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EVALUATION OF ELECTRON MICROSCOPY FOR PROCESS CONTROL IN THE ASBESTOS INDUSTRY

Industrial Environmental Research Laboratory
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EVALUATION OF ELECTRON MICROSCOPY FOR PROCESS CONTROL IN THE ASBESTOS INDUSTRY

by

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ABSTRACT

The overall objective of this study was to evaluate the transmission electron microscope and scanning electron microscope as potential tools for counting fine-particle asbestos fibers for process control in the asbestos industry. The capabilities and limitations of both instruments were defined in applications where asbestos specificity is not necessarily required and where cost of analysis must be minimal. It was shown that both microscopes are equally capable of counting all fibers in the full particle-size distribution. However, because of fiber agglomeration and difficulty in distinguishing fibers from background, each microscope is capable of observing only 75 percent of the distribution. In contrast, present standard light microscopy methods observe only the coarser 10 percent of the distribution; the fine fibers are not resolved.

Optimum asbestos fiber counting was carried out at 15,000 × magnification and at fiber concentrations on the filter of 40,000 to 80,000 fibers/mm^2. The minimum number of fibers counted to obtain high statistical confidence was 200 fibers per data point. Standard preparation techniques for the filter samples were found to have no effect for either instrument. Ashing of filters to remove nonasbestos fibers was responsible for an asbestos fiber loss of 85 percent.

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SECTION 1

CONCLUSIONS

Evaluation of air-pollution abatement measures in the asbestos industry has been hampered by the lack of a cost-effective asbestos fiber counting method that is capable of detecting fine asbestos fibrils. Previous studies have shown that the TEM and the SEM offer this capability in applications where all fibrous materials can be assumed to be asbestos. The objective of the present study was to evaluate the capability and limitations of the TEM and SEM for counting asbestos fibers from a particle distribution expected from effluents in process industries controlled by dust-abatement equipment.

The results of this study indicate that the SEM and TEM are equally capable of counting asbestos fibers in process control applications where fibrous materials can be assumed to be asbestos. The study was made on filter samples prepared specifically for each microscope; fiber length and diameter were measured on more than 60,000 asbestos fibers. A comparison of fiber counts on specifically selected filters showed that each instrument failed to observe 26 percent of the fibers observed by the other instrument. The SEM was not capable of resolving individual fibers in dense agglomerates; the TEM confused the asbestos fibers with the texture of support filters. If the same fiber distribution were measured by phase contrast light microscopy, 90 percent of the fibers would not be observed because of inadequate resolution.

In an evaluation of the statistical confidence in the data, it was determined that the standard deviation of individual measurements, defined as the fiber count of a single field of view, obeyed a chi-squared distribution with respect to the number of fibers counted. From this relationship, it was concluded that the deposition of fibers on a set of filters was uniform, and that the individual fiber counts were distributed in a normal distribution about the mean. It was also determined from this relationship that a count of 200 fibers represents the cost-optimum fiber count for maximum confidence in the data.

The particle size of all fibers counted was found to be log-normal in both fiber length and fiber diameter, with a mean fiber length of 1.5 μm and a mean fiber diameter of 0.17 μm. Only 16 percent of the fibers were longer than 5 μm; 1 percent were shorter than 0.1 μm.

A study of test parameters revealed that a magnification of 15,000 × is optimum for both SEM and TEM examination. At this magnification, the resolving power permits observance of the finest asbestos fibril, and the field of view is adequate for cost-effective fiber counting in routine analyses. A correlation between concentration of fibers on a filter and the accuracy of the fiber count showed that 40,000 to 80,000 fibers/mm^2 is the optimal concentration.

Results also indicate that neither SEM nor TEM standard preparation techniques affect the accuracy of the resulting count statistics. However, when filters were ashed to remove nonasbestos fibers, the resulting count indicated an 85-percent loss of the asbestos fibers on the filter.

For asbestos fiber counting in routine applications, it was shown that both the SEM and the TEM yield comparable results that are much better than those obtained with light microscopy methods. However, capital and operating costs for TEM analyses are 5 to 10 times higher than those for light microscopy. In contrast, SEM analyses can be performed for approximately twice the cost of light microscopy methods. Moreover, although TEM analyses are not amenable to new cost-saving techniques, automation of the SEM is possible; through use of newly developed instrumentation for image analyses, total costs for the SEM may be reduced to nearly those of light microscopy.

SECTION 2

INTRODUCTION

HEALTH EFFECTS

The health problem that results from inhalation of asbestos fibers by workers in the asbestos industry was first recognized in 1900. That this hazard could affect the general population was recognized little more than a decade ago (1, 2). A large quantity of information has since been published on the physiological effects of asbestos inhalation, but the mechanisms that cause these effects are still unknown (3).

Inhalation of asbestos has been shown to cause asbestosis (4-6), pleural or peritoneal mesothelioma (7-9), and bronchial carcinoma (10-12), each of which can be fatal. One obstacle to the detection and correlation of the diseases is the very long induction period (20 to 30 years) between initial exposure and evidence of biological effects. In addition, the incidence of these diseases among nonindustrial workers has created further uncertainty as to the causes (13). Because "asbestos" refers to two mineral groups that encompass several distinct fibrous minerals, the physiological effects of specific mineral types are not understood and have not been included in most medical reports (14).

A further complication was introduced by a recent study (15) that showed a higher incidence of lung cancer among cigarette-smoking asbestos workers than among nonsmoking asbestos workers. One possible explanation for many of the uncertainties about asbestos diseases is the belief (3) that asbestos serves as a carrier of carcinogenic agents, specifically polycyclic aromatic hydrocarbons. The source of these hydrocarbons could be the natural oils of the asbestos fibers (16), adsorbed hydrocarbons from the urban environment (17), or hydrocarbons adsorbed in cigarette smoking by *in vivo* asbestos fibers (3).

The presence of relatively high concentrations of asbestos fibers in urban environments and the uncertainty as to the cause of the associated diseases have prevented the establishment of a dose-response relationship between exposure levels and disease. Consequently, a safe level of asbestos fiber exposure cannot be determined (18). The problem is further aggravated by the presence of asbestos fiber in foods (19), drinking water supplies (20), and parenteral drugs (21), and the uncertainty with respect to the health effects.

The sources of asbestos fibers in the urban atmosphere are mines, manufacturing processes, and users (3). Abatement measures within the asbestos industry include elaborate ventilation systems that are designed to protect both workers and the local community (22). Because asbestos fibers that can be inhaled are of a size that renders them easily airborne (23), the effectiveness of abatement measures must be nearly absolute if adequate protection is to be provided.

PROBLEM DEFINITION

Although asbestos exposure was identified as a potentially fatal occupational risk as early as 1907, serious disease among asbestos workers still persists, and the disease is also found in the general population (24). A major reason for the persistence of the disease is the long period of latency between exposure and overt symptoms. Although industry has adopted measures to protect workers and the general public, insufficient knowledge of the physiological causes of the disease makes it difficult to ascertain whether or not present abatement measures are capable of preventing the disease by the year 2000. One reason for this uncertainty is the limited effectiveness of present techniques for measuring airborne asbestos. Although methods for determining concentrations of fibers have been developed for industrial hygiene purposes (25-26), the methods do not provide an understanding of the relative biological effect of different sizes of fibers, their concentrations, or the fiber mass (27).

Standard methods for asbestos measurement use light microscopy with phase-contrast illumination to count fibers collected on a membrane filter. In current practice, only fibers longer than 5 μm with diameters greater than 1 μm are counted. However, of the airborne asbestos particles, the long asbestos fibers that can penetrate to the alveolar regions are those with diameters less than about 3 μm.* Several investigations (Appendix A) have indicated that the mean particle diameter and mean length of airborne asbestos distributions lie at about 0.3 and 1 μm, respectively, depending on the source of the sample. Thus, more than 50 percent of the total fiber number in the airborne distribution may not be observed and is not counted by optical microscopy. These data indicate that standard light optic methods of asbestos fiber counting are capable of detecting only the coarser portion of the airborne asbestos distribution and, of the fibers counted, only those between 1 and 3 μm

*Several investigations (28, 29, 30) have determined 10 μm to be the maximum aerodynamic diameter of dust particles that are inhaled and remain deposited within the lungs. For asbestos fibers, identified by a length/diameter ratio greater than 3, the equivalent maximum aerodynamic diameter is 3.3 μm (31). Observation of fibers with lengths up to 200 μm deep within the lungs is not inconsistent with this size limitation, because the aerodynamic behavior of asbestos fibers is controlled by the fiber diameter rather than the length.

diameter have biological significance. Thus, the standard methods for determining fiber concentration may not provide control on a biologically significant portion of the airborne asbestos fiber distribution.

Most of the histological record has been concerned with the effect of long fibers, probably because techniques for the preparation of histological samples and their observation are made easier by long fibers. Because of the record, many investigators have evidently concluded that long fibers are more dangerous than short fibers. However, Hold (32) reported that these findings, i.e., very fine dust not seen in light microscopes, coupled with experience with rats, resulted in the inevitable conclusion that very small dust particles are at least as lethal as long fibers. Because the relationship between fiber size and asbestos disease has not been clearly established, and the mechanisms that contribute to asbestos diseases are not yet understood, it is not known whether or not the uncounted fibers contribute to the epidemiology.

Light microscopy with an oil immersion lens cannot resolve fibers with diameters less than 0.2 μm. Electron microscopy methods are capable of resolving to less than 0.001 μm with a transmission electron microscope (TEM) or to less than 0.01 μm with a scanning electron microscope (SEM). Both instruments can provide a more complete description of the asbestos fiber distribution. The U.S. Environmental Protection Agency recognized the potential role of electron microscopy, and in 1971 a procedure for TEM examinations of airborne asbestos was proposed (33). The major shortcomings of the TEM method are the long, tedious sample preparation and the high cost of analysis (typically ten times the cost of light optical microscopy). Additionally, where there is an unequivocal need for asbestos identification, the method involving electron diffraction is too expensive for routine use.

The SEM is a more recently developed instrument and has not been extensively evaluated for asbestos counting (34). The cost of analysis may be near that for the light microscopy, but this also has not been evaluated. The major shortcoming of the SEM is the high cost of distinguishing asbestos fibers from other fibers by x-ray analyses. (A scanning transmission electron microscope (STEM) combines the features of the SEM and TEM and permits the identification of asbestos by electron diffraction; however, this capability also increases analysis cost, which makes the method impractical for routine use.) Although this shortcoming may limit use of the SEM for ambient air monitoring, the instrument should be completely adequate for measuring the effectiveness of industrial control equipment and for certain epidemiological studies. In both of these applications, the electron microscope may be capable of measuring the total fiber distribution. This will assure adequate industrial control of asbestos fibers and provide a tool for gaining an understanding and control of asbestos diseases.

This study focused on evaluation of the TEM and SEM as tools for counting fine asbestos fiber in routine application for the subsequent evaluation of control equipment in the asbestos process industry. Alternative sampling and preparation techniques were investigated, and the two instruments were compared in order to determine the limitations and consistency of each. An expected result of this study, although not a study objective, is the establishment of a measuring technique for counting fine asbestos fiber that can provide a data base for the determination of the biologic effect of fine fibers.

SECTION 3

INSTRUMENTATION

Electron microscopy has been available as a research technique for 20 years, and the TEM has been recognized as a tool for asbestos counting for at least 10 years. Although the TEM provides the capability for observing and counting the smallest asbestos fibril, and development studies have been completed (33), the instrument has not been used for asbestos measurement, except for selected research, primarily because of the high costs involved.

During the last five years, however, the SEM has been developed sufficiently to be considered an alternative tool to the TEM for counting small fibers (34). Although the SEM does not offer the ultimate resolution of the TEM (7 nm versus 0.3 nm), the resolution of the modern SEM is sufficient to detect the smallest asbestos fibrils (20-nm dia). In addition, the SEM offers the advantage of much simpler sample preparation, which increases throughput and decreases the cost of routine asbestos examination and counting. The SEM, therefore, could fill the gap between the light microscope and the TEM, with all three instruments being used complementarily. Light microscopy, with the lowest initial equipment cost, can provide estimates of fiber concentrations. The SEM, with higher initial cost, provides the ability to count small particles. The TEM, with highest overall cost, permits research studies of individual fibrils and basic asbestos structures.

For a better understanding of each technique, it is important to know the basic differences between light and electron microscopes. For example, because of differences in their modes of operation, the two types of microscopes have different sample requirements. The electron microscope displays sample response to electrons rather than light; therefore, the electron image of a particular sample may be quite different than the light image of the same area. The samples observed in an electron microscope must be electrically conducting in order to receive the electron beam and avoid charge buildup that occurs when an electrically conductive path to ground does not exist. In addition, electron microscopy requires that the sample be examined in a vacuum. Thus, samples prepared for electron microscopy must be capable of withstanding both the vacuum environment during examination and the turbulence that may occur during chamber evacuation prior to examination.

