use, maintenance and calibration should be performed on a regular schedule and records of these should be kept.

Ordinarily, pumps should be calibrated in the laboratory. The accuracy of calibration is dependent on the type of instrument used as a reference. The choice of calibration instrument will depend largely upon where the calibration is to be performed. For laboratory testing, primary standards, such as a spirometer or a soapbubble meter, are recommended, although other standard calibration instruments, such as a wet-test meter or dry gas meter, can be used. The actual setups will be similar for all instruments.

Instructions for calibration with the soapbubble meter follow. If another calibration device is selected, equivalent procedures should be used. Since the flowrate given by a pump is dependent on the pressure drop of the sampling device, in this case a filter, the pump must be calibrated while operating with a representative filter in line. The calibration system should be assembled in this order: soapbubble meter, water manometer, filter, and pump.

(a) Check the voltage of the pump battery with a voltmeter to ensure adequate voltage for calibration and charge the battery if necessary.

(b) Turn on the pump and moisten the inside of the soapbubble meter by immersing the buret in the soap solution and drawing bubbles up the inside until they travel the entire buret length without bursting.

(c) Adjust the pump rotameter to provide the desired flowrate.

(d) Check the water manometer to ensure that the pressure drop across the sampling train does not exceed 13 inches of water at 1 liter/minute.

(e) Start a soapbubble up the buret and measure with a stopwatch the time required for it to move between calibration marks.

(f) Repeat the procedure in (e) above at least twice, average the results, and calculate the flowrate from the volume between the preselected marks divided by the time required for the soapbubble to traverse the distance.

(g) Record the volume measured, elapse time, pressure drop, air temperature, atmospheric pressure, serial number of the pump, the date, time, and name of the person performing the calibration.

(h) The rotameter reading should be corrected for temperature and pressure, if necessary.

XII. APPENDIX IV
ANALYTICAL METHOD - GRAVIMETRIC ANALYSIS

Principle of the Method

A known volume of air is drawn through a tared polyvinyl chloride filter to collect fibrous glass.

The sample-containing filter is removed from the cassette and dried over a desiccant to constant weight and weighed using a suitable microbalance. If the desiccated sample and filter exceeds the weight of the filter by more than 5 mg then the sample and filter is ashed in a platinum crucible. The crucible is heated to a constant weight and weighed using a microbalance.

Range and Sensitivity

Although this method has not been validated for fibrous glass, it has been validated for other substances, such as carbon black, that have a recommended environmental limit similar to fibrous glass. This method for fibrous glass has been validated for carbon black over the range of 1.86-7.7 mg/cu m at an atmospheric temperature and pressure range of 18-25 C and 749-761 mm Hg, using a 200-liter sample. Under the conditions of sample size (200 liters), the working range of the method is estimated to be 1.5-10 mg/cu m or a 0.3-2 mg total weight of material collected on the filter. It was also validated for a 100-liter sample over the range of 7.8-27.7 mg/cu m at atmospheric temperature and pressure conditons as above.

The method may be extended to higher sample concentrations by collecting a smaller sample volume; however, no more than 1.5 to 2 mg of material should be collected on any filter because greater amounts will be lost due to flaking.

Interferences

The presence of any other particulate material in the air being sampled will be a positive interference since this is a gravimetric method. Those materials that volatilize or combust at 600 C or less will not be interferences.

Information on any other particulate materials present should be solicited. If the concentration of other particles is known, then the fibrous glass concentration can be determined by the difference. If other particulate matter is known to be present and its concentration cannot be determined, then this method will not provide a limited measure of the fibrous glass concentration.

Precision and Accuracy

The precision and accuracy of the total sampling and analytical method has not been determined specifically for fibrous glass; however, it has been determined for other substances, such as carbon black, with a similar recommended limit. For carbon black, the coefficient of variation for the total analytical and sampling method in the range of 1.86-7.7 mg/cu m was 0.056. This value corresponds to a 0.20 mg/cu m standard deviation at the Occupational Safety and Health Administration (OSHA) carbon black

standard level. A collection efficiency of greater than 98.7% was determined for the collection medium at the 2X level; thus, no bias was introduced in the sample collection step. Likewise, no significant bias in the analytical method is expected other than normal gravimetric errors. The coefficient of variation is a satisfactory measure of both accuracy and precision of the sampling and analytical method.

Advantages and Disadvantages of the Method

The analysis is simple but the method is nonspecific and subject to interference due to presence of other nonvolatile or combustible particulates in the air being sampled.

