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C.R. FORTNEY

EQUIPMENT AND PROCEDURES FOR MOUNTING MILLIPORE FILTERS AND COUNTING ASBESTOS FIBERS BY PHASE CONTRAST MICROSCOPY

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FOREWORD

To determine concentrations of asbestos dust in air for comparison with established hygiene limits, a simple reproducible method is required. A simple and inexpensive sampling method uses disposable (reloadable if desired) plastic membrane filter holders, pre-loaded with membrane filters. These membrane filters may be rendered transparent and the asbestos fibers counted with a phase-contrast microscope at 430X (or 400X). This method has been used by the Asbestosis Research Council in Great Britain and the U. S. Public Health Service in the United States of America. It is specified in the proposed change for asbestos in the 1968 threshold limit values of the American Conference of Governmental Industrial Hygienists.

Uniform procedures must be used for carrying out these counts if reproducible results are to be achieved. Detailed equipment and procedures used by the U. S. Public Health Service are described here.

INTRODUCTION

For several years, a set method for counting and sizing airborne particulate matter, which has been deposited upon membrane filters, has been used. The filters used in this technique are 37 mm diameter cellulose ester membrane filters manufactured by Millipore Corporation, (cat. no. AAWP03700). The slide mounting technique refers only to these filters, and will not work for any others.

The filters may be purchased premounted in convenient field monitor cases, and are ready for sampling. The filters are nearly 100% efficient for any asbestos dust, even for fibers with diameters much smaller than the 0.8 micron (μ) pore size. Sample preparation for electron and optical examination are both relatively simple. Slide mounts of the filters are semi-permanent.

The following procedures and recommendations will produce accurate results, if each step is performed exactly as indicated.

EQUIPMENT LIST

1. Table
2. Adjustable chair
3. Phase contrast microscope
4. Ribbon filament illuminator or (built-in illuminator)
5. Eye piece reticle (Porton type)
6. Lens tissue
7. Small camels hair brush
8. Slides
9. Cover slips
10. Spatula
11. Tweezers
12. Scalpel
13. Wheaton Balsam bottle

LABORATORY SET-UP

It is important to provide a suitable counting area, for the room in which the counting is to be done has a great bearing upon the microsconist. It should be out of the way of normal traffic, and be kept as free from dust and smoke as possible. The tables on which the microscopes are placed should be as free from vibrations and shocks as possible. An adjustable chair will do much to add to the comfort of the counter.

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PRELIMINARY MICROSCOPE SET UP

Before any dust sizing or counting can be done, proper equipment must be at hand. This section describes the required equipment.

The illuminator should be small and reasonably compact. Ribbon filament illuminators are generally much easier to use. The illuminator must incorporate a condensing lens, so that the enlarged image of the lamp filament may be focused in the plane of the substage condenser diaphragm. The illumnator must also have an iris diaphragm located as near as possible to the condensing lens. The iris serves as a field diaphragm and is focused in the plane of the specimen.

The microscope used must have a substage condenser fitted with an iris diaphragm. This iris serves as an aperture diaphragm. The microscope should have a calibrated reticle inside the non-adjustable eyepiece. The reticle should be small enough that all the counting field is in focus (if the flat field objective and eyepieces are used, essentially the entire field will be in focus). The microscopes must be equipped with phase contrast accessories. Using phase contrast, even objects with almost the same refractive index as the mounting media can be seen. Light waves which travel through such a specimen are retarded by a fraction of a wavelength to produce a change in phase. The phase contrast optical system reveals these phase changes as light or dark contrast against the background.

This procedure was written by a user of Bausch and Lomb phase-equipped microscopes, so that microscope set-up procedures correspond with

these. However, any good microscope with phase contrast accessories will be satisfactory.

For continual routine examination of filters, the binocular type microscope is much more comfortable. The use of a first surface mirror is recommended, but not necessary. By utlizing a second surface mirror, multiple images of the illuminator field iris are formed which will detract from the crispness of the final image.

As mentioned previously, the microscope must contain a reticle. Any reticle, such as the Porton or Patterson Globe and Circle, which projects a constant counting area and has provision for sizing, will work. The Porton reticle which we use outlines a rectangle that fits easily within the periphery of focus. The rectangle is divided into two squares. The left square is divided into six rectangles which constitutes the counting area. Above and below the large rectangle are a series of circles in which every other circle doubles in diameter. Hence the third is twice the diameter of the first; the fourth is twice the size of the second, etc. The right half of the rectangle contains a scale for extending the size above the number nine circle. The formula $D=L\ 2^N$ describes the circles sizes. The diameter D is found from L which is the unit of length, and N which represents the number of the circle. L is determined by calibration with a stage micrometer. The entire length of the rectangle is 200 L units. By measuring the length and dividing by 200, L is obtained and the circle sizes may be calculated. Whenever a microscope is disassembled or cleaned, the reticle calibration should be checked. Another fact to take

into consideration is that changing the interpupillary distance of the binocular will change the calibration, so the microscopist may want to check the calibration at both extremes of adjustment. The counting area on the reticle should be kept as clean as possible, as dirt on the reticle is in focus and may be counted.

MICROSCOPE SET-UP AND THE PROCEDURE FOR OBTAINING KOHLER ILLUMINATION

By utilizing equipment that meets the requirements just mentioned, any counter may achieve comparable counts after some practice. This section will deal with setting the microscope up and obtaining Kohler illumination. It is extremely important that every step be followed exactly as indicated.

1. Place the microscope on a flat, level surface at a height such that the eyepieces may be observed without strain or discomfort.
2. Place a moderate dust sample upon the stage.
3. Place the illuminator directly in front of the microscope. Sight across the two and be sure they are aligned. For coil filament bulbs the illuminator iris should be ten inches from the center of the microscope mirror. For ribbon filament bulbs, the front of the filter holder should be seven inches from the center of the plane side of the mirror. Never use the curved side.
4. Remove all diffusing filters from the system and insert a neutral density filter and a clear blue filter. Blue or green colored filters may be used at the discretion of the microscopist.

NOTE: Where the microscope includes a built-in illuminator to give Kohler illumination, the above steps will not all be necessary.

REVIEW

A. Check microscope and illuminator alignment.

B. Check the distance between the microscope and the illuminator.

C. Be sure that you are using the flat side of the mirror.

D. Make sure that there are only two filters in the system.

5. By means of the focusing knob on the side of the substage condenser assembly, raise it until the upper lens nearly touches the botton of the slide. Remember that the free working distance of the condenser is in the order of 1.2 mm and that the slide is about 1 mm thick.

6. Turn on the illuminatr. Using the tilt controls, direct the beam onto the center of the mirror. Then, by tilting the mirror direct the beam upward into the condenser.