TRANSMISSION ELECTRON MICROSCOPE

The TEM can be thought of as an electron analog of the transmitted light microscope. The electron-light analog is basic to the optics of the two instruments, as shown in Figure 1(a). Both instruments use a focused beam to illuminate the sample, which, after passing through the sample, is further focused and enlarged. With the transmitted light microscope, the enlarged image is focused through an eye piece; with the TEM, the image is focused on a phosphor screen.

The sample for the transmitted light microscope must be translucent to the illuminating light; in the same manner, the sample for the TEM must be translucent to the electron beam. Thus, for TEM observation, a sample must be thin enough for electrons to pass through. With asbestos fibers, the normal method of sample preparation requires the transfer of particles collected on a filter to a sample holder that is translucent to the electron beam. The asbestos particles are then observed in silhouette against the translucent background. When the fibers are small, some of the electron beam can pass through the fiber and reveal high-resolution details of the morphological features of each fiber particle. The primary high costs of this technique are those of preparing the sample for observation. Previous work on developing TEM for asbestos counting has been directed toward sample preparation methods.

SCANNING ELECTRON MICROSCOPE

The SEM may be compared and contrasted with the reflected light microscope as shown in Figure 1(b). Both instruments can examine opaque specimens, allowing observation of a considerably broader range of specimens than can be observed with transmission systems. Sample preparation is usually simpler for reflected light microscopy than for transmitted light microscopy; in the same way, SEM sample preparation is simpler than TEM preparation. The electron-light analog can be carried a step further in that an opaque feature viewed by transmission microscopy will appear dark, whereas the same feature viewed by reflected microscopy will frequently appear light.

The SEM and reflected light microscope differ in that the reflected light microscope focuses light to form an image after the light has left the sample, whereas the SEM focuses the electron beam before it strikes the sample. The electrons that leave the sample as a result of the electron beam are collected and displayed on a cathode ray tube (CRT). The finely focused electron beam is scanned synchronously with the beam in the CRT, and each point on the sample is represented by a point on the CRT screen. This forms an image much like a television image.

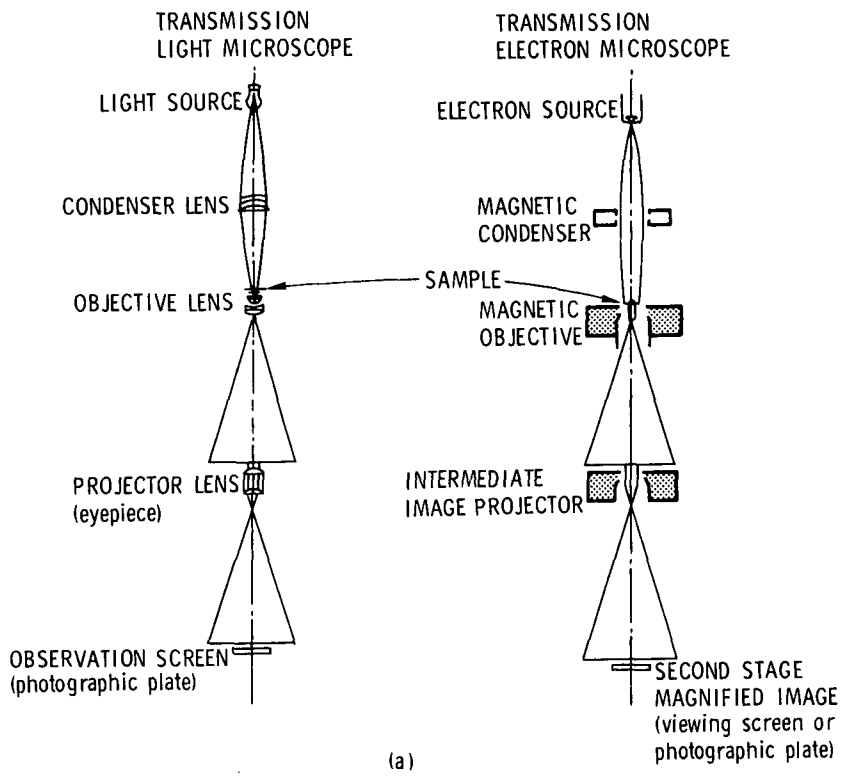

Figure 1. Schematics of scanning electron and transmission electron microscopes and reflected light and transmitted light microscopes.

SECTION 4

EXPERIMENTAL OBJECTIVES

The overall objective of this study was to evaluate both the TEM and SEM as potential tools for counting asbestos fibers in process control applications. Additional objectives were to make direct comparisons of the two instruments in order to determine their relative usefulness in process control applications, and to identify and develop suitable sample preparation methods in order to reduce operating costs.

PREPARATION TECHNIQUES

An important objective of this study was to determine the degree to which asbestos count statistics are affected by the specific preparation techniques required for each instrument. This investigation included evaluation of the individual stages in sample collection and handling procedures. It was generally believed that the probability of fiber loss increased as the number of steps (or complication of the step) increased during sampling and handling procedures.

SEM preparation is basically very simple. In this procedure, fiber loss is most likely to occur during handling in sample transportation and during evacuation of the instrument.

For TEM examination, removal of the asbestos fibers from the collection filter is basic to preparation because filters are too thick for electron transmission. In this procedure, loss of fibers is most likely to occur during dissolution of the filter. In addition, handling of the sample at each stage may contribute to fiber loss.

Ashing is not required for either SEM or TEM examination; however, ashing provides a means of removing most nonasbestos fibers from samples collected from the ambient air and is therefore part of the 1971 EPA-proposed TEM technique. Because the technique involves burning the supporting filter, there is a strong potential for loss or breakage of asbestos fibers. Further loss may occur during the refiltering operation following ashing. After ashing, the sample is filtered and ready for subsequent SEM or TEM preparation techniques.

Selection of a suitable filtering medium is important, because the proper filter will simplify counting and facilitate examination. An unsuitable

filter can contribute to excessive fiber loss during preparation and, therefore, to inaccurate results. Two types of filters are available commercially for airborne asbestos collection: cellulose fibrous filters and perforated plastic membrane filters.

Handling can result in sample loss or alteration at any stage of preparation. Thus, one objective of this study was to determine losses caused by handling and to identify procedures particularly susceptible to handling losses.

DETECTABILITY LIMITS

Another important objective of this study was to investigate limits of asbestos fiber detectability for each instrument and to determine whether or not the difference in ultimate detectability between the two instruments is significant in asbestos counting statistics. If a difference in detectability limits were found, it was expected to be caused by the difference in ultimate resolution between the two instruments. Whether or not the difference was significant would depend on the minimum size of asbestos fibers. If all fibers were large enough to be easily observed with both instruments, the difference in resolution was not expected to be significant for statistics on asbestos counting.

INSTRUMENT OPERATING CONDITIONS

An objective of this study was to establish optimum operating conditions for each instrument so that the use of electron microscopy for asbestos counting could be evaluated. Among the conditions to be investigated for each machine were instrument response to alternative specimen preparation techniques and to filter medium, most suitable magnifications for examination, and contrast enhancement methods. In all cases, an evaluation was made of standard operating conditions as well as newly identified conditions revealed in the course of the study.

Proper magnification selection is necessary to provide accurate counting statistics in minimal time. If magnification is too high, large fibers are not easily observed, and excessive time is needed to count the small fibers, as few fibers are visible in each frame. On the other hand, if magnification is too low, small fibers may not be resolved; this will skew the measured size distribution as well as the total concentration measurements.

Contrast enhancement is particularly important for the SEM, where fibers are viewed against a background that tends to obscure the fibers if insufficient contrast is available. Lack of contrast is generally less of a problem for the TEM than for the SEM, but it can be a problem if specimens are not prepared properly.

EFFECTS OF CONCENTRATION VARIATIONS

Variations in the amount of asbestos collected from the air onto the filter can influence the accuracy of fiber counting. If too many fibers are present, agglomeration or simple overlapping of fibers can occur, which complicates the counting procedures. If too few fibers are present, the precision of the counting statistics will be doubtful, and the counting procedure will require excessive time and cost. An important experimental objective, therefore, was to determine the effect of variations in fiber-counting statistics for each instrument.

SUMMARY OF OBJECTIVES

The effect of each of these four variables on asbestos fiber-count statistics was determined by evaluating and comparing total particle count, particle density, and particle size distributions. Comparison of the count statistics produced by each technique resulted in conclusions regarding the variables inherent in the operation of both types of electron microscopes. Through these comparisons and resulting conclusions, both the TEM and SEM were evaluated as potential tools for counting asbestos fibers in process control applications.

SECTION 5

EXPERIMENTAL PROCEDURE

The experimental plan of this investigation was divided into four discrete activities:

1. Samples that could be used for examination with both microscopes were produced. The intent was to provide both instruments with identical inputs that represented samples that might be collected from field sites.
2. Samples were prepared for observation. Because the preparation techniques are different for each microscope, the effects of these techniques were among the major variables to be evaluated.
3. The asbestos fibers were observed and counted by the method most suitable for each instrument.
4. The data were analyzed. Total fiber counts for each data set were corrected for geometric and magnification effects and normalized for direct comparisons of fiber concentrations and particle-size distributions.

SAMPLE PRODUCTION

All samples in this experiment were produced by aspirating samples of asbestos with a Royco model 256 aspirator and collecting the airborne fibers on filters held in a Nuclepore double filter holder. In all cases, the asbestos used was Canadian chrysotile from a standardized sample (Duke Standards, Inc., Palo Alto, California). In the standard sample, 70 percent of the fibers were 8 μm in length or less (determined by optical microscopy), and the particle-size range was similar to that expected in air samples from process industries. The immediate objective during sample generation was to deposit asbestos as uniformly and consistently as possible to provide equivalent samples for each technique. The samples prepared were free from nonasbestos fibrous contamination, as most process plant environments are expected to be, because consideration of specificity was not within the scope of the investigation.

Seventy-five percent of the filters used in the experiment were Nuclepore membrane filters (Nuclepore Corporation, Pleasanton, California),

characterized by a smooth surface with uniform, round holes. All Nuclepore filters were precoated with carbon to provide conductivity during examination. The remainder were Millipore filters (Millipore Corporation, Bedford, Massachusetts), characterized by a fibrous surface.

Asbestos from the aerosol generator was deposited on 36 filters. The filters were divided into three groups (I through III) of 12 filters each, with each group representing different fiber concentrations on the filter. Each group was composed of three replicate sets (1 through 3) of four filters per set; the four filters in each replicate set were labeled A through D. A schematic diagram of the test plan is presented in Figure 2, which shows the organization of the test matrix and the course of examination.

Group I was produced such that each filter had 150,000 fibers/mm^2. Each Group II filter had 80,000 fibers/mm^2, and each Group III filter had 40,000 fibers/mm^2. Within each set, filters A, B, and D were carbon-coated Nuclepore filters with 0.2-μm pore diameter. Filter C was a Millipore filter with 0.45-μm equivalent pore size. (The different pore size between Nuclepore and Millipore filters gave experimentally equivalent retention.) All four filters in a set received asbestos loading as part of the same aerosol generator run. Filters A and D of each set were the first and last, respectively, to receive asbestos during each run, and direct comparison was made between their fiber concentrations in order to ascertain that asbestos loading did not vary through the aerosol generator run.

Fiber deposition was visually uniform over the surface of each filter. Concentration, uniformity of deposition, and operating conditions for asbestos deposition (in particular, flow rates and deposition times) were established, with the SEM and carbon-coated Nuclepore filters used for calibration. Intentional fiber concentration differences were accomplished by varying deposition times; all other parameters were held constant. During generation of Group I filter samples, the glass nebulizer in the aerosol generator broke and was replaced. Only after all data were collected was it realized that the fiber length distributions were different as obtained from the two nebulizers.

PREPARATION FOR OBSERVATION

The preparation techniques for observation with the two microscopes are considerably different. For the SEM, an electrically conductive filter is used to collect asbestos from the ambient air. The filter is then removed from its sample collection holder, mounted on a sample mount for the microscope, and inserted into the SEM. The lack of processing after sample collection is made possible by the use of precoated filters. Where the filters are coated with carbon before sample collection, it is possible to achieve the electrical conductivity necessary for SEM observation without disturbing the sample. The procedure often provides better contrast, because the poorly conducting asbestos fibers produce a strongly charged image against the carbon-coated filter.

