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USPHS/NIOSH MEMBRANE FILTER METHOD FOR EVALUATING AIRBORNE ASBESTOS FIBERS

MARGINALIA REDACTED

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TR-84

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U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Health Services and Mental Health Administration
National Institute for Occupational Safety and Health
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FOREWORD

The United States Public Health Service through the National Institute for Occupational Safety and Health has established a sampling and evaluation procedure for determining airborne asbestos dust exposures. The sample is collected by drawing air through a membrane filter by means of a battery powered vacuum pump. The filter is changed into a transparent homogeneous plastic mount, and the fibers are sized and counted by phase contrast microscopy. This method was first used by the Asbestosis Research Council in Great Britain and later modified by the U.S. Public Health Service for asbestos dust studies in the United States. It is specified in the emergency asbestos standard as published in the Federal Register on December 7, 1971.

UNIFORM procedures must be used for sampling and evaluating asbestos dust if reproducible results are to be achieved. Detailed equipment and procedures used by National Institute for Occupational Safety and Health are described herein. Mention of commercial concerns or products does not constitute endorsement by the Public Health Service, the U.S. Department of Health, Education, and Welfare, or the National Institute for Occupational Safety and Health.

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1. SCOPE

1.1 This method describes the equipment and procedures for collecting, mounting, sizing and counting asbestos fibers on membrane filters in the evaluation of airborne asbestos dust.

1.2 The method has been successfully applied using 37 mm Millipore AA filters and small battery operated personal sampling pumps at a flow rate of about 2 liters per minute for time periods of about 5-240 minutes at concentrations normally encountered in industrial hygiene practice, e.g., in the vicinity of 5 fibers per milliliter greater than 5 micrometers in length. Large deviations from these conditions may result in filters with either too many fibers to count or too few fibers for good statistics.

1.3 This method considers only fibers with an aspect ratio (length to width) of 3 to 1 or greater and a length of 5 micrometers or more.

2. SUMMARY OF METHOD

2.1 The sample is collected by drawing air through a membrane filter by means of a battery powered personal sampling pump. The filter is transformed from an opaque solid membrane to a transparent, optically homogeneous gel. The fibers are sized and counted by phase-contrast microscopy.

3. SIGNIFICANCE

3.1 This method was first used by the Asbestosis Research Council in Great Britain [1] and later slightly modified by the U.S. Public Health Service for asbestos dust studies in the United States. [2] It has been specified as the method of test for the federal standard for asbestos in industrial air (29 C.F.R. Sec. 1910. 93a) [3], the U.S. Public Health Service Criteria document on occupational exposure to asbestos [4], and in sampling procedures

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of the Occupational Safety and Health Administration, Department of Labor. (5)

4. DEFINITIONS

4.1 For definitions of terms used in this method, refer to ASTM Definitions D 1356, Terms Relating to Atmospheric Sampling and Analysis.

5. INTERFERENCES

5.1 In some instances an asbestos environment may be contaminated with other airborne fibrous materials. When evaluating samples taken in this kind of environment it is suggested that all initial counts be made using the 3:1 (length to diameter) criteria. If concentrations fall within acceptable safe limits then no further evaluation is necessary.

On the other hand, if concentrations appear to be high, and there is some doubt to whether the concentration reflects a total asbestos exposure, it will be necessary to examine the sample by other analytical methods. Unfortunately, there is no simple method for identifing asbestos fibers. The most feasible and least time consuming methods would be microscopical dispersion staining and polarized light. If a transmission electron microscope is available this method of evaluation can also be utilized. Unlike dispersion staining, the electron microscope evaluation is more orientated toward fiber identification and less toward the ability to achieve an asbestos-to-other-types-of-fiber ratio. Keenan and Lynch (6), Crossman (7), and Julian and McCrone (8) have reported on the above procedures.

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6. APPARATUS

6.1 Sampling equipment. Equipment is described in the Air Sampling Instrument guide of the ACGIH.(9)

6.1.1. Personal sampling pump. The Mine Safety Appliances Co., Pittsburgh, Pa.; National Environmental Instruments Co., Fall River, Massachusetts; and Willson Products Division, Reading, Pennsylvania, products have been found satisfactory.

6.1.2 Collecting filter and holder. This is provided by the Millipore Corporation of Bedford Massachusetts as a unit consisting of a) three styrene sections, with the designation Millipore Aerosol Monitor.case., b) a 37 millimeter diameter plain white cellulose ester membrane filter designated Millipore AA (normal pore size 0.8 micrometer), c) a support pad, and d) plastic sealing caps. These are available in boxes of 50 as catalog number [illegible]. Equivalent filters from other manufacturers may be used.

6.1.3 Rubber tubing, 6 millimeter bore (1/4 inch), about 90 centimeters (3 feet) long.

6.1.4 Metal adapters for connecting tubing end to field monitors. These are available as catalog number LH/L from Bection, Dickinson, and Company of Rutherford, New Jersey.

6.1.5 Spring clip to hold rubber tubing and field monitor in place on lapel or shirt. This can be improvised or ordered as catalog number 1140 from the John F. Maguire Company, 120 Bacon Street, Pawtucket, Rhode Island 02860.

