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The Method Used by the U. S. Public Health Service for Enumeration of Asbestos Dust on Membrane Filters

George H. Edwards and Jeremiah R. Lynch*

Introduction

In the early epidemiologic studies of the asbestos industry by Fulton (1935) and Dreesen (1938), the impinger was settled upon as the standard method for appraising dustiness. Since few fibers were seen it was necessary to count both grains and fibers to obtain a statistically useful count despite the assumption that asbestosis was caused by fibers. This inconsistency notwithstanding, dust control based on impinger results has greatly reduced the incidence of asbestosis where it has been conscientiously applied in the asbestos textile industry. However, a number of investigators (Lynch and Smith, 1955; Doll, 1955) have suggested other health hazards associated with asbestos, and a more relevant and sensitive method is required for estimation of the hazard, especially in industries using only part asbestos in their product.

A further weakness in the impinger method is that it is not amenable to long personal samples. It is believed that personal samples best approximate the worker's actual exposure, and Roach (1966) has recommended a sample duration of ten work shifts for mineral dusts.

New methods were therefore needed, and among those studied were fiber counts at 430X phase contrast on membrane filters that had been made transparent. This paper deals with the development and standardization of this method.

The principal variable which needed to be considered was the mounting procedure in relation to maximum transparency and the effect of time on the dimensional stability of the mount. Methods using immersion oil, airplane glue or acetone were found to be either too cumbersome or resulted in an imperfectly cleared or mottled filter, particularly as viewed with phase contrast optics. In methods which involved the application of liquid over the dust deposit the movement of the meniscus across the deposit sometimes caused redistribution of the dust. The use of a 50-50

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ABSTRACT

Samples of air-borne mineral dusts are collected by drawing air through cellulose membrane filters. Particles smaller than nominal pore size are retained. Varying sampling rate and filter holder controls the distribution of dust across the filter face. Samples are mounted on standard microscope slides using a high viscosity solution of membrane filter material in a mixture of diethyl oxalate and dimethyl phthalate to render the filter transparent. The mount is stable for 28 to 30 days, but after this period migration of the dust particles causes a loss of areal concentration.

The dust particles, which lie on the surface of the filter, are counted with a phase contrast microscope, using 10X eyepiece and a 4 mm "high-dry" objective. The field area is delineated with a Porton reticle. Count data are recorded in a form allowing data reduction and statistical analysis by electronic computer. This method is suitable for experimental studies of most mineral dusts, but cannot at present be used in connection with Threshold Limit Values. This method is now included as acceptable in the TLVs (PWM - May 1969)

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mixture of diethyl oxalate-dimethyl phthalate was attempted and found to result in satisfactory clearance. However, since this medium dissolved the filter, the migration of particles resulted in changing counts with time and needed to be examined to evaluate the method.

Sampling Procedure

Samples for count are collected on Millipore^{R} type AA filters by personal samplers operated by battery powered pumps worn by the employees, and as simultaneous samples collected in conjunction with impingers. The filters are contained in plastic filter holders and are supported on thick pads which also aid in controlling the distribution of air through the filter. Several methods of admitting air to the filter have been attempted. With the face cap removed and the filter completely exposed, the air may enter the filter evenly, but large high velocity particles and foreign objects may also hit the filter and possibly cause damage. As an alternative, the face cap may be left in place and the small plug removed. The passage of air through this 4 mm opening at a flow rate of 2 liters per minute produces a velocity of 265 cm/sec, which yields an impaction parameter of 0.081 for 1 micron (μ) unit density spheres. Although this impaction parameter would predict a very low collection efficiency of 1 μ particles by impaction, some larger particles would tend to be impacted on the center of the filter. This was found not to affect count distribution, which is dominated by small particles; however, it does result in obscuring a small portion of the filter, particularly if many large particles are present, as in friction product plants. The third alternative was to drill six additional 4 mm holes in some face caps and to fit these perforated caps to the filter holder during sampling. This reduced the velocity to 38 cm/sec thus eliminating the impaction problem while still protecting the filter from damage.

Mounting the Sample

The mounting medium used in this method is prepared by dissolving 0.1 gm of membrane filter material per ml of a 1:1 solution of dimethyl phthalate and diethyl oxalate. The exact proportions of the 3 components are not critical, but the medium must have as high a viscosity as possible without being difficult to handle. The index of refraction of the medium thus prepared is $N_D = 1.47$.

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) microscope slide, using a dropper or applicator. The volume of the drop is approximately 0.05 ml. A wedge-shaped piece about 1 cm by 2 cm is excised from the filter using a scalpel and forceps, and placed dust side up on the drop of mounting solution (Figure 1). A #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.

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Clearing of the filter with this method is slow, requiring about 15 minutes. The sample may be examined as soon as the mount is transparent.

