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Recommended Procedures for Sampling and Counting Asbestos Fibers

Procedures for the Evaluation of Occupational Exposures to Airborne Asbestos

Joint AIHA-ACGIH Aerosol Hazards Evaluation Committee

FIBROUS ASBESTOS HAS BEEN IDENTIFIED as the causative agent of asbestosis and has been associated with an increased cancer incidence. The American Conference of Governmental Industrial Hygienists (ACGIH) has established (1974) a Threshold Limit Value (TLV) for asbestos of 5 fibers greater than 5 micrometers in length per milliliter of air. A TLV footnote specifies the determination shall be made by the membrane filter method at 400× to 450× magnification and with phase contrast illumination. These procedures define a standard of sampling and of processing the collected samples in order to evaluate occupational exposure to asbestos fibers.

Sampling

Airborne samples of asbestos must be collected on membrane filters which retain at the surface essentially all of the particles in excess of 0.5 μm in diameter. The filter must be rendered transparent by the mounting medium. The filter should be packed in a sealed holder which is capable of being readily opened for sampling purposes and of being resealed after the sample has been collected. The filter must be fully exposed during sampling. (Recommended filters and filter holders are listed under **Supplies**). The filter must be practically dust-free with an average background count of less than 25 fibers per square centimeter. At least 2% to 4% of the filters intended for collecting samples should be set aside to determine the background count. (See **Counting** and **Calculations** sections). In addition, the filter resistance should not exceed 3 mm of mercury when filtering air at the rate of 0.3 liters per minute (lpm) per square centimeter of filter.

When collecting the sample, care must be taken to prevent dust from falling or from being projected onto the open filter. This may be accomplished by pointing the filter head downward. A guard or shroud should be attached to the filter holder to further protect the open filter from contamination as well as optimize uniform deposition on the filter. Its diameter should be about the same as the holder so that it can be attached tightly to it and it should project at least 1½ times the filter diameter in front of it. A protective device is particularly important if the filter head is to rest on contaminated clothing. (See Figure 1.) The filter holder may be stored for a limit of six months once it is resealed.

The sample should not be too dense, since samples in which particles overlap must be rejected as uncountable. Similarly, a large enough volume of air should be sampled so that there are sufficient numbers of fibers in each field. Experience indicates that more than 150 particles per field, including non-fibrous background, may interfere with counting the sample. It is desirable that there be no more than 10 fibers per field. Two or three samples should be collected at each sampling location. The results should

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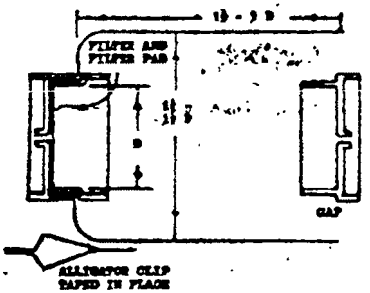


Figure 1. Diagram of shroud for membrane filter holder. The shroud may be constructed from a plastic bottle by cutting off the neck and drilling a hole in the bottom. To use, push the filter holder through the hole and reach in through the top to remove the holder cap. To remove the holder, replace the cap and push the filter holder forward through the hole.

be averaged to obtain the fiber concentration.

Breathing zone samples should reflect the worker's entire normal work day. They must be collected as close to the worker's nose and over as long a time period as possible. Calibrated battery powered personal pumps (see Appendix III, Pump Calibration) are satisfactory air movers. They can be worn by the worker and supported at the belt by means of a clip. The sampling head, which can be pinned to the collar, is connected to the pump by flexible tubing. Such pumps can be operated continuously for about eight hours and recharged overnight.

If a 37-mm filter is used to collect the sample, the pump should be adjusted to sample between 1 and 2 liters per minute. The volume of air sampled should be adjusted so that an optimum density of dust is collected on the filter. Since the dust concentration is not known before sampling, the optimal sampling period must be determined by trial and error. It is suggested that, unless experience indicates otherwise, the first sample should be collected from 20 liters of air, the second from 40 and the third from 80 liters. If a filter other than 37-mm is used, the volume of air sampled should be altered in direct proportion to the open filter area. Samples collected during short periods of exposure should be collected at a high flow rate and if necessary, for the entire exposure period. In all cases the count should be adjusted with regard to time, so that the exposure is expressed as an 8-hour time-weighted average exposure. For compliance evaluations with short term exposures, 37-mm filters samples should be collected for 15 minutes at a minimum of 2 lpm.