6.1.6 Field assembled monitors. If a great number of samples is to be taken, it will be less expensive to reuse plastic cases. However, great care must be taken in the cleaning and reassembly process. Millipore filters and pads 100/box are available as catalog numbers

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AANP 03700; empty Aerosol Monitors as catalog number M 00037AO, and "shrink-fit" sealing bands from the Walter H. Jelly and Company, 2822 Birch Street, Franklin Park, Illinois 60131, as white, opaque, cellulose bands, 41 x 25, number 28.

6.2 Optical equipment. For general guidance on microscopes, consult Needham [10] and Clark [11] - Phase contrast microscope and accessories.

6.2.1 Microscope body with binocular head.

6.2.2 10X Huygenian eyepiece (s).

6.2.3 Koehler illumination (preferably built in and having provisions for adjusting light intensity) (see Fig.1).

6.2.4 Porton reticle (see Fig.2).

6.2.5 Mechanical stage.

6.2.6 Abbe, Zernike, or Heine phase-contrast condenser.

6.2.7 40-45X (165 N.A. at least) positive phase-contrast objective.

6.2.8 Phase-ring centering telescope.

6.2.9 Green filter.

6.2.10 Stage Micrometer, 0.1 and 0.01 millimeter divisions (see Fig. 4).

NOTES: Most manufacturers sell a basic body unit with a built-in illumination system. Phase-contrast accessories can usually be purchased as a kit consisting of a 10X , 40X, and 90X objectives, a cordenser, 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 is given as Table 2 to aid in selecting a proper instrument.

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6.3 Filter Mounting Equipment. Experience has shown that certain equipment is useful for efficient sample mounting. The following items are recommended for extracting and placing a portion of the filter upon the slide..

6.3.1 Scalpel or scissors: A scalpel or scissors is needed to remove a portion of the filter. Either a pair of microscissors or a number-ten-curved-blade scalpel works best.

6.3.2 Tweezers: A pair of fine-tipped tweezers is used to remove the membrane filter slice from the field monitor and place it upon the slide.

6.3.3 Slides: 2.5 by 7.5 cm (one inch by three inches) glass slides are most commonly used. Sample number, data, initials, etc. can be conveniently written on a frosted end slide.

6.3.4 Cover Slips: Cover slips are a necessary part of the slide mount and optical system. The shape should be appropriate for the size of the filter wedge. The thickness used is dependent on the correction factor built into the objective. Nearly every 4 mm phase contrast objective is optically corrected for a #1½ (0.17 millimeter) thickness cover slip. Improper cover glass thickness will detract from the final image quality.

6.3.5 Lens Tissue: To insure cleanliness, use a lint-free lens tissue. This tissue should be used also for wiping mounting tools and for cleaning slides and cover slips.

6.3.6 Glass Rod: A spatula or fire-polished glass rod is needed to spread the mounting solution on the slide.

6.3.7 Wheaton Balsam Bottle: This special glass container has a glass top which hinders contamination of the solution. A

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glass rod is included for dispensing the solution. This is available as catalog number 2244 from the Arthur H. Thomas and Co., P.O. Box 779, Philadelphia, Pennsylvania 19105.

7. REAGENTS

7.1 Dimethyl phthalate.

7.2 Diethyl oxalate. These should be reagent grade, free from particles and color, conforming to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.

8. SAFETY PRECAUTIONS

8.1 Avoid getting large amounts of the mounting solution on the skin.

9. SAMPLING

9.1 General information. Refer to the Recommended Practice D 1357, for Planning the Sampling of the Atmosphere and Recommended Practice D 2009, for Collection by Filtration and Determination of Mass, Number, and Optical Sizing of Atmospheric Particulates. These guides are cited only for general principles, as they are intended primarily for air pollution measurements at fixed sampling stations.

9.2 Calibration. The personal sampling pump should be calibrated against a bubble meter, wet test meter, spirometer, or similar device at 2.0 liters per minute and fully charged prior to sampling.

9.3 Mounting the sampler. Fasten the sampling pump to the worker's belt and field monitor to the lapel or shirt front (as close to the face as is practical). Remove the cover of the plastic monitor making certain the exposed filter is facing downward. Turn on the pump

pump and adjust to 2 liters per minute.

9.4 Optimum sampling times. Leidel (12) has described the requirements for optimum sampling times for airborne asbestos fibers. A nomogram to aid calculation of these times is shown as Fig. 3. Note that for typical microscope field areas and pump flow rates that the optimum collection times are about 50 to 80 minutes duration.

9.5 End of the sampling period. Remove the field monitor, replace the the plastic top cover and the small end caps, and store the monitor filter-side up in a covered container. If an 8-hour average is being sought, ~~insert~~ attach a new monitor immediately. Always shut off the pump when changing monitors to avoid contaminating the pump.

9.6 Blanks. With each batch of samples submit two filters which have been subjected to exactly the same handling as the samples except that no air has been drawn through them. Label these as blanks.