7. Close the substage iris completely. Open the illuminator iris all the way.

8. By means of the focusing controls on the side of the illuminator, focus the image of the filament on the botton of the substage iris. This may be done by leaning over the microscope and observing the substage iris in the microscope mirror. (On such models as the Bausch & Lomb PR-27, there is a fine focus knob at the rear of the illuminator). In that case, move the condensing lens all the way forward and trim the focus with the fine focus knob.

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9. Open the substage iris about half way.

REVIEW

A. Check the distance between the slide and condenser.

B. Be sure that the beam is striking the center of the mirror, and that it is being deflected upward into the condenser.

C. Please repeat step 7.

D. Check step 8. If the image is not in focus now, correct it. Do not continue until the filament is correctly focused.

E. Please repeat step 9.

10. Put the 10X objective in place and focus on the sample. This is done as follows: First, looking from the side of the microscope, lower the objective until it gets very near to the slide. Then, by looking through the eyepieces, focus up. Never focus down with coarse focus when looking through eyepieces.

A word of caution: The objective lenses are very expensive, so be careful not to grind them through the slide. Always focus up. Now trim the focus with fine focus knob. Focus sharply on the sample. Secondly, close the field iris fully. If the condenser is nearly in focus, you should see a bright spot of light. By using the condenser focus knob, bring the field iris into sharp focus. Now, both the field iris and the objective are in focus on the sample. If there appear to be multiple images of the field iris, it is because of the second surface mirror. Some of the light waves are reflected from the first surface, resulting in secondary images.

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12. If the image of the field iris is not centered, re-center it by tilting the mirror. If the color around the field iris is not uniform, recheck the tilting of the illuminator.

REVIEW

A. Check and be sure you are focused on the sample. 10X objectives have a relatively long focal length and it may possible to focus on: (a) the top of the cover slip. (b) the sample. (c) the botton of the filter. (d) the top of the slide. (e) the bottom of the slide. (f) the top of the condenser. So be sure of the focal plane.

B. Be sure that the field iris is focused in the sample plane. Check this often when examining samples. As the sample focus changes, so does the condenser focus.

C. Recheck the centering and tilt of the illuminator.

DETAILED INSTRUCTIONS FOR BAUSCH AND LOMB BINOCULAR MICROSCOPES

1. Leave the microscope phase rind on 0. Insert the telescope that is supplied with the kit into the right eyepiece tube. Focus on the dark phase ring. Open and close the substage iris to be sure that it is centered in relation to the objective phase ring. If it is not in the center, loosen the set screw on the condenser mount and properly seat the condenser. Then re-tighten the set screw. This should very seldom be necessary. Do not force anything and be careful and patient. When you are satisfied that the condenser is aligned, open it all the way.

2. Turn the phase wheel to 10. This shows that the 10X phase

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ring is in place. Look through the telescope again. By using the adjustment screws on the side of the condenser, move the rings until they are perfectly concentric. When changing phase rings, move them carefully, so as not to knock the condenser out of alignment.

3. Turn to the 43X objective. Carefully turn the wheel to the 43 phase ring. Align the two rings in the same manner as performed in step 2.

4. Remove the telescope and replace the eyepieces. Again, check the condenser focus. You are now ready to examine the sample at 430X, assuming that you are using 10X eyepieces.

FILTER MOUNTING

Set-up Prior to Mounting

Have available at hand a quality scalpel and a supply of no. 10 curved blades, a pair of tweezers (fine pointed are preferred), a spatula or fire polished glass rod and a box of lens tissue. Slides (1" x 3" or 25 x 75mm) with frosted ends are best because one may label the slide with the date, sample number, etc. It is advisable to use quality number 1-1/2 cover slips. Most dry objectives are corrected for this thickness cover glass. The mounting media should be kept in a Wheaton balsam bottle. The edge around the lid should be coated with stopcock grease to keep out the surrounding air.

The mounting solution may now be prepared. A one to one solution by volume of dimethyl phthalate and diethyl oxalate is poured into the balsam

bottle. Then add 0.05 gram of filter material for each milliliter of solution. The added filters increase the viscosity of the solution. Before using the solution, all of the filter material must be dissolved and the solution must be of uniform consistency. This may require a day or so with frequent stirring. Refrigeration may add to the shelf life of the solution.

Mounting Procedure

Keep in mind that cleanliness is important. First, lay down two pieces of lens tissue; one directly in front of you and the other to the left or right for the mounting tools. Next, wipe the scalpel, tweezers and glass rod or spatula with lens tissue. Holding a slide by the frosting, wipe it clean. Lay it down with the frosted end toward you. Even pre-cleaned slides must be cleaned this way. Hold a cover slip by the edges between the index finger and the thumb, and clean it in the same manner as the slide.

Place the cover slip so that one edge rests on the unfrosted end of the slide and the other edge rests on the lens tissue. Be sure that the lower surface does not come into contact with the lens tissue.

Using the glass rod, or dropper, dispense a small drop of the mounting media onto the center of the slide. Replace the rod or dropper into the solution bottle and cover it. Using the spatula, spread the solution into a triangular shape. While the fluid is spreading, cut a wedge of corresponding size from the filter. Place it upon the mounting media, sample side up. Pick up the cover slip and place it on top of the filter. Press lightly on the cover slip to be sure the mounting media has made contact with it. Label the slide before completing any other

slide. Sometine, after an hour or so, the filter may not have cleared. Usually, this can be cured by pressing lightly on the cover slip with a pencil eraser that has been wrapped with lens tissue. If the filter has been used to filter liquids or is damp, it will require drying, for it will not clear unless completely dry.

Use only enough solution to clear the filter. If too much solution is used the sample may spread and give an erroneous count per unit area. The filter should be counted as soon as possible because the solution may eventually form crystals that look like fibers and may be mistaken for part of the sample.

After a little practice and experience, several samples may be mounted at the same time with excellent results.

Remember to keep the area in which mounting is done as clean as possible to prevent contamination of the filter.

MICROSCOPE PRECAUTIONS AND MAINTENANCE

Quality lens tissue is a must. The microscopist should use it for any kind of lens cleaning. It should be as lint free as possible. It may be wrapped around the blunt end of a small camels hair brush and used to clean the eyepieces. Never wipe the inside of the microsocpe body. If it is necessary to remove dirt from the inside of the tubes, blow it out with an aspirator bulb. Whenever you are using immersion oil, be sure that you wipe all surfaces that were in contact with the oil.

Generally, solvents should not be used when cleaning lenses. Alcohol, like most solvents will dissolve the lens mounting cement, which may ruin

the lens system. Xylene should be used sparingly to remove immersion oil.

Use a camels hair brush to clean first-surface mirrors and non-critical lens surfaces. Nevet wipe first-surface mirrors with lens tissue.