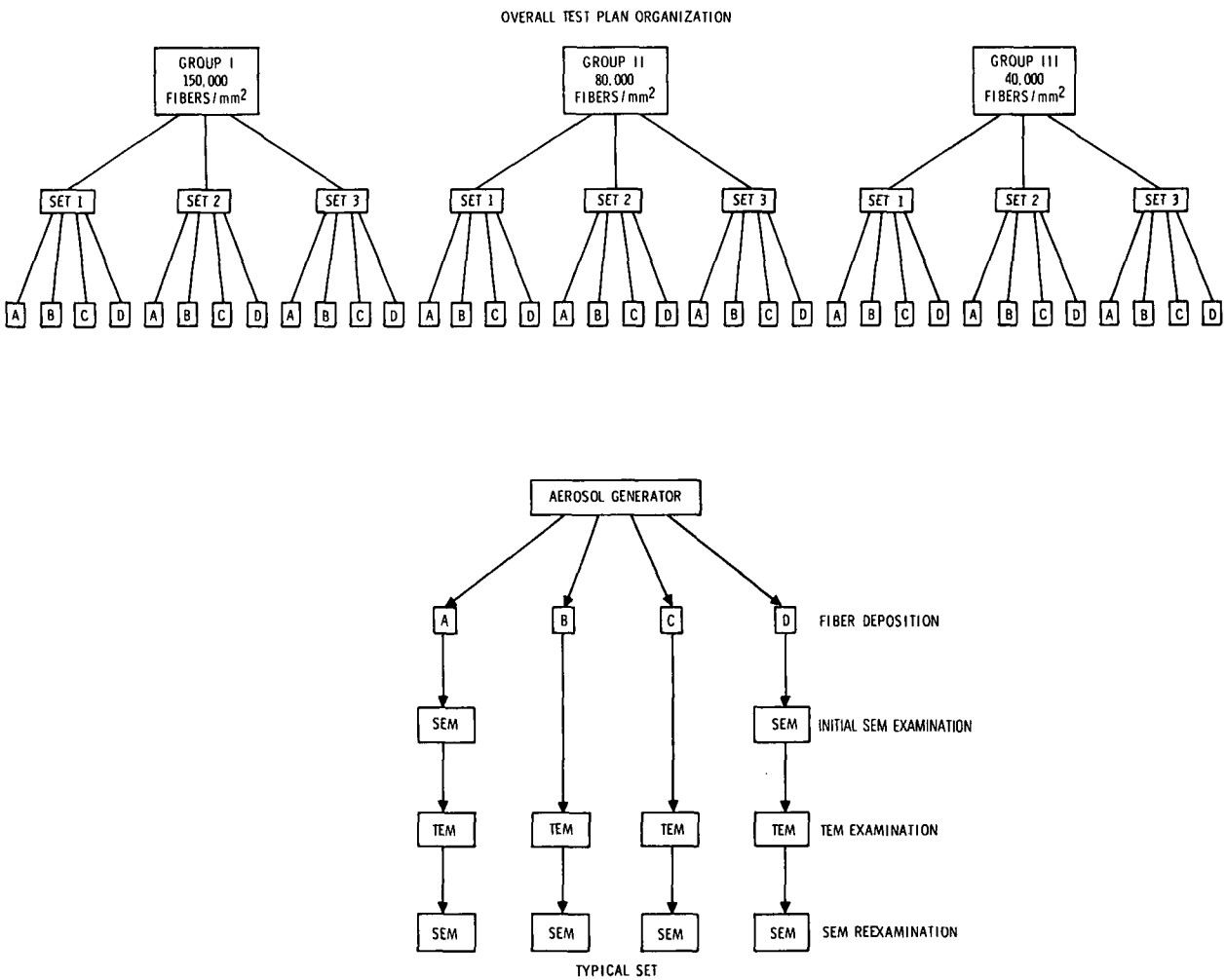

Figure 2. Schematic of general test plan and examination plan for typical set.

The preparation needed for TEM observation is considerably more involved. As a starting point, the TEM preparation technique recommended in 1971 by the EPA (33) was used to prepare specimens for TEM observation. Briefly outlined, the procedure consists of (1) ashing the filter, (2) redepositing on a second filter, (3) carbon coating the asbestos-bearing filter, (4) dissolving the filter away, (5) floating the remaining asbestos-bearing carbon film on water, and (6) depositing the film on a TEM grid for observation. Ashing is not an essential step in the TEM technique and is primarily intended to eliminate nonasbestos material. Ashing, therefore, was used for only two sets of Group I filters processed for TEM examination; this permitted observation and evaluation of the effects of ashing on the asbestos samples. Because ashing of TEM samples is not required, all other preparation involved carbon coating of the asbestos-bearing filter, followed by dissolution of the filter. The carbon film bearing the asbestos was then water-floated and placed on a TEM grid for subsequent examination.

OBSERVATION AND COUNTING

Observation and counting with the SEM was accomplished by inserting the prepared sample into the evacuated SEM chamber through an airlock and observing a number of areas on the filter surface. The filter was held perpendicular to the electron beam, and 45 predetermined, arbitrary locations were photographed on each filter. Asbestos fibers that appeared in the photographs were then manually counted and categorized by length, width, and aspect ratio.

Most fibers were counted and categorized while they were being observed in the TEM; the rest were measured from photographs. All fibers in five arbitrarily selected 80- by 85-μm TEM grid squares were counted and categorized as with the SEM. In addition, 20 TEM photographs were taken for subsequent direct comparison with the SEM.

Initial SEM Examination

Filters A and D from each set were examined with the SEM for a check of deposition distribution and uniformity. In addition, examination of these two filters provided a baseline for comparison of changes caused by subsequent handling. Both A and D filters were photographed 20 times at 6000 × magnification and 25 times at 15,000 × magnification. The 6000 × photographs were taken in a 4 by 5 matrix. Ten of the photographs taken at 15,000 × were of areas already photographed at 6000 ×. This provided more detailed information on fiber size, detectability limits, and possible tradeoffs between these limits and the advantages offered by the larger field of view in low-magnification views. The 15 remaining photographs taken at 15,000 × were of sites other than those used for the 6000 × photographs.

TEM Examination

Subsequent to SEM examination of filters A and D of each set, all four filters in each set were prepared for TEM examination in accordance with the described technique (except for sets 1 and 2 of Group I, which were first ashed). The filters prepared by this technique were examined in the TEM at 30,000 ×. All fibers present in five grid squares were counted. In addition, for several filters, all particles in each of the five squares were also counted and characterized from photographs as a check on the technique. For all filters, photographs were taken at 10 preselected locations within each square at 30,000 × magnification, and 10 photographs were taken at 15,000 × at these same locations.

SEM Reexamination

After TEM examination, each TEM sample was reexamined in the SEM at both 15,000 × and 30,000 × and in the same locations as in the TEM examinations. Count statistics were compiled and photographs taken that corresponded to those compiled and taken during TEM examination. This procedure provided a direct one-to-one comparison between the SEM and TEM when the same sample was examined.

DATA ANALYSIS

For most TEM analyses, data were generated and collected by means of in situ fiber counting methods in accordance with recommended procedures from a previous study (33). Manual counting from photographs was used for the remaining TEM analyses and for all of the SEM analyses. Each fiber was categorized by length and diameter, and approximately 25 percent of the fibers on filters A and D were also categorized with respect to aspect ratio. Fiber data from each photograph were corrected for magnification differences to convert all data to a common base (Appendix B). These data were then normalized with respect to total fiber count for development of length, diameter, and aspect ratio distributions. The distributions were then used in comparisons and for correlations of the various experimental parameters evaluated in this study. For each filter, a value of fiber concentration was determined from a total of all areas examined. Standard deviations of fiber concentration were then calculated from each sample, defined as either individual photographs or groups of photographs. Standard deviation of fiber concentration was an additional test parameter used in comparing and evaluating data.

Effects of Fiber Deposition Uniformity

The uniformity of fiber deposition was determined by comparing the fiber concentration measured between filters A and D of each filter set. The difference in fiber concentration between the A and D filters in each set was evaluated with respect to the distribution of concentration differences among all sets.

Effects of Magnification

The comparison of fiber-count statistics between filters observed at different magnifications was used in determining the effect of viewing magnification on counting accuracy. This comparison was also used in determining the relationship between instrument detectability limits and observation of the smallest fibrils.

Effect of Preparation Procedures

The fiber concentration and size distribution as determined from SEM analyses on filters A and D were compared with those values determined from TEM analyses on filters A, B, C, and D of each set. This comparison provided a measure of the effect of preparation procedures and included data with and without ashing in the TEM procedure. Count statistics from filter B (Nuclepore) were compared with those from filter C (Millipore) to determine the effect of filtering media.

Effect of Asbestos Fiber Concentration

The concentration of the asbestos fibers on the collection filter may affect the accuracy of the fiber count. For high concentrations, the individual fibers may pile up and overlap so that an accurate count cannot be made. For concentrations that are too low, the statistical significance of the fiber count may be inadequate. For a determination of the effect of fiber concentration on count accuracy, the standard deviation in fiber concentration and particle-size distribution of each set was evaluated as a function of the concentration of fibers on each filter.

Effects of Instrument Selection

The relative count accuracy of the two electron microscopes was evaluated by comparing the count statistics from filters A, B, C, and D as measured on the TEM with the count statistics from these filters as reexamined from grids on the SEM. Additionally, the count statistics from filters A and D in the initial SEM examination were compared with the count statistics from these same filters in the SEM reexamination.

SECTION 6

EXPERIMENTAL RESULTS

The fiber-count statistics gathered in this study are presented in Tables 1 through 3 for Groups I through III, respectively. Fiber concentration and length and diameter distributions are expressed in units of thousands of fibers per square millimeter and calculated directly from fiber counts and viewing areas in accordance with the method described. Each data point presented in these tables from 6000 × magnification is an average value that represents the summation of counts from 20 photographs. Each data point from 15,000 × magnification is an average value that represents the summation of counts from 25 photographs. The 30,000 × data are average values of all fibers counted in five TEM grid squares. Also included in these tables are the mean values of each statistic calculated from the three replicate sets. Because set 3 in Group I was not a replicate of sets 1 and 2, a separate summation is given.

For evaluation of the experimental significance of these data, the variation in fiber concentration, normalized to percentage, was examined as a function of the number of fibers counted.

The percent standard deviation of fiber concentration for each sample, calculated from the mean of all samples from a particular filter, is plotted in Figure 3 as a function of the total fibers counted on each filter. (A sample is either an individual photograph or a group of photographs from a single filter.) These data show that the statistical variation in fiber density obeys a chi-squared distribution function with one degree of freedom. The distribution may be represented by

$$\% \text{ standard deviation} = \left(\alpha^2 + \frac{\beta}{n \exp(n)}\right)^{1/2}$$

where $\alpha = 10$, $\beta = 1 \times 10^{-7}$, and n is the number of fibers counted, normalized to the median count ($n = N/\tilde{x}$, $\tilde{x} = 260$, N = fiber count). The α term represents the effect of variations due to uncertainty in magnification and is reflected in the minimum value of the function that asymptotically approaches 10-percent standard deviation rather than zero (Appendix B). The constant β in the preceding equation was empirically calculated from the data in Figure 3. The agreement between the measured data and the chi-squared distribution indicates that the fiber concentration variation from the mean is

TABLE 1. FIBER COUNT FOR GROUP I*

Filter	Magnification	Instrument	Length (μm)			Diameter (μm)			Total fiber densities
			<1.0	1.0-2.5	>2.5	<0.1	0.1-1.0	>1.0	
			Set 1						
A	6,000	SEM	19.4	23.5	37.2	32.2	46.6	1.3	80.1
A	15,000	SEM	18.6	28.0	40.4	23.8	61.8	1.4	87.0
A	30,000	TEM	2.3	3.3	2.1	2.7	4.4	0.6	7.7
B	30,000	TEM	14.4	3.0	0.9	0.3	16.9	1.1	18.4
C	30,000	TEM	3.5	1.4	1.2	0.2	5.1	0.8	6.0
D	30,000	TEM	4.2	4.4	1.8	3.6	6.4	0.4	10.4
D	6,000	SEM	12.5	26.3	36.7	23.5	51.8	0.2	75.5
D	15,000	SEM	26.6	28.0	29.5	9.7	73.8	0.7	84.1
			Set 2						
A	6,000	SEM	15.4	17.9	38.7	16.5	54.8	0.9	72.0
A	15,000	SEM	7.4	16.1	34.9	6.7	50.4	1.3	58.4
A	30,000	TEM	2.0	2.6	1.1	0.5	4.7	0.5	5.7
B	30,000	TEM	2.8	3.0	1.1	1.7	4.9	0.2	6.9
C	30,000	TEM	4.3	7.0	3.6	3.5	11.1	0.5	15.1
D	30,000	TEM							
D	6,000	SEM	7.7	14.9	22.1	12.5	32.2	0	44.7
D	15,000	SEM	12.6	13.6	22.9	10.8	38.4	0.7	49.1
			Set 3						
A	6,000	SEM	14.1	59.7	35.6	27.0	81.8	0.5	109.4
A	15,000	SEM	55.9	59.8	47.5	44.3	118.4	0.5	163.2
A	30,000	TEM	26.4	21.5	7.1	20.3	34.4	0.3	55.0
B	30,000	TEM	9.3	43.2	9.0	16.4	44.6	0.6	61.5
C	30,000	TEM	7.7	48.0	18.5	15.0	57.9	1.2	74.1
D	30,000	TEM	3.0	12.8	5.7	7.8	13.2	0.5	21.5
D	6,000	SEM	22.1	32.9	35.6	22.6	66.9	1.1	90.6
D	15,000	SEM	37.5	51.3	46.6	24.9	110.5	0	135.4
			Mean						
Sets 1 and 2	6,000	SEM	13.8	20.7	33.7	21.2	46.3	0.8	68.2
Sets 1 and 2	15,000	SEM	16.3	21.4	31.9	12.6	56.3	0.9	69.6
Sets 1 and 2	30,000 (Ashed)	TEM	4.8	3.5	1.7	1.8	7.6	0.6	10.0
Set 3	6,000	SEM	18.1	46.3	35.6	24.8	74.4	0.8	100.0
Set 3	15,000	SEM	46.7	55.6	47.1	34.6	114.5	0.3	149.4
Set 3	30,000	TEM	11.6	31.4	10.1	14.9	37.5	0.7	53.0