9.7 Shipping. The field monitors in which the samplers are collected should be shipped in a rigid container with sufficient packing material to prevent crushing.

10. CALIBRATION AND STANDARDIZATION

10.1 Porton reticle. The asbestos fiber count procedure consists of comparing fiber length by comparison with calibrated circles, and counting all fibers greater than 5 micrometers in length within a given counting field area. This is done with the use of a Porton reticle (Figure 2). The Porton reticle is a glass plate inscribed with a series of circles and rectangles. The square on the left, divided in to six rectangles, is defined as the counting field.

10.2 Placement in eyepiece. The Porton reticle is easily placed inside a Huygenian-type eyepiece where it rests on the field-limiting diaphragm. A split ring is included with the reticle to aid

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mounting when there is no aperture present. The reticle should be kept clean, since dirt on the reticle is in focus and will complicate the counting and sizing process.

10.3 Stage micrometer. The Porton reticle cannot be used for counting until it has been properly calibrated with a stage micrometer. A stage micrometer is a glass slide with a scale unit which can be seen only under a microscope. Most stage micrometer scales are about two millimeters long and are divided into units of one-hundreth of a millimeter or ten micrometers (see Figure 4). Before calibration, the microscope must be set up according to the manufacturers directions.

10.4 Microscope adjustment. The microscopist will find the following to hold true for most microscopes. The enlarged image of the illuminator's filament must be focused either on the condenser's fully closed iris or at the back focal plane of the objective lens. It is important that the phase-shifting element ring and annular diaphragm be concentric. Most microscopes have centering controls which allow manual correction if necessary. Next, close the illuminator's field iris, and focus the condenser's image in the sample's focal plane.

10.5 Reticle calibration procedure. Each eyepiece-objective-reticle combination on the microscope must be calibrated. Should any of the three be changed (disassembly, replacement, breakage) the combination must be re-calibrated. For proper calibration, the following procedure should be closely followed.

With the 10X objective in place, place the stage micrometer on the mechanical stage, focus, and center the image. Change to the 4 mm (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 dimension of the large rectangle, estimating any portion of the final division (0.204 mm in Figure 4). This measurement represents 200 L

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units so one divides by 200 to find "L". The large rectangle is 100 L units long on the short dimension. This calculated "L" is inserted into the formula $D = L\sqrt{2^N}$ where "D" is the circle number (indicated on the reticle). 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 diameter of number two. When the circle sizes have been determined, the counting field area consisting of the left six rectangles can be calculated from the relation $10{,}000\ L^2$. This completes the reticle calibration for this specific objective - eyepiece - reticle combination. Table 2 shows a calibration card for three different objective lenses.

11. PROCEDURE

11.1 Mounting. A very important part of the sample evaluation is the mounting process. This process involves a special mounting medium of prescribed viscosity. The proper viscosity is important in order to expedite filter clearing and minimize particle migration. Once the sample has been mounted, an elapsed time of approximately fifteen minutes is needed before the sample is ready for evaluation.

11.2 Preparation of the Mounting Solution. Combine the dimethyl phthalate and diethyl oxalate in a one to one ratio by volume and pour into a Wheaton balsam bottle. Add 0.05 gram of clean filter material per milliliter of solution to reach the prescribed viscosity. The solution must be stirred or shaken until the filters have dissolved and a homogeneous mixture was formed. The normal shelf life of the mounting solution is about six months, after which time fiber-shaped crystals may begin to form. The crystallizing process may be retarded by keeping the solution in the Wheaton balsam bottle and in a cool place. Twenty milliliters of mounting solution will prepare approximately 300 samples.

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11.3 Sample Mounting: Cleanliness is important! A clean working area will help achieve better results. The following steps should be followed when mounting a sample.

11.3.1 Clean the slides and cover slips with lens tissue. Lay the slide down with the frosted end up. It is a 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 the bottom surface (the one which contacts the filter) from becoming contaminated.

11.3.2 Wipe all the mounting tools clean with lens tissue and place them on a clean surface (i.e., lens tissue). When mounting a series of filters the scalpel or scissors should be wiped clean before cutting each sample.

11.3.3 Using the glass rod which is supplied with the Wheaton balsam bottle, apply a small amount of mounting solution (usually less than a drop) into the center of the slide. Do not touch the slide with the rod, this can contribute to the contamination of the mounting solution. It may be necessary to adjust the quantity of solution used. 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 will occur.

11.3.4 With the 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.

11.3.5 Separate the middle and bottom sections of the field monitor case to expose the fragile filter. Cut a triangular wedge from the filter using the scalpel or microscissors. The size of the wedge should approximate one-eighth of the filter surface.

11.3.6 Grasp the filter wedge with the tweezers in the area which was

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sandwiched by the monitor case sections. Do not touch the filter with your fingers. Place the wedge, sample side up, upon the mounting medium.

11.3.7 Pick up the cover slip with the tweezers filter/media combination. Press lightly on the cover until it makes contact with the now-dissolving filter. Once this contact has been made, do not reposition the cover slip.