Avoid touching a lens surface with fingers as the fingerprint has a tendency to etch into the lens surface.

COUNTING PROCEDURE

Place the sample on the stage and focus on it. Since the sample is retained in the top ten fifteen microns of the filter, be sure that you are focused in the proper plane. The reticle in the left eyepiece is used to define the counting area. The reticle should be calibrated prior to being used. The total fiber count (a fiber is anything three times as long as it is wide) should be at least 100 fibers, or twenty fields, whichever is less. It is advisable to made as large a count as practical to get an accurate average. If a fiber crosses the limits of the counting field, only those crossing either or both of two adjacent sides are counted. For example, a fiber crossing the top or right, or possibly both sides would be counted. The sides chosen are at the discretion of the counter, but any counter must always use the same sides. The fibers counted are estimated as to length,using the circles at the top of the reticle. Our practice has been to record all fibers seen, all fibers longer than five microns and all fibers longer than 10 microns. However, for comparison with the ACGIH TLV (1968), only the longer than 5μ fibers need be counted.

FIELD MONITOR DISCUSSION

Membrane filters may be purchases pre-loaded in plastic holders (Aerosol Analysis Monitors). The Monitors consist of three sections. The filter is held between the middle and bottom sections. Between the membrane filter and the bottom section lies a support pad. The calibrated pump, designed to draw a known and constant supply of air, is always connected to the bottom of the field monitor case. When many loaded cases are taken into one particular area for sampling, a blank or control filter (meaning a loaded field monitor case) should be taken out to the area, brought back unused and counted. This will determine what degree of general contamination is present on the filters. We have found that banding the field monitor cases with cellulose bands greatly reduces the general contamination of the filters. The bands may be purchase from Walter H. Jelly and Company, Inc. They are white, opaque, size 41 x 25 and come packed in solution S-132. Upon removal from the solution, the bands are slipped around the middle and bottom joints, or around all joints, depending upon whether the sample is going to be drawn through the top section plug hole, or the entire top section removed for "open" sampling. The latter procedure is recommended for asbestos dust sampling since it results in a more even sample distribution over the entire exposed filter surface.

Convenient personal sampling pumps, such as those distributed by Mine Safety Appliances Co., Willson Products Division or Union Industrial are excellent for short term, low rate (around 2 lpm) sampling. A sampling pump is connected to a 3 foot length of non-collapsable, one quarter inch, rubber tubing with a male Leur slip adapter (stock number LL/L, available from Becton, Dickson and Co.) inserted into the other end. This enables one to

connect the hose directly to the case by simply inserting the adapter into the plug hole at the bottom of the case.

Field monitor cases may easily be re-used. The plugs and all sections are separated and placed in warm detergent water. The parts should be scrubbed thoroughly and rinsed in tap water. However, if the filters are to receive any chemical analysis, the cases should also be rinsed in distilled water. The cases should be allowed to dry in a cleann area (a clean room is preferable), to reduce contamination. The clean area is equally important when loading the filters into the cases. The filter pad (the thicker, fibrous disc) is placed in the bottom section. The filter is placed directly on the pad. The middle and top sections are added, the case is pressed tightly together, and the top plug is inserted. Replacement of the bottom plug is not necessary. A cellulose band is then place around the case and after the band dries, the unit is ready for sampling. Sample code numbers, etc. may be written on the band or upon the filter case, first making sure the markings can be removed when and if it is necessary.

Additional information concerning filters and field monitors may be obtained from Millipore Corporation.

CALCULATION OF CONCENTRATION

When a sample is taken, pertinent information about the sample should be recorded at the sampling site. First, the sample number should be written on the field monitor case and the sample ticket. Next, enter a description of his operation in the appropriate space. If the sample is a general air sample and does not pertain to any particular employee,

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write "general air sample" in the employee's name space. Information about the area may be written in the "description of operation" space. If you have several pumps which are identical in appearance, but have different flow rates, it would be a good idea to assign them instrument numbers. This enables you to keep accurate records of maintenance and califbration. When instrument numbers are kept it is not necessary to enter the flow rate at the sampling site. It may be entered later from the record cards. Otherwise, enter the flow rate at the sampling site. When a number of filters are taken into a general area a control (or blank) is taken along. This should be assigned a number and entered in the blank number space. Do not assign blank filters duplicate numbers.

When the sampling pump is turned on, enter the time in the "time on" space. While the sample is being taken make notes of controls, i.e. exhaust hoods, fans, window ventilation, etc. These notes should be entered in the "description of controls". The sampler should then sign his name or initials at the bottom of the sheet.

After sufficient time has passed, the sampling instrument should be turned off and the time recorded in the "time off" space. The difference between "time on" and "time off" space. The "total air volume" may be found by multiplying the flow rate (expressed liters per minute) by the total time and recorded in the appropriate space.

After the sample has been taken, brought back into the lab and mounted, the counting procedure is next.

The microscope must be properly set up. Place the slide to be counted on the mechanical stage. Focus on the sample a short distance away

from the edge. Never count close to the edges of the filter because the filter will spread a little and lower the count. Now, enter the date, sample number, blank number and field area on the count sheet. The counter should initial the form in the "initial" space. The sized fibers are entered from right to left across the form, first entering the >10μ fibers, then the >5μ fibers and finally, all fibers seen, called the total. After 100 fibers or 20 fields are counted and recorded, all of the vertical columns are added up and the totals are entered under their corresponding columns. Divide the totals at the bottom by the number of fields counted to determine the average number of fibers per size range per field. By using information on these two sheets the concentration in fibers per cubic milliliter may be calculated and entered in the "con." space on the count sheet.

$$\text{Concentration} = \frac{(\text{Av. Fiber Cnt.})\ (\text{filter area})}{(\text{field area})\ (\text{Sample volume})\ (1000)}$$

Av. Fiber Count:	Average fiber count per size range
Filter area:	855 mm^2 for 37 mm dia. membrane filters
Counting area:	Reticle calibrated in mm^2
Sample volume:	total time x flow rate, express in liters
1000:	Converts liters to milliliters

If blanks are used, subtract the average blank count of the same size range from the average fiber count.