*Fibers/mm^2 (in thousands)

TABLE 2. FIBER COUNT FOR GROUP II*

Filter	Magnification	Instrument	Length (μm)			Diameter (μm)			Total fiber densities
			<1.0	1.0-2.5	>2.5	<0.1	0.1-1.0	>1.0	
			Set 1						
A	6,000	SEM	14.2	22.9	40.4	28.9	48.5	0.2	77.5
A	15,000	SEM	31.0	29.0	24.0	36.6	47.4	0	84.0
A	30,000	TEM	21.0	22.6	15.3	17.6	40.8	0.5	58.9
B	30,000	TEM	15.2	25.1	20.2	14.6	44.9	0.9	60.4
C	30,000	TEM	13.6	18.4	14.3	7.9	37.9	0.5	46.2
D	30,000	TEM							
D	6,000	SEM	17.5	19.0	20.5	20.7	36.3	0	57.0
D	15,000	SEM	22.9	19.3	10.1	14.5	37.8	0	52.3
			Set 2						
A	6,000	SEM	18.0	31.6	35.3	22.1	62.4	0.4	84.9
A	15,000	SEM	36.8	30.9	26.6	33.6	60.7	0	94.3
A	30,000	TEM	19.1	18.8	22.4	20.5	39.6	0.3	60.4
B	30,000	TEM	15.4	20.4	19.1	16.0	38.4	0.5	55.0
C	30,000	TEM	24.5	21.3	18.1	6.5	57.3	0.1	63.9
D	30,000	TEM	22.0	20.6	25.6	18.9	49.2	0.1	68.2
D	6,000	SEM	24.8	38.5	38.0	32.9	68.2	0.2	101.3
D	15,000	SEM	36.4	25.8	21.3	19.3	64.3	0	83.5
			Set 3						
A	6,000	SEM	15.7	15.0	12.3	9.0	34.0	0	43.0
A	15,000	SEM	16.4	18.7	15.5	10.8	39.9	0	50.7
A	30,000	TEM	21.7	18.8	16.7	13.6	43.5	0	57.2
B	30,000	TEM	53.9	39.5	19.8	21.9	90.9	0.3	113.1
C	30,000	TEM	11.5	13.6	14.5	3.2	35.7	0.8	39.6
D	30,000	TEM	41.0	33.3	22.2	14.8	81.5	0.3	96.5
D	6,000	SEM	31.9	21.5	17.1	22.7	47.6	0.2	70.5
D	15,000	SEM	22.1	28.4	21.8	12.0	60.3	0	72.3
			Mean						
	6,000	SEM	20.4	24.8	27.3	22.7	49.5	0.2	72.4
	15,000	SEM	27.6	25.4	19.9	20.6	52.3	0	72.9
	30,000	TEM	23.5	22.9	18.9	14.1	50.9	0.4	65.4

*Fibers/mm^2 (in thousands)

TABLE 3. FIBER COUNT FOR GROUP III*

Filter	Magnification	Instrument	Length (μm)			Diameter (μm)			Total fiber densities
			<1.0	1.0-2.5	>2.5	<0.1	0.1-1.0	>1.0	
			Set 1						
A	6,000	SEM	12.5	14.2	11.7	14.9	23.5	0	38.4
A	15,000	SEM	15.3	17.9	10.8	6.4	37.6	0	44.0
A	30,000	TEM	14.4	16.4	16.4	10.9	36.0	0.2	47.2
B	30,000	TEM	28.1	15.7	17.6	17.7	43.6	0	61.4
C	30,000	TEM	15.6	15.0	14.9	9.0	36.2	0.2	45.5
D	30,000	TEM	12.1	10.5	16.7	11.7	27.3	0.3	39.3
D	6,000	SEM	9.6	9.9	12.3	6.1	25.7	0	31.8
D	15,000	SEM	16.7	11.9	9.7	7.6	30.7	0	38.3
			Set 2						
A	6,000	SEM	15.0	14.0	14.8	11.9	31.9	0	43.8
A	15,000	SEM	17.9	17.0	12.6	7.7	39.8	0	47.5
A	30,000	TEM	11.7	13.0	17.0	13.4	28.3	0	41.7
B	30,000	TEM	15.2	13.2	16.7	14.9	30.1	0.2	45.1
C	30,000	TEM	17.6	11.8	15.8	12.0	33.0	0.2	45.2
D	30,000	TEM	22.2	13.8	17.1	17.1	35.9	0.2	53.1
D	6,000	SEM	16.1	14.0	12.7	9.3	33.5	0	42.8
D	15,000	SEM	11.7	12.5	15.3	10.7	28.8	0	39.5
			Set 3						
A	6,000	SEM	17.9	14.8	17.6	19.4	30.9	0	50.3
A	15,000	SEM	14.9	13.3	16.5	11.3	33.4	0	44.7
A	30,000	TEM	21.3	16.9	17.7	18.8	37.0	0.2	55.9
B	30,000	TEM	18.6	12.7	15.3	15.6	30.9	0.1	46.6
C	30,000	TEM	16.7	11.4	7.0	9.8	24.9	0.2	35.1
D	30,000	TEM	16.7	12.2	15.4	17.7	26.5	0.2	44.4
D	6,000	SEM	14.0	15.0	17.2	11.2	35.0	0	46.2
D	15,000	SEM	11.3	4.3	13.9	9.2	20.3	0	29.5
			Mean						
	6,000	SEM	14.2	13.7	14.4	12.2	30.2	0	42.2
	15,000	SEM	14.6	12.8	13.1	8.8	31.8	0	40.6
	30,000	TEM	17.5	13.6	15.6	14.1	32.5	0.2	46.7

*Fibers/mm^2 (in thousands)

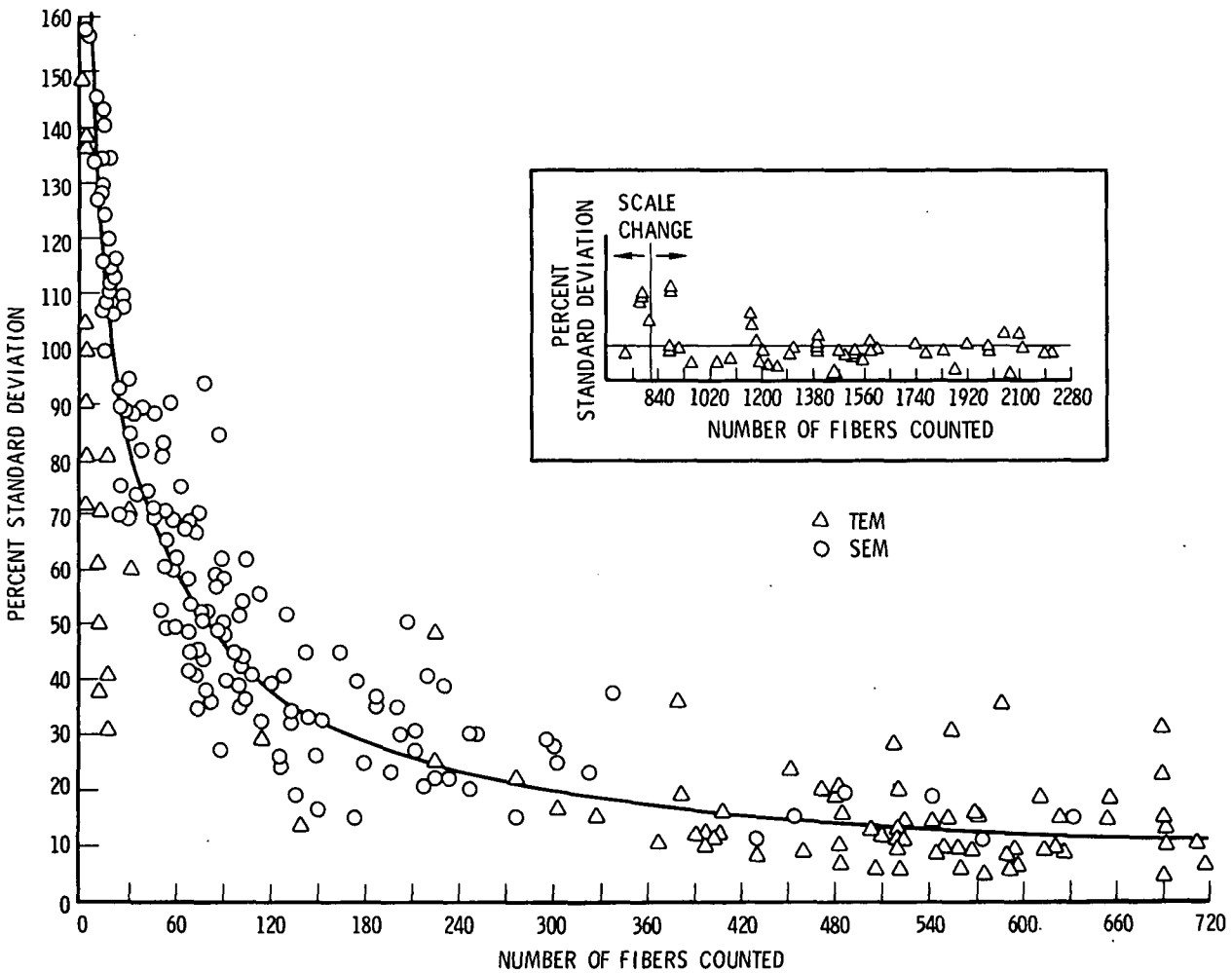

Figure 3. Percent standard deviation vs fibers counted per sample.

normally distributed and that, except for the displacement due to uncertainty in magnification, no systematic variation is present.

For sample sizes larger than 200 fibers, the precision in the measured fiber concentration is reasonably invariant; for sample sizes less than 200 fibers, the precision in measured concentration is strongly affected by the total fiber count.

The distribution in the total fiber concentration for each group with respect to the examining instrument and magnification is shown in Figure 4, where Group I is divided into two parts because set 3 did not replicate sets 1 and 2. The distributions of fiber length and fiber diameter for all fibers counted are shown in Figures 5 and 6, respectively. These distributions show that the fibers counted are representative of airborne distributions; most of the fibers are less than 1 μm in diameter, and only a small number are longer than 5 μm. These distributions were replotted on a logarithm-probability graph paper in Figure 7, where both length and diameter distributions are shown to be log-normal; the mean fiber length is 1.5 μm, and the mean fiber diameter 0.17 μm. This distribution shows that virtually all fibers counted were biologically significant (< 3.3 μm). Only 16 percent of the fibers were longer than 5 μm, and 1 percent were shorter than 0.1 μm. A discussion of distributions, from the literature, is presented in Appendix A.

The distributions of aspect ratio for the magnifications used in the study are plotted in Figure 8, where the aspect ratio distributions are shown to be relatively constant even after ashing. Ashing, however, appears to reduce the percentage of long fibers to some degree. The data for this figure were obtained from reexamination of photographs and represent only a portion of all fibers counted.

The bar graphs in Figures 9 through 11 show fiber length distributions by set for Groups I through III, respectively. Each of three length categories is represented by a bar. Three such graphs, each representing the distribution of a single set, are then superimposed to form a nine-element graph. The data for instrument and magnification are represented by nine-element graphs, and three such graphs represent the group. Figures 10 and 11 also show the average value for the three sets by an additional bar that is more prominently displayed than that of the individual sets.