Sample Preparation

A solution one-to-one by volume of dimethyl phthalate and diethyl oxylate in which has been dissolved 50 milligrams of membrane filter material per milliliter of solution is the preferred counting medium. The chemicals used to prepare the medium should be examined microscopically to be certain that they are dust free. The fiber material to be dissolved should not be marked and should have a maximum background count of 25 fibers per square centimeter of filter. The counting medium may be stored in a wide-mouthed Wheaton Balsam bottle and should be applied with a glass rod. The counting medium has a refractive index of 1.461 at 25°C. The medium should be made in small quantities since it has a 6 month shelf life.

Asbestos fibers are counted on wedge shaped sections cut from the filter. The wedge should be reasonably sized so that it can be mounted on a 25 x 75 mm (1 x 3 inch) glass microscope slide. The slide should be cleaned of dust before it is used. A drop or two of counting medium is placed on the slide and the filter wedge is placed dust side up on top of the medium. The wedge is covered by a No. 1½ coverslip. Care must be taken while lowering the coverslip to avoid trapping air under it. Air bubbles may be forced out by exerting slight pressure on the cover slip with a pencil eraser. Extreme care must be taken to avoid

8002 0005

too much pressure, since this causes distortion and stretching in the filter.

The wedge is usually cleared within 15 minutes; however, a residual background granularity may be noted. This will disappear within a day. Counts must be completed within two days since there is a tendency for fiber migration and crystal growth. The prepared medium should be checked periodically for a background count. (See **Counting** and **Calculations** sections.)

An alternate medium may be used when the samples must be counted immediately. It is prepared in the same manner as the preferred viscous medium, except that the added membrane filter is omitted. Since this medium is much less viscous it should be stored in narrow-mouthed bottles. This medium is applied with a glass dropper after the filter wedge has been placed dust side up on the slide. However, a drop should be placed on the slide adjacent to but not on top of each corner of the wedge. This medium acts very rapidly and the No. 1½ coverglass should be lowered within a minute. Slides made up with this "alternate medium" are ready for counting within five minutes and the count must be completed within two hours after preparation.

Filters which have been used to collect samples and which have been resealed in a holder should be removed only in a clean environment. They should not be held with the fingers. They may be held with tweezers and cut with either a scalpel or scissors. Bottles containing media must be tightly closed except during use. All bottles, tools used for handling and cutting filters, and containers used for storing tools must be thoroughly cleaned and dried. Tweezers, scissors, scalpels, etc. should be set aside for this purpose exclusively. Although slides and coverslips are purchased pre-cleaned, they should be wiped lightly with clean lens tissue to remove traces of dust. Do not attempt to reuse slides or coverslips.

Microscope

A microscope equipped with a phase contrast substage condenser, a 4 mm "high dry" phase-contrast objective (40× to 45×) and a 10× eyepiece is used to count the sample. It should have either a pre-focused built-in illuminator supplied with an iris diaphragm or be lighted by a separate bright illuminator-flat mirror combination. If a separate illuminator is used it should be equipped with a condensing lens, iris diaphragm, and means for adjusting both the illuminator level and the distance between the condensing lens and the bulb. Zoom microscopes may be used for counting asbestos fibers, provided that the total magnification is within the 400-450× range.

The illuminator should be equipped with an adjustable constant voltage transformer. Both the microscope condenser and stage require close adjustment and both should be rack and pinion mounted. The stage must move along two perpendicular axes in a horizontal plane, while the condenser must move up and down in a vertical plane. Lighting must be adjusted for optimum balance. The Kohler method of illumination is recommended. (See Appendix I for procedures required to establish Kohler illuminator.)