ADDRESSES

Millipore Field Monitor Cases (complete)
Cat. No. MAWP 037A0
Millipore filter (W. Pads)
Cat. No. AAWP03700
Millipore Filter Corporation
Bedford, Massachusetts ~~07130~~ 01730

Porton Reticle
Cat. No. 30084
Patterson Globe & Circle Reticle
Cat. No. 30083
Edmund Scientific Co.
701 Edscorp Building
Barrington, New Jersey 08007

Wheaton Balsam Bottle
Cat. No. 2244
Arthur H. Thomas Company
Post Office Box 779
Philadelphia, Pennsylvania 19105

Adapters, hose end for 1/4" tubing to male leur slip
Cat. No. LH/L
Becton, Dickinson and Company
Rutherford, New Jersey

White, opaque, cellulose bands, 41 x 25
No. 28
Walter H. Jelly & Co., Inc.
2822 Birch Street
Franklin Park, Illinois 60131

Personal Sampler Mk. II
Willson Products Division
Reading, Pennsylvania

Monitaire Sampler
Mine Safety Appliances Co.
Pittsburgh, Pennsylvania

Unico Telematic Model C-110
Unico Environmental Industries, Inc.
Fall River, Massachusetts

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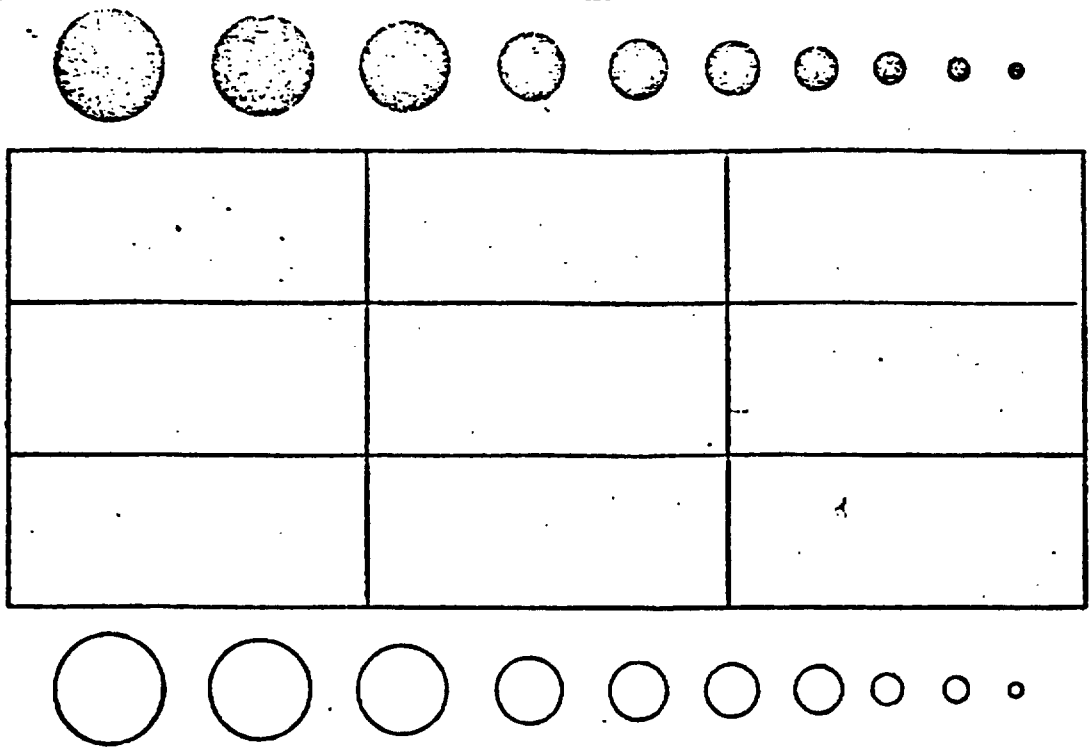
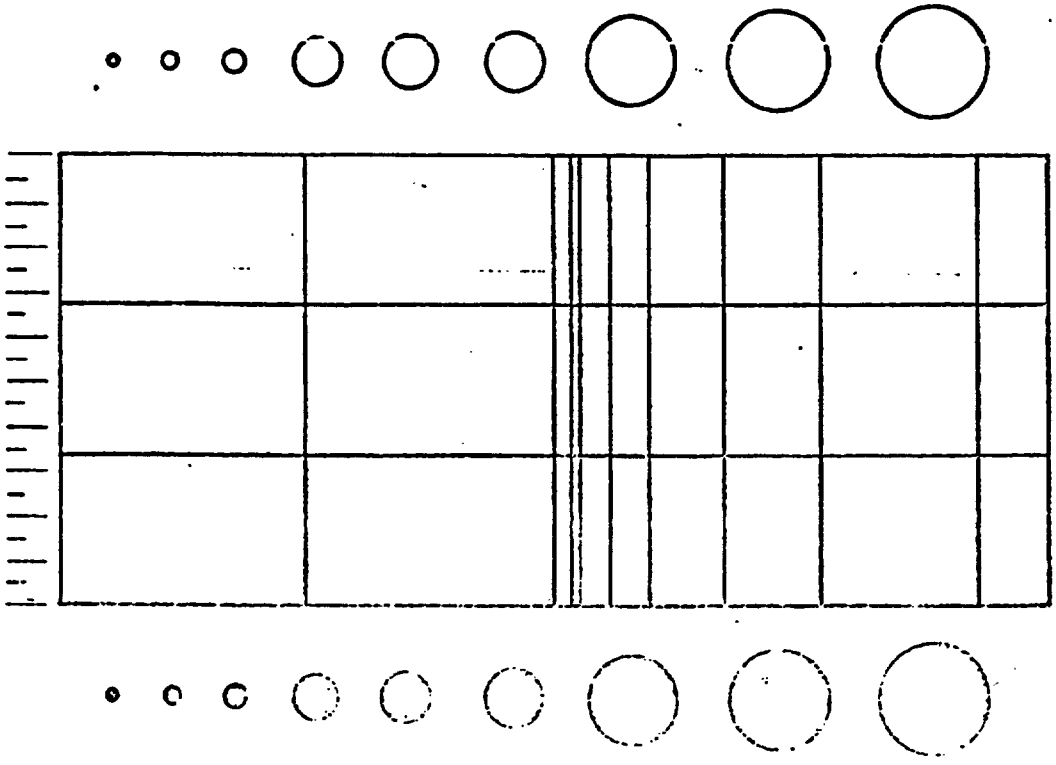


PATTERSON GLOBE AND CIRCLE



PORTON RETICLE

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SAMPLE _____ DATE _____

INST. _____ FLOW RATE _____ BLANK _____

TIME ON _____ TIME OFF _____

TOTAL TIME _____

TOTAL AIR VOLUME _____

EMPLOYEES NAME _____

JOB TITLE _____

DESCRIPTION OF OPERATION

DESCRIPTION OF CONTROLS

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INITIALS _____

SAMPLE TICKET

DATE _____ SAMPLE _____

FIELD AREA _____ BLANK _____

INITIALS _____ CONCENTRATION _____

	TOTAL	FIBERS >5 μ	>10 μ
TOT.			
AV.			