The bar graphs in Figures 12 through 14 represent the distribution of fiber diameters for each of the three groups. Each magnification is shown separately, and the range of diameters is divided into four parts. The distributions for Group II and III are nearly identical. Group I distributions are dissimilar, and the reasons for this effect are discussed in Section 7 under Effect of Fiber Concentration.

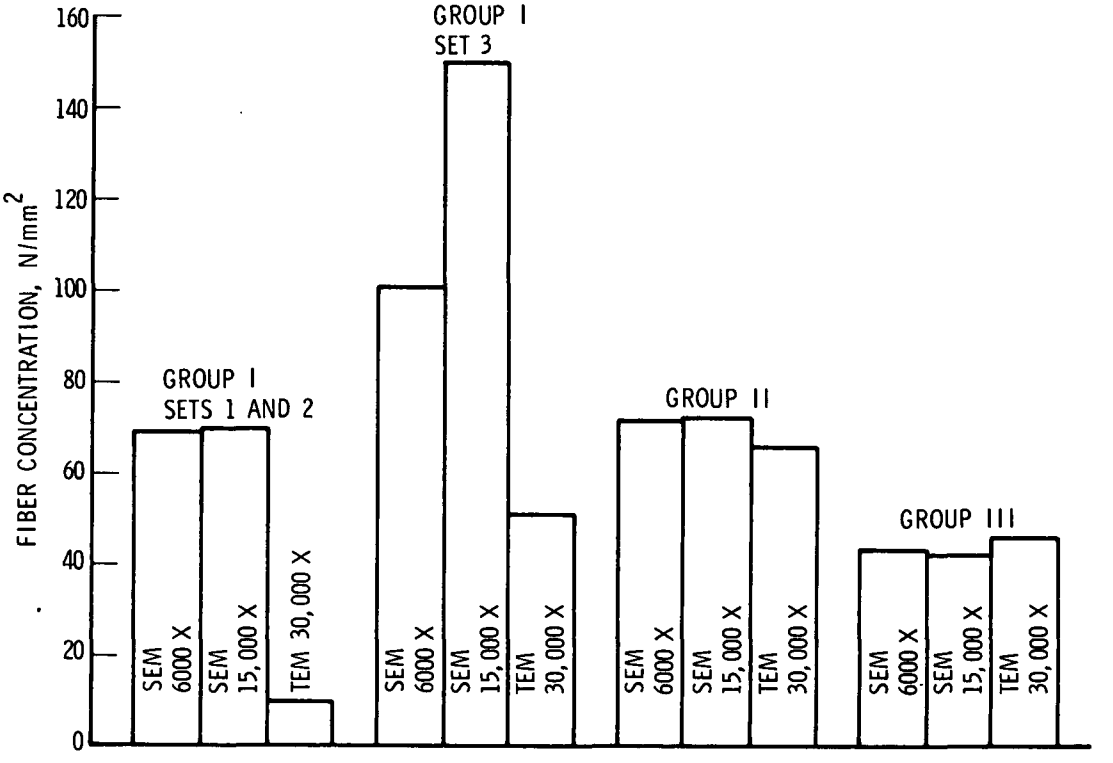

Figure 4. Comparison of measured fiber densities vs magnification for each group.

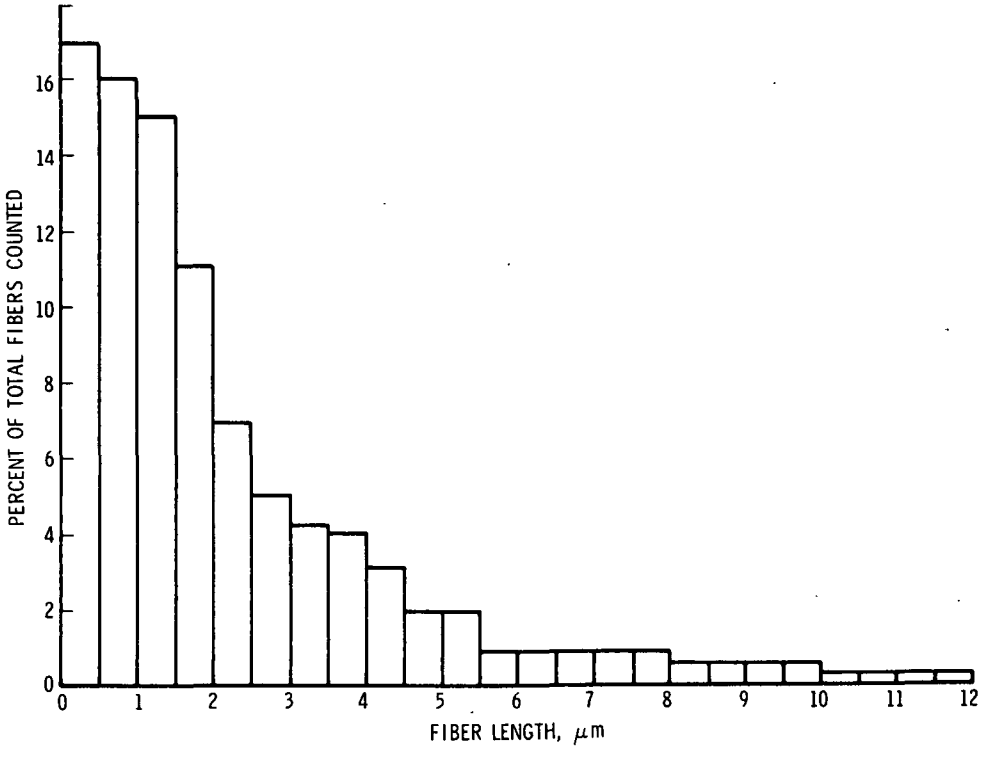

Figure 5. Distribution of fiber lengths for all fibers counted. Set 3 of Group I is excluded.

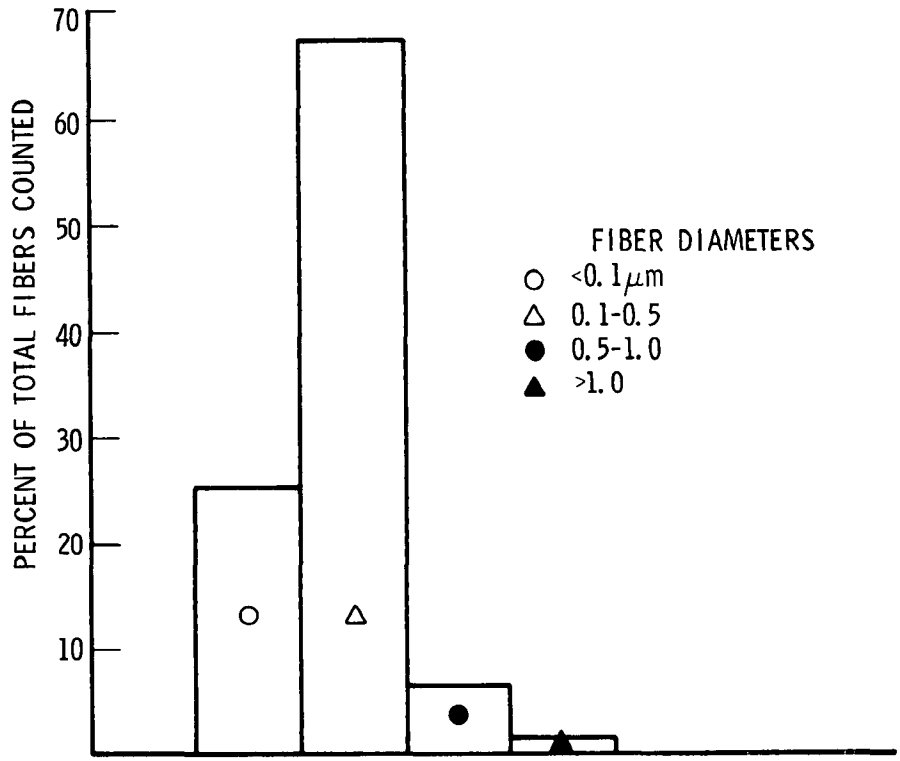

Figure 6. Composite fiber diameters for all groups and magnifications.

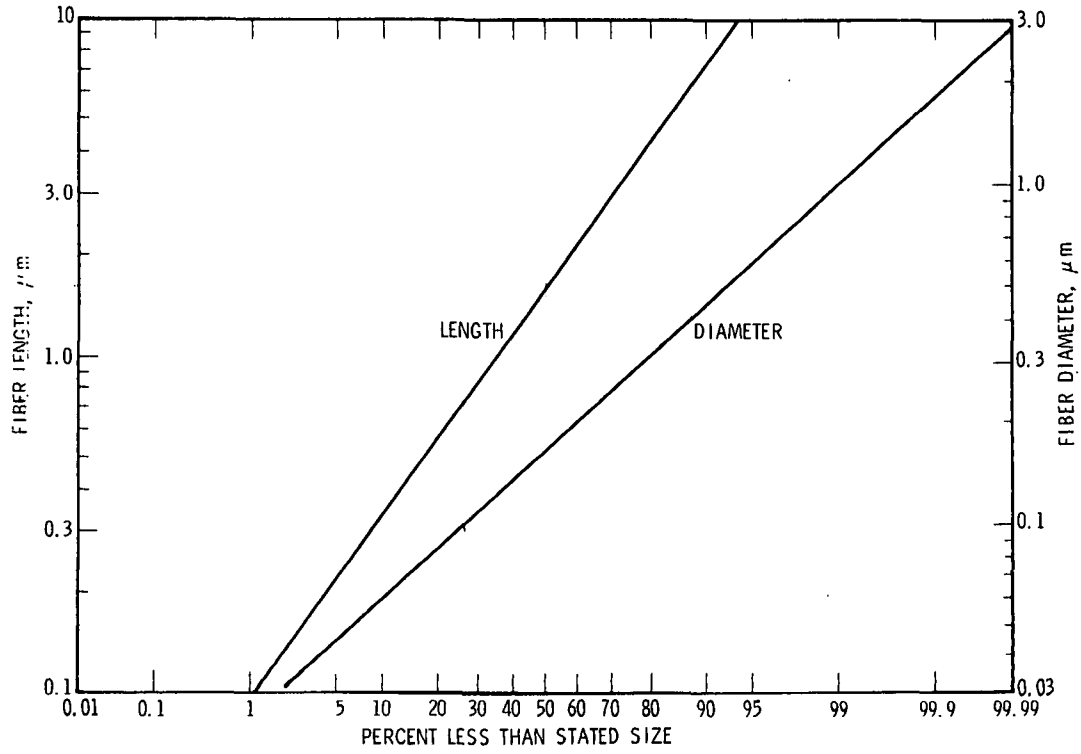

Figure 7. Total fiber length and diameter distributions. Set 3 of Group I is excluded from length data.

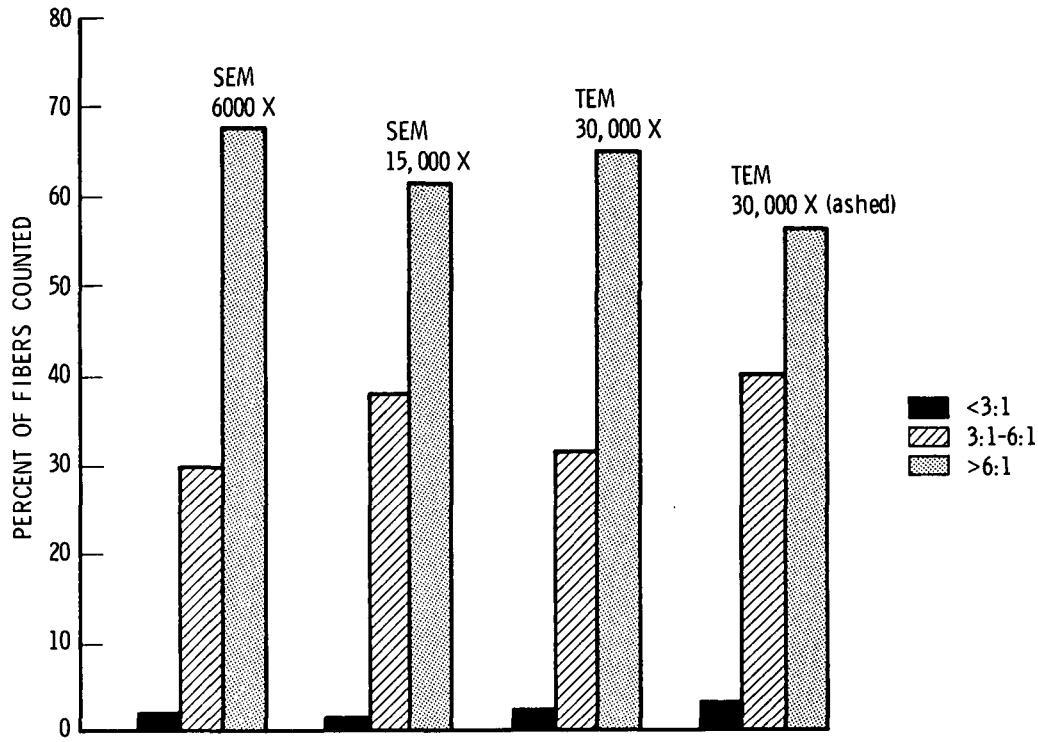

Figure 8. Distribution of aspect ratios for each magnification and preparation.