11.3.8 Label the slide with the sample number and current date before proceeding to the next filter.

11.3.9 The sample should become transparent after about fifteen minutes. If the filter appears cloudy, it may be necessary to again press very lightly on the cover slip.

11.3.10 Discard the sample mount after three days if it has not been counted. Crystals which appear similar to asbestos fibers may begin to grow first at mounting solution/air interfaces. They seldom present any problems if the slide is examined before three days. In any case, stay away from the filter's edges when counting and sizing.

11.4 Counting and sizing

11.4.1 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 ten to fifteen micrometers of the filter surface. When counting and sizing, frequent use of the fine focus control will help to insure that nothing is missed. Start counting from one end of the wedge and progress in a straight line to the other end (count in either direction from circumference to wedge tip). To determine the average fiber deposition per field one must count as many fields as necessary to yield 100 fibers. A minimum of 20

fields must be counted and in general one would stop counting at 100 fields. Random fields are selected, without looking into the eyepieces, by slightly advancing the slide with the mechanical stage control.

11.5 Achieving comparable results

11.5.1 Size only fibers with an aspect ratio (length to width) greater than or equal to 3:1.

11.5.2 Count only fibers greater than five micrometers in length. (Be as accurate as possible in sizing fibers near this length).

11.5.3 Count as many fields as required to yield at least 100 fibers: generally a minimum of 20 and at most 100.

11.5.4 Select the field of view without looking through the microscope's eyepieces to eliminate unconsciously selected "heavy" or "light" areas.

11.5.5 The fields are selected in a straight line running from the outside perimeter and progressing toward the tip of the wedge.

11.5.6 (Generally) Do not count a filter with fields containing more than twenty fibers each. Such a filter could be reported as "too heavily loaded" and not as "greater than X fibers/ml."

11.5.7 When an agglomerate (mass of material) covers a significant portion of the field of view (approx. 1/6 or greater) reject the field and select another. (Do not include it in the number of fields counted). However, report the fact as it may have meaning to sampling or medical personnel.

11.5.8 Count and size only free fibers - that is, fibers that are unattached. One must be able to see both ends of the fiber (Figs.15,16 and 17).

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11.5.9 Count any fiber greater than 5 micrometers in length if:

a) it is entirely within the counting field (Figs. 5,10, 14, 16, and 17).

b) or if some portion of the fiber is inside the counting field and the fiber crosses one or both of the pre-selected adjacent sides. The intent of this rule is to exclude potentially eligible fibers that are either outside the field of view or that cross either of the remaining sides (even if the fiber also crosses one of the selected sides.) (Figs. 6,7,8,9,11,12, and 13).

12. CALCULATIONS

12.1 Calculation of airborne concentration. Asbestos fiber airborne concentration may be calculated from the following formula:

$$C = \frac{(AFC-BAFC)(FA)}{(A)(V)}$$

Where:

C = Airborne concentration in fibers/milliliter

AFC = Average fiber count in fibers/field.

BAFC = Average fiber count of blank (s) or control filters(s), (It is subtracted to eliminate the error or background contamination.) in fibers/field.

FA = 855 mm^2 for 37 mm diameter filter (the portion of the Millipore filter which is exposed when mounted in the field monitor case, i.e., the effective filter area).

A = The area of the counting field of a calibrated Portion reticle expressed in mm^2/field.

V = Total air volume collected through filter expressed in milliliters.

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13. PRECISION AND ACCURACY

13.1 An accuracy and precision study of the membrane filter counting procedure for asbestos has been conducted by Holland and Conway (13). Their study was an intralaboratory one involving six counters. Three of the counters were experienced while the other three had only a short familiarization with the rules for fiber counting. The conclusions of the study included:

13.1.1 The precision of the procedure for filters not containing an abundance of five fibers can be estimated by a standard deviation of 16.2%. This value includes variation among counters and observed interaction effects.

13.1.2 The accuracy of the procedure for similar filters may be estimated for a 100-fiber count by a standard deviation of 21.4%. This assumes that the contribution of the overall variance from the non-uniform fiber distribution is additive.

13.1.3 The fiber concentration varies significantly between angular sectors on a given filter. Where approximately 100 fibers are counted, the standard deviation of the fiber count distribution for the whole filter appears to be represented by 1.6 $(n)^{\frac{1}{2}}$, rather than $(n)^{\frac{1}{2}}$ which is a property of the Poisson distribution.

13.1.4 A high percentage of very fine fibers on the filter can significantly affect the standard deviations and confidence limits for counts by different counters. After combining variations in fiber concentrations over the entire filter with those for different observers it was concluded:

a) For filters with a low concentration of five fibers, the standard deviation is estimated at 21% and the 95% confidence interval is ± 43%.

b) For fibers with a high concentration of five fibers, the standard deviation is estimated at 25% and the 95% confidence interval is ± 50%.