COUNT SHEET

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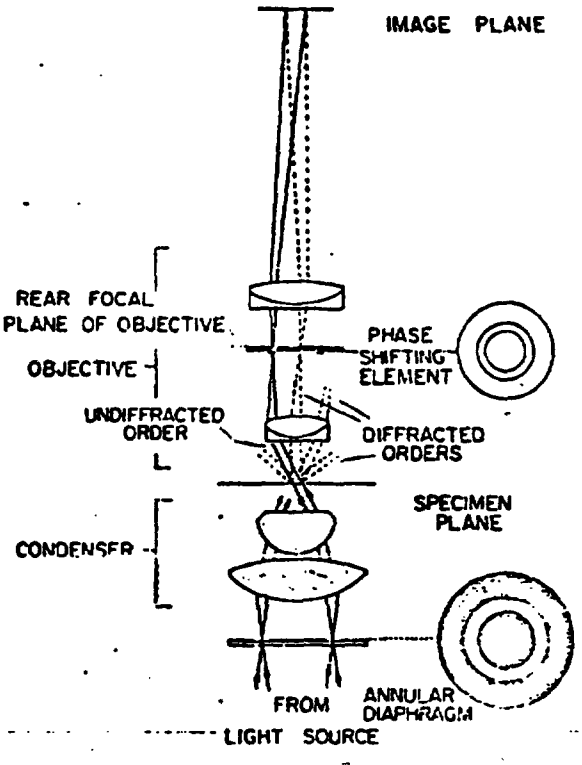

PHASE CONTRAST MICROSCOPE

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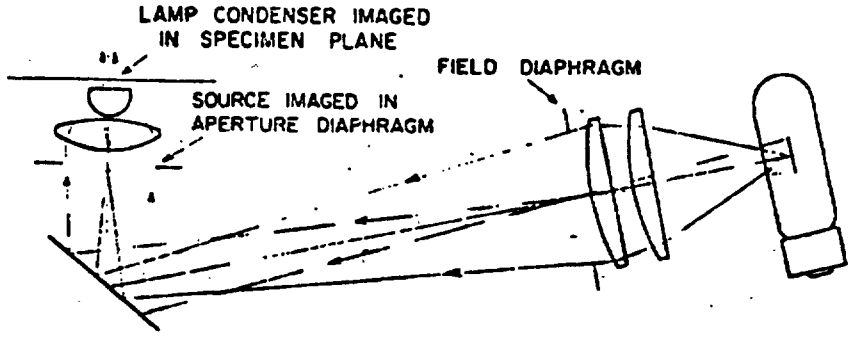

KOEHLER ILLUMINATION

VIII. APPENDIX I

Air Sampling Methods

In the study of asbestosis conducted by Dreessen et al.[63] midget impinger count data were used as an estimate of dust exposure. All of the dust particles seen, both grains and fibers, were counted since too few fibers were seen to give an accurate measurement. The resulting count concentration was a measure of overall dust levels rather than a specific measurement of the asbestos concentration. This method was satisfactory at that time since exposures were massive and the control measures installed to reduce overall dust levels also reduced the asbestos dust levels.

As dust levels were reduced, it became necessary to measure the biologically appropriate attribute of the dust cloud. At equal levels of overall dustiness, the concentration of asbestos could vary considerably from textile manufacture (75-85%) to insulation (5-15%). Furthermore, if the limit were lowered below the 5 mppcf used previously and dust counts taken by the impinger technique, it would be necessary to consider the effect of background dust, which could be as high as 1 mppcf.

A number of methods for measurement of asbestos dust concentrations have been used in the NIOSH epidemiological study of the asbestos product industry.[73,74,75,76] Based on these data, the preferred index of asbestos exposure is the concentration of fibers longer than 5 μm counted on membrane filters at 430X with phase contrast illumination.[71,72] This index is utilized in the method adopted as the standard field sampling method by the Public Health Service.

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fibers longer than 5 μm in length are counted in preference to counting all fibers seen in order to minimize observer/microscope resolving power variability. Furthermore, the British define a "fibre" as a particle, "of length between 5 μm and 100 μm and having a length-to-breadth ratio of at least 3:1, observed by transmitted light by means of a microscope at a magnification of approximately 500X."[62]

Although the British have refrained from standardizing on a single method of measurement, recent measurements have been performed by a method essentially identical to the fiber-count method described in detail below, and the British hygiene standards for use with their asbestos regulations are stated in these terms.[62]

Principles of Sampling

A dust sampling procedure must be designed so that samples of actual dust concentrations are collected accurately and consistently. The results of the analysis of these samples will reflect, realistically, the concentrations of dust at the place and time of sampling.

In order to collect a sample representative of airborne dust, which is likely to enter the subject's respiratory system, it is necessary to position a collection apparatus near the nose and mouth of the subject or in his "breathing zone".

The concentration of dust in the air to which a worker is exposed will vary, depending upon the nature of the operation and upon the type of work performed by the operator and the position of the operator relative to the source of the dust. The amount of dust inhaled by a worker can vary daily, seasonally, and with the weather. In order to obtain representative samples of workers' exposures, it is necessary to collect samples under varying conditions of weather, on different

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days, and at different times during a shift.

The percentage of working time spent on different tasks will affect the concentration of dust the worker inhales since the different tasks usually result in exposure to different concentrations. The percentage can be determined from work schedules and by observation of work routines.

The daily average weighted exposure can be determined by using the following formula:

$$\frac{(\text{Hours X conc. task A}) + (\text{Hours X conc. task B}) + \text{etc.}}{8 \text{ Hours (or actual hours worked)}}$$

The concentration of any air contaminant resulting from an industrial operation also varies with time. Therefore, a longer sampling time will better approximate the actual average.

With the following recommended sampling procedure, it is possible to collect samples at the workers' breathing zones for periods from 4 to 8 hours, thus permitting the evaluation of average exposures for a half or full 8-hour shift--a desirable and recommended procedure. Furthermore, dust exposures of a more normal work pattern result from the use of personal samplers. In evaluating daily exposures, samples should be collected as near as possible to workers' breathing zones.

Collecting Sample

The method recommended in this report for taking samples and counting fibers is based on a modification of the membrane filter method described by Edwards and Lynch.[71]

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The sample should be collected on a 37-millimeter Millipore type AA* filter mounted in an open-face filter holder. The holder should be fastened to the worker's lapel and air drawn through the filter by means of a battery-powered personal sampler pump similar to those approved by NIOSH under the provisions of 30 CFR 74. The filters are contained in plastic filter holders and are supported on pads which also aid in controlling the distribution of air through the filter. To yield a more uniform sample deposit, the filter-holder face-caps should be removed. Sampling flow rates from 1.0 liter per minute (lpm) up to the maximum flow rate of the personal sampler pump (usually not over 2.5 lpm) and sampling time from 15 minutes to eight hours are acceptable provided the following restraints are considered:

(a) In order to obtain an accurate estimate of the number of fibers the statistical error resulting from the random distribution of the fibers must be kept to an acceptably low level. Since fiber counts follow a Poisson distribution, a count of 100 fibers in a sample would have a standard deviation of $\sqrt{100}$ or 10 fibers or $\pm$ 10%. Thus the 95% confidence limits would be approximately 2 standard deviations or $\pm$ 20%. Since the 37 mm filter has an effective collecting area of 855 mm^2 and the projected field area of the Porton reticle is 0.005 mm^2, each field represents 1/171000 of the sample. Based on this ratio the following number of fields must be counted to measure the various limits in various sampling times:

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*Mention of commercial products does not constitute endorsement by the Public Health Service or U. S. Department of Health, Education and Welfare.