The transition of the dust-supporting substrate from translucent, solid membrane filter cellulose ester material to a transparent, optically homogeneous gel is very gentle, and at no time does a meniscus sweep across the dust deposit. Rather, the substrate slowly softens beneath the dust, and there is no opportunity for washing away or redistribution of the dust.

The optical homogeneity of the resulting mount is nearly perfect, with only a slight background granularity under phase contrast, which disappears within 1 or 2 days.

Stability

Observers noted a reduction with time in apparent concentration for samples mounted with only dimethyl phthalate and diethyl oxalate. By observation, this loss was due to the migration of the dust particles outward from the center through the gelatinous material of the fixed mount, with a decrease in areal concentration. To determine the magnitude of this loss, a group of samples was mounted using 1:1 dimethyl phthalate and diethyl oxalate only, and counted at one day to three-day intervals for periods ranging up to 76 days. Counts were displayed as fractions in percent of the original, or first day counts (Figure 2). At the end of one week, samples mounted by this method yielded only 75% of their original values. By 30 days after mounting, the counts using this method had declined to 40% of their first day value, and continued at this level with no further decline until counting was terminated 76 days after mounting.

A group of samples mounted in the method described in the section "Mounting the Sample" using high-viscosity mounting medium were counted at one-day intervals for eleven days. No loss of areal concentration was apparent over this short period and a second group was similarly mounted and counted for a period of forty-six days. The composite of these two series is shown in Figure 2. No loss of concentration was detectable during the first 30 days, but the count declined to 85% of its original value by the 46th day, when the series was terminated.

A 6 mm square of membrane filter with a vacuum-deposited film of carbon was mounted in the manner described to provide a macroscopic observation of the distortion of the filter areas. After a few days, the square began to disintegrate at the edges, but the major portion showed no discontinuity during the initial 30 day period. By the end of 62 days, most of the square had disintegrated and the fragments migrated over an area 16 mm by 17 mm.

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Evaluation

The filter samples mounted in the manner previously described are evaluated in terms of grain and fiber concentration, and fiber size. Binocular research microscopes equipped with fixed mechanical stages have been used. Phase contrast optics fitted to these instruments include an Abbe condenser with rotating phase turret and a 4 mm "high-dry" achromatic objective used for all membrane filter determinations. In all cases 10X Huygens eyepieces, one of which contained a Porton reticle at the level of the field-limiting diaphragm, were used. The left half of the Porton reticle field served to define the counting area or field.

A ribbon filament illuminator was critically adjusted to provide Kohler illumination through a clear blue filter.

Twenty field located at random on the sample were counted and total grains, total fibers, fibers greater than 5 μ, and fibers greater than 10 μ were recorded. Any particle having an aspect ratio of 3 or greater was considered a fiber.

Recording of Data

Since it was recognized early in the study of the asbestos products industry that the large number of samples to be collected would present a burdensome data processing problem, the data were collected on forms designed for transcription to punch cards (Figure 3). Computer programs were then written to calculate dust concentrations and to produce statistical summaries consisting of mean, standard deviation, median, etc., for any group of samples by plant, operation, job, type, etc. These programs, written in Automath (Fortran II) are applicable to any problem using the sample and count forms as source documents. Additional analyses possible are correlation and regression of simultaneous samples and Poisson index of dispersion of field counts for various counters.

Summary

A method for sampling and counting asbestos dust using membrane filters has been developed and evaluated. The mounting medium developed clears the filter gently so as to not redistribute the dust deposit, and produces a mount with adequate dimensional stability for a period of thirty days. This method has been used in developing environmental data in connection with the Public Health Service study of the asbestos products industry, and is recommended for research studies in other dusty trades although not for application to Threshold Limit Values. A system of data handling involving electronic computer processing, which is also applicable to other studies, was discussed.

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NOTE:

Millipore® filters are named in this paper since the method was developed using this brand of filters. However, this does not imply indorsement by the United States Department of Health, Education, and Welfare or the Public Health Service. Similar techniques may be developed for other filter brands.