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13.2 Lynch, Kronoveter, and Leidel (14) have also reported on the precision of the method. Their intralaboratory study utilized the data from a large number of dust counts made by different methods by experienced counters over a period of years in an epidemiologic study of the asbestos products industry. They concluded that the standard deviation of counts of fibers longer than 5 micrometers on membrane filters could be estimated from the relation $\sigma = (n)^{0.591}$. Thus, for counts of about 100 fibers, the standard deviation could be estimated at about 15.2% and the 95% confidence limits at ± 30.4%. This agrees very well with the precision values reported by Holland and Conway.

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Rockleigh, N.J. 07647

Nikon, Inc.
Instrument Division
Garden City, N.Y. 11530

Olympus Microscopes
Micro Optics Company
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Southfield, Mich. 48075

American Optical Corporation
Reichert Products
Buffalo, N.Y. 14215

Unitron Instrument Company
Microscope Sales Division
66 Needham Street
Newton Highlands, Mass. 02161

Carl Zeiss
444 Fifth Avenue
New York, N.Y. 10018

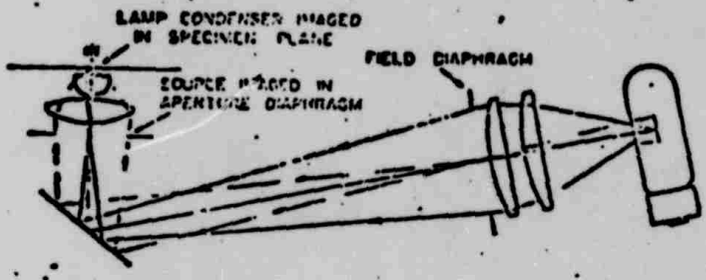
Table 1 - Manufacturers of phase-contrast microscopes.

#1642 B & L DYNOPTIC 10X HUYGENIAN EYEPIECES

	10x	43x	97x
200L	0.632mm	0.1[illegible]mm	0.0065mm
100L	0.316	0.074	0.0325
1L	0.00316	0.00074	0.000325
Diam. Circle #1	4.46μ	1.05μ	0.46μ
2	6.32μ	1.48μ	0.65μ
3	8.92	2.10	0.92
4	12.64	2.96	1.32
5	17.84	4.20	1.84
6	25.28	5.92	2.54
7	35.68	8.40	3.68
8	50.56	11.84	5.28
9	71.36	16.80	7.36
10	101.12	23.68	10.56
11	142.72	33.60	14.72
12	202.24	47.36	21.12
13	285.44	67.20	29.44
Field Area	0.0990mm^2	0.0055mm^2	0.00106mm^2

Table 2 - Porton reticle clibration card for three eyepieces.

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KOEHLER ILLUMINATION
Figure 1