Apparatus

(a) Sampling Equipment

The sampling unit for the collection of personal air samples for the determination of fibrous glass has the following components:

(1) The filter unit, consisting of the filter media, cellulose supported pad and 37-mm three-piece cassette filter holder.

(2) Personal sampling pump: A calibrated personal sampling pump whose flow can be determined to an accuracy of +5% at the recommended flowrate. The pump must be calibrated with a filter holder and filter in the line.

(3) Thermometer.

(4) Manometer.

(5) Stopwatch.

(b) Polyvinyl chloride membrane filter; 37-mm diameter, 0.8-micrometer pore size.

(c) Plastic Petri dish-filter holder or equivalent for storage and weighing.

(d) Desiccator.

(e) Platinum Crucible.

(f) Platinum-tipped or Nichrome Forceps.

(g) Platinum or Silica Triangles.

(h) Microbalance capable of weighing to 10 micrograms. Particular care must be given to proper zeroing of the balance. The same balance should be used for weighing filters before and after sample collection.

Reagents

Drierite or any other suitable desiccant.

Analysis of Samples

(a) Preparation of Filters

All filters must be dried and weighed prior to use.

(b) Sampling Requirements and Shipping of Samples

(1) To collect fibrous glass, a personal sampler pump is used to pull air through a polyvinyl chloride membrane filter. The filter holder is held together by tape or a shrinkable band. If the filter holder is not tightened snugly, the contaminant will leak around the filter. A piece of flexible tubing is used to connect the filter holder to the pump. Sample at a flowrate of 1.5 to 2 liters per minute. After sampling,

replace small plugs to seal filter cassettes.

(2) Blank

With each batch of ten samples submit one filter from the same lot of filters which was used sample collection to exactly the same handling as the samples except that no air is drawn through it. Label this as a blank.

(3) Shipping

The filter cassettes should be shipped in a suitable container designed to prevent damage in transit.

(c) Analysis of Samples

(1) If the outer surface of the cassette filter holder is heavily coated with dust, carefully swab the outer surface with a moist paper towel before opening the cassette so as to minimize sample contamination. Discard paper towel.

(2) Open the cassette filter holder and carefully remove the polyvinyl chloride membrane filter from the holder and cellulose support pad with the aid of filter tweezers. Transfer filter to a filter holder.

(3) Dry the filter to constant weight in a desiccator containing a dessicant. This takes about 12 hours.

(4) Weigh the filter using a microbalance. If the weight of filter contents exceeds 5 mg then put filter and contents in a clean, dried, and tared crucible.

(5) Put the crucible in a muffle furnace and ash at 600 C to constant weight. When handling the platinum crucible platinum-tipped or nichrome forceps should be used. If it is necessary to hold or stabilize

the crucible platinum or silica triangles should be used. Iron forceps should never be used for crucibles that are above 500 C because iron will alloy with platinum. Very hot crucibles should not be put into the desiccator. The crucible should be allowed to cool in the air until the temperature has fallen below 100 C. Then it may be placed in the desiccator.

- (6) Weigh the crucible using a microbalance.

Calibration and Standards

The microbalance should be properly zeroed for all weighings and preferably the same microbalance should be used for weighing filters before and after sample collection. The balance should be maintained and calibrated with National Bureau of Standards (NBS) Class M weights.

Calculations

- (a) Record the tare weight, in μg , of the dry filter before sampling.
- (b) Record the weight, in μg , of the dried, sample-containing filter.
- (c) The difference between these two weights represents the μg of sample.
- (d) Corrections for the blank must be made for each sample. (If found to be necessary, corrections should also be made for other particulate matter.)

$$\mu\text{g sample} = \mu\text{g found in sample filter}$$

$\mu\text{g blank} = \mu\text{g found in blank filter}$

(e) The concentration of the analyte in the air sampled can be expressed in mg per cu m ($\mu\text{g/liter} = \text{mg/cu m}$) by the following equation:

$$\text{mg/cu m} = \frac{\mu\text{g found (section (d))}}{V_s}$$

V_s = Volume of air in liters at 25 C and 760 mm Hg

(f) If the ashing procedure is to be performed record the tare weight, to the nearest μg , of the dry crucible before adding the filter.

(g) Record the weight, to the nearest μg , of the crucible and contents after the sample-containing filter has been ashed.

(h) The difference between these two weights represents the μg of sample.

(i) Corrections for the ashed blank filter must be made for each sample.