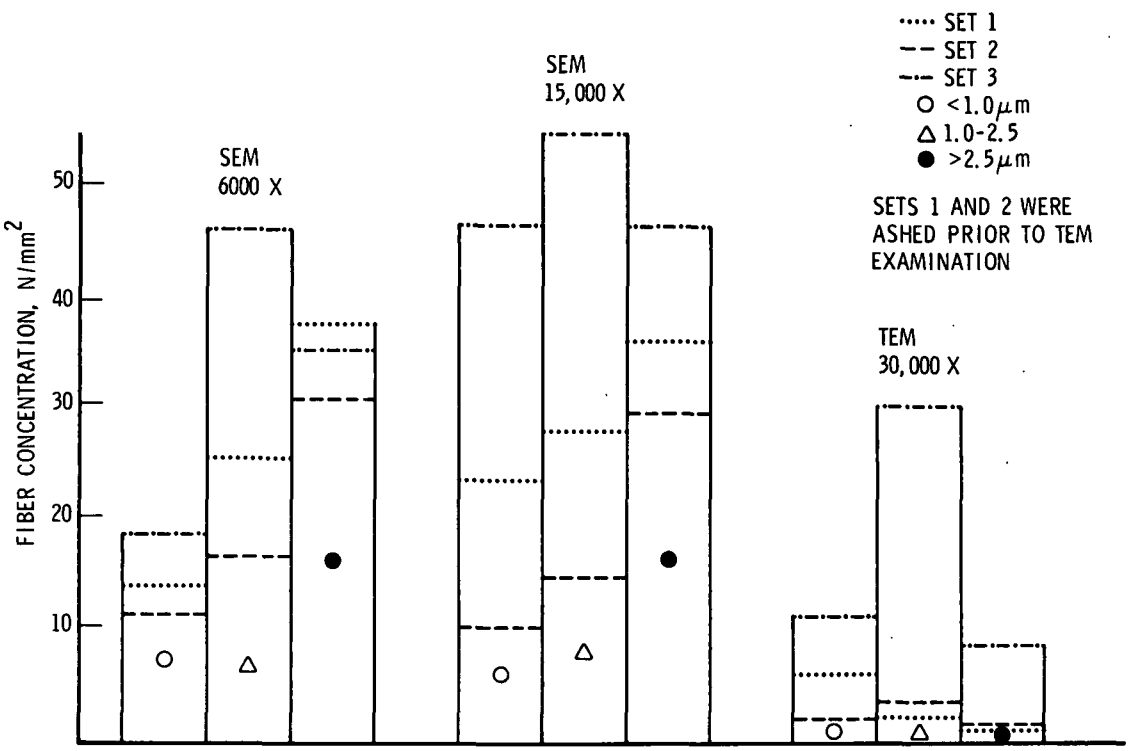

Figure 9. Group I fiber length distributions.

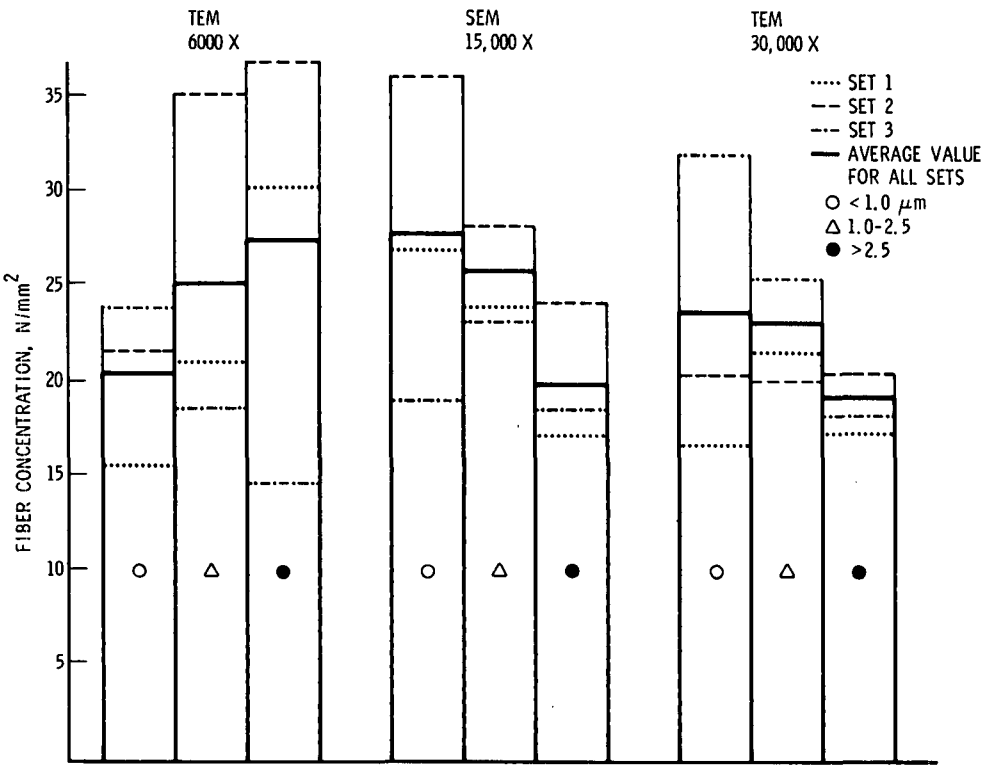

Figure 10. Group II fiber length distributions.

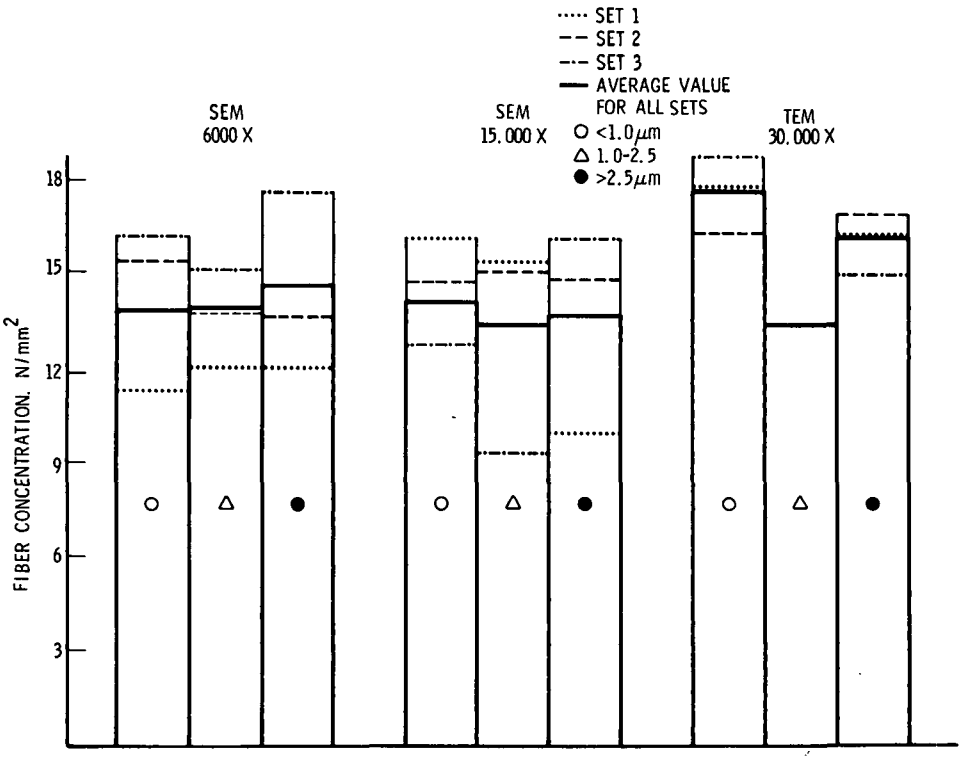

Figure 11. Group III fiber length distributions.

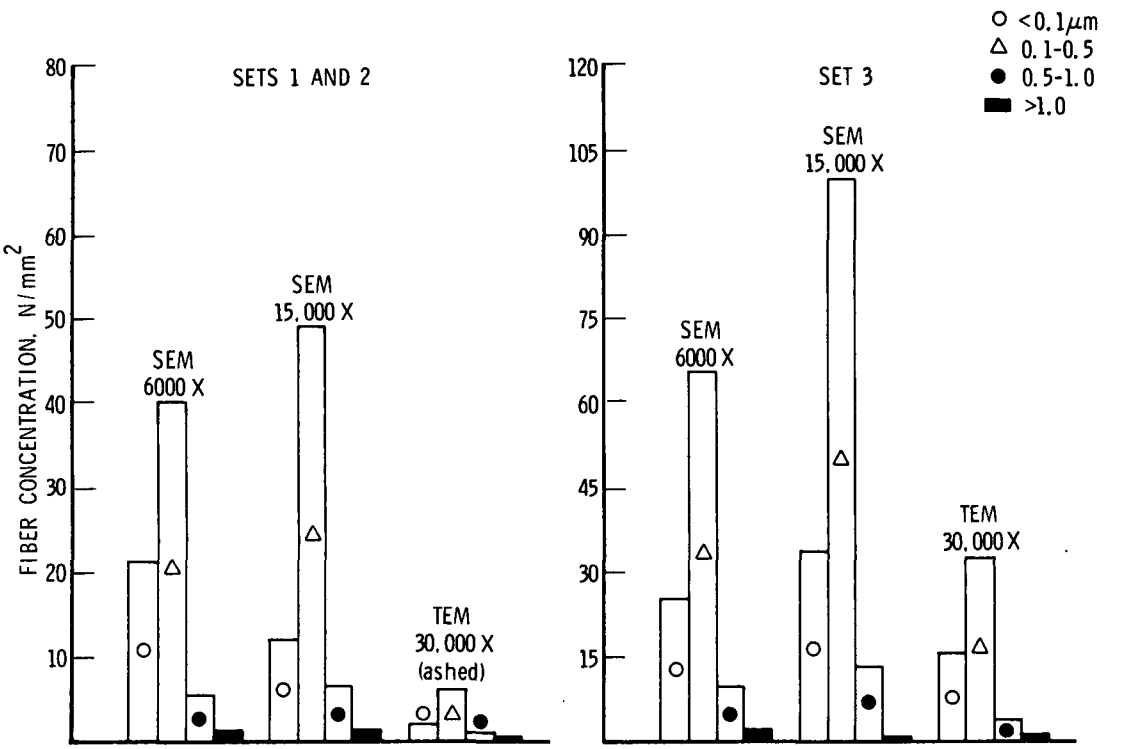

Figure 12. Group I fiber diameters.

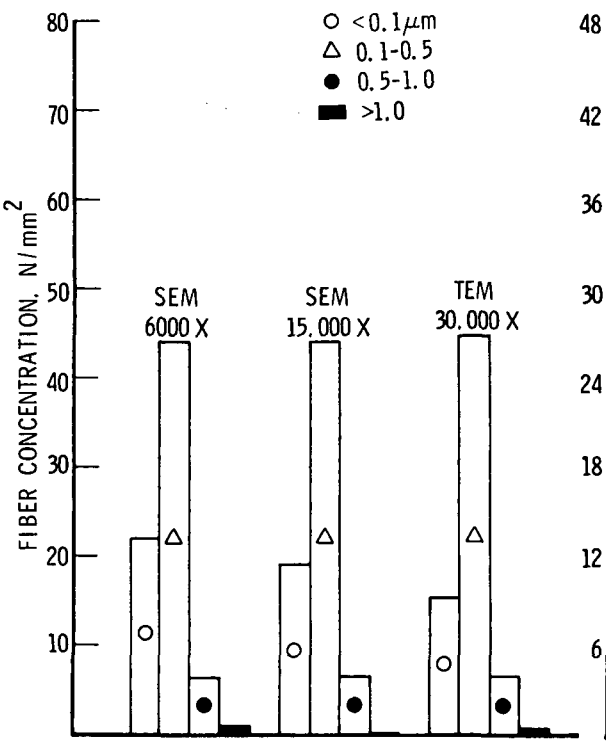

Figure 13. Group II fiber diameters.