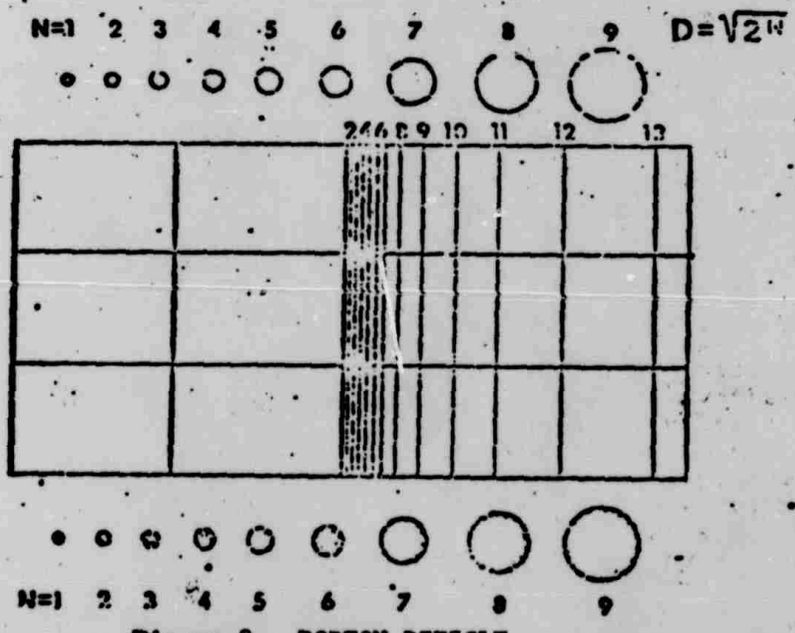


Figure 2. PORTON RETICLE

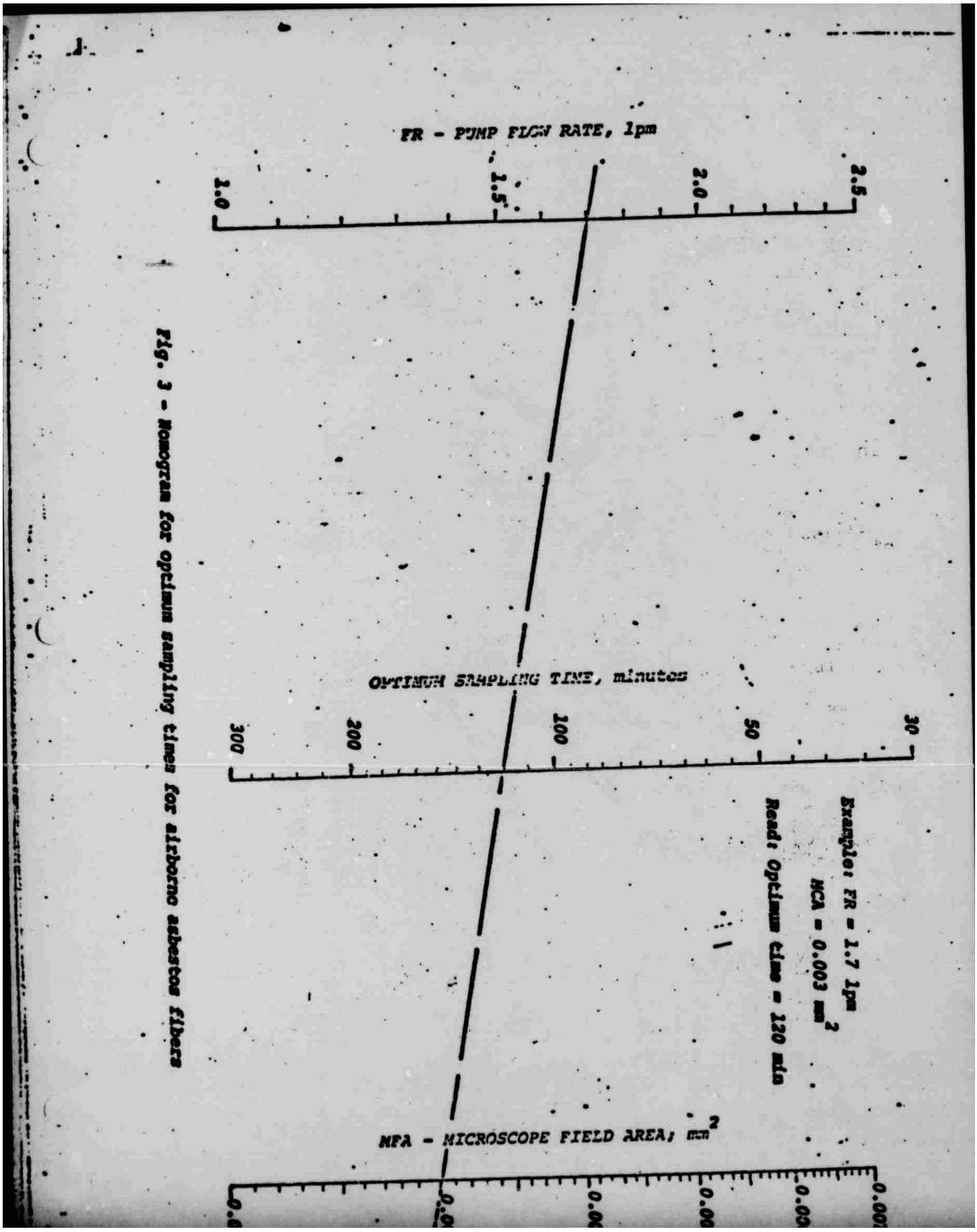


Fig. 3 - Nomogram for optimum sampling times for airborne asbestos fibers

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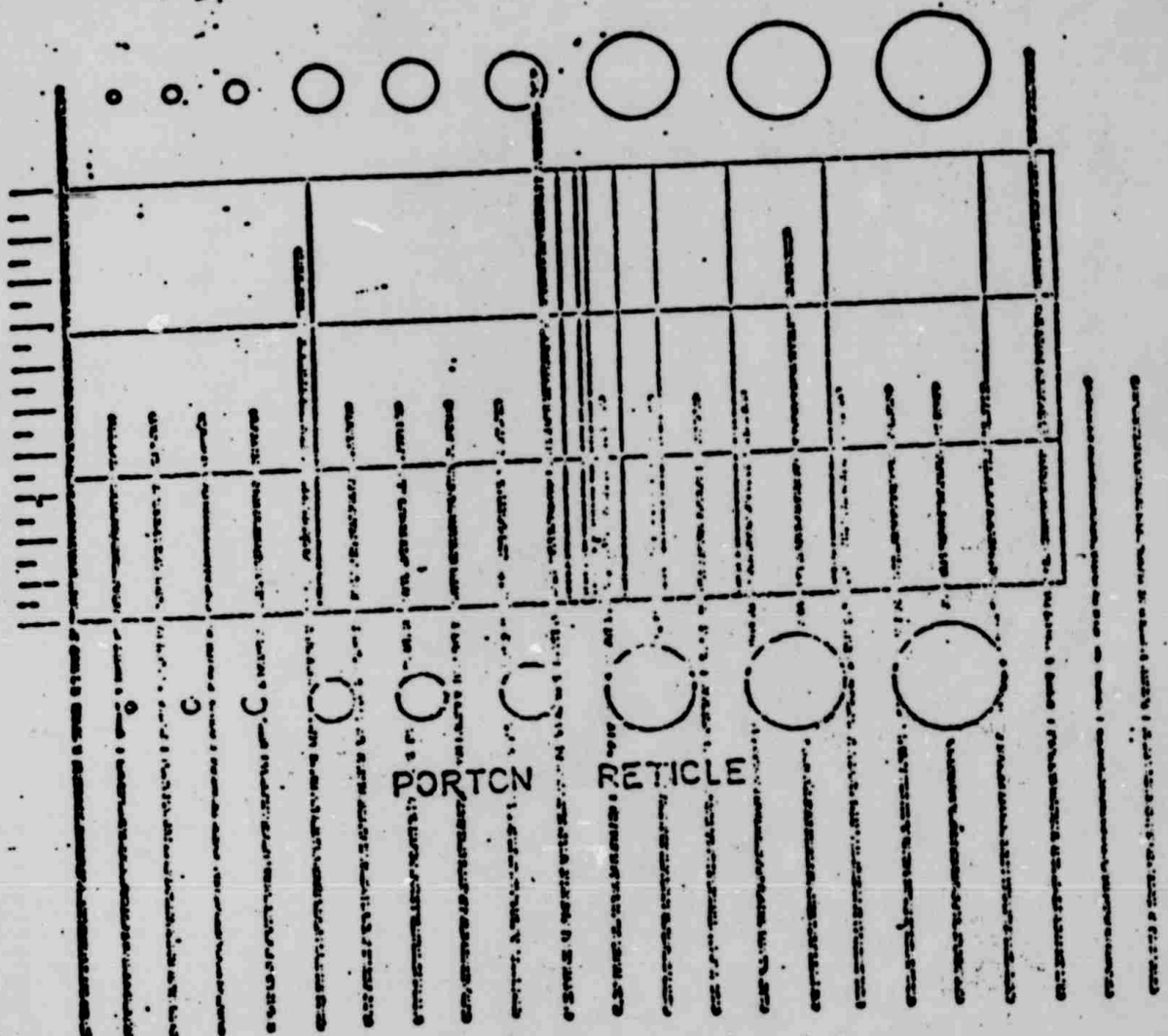


Figure 4 - Porton reticle and stage micrometer.

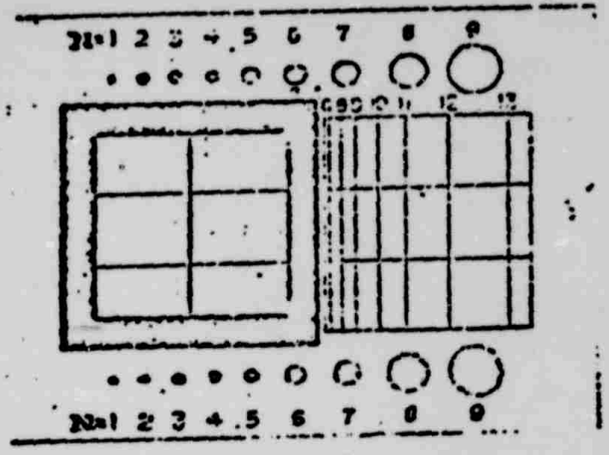