Sampling Time Minutes	Flow Rate lpm	Number of Fields for 100 Fibers 0.2 fibers/ml	2.0 fibers/ml	10 fibers/ml
10	2	4350	435	91
15	2	2860	286	58
30	2	1430	143	29
90	1	1000	100	20
90	2	500	50	10
240	1	260	26	7
240	2	180	18	4
480	1	180	18	4

(b) Do not count a field containing over 20 fibers because in addition to the fibers being counted, there are also present a number of grains, which interfere with the accuracy of the count.

Based on these restraints, i.e., number of fields to be counted and maximum number of fibers per field, acceptable sampling parameters for the various limits are underlined in the above table.

The following conclusions may be drawn from this analysis:

(1) The short-term limit should be for a period of at least 15 minutes and preferably 30 minutes.

(2) The 2.0 fiber/cc limit may be evaluated over periods of from 90 to 480 minutes.

As many fields as required to yield at least 100 fibers should be counted. In general the minimum number of fields should be 20 and the maximum 100.

Mounting Sample

The mounting medium used in this method is prepared by dissolving 0.05 g of membrane filter per ml of 1:1 solution of dimethyl phthalate

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and diethyl oxalate. The index of refraction of the medium thus prepared is ND = 1.47.

To prepare a sample for microscopic examination, a drop of the mounting medium is placed on a freshly cleaned, standard (25 mm X 75 mm), microscopic slide. A wedge-shaped piece with arc length of about 1 cm is excised from the filter with a scalpel and forceps and placed dust-side-up on the drop of mounting solution. A No. 1-1/2 coverslip, carefully cleaned with lens tissue, is placed over the filter wedge. Slight pressure on the coverslip achieves contact between it and the mounting medium. The sample may be examined as soon as the mount is transparent. The optical homogeneity of the resulting mount is nearly perfect, with only a slight background granularity under phase contrast, which disappears within one day. The sample should be counted within two days after mounting.

Evaluation

The filter samples mounted in the manner previously described are evaluated in terms of the concentration of asbestos fibers greater than 5 μm in length. A microscope equipped with phase-contrast optics and a 4-mm "high-dry" achromatic objective is suitable for this determination. 10X eyepieces, one of which contains a Porton or other suitable reticle at the level of the field-limiting diaphragm, should be used. The left half of the Porton reticle field serves to define the counting area of the field. Twenty fields located at random on the sample are counted and total asbestos fibers longer than 5 μm are recorded. Any particle having an aspect ratio of three or greater is considered a fiber.

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The following formulae are used to determine the number of fibers/ml:

(1) $\frac{\text{Filter area } (mm^2)}{\text{Field area } (mm^2)} = K$

(2) $\frac{\text{Average net count} \times K}{\text{Air volume sampled (ml)}} = \text{fibers/ml}$

For example, assume the following: area of the filter used was 855 mm^2, counting area of one field under the Porton reticle was 0.005 mm^2; average net count per field of 20 fields was 10 fibers; and sample was collected at 2 liters per minute for 90 minutes: Then:

$$\frac{855 mm^2}{0.005 mm^2} = 171{,}000 \text{ (K)}$$

$$\frac{10 \text{ fibers} \times 171{,}000}{2{,}000 \text{ ml/min} \times 90 \text{ min}} = 9.5 \text{ fibers/ml}$$

Calibration of Personal Sampler

The accuracy of an analysis 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 somewhat dependent on the use, care, and handling to which the pump is subjected. Pumps should be calibrated if they have been subjected to misuse or if they have just been repaired or received from a manufacturer. If hard usage is given the instrument, more frequent calibration may be necessary.

Ordinarily, pumps should be calibrated in the laboratory both before they are used in the field and after they have been used to collect a large number of field samples. 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, a 1-liter burette or wet-test meter should be used. In the field, a rotameter is the most convenient

instrument used. The actual set-up will be the same for all of these instruments. The calibration instrument will be connected in sequence to the filter unit which will be followed by the personal sampler pump. In this way, the calibration instrument will be at atmospheric pressure. Connections between units can be made using the same type of tubing used in the personal sampling unit. Each pump must be calibrated separately for each type of filter used, if, for example, it has been decided to use a filter with a different pore size. The burette should be set up so that the flow is toward the narrow end of the unit.

Care must be exercised in the assembly procedure to insure adequate seals at the joints and that the length of connecting tubing be kept at a minimum. Calibration should be done under the same conditions of pressure, temperature and density as will be encountered. The rotameter should be used only in the field as a check if the diaphragm or piston pumps are not equipped with pulsation dampeners. The pulsating flow resulting from these type pumps causes the rotameter to give results which are not as accurate as that obtained with a burette or wet-test meter. Calibration can be accomplished with any of the other standard calibrating instruments, such as spirometer, Marriott's bottle, or dry-gas meter. The burette and wet-test meter were selected because of their accuracy, availability, and ease of operation.

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2/3 used in cement products
fire-proop textiles, brake linings and gaskets
asbestos paper and mullborad, roof tiling, asbestos sheeting
paints, floor tiles, plastics

PRODUCT

CHEMICAL NAME

Filters in chem; food, drug, beer industries

Asbestos

TRADE NAME, SYNONYMS

90% of asbestos held is of chrysatile variety

CHEMICAL FAMILY

MANUFACTURER

FORMULA

MOL. WT.