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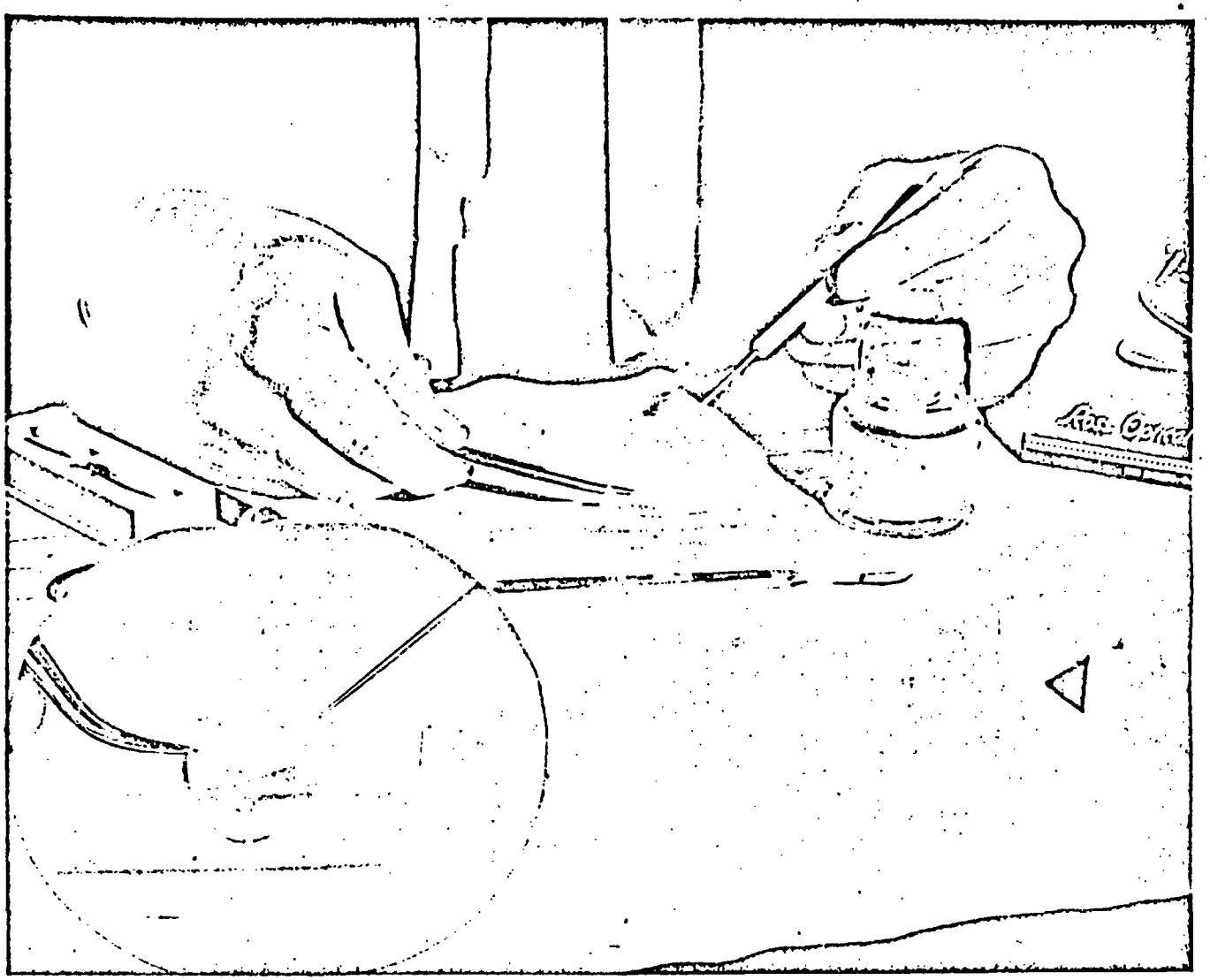

Figure 1. The wedge-shaped piece of membrane filter is placed dust side up on a drop of mounting medium which has been applied to a standard microscope slide. The mount will be covered with a #1-1/2 coverslip.