Fig.5-Boundaries of counting field area.

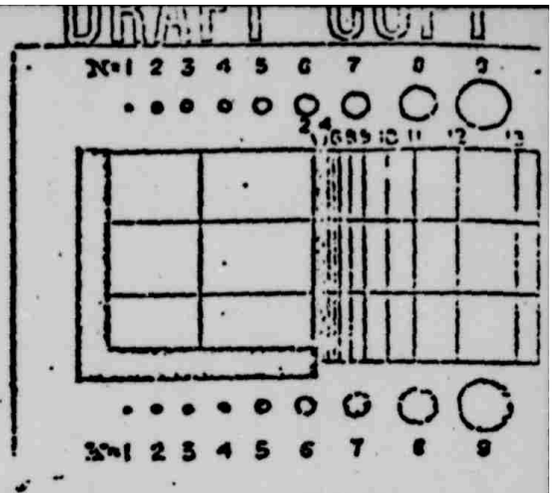


Fig.6-Pre-selected adjacent sides.

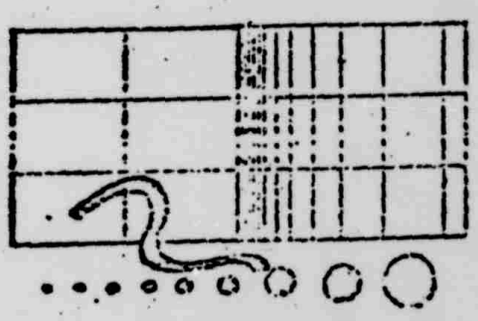


Fig.7-DO COUNT, part of fiber is within the field area and crosses one of the pre-selected sides.

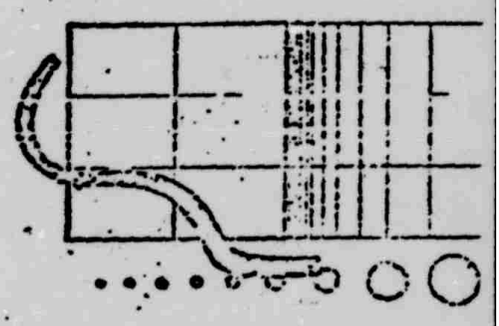


Fig.8-DO COUNT, fiber crosses both pre-selected sides and part of fibe is inside field area.