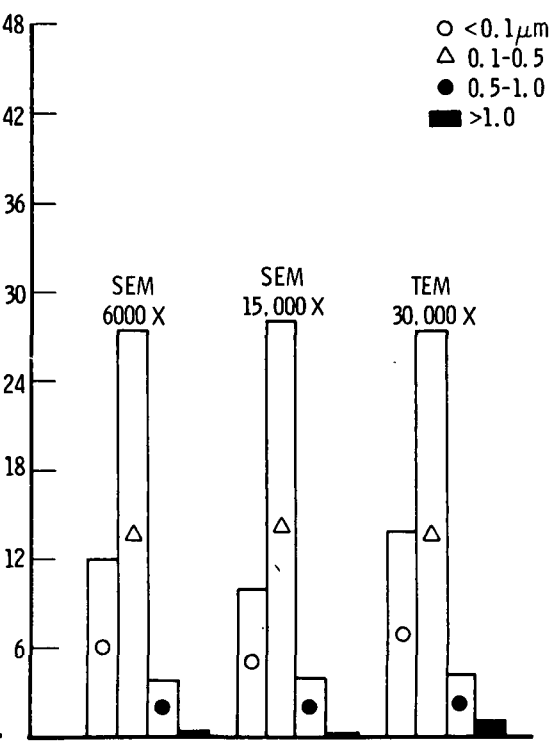

Figure 14. Group III fiber diameters.

SECTION 7

EFFECTS OF TEST PARAMETERS

FIBER DEPOSITION UNIFORMITY

The first and last filters from each deposition run (filters A and D of each set) were examined and compared in order to establish the consistency of asbestos deposition on the sample filters. Filters A and D from each set of four filters were initially examined in the SEM before any other operation was performed. Because all four filters in a set were sequentially exposed to the asbestos generator air stream, without alteration of deposition parameters, comparison of the first and last filter in each set provided a measure of any systematic variation in fiber deposition in each set. Comparison of fiber concentration variations on filters A and D from all sets provided a measure of both the random and systematic variation in fiber deposition on all the filters.

Fiber counts at both 6000 × and 15,000 × magnifications were made, and fiber concentration variations were calculated from the total areas observed. The fiber concentrations from all nine filter sets are given in Table 4. The difference in fiber concentration between filters A and D for each set was calculated, divided by the sum of the fiber concentration from both filters, and expressed in percent. The fiber concentration variation among the nine sets ranged from 1 to 24 percent of the average fiber concentration for each set.

The mean value for the variation in the percent fiber deposition from all sets was 5 percent and represents the systematic variation from zero. The standard deviation calculated from all sets at 12 percent represents the random variation. Because the systematic variation was small relative to the random variation, it appears that there is no significant systematic variation in fiber deposition among the filter sets. The variation in fiber deposition for filters in any group was not significantly different than that for all groups; it was concluded, therefore, that fiber deposition variation is not a function of the actual fiber concentration.

The experimental significance of the random variation in fiber concentration on the total experimental program was further evaluated by comparing the data of this experiment with the total data base presented in Figure 3. The number of fibers counted on filters A and D among all sets varied between 200 and 600 fibers, from which the expected percent standard deviation in accordance with Figure 3 would decrease from 20 to 10 percent.

TABLE 4. SEM COMPARISON OF FILTERS A AND D

	Fiber concentration variation			
	6000×		15,000×	
	f*	Percent	f*	Percent
Group I				
Set 1	4.6/155.6	3	2.9/171.1	2
Set 2	27.3/116.7	23	9.3/107.5	9
Set 3	18.8/200.0	9	27.8/298.6	9
Group II				
Set 1	20.5/134.5	15	31.7/136.3	22
Set 2	-16.4/186.2	-9	10.8/177.8	5
Set 3	-27.5/113.5	-24	-21.6/123.0	-18
Group III				
Set 1	6.6/70.2	9	5.7/82.3	7
Set 2	1.0/86.6	1	8.0/87.0	9
Set 3	4.1/96.5	4	15.2/74.2	20

*f = A-D/A+D, where A = fiber concentration on filter A of a set, and D = fiber concentration on filter D of the same set (See Tables 1 through 3.) Mean = 5 percent, standard deviation = 12 percent.

The measured standard deviation of 12 percent from all sets lies within this range; therefore, it may be concluded that the measured random variation does, in fact, represent the true variation in fiber deposition on the filters, and that no systematic variation in fiber deposition exists either within a set or among the sets. It may also be concluded that an accurate value of fiber concentration from each set can be determined from a sample size that consists of 200 fibers or more.

EFFECT OF MAGNIFICATION

The choice of magnification involves a tradeoff between effective resolving power and area of coverage. Magnifications ranging from 6000 to 30,000× were used to establish reasonable guidelines for effective measurement. At 6000× on the SEM, even the smallest fibril could be observed; however, as the concentration of the fibers on the filters increased, fiber clusters were created, and the 6000× magnification was not adequate for resolution of the smallest fibrils when they were partially obscured by other fibers. With the SEM, 15,000× magnification was usually sufficient for resolution of fine particles, and an increase to 30,000× did not significantly increase the instrument's ability to resolve fine fibrils partially obscured by agglomeration. Because of its higher resolution, the TEM was better able to distinguish individual fibers in an agglomerate at 15,000× magnification; an increase to 30,000× magnification was not required for further improvement.

EFFECT OF SAMPLE PREPARATION PROCEDURES

The fiber density on filters A through D was determined to be uniform within each set. When the SEM fiber-count statistics for filters A and D were compared with the TEM data for these same filters, it was possible to evaluate the effect of the TEM's extensive preparation procedures on fiber count and fiber distribution. But, because both filters were previously examined in the SEM, filter B was included in order to eliminate possible effects of the SEM examination. TEM data from filter B were then compared with the TEM data from filters A and D. Filter C, a fibrous filter, was added to the study to permit comparison between the two types of filters. (Filters A, B, and D are membrane filters.)

A comparison of fiber concentration and fiber distribution is presented in Table 5. There is no discernible difference between SEM results from filters A and D and TEM results from these same filters. In addition, these results are generally in good agreement with the results from filter B. The data from filter C also agree well with all other data. However, results from Group I filters with high fiber concentrations did not agree.

It was concluded that filter preparation techniques for either the SEM or TEM (without ashing) had no discernible effect on the results. Moreover, type of filter did not affect the TEM results, and the data are identical to the SEM results obtained with a membrane filter. (Fibrous filters are not easily used for SEM examination.) Thus, neither sample preparation nor

TABLE 5. COMPARISON OF FIBER STATISTICS FOR FILTERS PREPARED FOR SEM AND TEM EXAMINATION

Group	Set	Filter*	Instrument	Fiber length (μm)			Concentration
				<1.0	1.0-2.5	>2.5	N/mm^2
I	3	A + D	SEM	32.4	50.9	41.3	124.7
		A + D	TEM	14.7	17.2	6.4	38.3
		B	TEM	9.3	43.2	9.0	61.5
		C	TEM	7.7	48.0	18.5	74.1
II	1	A + D	SEM	21.4	22.6	23.8	67.7
		A + D	TEM	21.0	22.6	15.3	58.9
		B	TEM	15.2	25.1	20.2	60.4
		C	TEM	13.6	18.4	14.3	46.2
	2	A + D	SEM	29.0	31.7	30.3	91.0
		A + D	TEM	20.6	19.7	24.0	64.3
		B	TEM	15.4	20.4	19.1	55.0
		C	TEM	24.5	21.3	18.1	63.9
	3	A + D	SEM	21.5	20.9	16.7	59.1
		A + D	TEM	31.4	26.1	19.5	76.9
		B	TEM	53.9	39.5	19.8	113.1
		C	TEM	11.5	13.6	14.5	39.6
III	1	A + D	SEM	13.5	13.5	11.1	38.1
		A + D	TEM	13.3	13.5	16.6	30.0
		B	TEM	28.1	15.7	17.6	61.4
		C	TEM	15.6	15.0	14.9	45.5
	2	A + D	SEM	15.2	14.4	13.9	43.4
		A + D	TEM	17.0	13.4	17.1	47.4
		B	TEM	15.2	13.2	16.7	45.1
		C	TEM	17.6	11.8	15.8	45.2
	3	A + D	SEM	14.5	11.9	16.3	42.5
		A + D	TEM	19.0	14.6	16.6	50.2
		B	TEM	18.6	12.7	15.3	46.6
		C	TEM	16.7	11.4	7.0	35.1

*Filters A, B, and D were membrane filters; filter C was a fibrous filter.

filter is a variable in the measurement of asbestos fibers by either microscope (exclusive of ashing for the TEM preparation). However, the membrane filter is more difficult to use for TEM and increases the probability of fiber loss.

The effect of ashing was studied in a second series of experiments on sets 1 and 2 of Group I filters. The data from this study (Table 6) show a marked difference in both fiber concentration and fiber distribution as a result of ashing, which was used in TEM sample preparation to remove nonasbestos fibers from the filter. The asbestos fiber concentrations from the TEM examination are only 15 percent of those obtained with the SEM. It was concluded that ashing is responsible for a nominal 85-percent loss of asbestos fibers.

TABLE 6. COMPARISON OF FIBER STATISTICS FOR FILTERS PREPARED FOR SEM EXAMINATION AND BY ASHING FOR TEM EXAMINATION

Group	Set	Filter*	Instrument	Fiber length (μm)			Concentration
				<1.0	1.0-2.5	>2.5	N/mm^2
I	1	A + D	SEM	19.3	26.4	36.0	81.7
		A + D	TEM	3.3	3.8	2.0	5.8
		B	TEM	14.4	3.0	0.9	18.4
		C	TEM	3.5	1.4	1.2	6.0
	2	A + D	SEM	10.8	15.6	29.7	56.1
		A + D	TEM	2.0	2.6	1.1	5.7
		B	TEM	2.8	3.0	1.1	6.9
		C	TEM	4.3	7.0	3.6	15.1

*Filters A, B, and D were membrane filters; filter C was a fibrous filter.

EFFECT OF FIBER CONCENTRATION

The degree to which fiber-count accuracy is affected by fiber concentrations on filters can be seen in the length distributions plotted in Figures 9 through 11. Group I results (Figure 9) were highly erratic, complicated by an accidental departure from the test plan. Group II results (Figure 10) indicate reasonably good agreement between the distributions obtained from the TEM analyses at 30,000× and those obtained from the SEM analyses at 15,000×. The SEM analyses at 6000× show a lower proportion of short fibers and a higher proportion of long fibers than the other two analyses. Close examination of the SEM photographs revealed that the difference

between the 6000× and 15,000× magnifications was a result of the smaller fibers being obscured by larger ones at the lower magnification. At the higher magnification, it is possible to separate some of the fibers that would otherwise appear as an agglomerated mass at lower magnifications. The average value of fiber concentration for all Group II filters was 70,300 fibers/mm^2, which is within 12 percent of the intended value. The standard deviation from the mean concentration for individual samples was 25 percent.

Group III data (Figure 11) show good agreement among all three distributions, which indicates that, at this concentration, there is no discernible effect from either instrumentation or magnification. The average fiber concentration from all Group III filters was 43,200 fibers/mm^2, which is within 8 percent of the intended value. The standard deviation from the mean concentration for individual samples was 15 percent.

In both Group II and Group III, the individual distributions of each set are similar and are well represented by the average. Moreover, agreement among sets provides a measure of their accuracy and reliability. Group I data, however, did not show the agreement seen in Groups II and III.

Group I distribution analyses (Figure 9) can be divided into two parts. The first, composed of sets 1 and 2, had average fiber concentrations of 68,900 fibers/mm^2, 54 percent from the desired value, and a 23-percent standard deviation from the mean concentration for individual samples. The second part, composed of set 3, had an average fiber concentration of 125,700 fibers/mm^2, 17 percent from the desired value, and a 25-percent standard deviation from the mean concentration for individual samples. The particle-size distribution from set 3, with the highest fiber concentrations, produced the most erratic results. The three distributions from the same filters differ in total concentration, and the individual fiber length distributions also differ. Figure 9 shows that the SEM analyses at 15,000× magnification had the highest total concentration, 140,400 fibers/mm^2, and a nearly uniform size distribution. The SEM analyses at 6000× had an average concentration, 100,000 fibers/mm^2, and were noticeably deficient in the proportion of fibers less than 1 μm in length. The obscuration of the finer fibers by the larger ones, as discussed for Group II, appears to be the primary reason for this observed distribution. Moreover, because of the high concentration of fibers on the filter, even larger fibers were obscured at the lower magnification and were not individually resolved; thus, a lower total concentration is observed. The average concentration from the TEM analyses was only 53,000 fibers/mm^2 and was deficient in all size categories. The TEM results were caused by fiber pileup. When the carbon coating is applied to a heavily laden filter, the uppermost fibers shield underlying fibers from the coating. Subsequently, when the filter is dissolved, the previously protected fibers are flushed away and are not available to the TEM for observation.