The counting field is defined by a reticle mounted at the level of the field-limiting diaphragm in the 10× eyepiece. The Porton reticle (Figure 2) is recommended. However any reticle which contains markings which in addition to outlining an area of about 0.005 square millimeters, as determined by

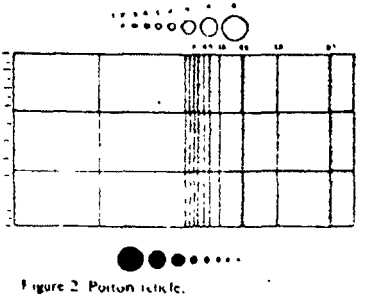


Figure 2. Porton reticle.

the combination of eyepiece and objective, and which defines linear distances that may serve as measuring aids, is satisfactory. The reticle must be calibrated prior to use and recalibrated each time it is removed from the microscope eyepiece or when the microscope tube length or interpupillary distance is changed. Zoom microscopes must be recalibrated each time the zoom position is changed. (See Appendix II for recommended calibration procedures.)

Counting

A fiber is defined as a particle whose length is at least three times greater than its diameter. All fibers longer than 5 μm within the area delineated by the reticle, or which enter it from either of two adjacent sides, are counted. Fibers entering the area from either of the other two sides, *i.e.*, those not arbitrarily chosen as "counting" sides are not counted, nor fibers in excess of 5 μm in diameter. Touching fibers, *i.e.*, one of whose ends touch another fiber regardless of the resulting angle, are considered as one. Fibers that cross each other are counted individually. Fibers that pass through the delineated area are counted provided that they cross at least one of the arbitrarily chosen "counting" sides. (See Figure 3.)

Fiber length should be determined by measuring against standards such as a circle or a space defined by markings on the calibrated reticle. If the fiber is curved or wavy the total length should be estimated by measuring along the curve. Once sufficient experience has been gained in judging length, only fibers whose length or diameter are in question need be measured.

A routine for choosing counting fields must be selected so that fields are not counted more than once and that a representative fraction of the total filter area is viewed. A convenient procedure which may be used is to select a series of microscope viewing fields along a radial line extending from the apex of the filter to the outer edge of the sample wedge. The viewing fields must be chosen without preference to a particular area and should be approximately equally spaced along the line. The first field should be located a little bit in from the point at which matter is deposited on the filter. If the required number of viewing fields cannot be located on a single radial line, additional lines parallel to the first should be chosen.

Preferably 100 fibers should be counted but all the fibers in 20 fields should be counted even if there are more than 100 fibers. The counting may be terminated after 100 fields have been searched even if 100 fibers have not been counted.

Calculations

The concentration of asbestos fibers in air can be expressed as:

$$\text{Asbestos Conc.} = \frac{\text{Fibers} \times R}{\text{Fields} \times \text{Vol}}$$

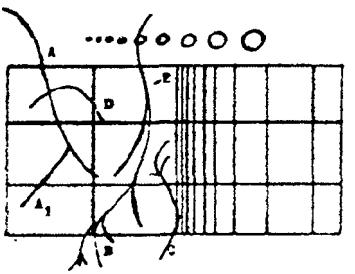


Figure 3. Illustration of fiber counting with a Porton reticle. The top and left side of the large box have been chosen as "counting sides". Circle 6 is 5 μm in diameter. (A) Fiber A crosses the top of the box and is counted, therefore. One end of Fiber A_1 touches Fiber A and thus is considered as part of it and is not counted separately. (B) Fiber B is a bundle containing many spikes. It passes through the box crossing bottom and top. It is counted as one fiber. (C) Fiber C passes through the box but it crosses the right side and bottom. It is not counted. (D) Fiber D is entirely within the box and crosses over Fiber A. It is not part of Fiber A, therefore it is counted as a separate fiber. (E) Fiber E is less than 5 μm in length. It is not counted.

8002 0005

where:

Fibers = total number of fibers counted

Fields = total number of fields counted

$$R = \frac{\text{filtration area}}{\text{area of a counting field}}$$

Vol = sampling rate (1pm) x time (min) x 10^3

= sample volume in ml of air

Since both the filtration area on the membrane filter and the area of the counting field are normally constant, the ratio of areas, R, is constant for the given conditions.