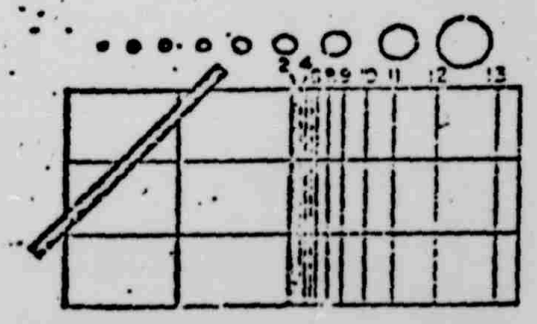


Fig.9-DO NOT COUNT, fiber crosses one of the wrong sides.

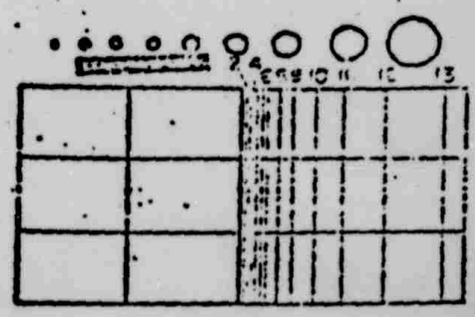


Fig.10-DO NOT COUNT, fiber is enti outside field area.

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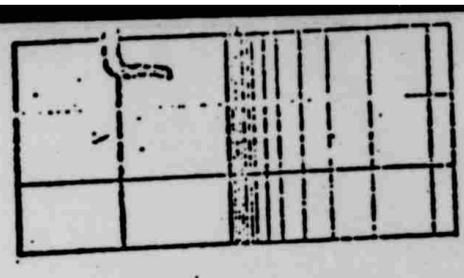


Fig.11-DO NOT COUNT, fiber crosses one of wrong sides.

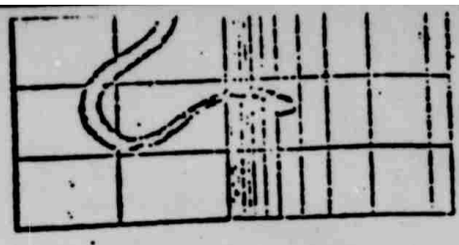


Fig.12-DO NOT COUNT, fiber crosse both wrong sides.

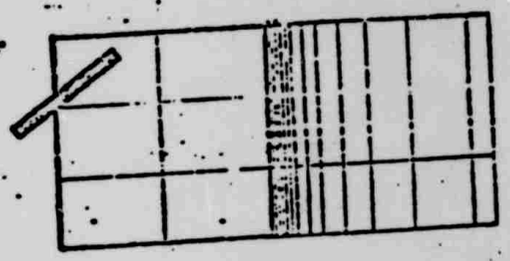


Fig. 13-DO COUNT, fiber crosses one of the pre-selected sides.

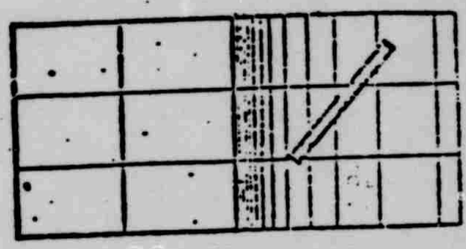


Fig.14-DO NOT COUNT, fiber is entirely outside field area.

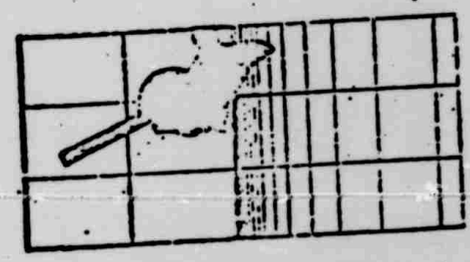


Fig.15-DO NOT COUNT, one end of fiber is not visible.

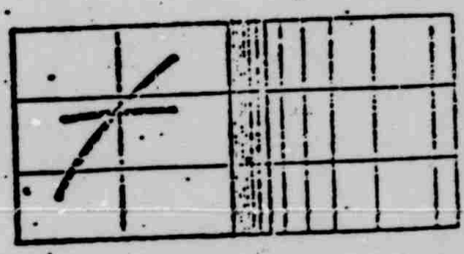


Fig.16-DO COUNT, as two fibers, both ends of both fibers are vi

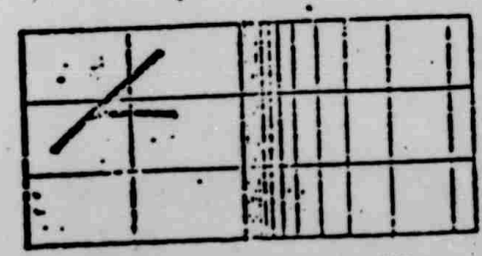


Fig.17-DO COUNT, as one fiber, one end of a fiber is not visible.

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