Magnesium Calcium Silicate

STRUCTURAL FORMULA

HAZARD CLASSIFICATION

( ) EXPLOSIVE ( ) POISON ( ) CORROSIVE
( ) FLAMMABLE LIQUID ( ) COMBUSTIBLE LIQUID
( ) IRRITANT ( ) OXIDIZING AGENT ( ) _____

TOXICITY RATING

SKIN

GI

LUNG

0 = None Acute Local

1 = Slight Chronic Local

2 = Moderate

3 = High Acute Systemic

U = Unknown Chronic Systemic

f 42 cases of mesotheliomas (rare cancer of pleura and petitoneum) one syr. period, all but 11 ad some exposure to asbestos (10 worked in asbestos plant, 8 lived or worked close to asbestos lant, 3 were family members of asbestos workers, 10 had history of assumed exposure, 10 no nown history of exposure) (5)Long-continued inhalation asbestosis (intestinal polmonary fibrosis) isease is characterized by asbestos bodies in lungs and sputum. Based on x-rays can be lassified as minimal, moderate and advanced. It is a serious disease in some misfones but ere usually just disabling unless some other serious disease sequences (6) Chief symptons f advanced asbestosis are variable cough, dysynea, substernal chest pains, cher. chest expansion, etness, emaciation, clubbed fingertips and curved fingernails. Any appreciable dec. in am't f asbestos dust in breathing atmos. will cause dec. in incidence and severity of asbestosis. ndividual susceptibility varies (7, Hyg. Guide)

Neoplasms, such as pleural and peritioned mesothelioma, occur in excess even among asbestos orkers with little nino radiological evidence of asbestosis, suggesting that exposures insufficient to cause asbestosis may still produce neoplasia. (22,23)

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PHYSICAL AND CHEMICAL PROPERTIES See Mineralogical Appendix of (36).

Physical State-Masses, either compact or of long silky fibers, flax-like and readily separa
Color white, greyish or greenish
Odor
Vapor Density (air = 1)
Vapor Pressure (mm Hg)
Specific Gravity (H_2O = 1)
Melting Point
Boiling Point
Flash Point
Auto - ignition temp.
Viscosity
Surface Tension
Evaporation Rate
lel
uel
pH
pka
Partition Coefficient
Solubility Coefficient

Solubility - H_2O

lipids

Stability

Chemical Reactivity chem. inert and resists attach by most chem.

Decomposition & Polymerization Products

Materials & Conditions to Avoid

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GENERAL INFORMATION

Ingredients (if mixture or contains sig impurities) % TLV

Chrysotile (hydrated magnesium silicate) from serpentine = 95% of commercial asbestos
$2H_2O(Mg_3Si_2H_4O_9)$

Crocidolite (sodium iron silicate)
Amosile (iron magnesium silicate)
Tremolite (0-20 mole % of $(OH)_2Ca_2Fe_5Si_3O_{22}$)
Anthophyllite
Actinite

FIRE & EXPLOSION HAZARD

Fire - fighting Procedures

Personnel Protection (Clothing, equipment, ventilation)

Storage & Handling

Clean - up and Disposal

HEALTH HAZARDS

LD_{50} (lethal dose 50)

LD_{ca} (approx. lethal dose)

LC_{50} (lethal conc. 50)

LC_{ca} (approx. lethal conc.)

TDD (toxic dose)

TXC (toxic conc.)

TEF (toxic effects)

TLV	ppm	mg/m^3	Sampling period (1/10 t/12)	Biol. half-time	Ref.
EC	5 fibers/ml (longer than 5)				
	5MPPCF				(12,11) (1
Some feeling this is too high (See TLV documentation and 17)					(13,14,15,
over)					

004260

riteria for limiting chrysotile exposure assuming 1% of asbestos during 50 yr. working exposure,

ust category	Chrysotile Conc. (av. of 3 hrs) fivers in length m
eglibible	0.5
ow	0.5-2.0 limit of 100 fibers/ml yrs
edium	2-10
igh	10
	5 mppcf would provide inadequate protection
	2 might not be substantiated

ypes of Asbestos (21)
hrysotile - widely used in food processing industries (sugar, lard, beer),
re bundles of fibrils, each 200-400A dia and 1000 A to several micrometers long
harmaceutical use as filter
rocidolite - gas mask and cigarett filters
nosite
rithophyllite
remolite
mount of asbestos in some samples of dvvgs exceeded those in other env. circumstances
such as in (21)) These were drugs to be given on IV, IM in IP.

ources of Asbestoc incl. serpentine rock (chrysotile).. Industrial controls are necessary
o prevent air pollution, particularly in construction products, ship building and repair.(22)

	Prod. (1962) 1000 tons	Color	Composition (%) Si	Mg	Fe	Source
hrysotile ccurs in erpentine rock	1700	white	40	38	2	Canada, S. Africa, S. Rhodesia, U.S.A. Italy, Cyprus
rocidolite ccurs in banded ron stone	132	blue	50	--	40	S. Africa (Cape) Australia
nosite	75	brown	50	--	40	S. Africa (Transuaal
rthophyllite	11	white	58	29	6	Finland, Italy

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Blood TLV

Urine TLV

Breath TLV

Absorption Rate

Excretory Rate Am't of asbestos recovered from lungs of persons occupationally exposed as ranged from 0.6-0.001% of lung wt. (24), with chrsotile asbestos fiber low and amphibole sbestos fiber generally high (25)

Critical Site (Locus of Action)

nhalation, Alreolar membrane responds with production of chronim interstitial pnemonitis (35) ee also 34)

Biotransformation Chrysotile asbestos tends to break down chem. and physically after pro-onged bid. residence. Is chem. unstable every in distilled H_2O, and ag. solu < 0.8 pH. Some f its magnesium will be leached out (24,25,26). The bundle of fibrils breaks open and the ndividual unit fibrils are separated (27) (Not seen with amphibote asbestos) (often <0.5u)

Susceptible Individuals

Conc. X Time	Response or Effect	Species	Ref.
atural chrysotile			
.05-0.2μ-10.5 mg + 14 mg	90% mortalilz in 18 mos.	55 rals.	3
.2-1 μdia.	100% mortalilz in 24 mos.		
ynthetic chrysotile firbrous	also macrophage reaction with minimal stromal participation did ot collagenize		
.02-0.04 μ dia. 14 mg + 45 mg.	22% mortalilz in 12 mos. 53% mortalilz in 24 mos.		3
alc (tremolite			
.1-0.2 μ dia 25 mg to 50 rats injected intratracheally	40% mortality in 6 mos		3
0.2 μ = high natural nickel content	44% mortality in 6 mos		
0.1 μ = low natural nickel content			
lso polypoid proh teratiose inflammuration which in time become callogenous scaes deforming ir ways amorphous magnesium silicate	75 mg intratrachally in 10 rats 10% mortality in 6 mos.		3
roliferative inflammation considered artificial			

SYMPTOMS

Acute

Chronic Mesothelioma of the pleura occurred in 33 cases in S. Africa, all but one with a probable exposure to crocidolite asbestos (ape Blue) (32) Pleural calcification from asbestos exposure may occur. Appears usually bilaterally in pauetal pleura (x-ray) with no clinical objective or subjective resp. symptons. (36)

Diagnostic Procedures A characteristic finding in asbestosis is asbestos bodies in lung and sputum. The care of the body of an asbestos fiber is surrounded by protein deposits; unstained specimens are golden yellow or golden brown. They (in lung or sputum) are not stained by ordinary histological stories but may be demonstrated by Prussion blue staining procedure (S). See also (9). After death when lungs examined by naked eye they are large and densely fibrotic. Often the lung is completely adherent to chest wall and in advanced cases to diaphrogan with formation of a thick and extremely dense layer of fibrous tissue. 4 main complications and segutae of polmonary asbestosis are purulent bronchitis, bronchial pneumonia, pulmona [redacted] TB and emphysema (8)

Treatment

Removal from exposure.