To illustrate: Air is sampled at 1 liter/minute for 50 minutes on a 37-mm membrane filter (filtration area 855 mm^2). A total of 60 fibers each longer than 5 μm are counted in 100 fields each 0.003 mm^2 in area:

$$R = \frac{855\ mm^2}{3 \times 10^{-3}\ mm^2} = 2.8 \times 10^5$$

and

$$\text{Asbestos Conc.} = \frac{2.8 \times 10^5 \times 60\ \text{Fibers}}{100\ \text{Fields} \times \frac{1 \times 10^3\ ml}{\text{min}} \times 50\ \text{min}}$$

= 3.4 Fibers/ml

Since the precision of this method is limited, the averaged results of the several samples should be reported to the nearest significant figure.

Appendix I

Recommended Procedure for Establishing Kohler Illumination

The microscope is set on a level surface at a convenient height and in such position that sighting through the eyepieces is possible without undue strain or discomfort.

The illuminator is placed directly in front of and aligned with the microscope. If the illuminator is equipped with a coil filament bulb, its iris should be ten inches from the mirror. If a ribbon filament bulb is used, the front of the filter holder should be at least seven inches from the mirror. Only the plane surface of the mirror should be used. All filters should be removed from the system except that if desired, neutral density filters may be used to reduce the light intensity. If the microscope is equipped with a built-in illuminator, proper illuminator positioning has already been accomplished and need not be of further concern.

Fix a mounted sample on the stage and recheck the alignment. Raise the substage condenser until it nearly touches the bottom of the slide. Turn on the illuminator and use the tilt controls to direct the light to the center of the mirror. Tilt the mirror, directing the beam upward into the stage condenser. Close the microscope substage iris and the illuminator iris. Use the illuminator focus control to focus the image of the filament on the bottom of the substage iris. The reflection of the iris may be viewed in the mirror by leaning over the microscope.

After adjusting the illuminator condenser so that there is a sharp image of the filament on the substage iris, open it about halfway. Recheck proper position of the mirror and then put the objective in place and focus sharply on the sample. It may be necessary to open the substage iris during focusing.

After the sample is in focus, fully close the iris. Readjust the condenser height so that the edge of the iris leaves are in sharp focus and there is a bright spot within the iris. Readjust the mirror to obtain maximum brightness. If the color around the iris is not uniform, recheck the illuminator tilt and focus. If the color is uniform and maximum brightness has been achieved, open the field iris until the blades just pass out of the field of view.

The proper phase stop is inserted or rotated into place in the substage condenser. The phase objective is rotated into place. The eyepiece is replaced with the phase ring centering telescope. The telescope is adjusted for sighting. The location of the phase ring is observed through the telescope. It may be adjusted by using the two rotating shafts provided with the phase condenser. When the ring is centered it appears as a bright annular ring mounted on top of a

gray ring. The center is black. There should be no overlap of the bright ring into the center. When the phase setting is obtained the telescope is removed and the eyepiece is replaced. The microscope is now ready for use.

Appendix II

Recommended Procedure for Calibrating Reticles

The reticle must be slightly smaller in diameter than the eyepiece in which it is to be used. It fits into the tube and is held in place at the eyepiece focal point by spring pressure. The focal point is marked by an internal collar which prevents the reticle from passing further into the tube. Some manufacturers locate the focal point just below the collar while others prefer a point immediately above it. Thus it may be necessary to insert the reticle between the lens and the collar on one microscope and below the collar on another. In either case the projected image of the reticle on the field must be clear and sharp.

The reticle must project a constant counting area as well as provide markings for sizing. Many types of reticles are satisfactory. The Porton reticle illustrated in Figure 2 is recommended. It outlines a large rectangle that fits easily within the field of vision. The rectangle is divided into two equal squares. The left square is further divided into six rectangles. Each side of each square has a length equal to 100 L, then the large rectangle is 200 L units long and the small ones 50 L units long and 33⅓ L units high. To the left of the left square is a scale which divides the side into 20 equal increments of 5 L units each. The right square is divided horizontally in thirds corresponding to the small rectangles and vertically into a series of increasing spaces. The distance between each vertical line and the center of the large rectangle corresponds to the diameter of a circle. Circles of sizes 1 through 9 are located above and below the large rectangle. The diameter of each circle is related in terms of L units of length in accordance with the formula:

$$D = L\sqrt{2^N}$$

where D is the diameter of the circle or the width of the space and N is the number of the circle, or space.