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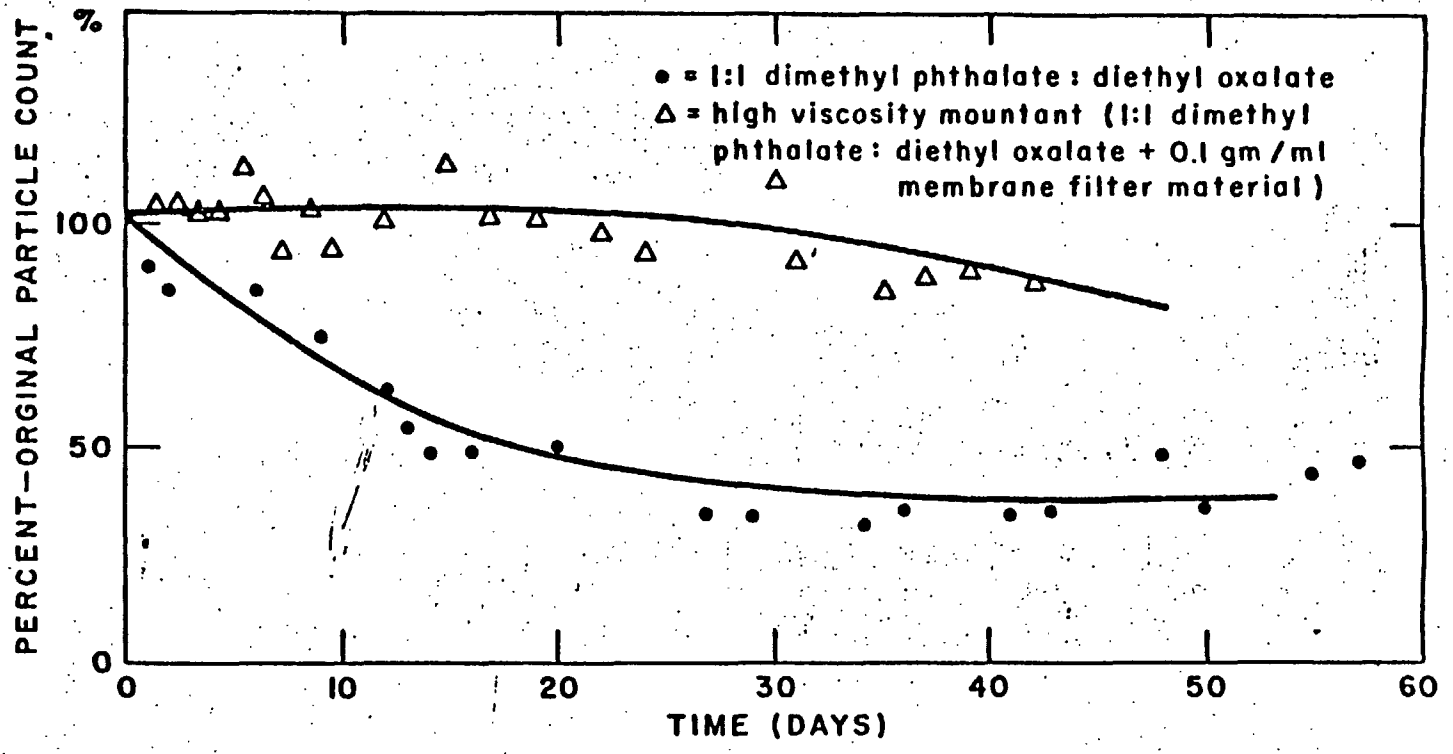

Figure 2. Decrease in percentage of original particle count with time for the original 1:1 dimethyl phthalate: diethyl oxalate mixture and for the high viscosity mixture containing 0.1 gm/ml of membrane filter material.

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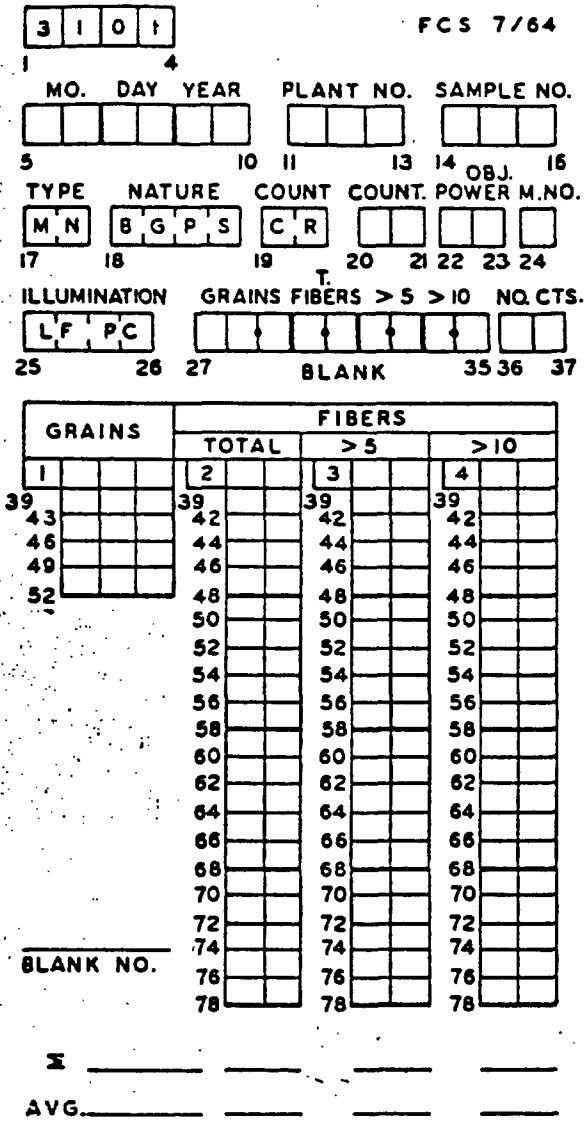

Figure 3. Count tabulation form used for the evaluation of dust samples taken on membrane filters. M = Membrane filter, U = Elutriated membrane filter, B = Breathing zone sample, G = General a sample, P = Personal sample, S = Sequential sample, C = Count (1st), R = Recount. "COUNT" provides for the technician's initials, "OBJ POWER" indicates the magnification, "M.NO" provides for the microscope number for calibration purposes, and LF and PC are light-field and phase contrast.

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