Medical Surveillance

Periodic clinical exams for signs and symptons of asbestosis, mil-examination of sputum for asbestos bodies and chest x-rays (hyg. Guide) In general the lapsed period between onset of asbestos air pollution and appearance of neoplasin is 20,30, 40, or more yrs (22). Possible variable parameters mid-intensity of exposure, fiber variety, size, competitive risk of asbestos, cofactors such as cigarette smoking, trace elements . . . (22)
Latent period rarely less than 10-20 yrs (30).

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INDUSTRIAL HYGIENE

Detection of Odor

Environmental Concentrations (including by-products)

Operations Being Used

Plants Being Used:

Potential Hazard O.K. if Average Conc. + $\frac{\text{Range of Conc.}}{\sqrt{n-1}}$ TLV

Analytical Procedures

. Qual. Analysis of Minerals by Solid Infrared Spectroscopy for mineral identification supplements used instead of X-ray diffraction. Qualitative infrared spectra for amosite, crocidolite, tremolite, serpentine, chrysotile. (4) Procedure for X-ray diffraction fo chrysotile, rocidolite and amosite and applicable to quantitative determination in 1-8 mg range latte and 1-10 mg range for chrysoh6 (37) (39,40)

. Dust may be sampled with electrostatic precipitation or by impinges method using alcohol or lcohol and water as collecting medium (1,8) The index of refraction of chrysolite is only lightly greater than Canada balsam, so relief is low. Other forms of asbestos have higher ndices of refraction. Extinction is parallel except in case of tremolite which has oblique xtinction. Bizefungence of chrysotile is moderate $n_\gamma - n\alpha = 0.03$. Max interferences color s yellow of the 1st order. (8, see 10). TLV is bored on impinger sampling procedure.

Environmental Monitoring TLV is based on fiber count, but LM observations that would be ounted as a article, by the EM is seen as numerous fibers (fibrils) attached to the surface f particles. In such circumstances any biol. effect of the fibers could be incorrectly ttributed to particles (22). Each asbestos fiber variety is quite different. Chrysotile iber for ex. is not a unit whole but is composed of a number of 300-400 A fibrils. In ndustrial situations one has some idea of the ratio of optically visible fibers/total chrysotile op. Not so in ambient air. Probably region EM to get this ratio and then use dp M in ccupational setting. Ambient air reguines EM with magnification of at least 20,000 and probably ver 40,000 x (22) What is seen by light microscopic methods often differs from EM observations or 1/100 fibers present in air could be seen with LM as compared to EM) and that resp. size fraions need not have some size and ist or properties as those in gross overall sample (24, 28)

over)

004264

3. Gravimetric method using Harlet sampler (31)
4. Rayco Electronic Counter - continous series of dust cannot of predetermined size (31)

(38) reports a procedure for separating respirable dust fraction (< 10μ) from settled dust, dust collected with hi-vol and electrostatic precipitatas samplers. The chem. and physical natures of the respirable fraction can then be analyzed. This method has been applied to asbestos and has shown that the chemical composition of the fraction < 10μ may be quite different from the parent material.

The results of our surveys of 9 asbestos textile plants reveal that dust conc. as measured by impinger are generally below TLV. Estimates of relative dustiness from membrane filter samples were in good agreement with those made from impinger samples by either conc. or body burden methods of graying. Both show approx. some variance. A gross airborne mass conc. of asbestos equivalent to 5 mppct would be on the order of 1 mg/m^3, and respirable mass conc. as determined by elutriator and cyclone, would be in the order of 0.5-0.6 mg/m^3 and 0.3-0.4 mg/m^3 respectively. The impinger is as convenient as any other method of sampling and no less accurate. However the hygiene criteria is based on largely grain counts while the fibers are thought to be the cause of disease. For this reason the counting of fibers on membrane filters appears to have an advantage, esp. in industries where asbestos is only a small fraction of the aribaine dust. (41)

Sampling
The impinger method is not adequate for obtaining a statistically useful index of auharne fiber conc. i.e., that part which is most relevant to disease-causing mechanism. The impinger has a collection eff. related to aerodynomic size of particles and is not efficient for particles with falling speeds < 1 μ unit density spheres. Further the 10x objective, light field, counting technique used does not resolve particles much smaller than 1 μ. It is est. that ∿1/100 fibers in the air as seen in EM was seen in impinger samples. Impinger counts are based to large extent on grain counts. But the falling speed of fibers dependent on dia only when aspect ratios 710; It is possible for much larger and heavier objects to penetrate deep into the lung if shaped as fibers than in the shape of grains.

Using membrane filters pare sizes of 0.8 μ, almost 100% efficiency is obtained for fibers down to several hundreds of a micron. The fibers are rendered transparent witn 1:1 dipthyloxalate and dimethylphthlate I counted with 4 mm objective (430x) under phase contract illumination.

When impinger samples give conc. of a 5 mppcf, membrane filters conc. are as follows: total fibers = 50 fibers/cc.
fibers longer than 5 μ = 30 " "
fibers longer than 10 μ = 15 " "

A goal of 4 fibers longer than 5 μ/cc has been mentioned for English asbestos textile mills, approx, equivalent to 1 mppcf by impinger count

which is very near the lower practical limit of sensitivity for the impinger. Wt. method of analysis suffers from some defect as impinger.

Chrysotile asbestos fibers are commonly found in lungs of urban dwellers with no occupational exposure to asbestos (22). Asbestotic pleural calcifiation has been associated with use of rock with high asbestos (anthophyllite) content in various parts of world (Bulguin, Finland, Czechoslavakia?) Ref. 1522.

Recovery of chrysotile asbestos from lung tissue always low; recovery of amphibole asbestos generally high in human lungs (25).

Asbestos insulation workers have comparatively light asbestos exposures as asbestos trades go. Many of the material they use contain little or no asbestos and others 5-15%. What few dues exposure surveys have been done have generally been within the 5 mppcf TLV. When exposure is reduced, eearly death from asbestosis decreases, and lung cancer becomes more common. Light exposure may come from industrial exposure and/or community asbestos air pollution. In this study it was shown that there is an extraordinary risk of developing and dying from lung cancer for asbestos workers who smoke cigarettes regularly. In the group studied, the combination of asbestos exposure and cigarette smoking $\sim$ 90x compared with men who neither work with asbestos nor smoke.(29).