The unit L is determined by measurement. A stage micrometer is the primary ruler. Any one or more of the definitive linear dimensions may be measured. If the length of the large rectangle is measured then L equals 1/200th of that value.

If the stage micrometer is marked in hundredths of millimeters the value of L is in hundredths of millimeters. If the left square is used as a counting area, its area is equal to

$$(100 \times 0.01\ L)^2\ \text{mm}^2$$

The filtering area of a 37-mm filter is 855 mm^2 and thus

$$R = \frac{855}{L^2}$$

If other areas are used as counting fields or other sized filters are used, the value of R must be calculated for the individual condition.

It is wise to prepare a chart showing each linear dimension and area for ready reference while counting.

Appendix III

Pump Calibration

Personal sampling pumps should be calibrated for flowrate frequently. They may be calibrated against a primary meter such as a spirometer or against a previously calibrated secondary standard such as a wet gas meter or dry gas meter. The train should be arranged so that the pump is operating against the same resistance it would normally operate against and that the calibrating meter is at atmospheric pressure. Figure 4 illustrates the recommended arrangement. Air flow through the filter may be limited by both restricting the air flow to the pump as well as by admitting secondary air into

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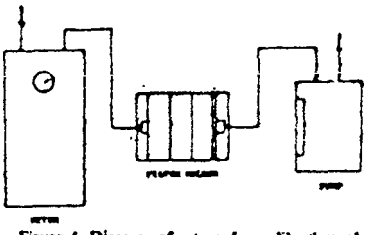


Figure 4. Diagram of set-up for calibration of pump.

the pump. Most personal pumps are constructed with adjustment valves that permit both actions. Calibration should be repeated after each 24 to 36 hours of pump use. If the pump has not been used for a prolonged period of time it should be recalibrated. Temperature and attitude corrections should be made.

Personal sampling pumps should not be used to such an extent that the batteries are allowed to run down below normal operating voltage. Since battery life varies, it is wise to occasionally check the life of the battery by allowing the pump to run down to the first indicated change in voltage under timed simulated conditions.

Supplies

Filters and Filter Holders

Millipore Corp.[a] Field Monitor filter holders preloaded with 37-mm Type AA filters, either white plain (catalog #MAWP037AO) or gridded (#MAWG037AO) are recommended. These are sold with a guaranteed average particle background and can be used without additional background checks.

It is less expensive to purchase unloaded field monitors (#MABG037AO) to be loaded with plain or gridded filters purchased separately in packages of 100 (#AAWP03700, and AAWG03700). The field monitor can then be reloaded after each use. However, it is necessary to carefully clean the empty monitor and to check the background count on filters in the reloaded monitor.

Type AA filters can be used in any diameter in any non-leaking open-type filter holder, as long as proper adjustment is made in the calculations for the change in filtering area, and periodic background checks are made.

Membrane filters of other types and manufacturers cannot be recommended at this time, either because they do not become optically clear in the mounting medium or because of excessive particle background.

Personal Battery Powered Sampling Pumps

(1) Casella[b] MK II or MK III Personal Sampler
(2) MSA[c] Monitaire Sampler, Model G.
(3) Bendix[d] VM 22 or Micronair Personal Sampler or C-115.

Microscopes: Phase Equipped

(a) microscope body with a fine focus accuracy of 0.006 mm;
(b) 10× eyepiece;
(c) mechanical stage;
(d) illuminator (preferably built in and having provisions for adjusting light intensity);
(e) 40× to 45× (0.65 N.A. at least) positive (bright field) phase-contrast objective;
(f) annual ring condenser diaphragm (corresponding to the objective); and
(g) phase ring centering telescope.

(*Note.* Most manufacturers sell a basic body unit and built-in illumination system as a unit. Phase-contrast accessories can usually be purchased as a kit consisting of 10×, 40× and 90× phase objectives, a phase condenser containing appropriate annular ring diaphragms, a phase ring centering telescope, and a green filter. It is to the microscopist's advantage to purchase the kit.)

A list of manufacturers of phase-contrast microscopes[e] and reticles[f] is given below to aid in selecting a proper instrument.

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[a] Millipore Corp., Bedford, Mass. 01730.
[b] William Products Div., P.O. Box 622, Reading, Penna. [illegible]