Although sets 1 and 2 of Group I had concentrations nearly equivalent to those of Group II, the distributions of fiber lengths were sufficiently different to justify giving individual consideration to the data from the two

groups. In SEM analyses, there was good agreement between the fiber length distributions and concentrations from sets 1 and 2 of Group I at both magnifications. Individual sets of Group II at a similar concentration also exhibited good agreement. With respect to fiber length distribution, sets 1 and 2 of Group I were low in short fibers and high in long fibers relative to Group II. Although fiber obscuration is likely at the lower magnification, as observed in Group II, the appearance of the same distribution at higher magnification indicates that the fiber length distributions were different than Group II distributions because of deposition, not obscuration.

It was concluded that fiber concentration on filters affects both observed concentration and distribution. At concentrations of 40,000 fibers/mm^2, reliable, accurate measurements can be made with either the SEM or TEM and at magnifications of from 6000× to 30,000×. At concentrations of 80,000 fibers/mm^2, the SEM analyses at 6000× showed a loss of fine fibers, although both the SEM analyses at 15,000× and the TEM analyses at 30,000× provided accurate results. At concentrations greater than 80,000 fibers/mm^2, no reliable data were obtained.

EFFECT OF INSTRUMENT PERFORMANCE

Samples prepared for and examined with the TEM were reexamined with the SEM to determine if the SEM data differed from the TEM data, exclusive of sample preparation techniques. From photographs taken of identical areas with both microscopes at 15,000× and 30,000×, every identifiable fiber was individually noted, as shown in Figures 15 and 16. Three types of discrepancies between matching pairs of photographs were considered: agglomeration, confusion, and absence. These data are given in Table 7.

An agglomeration is a cluster of fibers lying so close together that some cannot be distinguished. Confusion arises when the microscope cannot distinguish asbestos fibers from the background. When a fiber is not visible in one instrument but can be seen with the other, the term "absence" is used.

The overall performance of both instruments was comparable in that each microscope failed to observe 26 percent of the fibers observed by the other microscope. Of the fibers observed by the TEM but not by the SEM, 84 percent were not observed because of agglomeration. Of the fibers observed by the SEM but not by the TEM, 97 percent were not observed because of confusion. The TEM was better able to resolve individual fibers in agglomerates because of its superior resolution; however, because the TEM also views both sides of the carbon film that supports the asbestos, fibers become confused with background. This is shown in Figures 15 and 16 for both membrane and fibrous filters.

Fiber absences in each microscope accounted for 3 percent of the total discrepancy between the instruments. Absences may be caused by extreme agglomeration or confusion, by a loss (or gain) of fibers in handling between the instruments, or by insufficient resolving power.

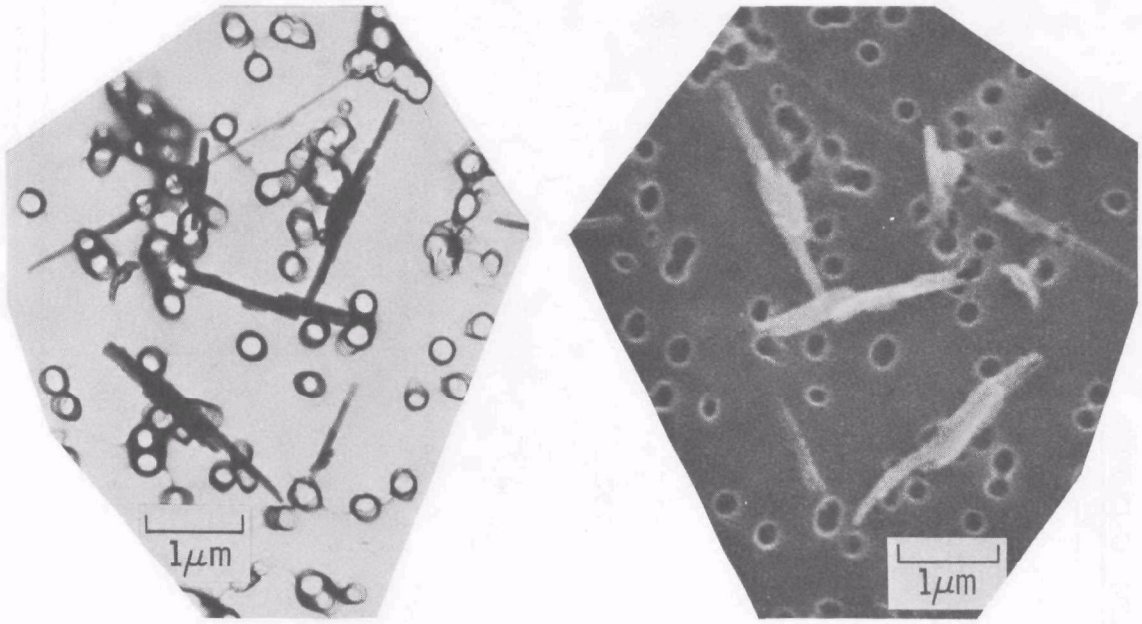

Figure 15. TEM photomicrograph (a) and SEM photomicrograph (b) from Nuclepore filters. Note confusion due to filter texture and ease of agglomerate separation in (a).

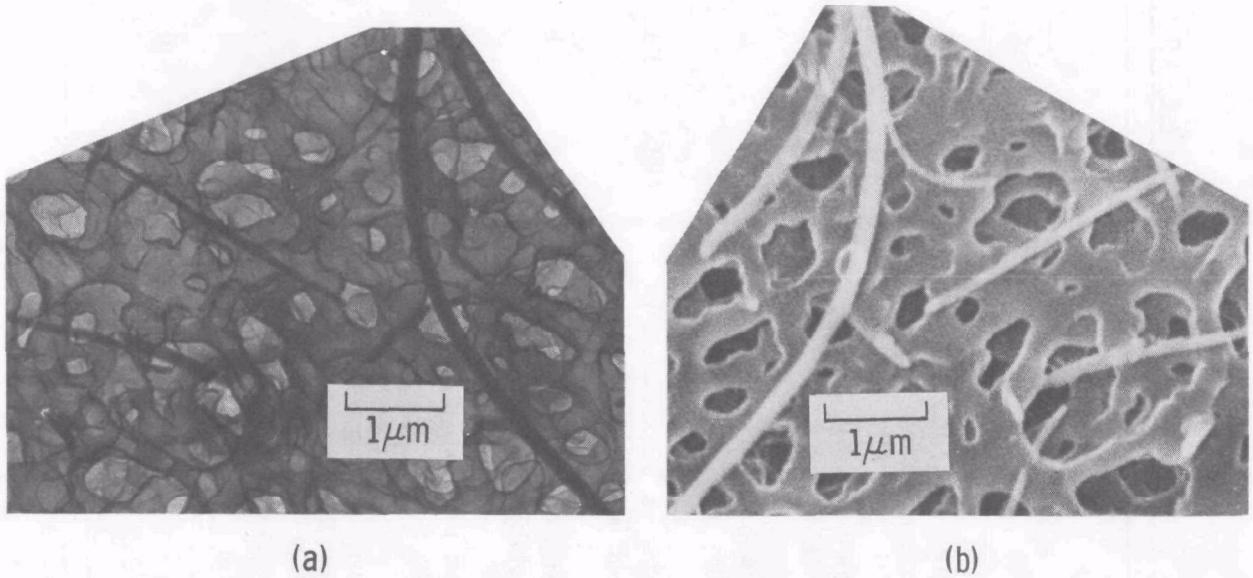

Figure 16. TEM photomicrograph (a) and SEM photomicrograph (b) from Millipore filters. Again note confusion due to filter texture in (a).

TABLE 7. SAMPLE AS OBSERVED BY SEM AND TEM

	Total fibers	Group			Percent of total fibers	Percent of fibers lost (each instrument)
		I	II	III		
Total number of fibers seen by either instrument	837	35	349	454	100	
Total fibers seen by TEM but not by SEM	215	13	95	107	26	100
Total fibers seen by SEM but not by TEM	217	8	85	124	26	100
Agglomerations separated by TEM but not by SEM	181	13	72	96	22	84
Agglomerations separated by SEM but not by TEM	2	1	0	1	0	1
Fibers seen well in TEM but confusing in SEM	27	0	16	11	3	12
Fibers seen well in SEM but confusing in TEM	210	7	82	119	25	97
Fibers seen in TEM but not seen in SEM	7	0	7	0	1	3
Fibers seen in SEM but not seen in TEM	7	0	3	4	1	3

As shown in Table 7, the distribution of fiber loss among the three groups was proportional to the number of fibers counted in each group. It is concluded, therefore, that the concentration of fibers on the filter does not contribute to fiber loss by any of the three identified discrepancies; however, at high fiber concentrations, large loss occurs in TEM preparations. No difference could be noted in comparisons of the relative loss at 15,000× and 30,000× from both microscopes. At both magnifications, fine fibers at high filter concentrations were affected equally.

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APPENDIX A

SIZE DISTRIBUTIONS OF ASBESTOS DUSTS

The size distribution of asbestos dusts has been measured by several investigators. Although similarities exist among the distributions, each is strongly dependent on the method of measurement. In many cases, an optical microscope was used; consequently, the distribution did not include the finer fibers not resolved with this instrument. Other instruments used were also insensitive to the finer sizes, and accurate correlation between studies could not be made.

A distribution of the lengths of airborne asbestos fibers of chrysotile, amosite, and crocidolite was measured by Wagner and Skidmore with a thermal precipitator (28). For each of the minerals, 90 percent of the fibers had particle lengths less than 7 μm. At greater fiber lengths, the proportion of long fibers was greater for chrysotile than for the amphibole minerals. Less than 0.1 percent of the amphibole fibers were longer than 30 μm, and about 0.01 percent were longer than 75 μm. For chrysotile fibers, 0.1 percent were longer than 50 μm, and about 0.01 percent were longer than 100 μm.

Roach (29) used a thermal precipitator and oil immersion objective lens to measure the length and diameter distributions of amosite fibers in the vicinity of a bagging operation. He found that 85 percent of the visible particles were shorter than 1 μm and only 5 percent were longer than 5 μm. When the same particles were sized by diameter, 94 percent had diameters less than 0.5 μm; only 0.6 percent were thicker than 1 μm. The results of Wagner and Skidmore and Roach are essentially the same, inasmuch as Roach may have measured a slightly finer portion of the airborne distribution.

Addingley (30) measured chrysotile fibers in the workrooms of weaving, doubling, and carding operations, using a Royco particle counter at equivalent sphere diameters between 0.3 and 10 μm in 15 separate size ranges. The distributions for the weaving and doubling operations were nearly identical. About 50 percent of the fibers were in the 0.5- to 0.7-μm range; less than 2 percent were greater than 1 μm. Below 0.5 μm, a decrease in frequency is observed that may correctly define the distribution or may instead be a consequence of a loss of instrument sensitivity at the finer sizes. Because the Royco particle counter measures an equivalent fiber diameter based on the cross section of a fiber, direct correlation is not possible.

A maximum in the particle size distribution of chrysotile fibers was observed by Craig et al. (35), who used an Anderson Cascade Impactor and mass calculations, and by Lynch et al. (36), who worked with a transmission electron microscope and a sample collected on a membrane filter. Craig found that the aerodynamic mass mean diameter lies between 1 and 3 μm and that 5 percent of the fibers had equivalent diameters greater than 7 μm for three samples measured. Lynch measured asbestos fibers in the textile, friction, and pipe industry. Median fiber lengths varied from 1.4 μm for the carding operation to 0.7 μm for the pipe finishing operation. The percentage of fibers longer than 5 μm ranged from 4 percent in the carding operation to 1 percent in the pipe finishing operation. For all other operations monitored, the median fiber length was 0.9 μm, with 2 percent of the fibers longer than 5 μm.

If the size distributions of the studies are correlated (giving recognition to the many qualification required), a log-normal distribution of asbestos (chrysotile) fiber size might be defined: a maximum number of fibers in the distribution are between 0.5 and 1 μm in length; less than 5 percent are more than 5 μm in length; and, if a reasonably symmetric distribution is assumed, about 5 percent are less than 0.1 μm in length. The works of Addingley, Craig et al. and Lynch et al are in reasonable agreement with this correlation, as are those of Roach and Wagner and Skidmore if account is made of the fine fibers not visible by optical microscopy methods.

Equally important in the evaluation of measuring devices is the relationship of asbestos size to its deposition in the respiratory system. The deposition of inhaled airborne dust of different sizes has been evaluated by several investigators (29, 37, 38). The remarkable agreement in the results indicates that the maximum aerodynamical equivalent diameter (AED) is about 10 μm. Thus, if the factor of 1/3 is used to define the diameter of asbestos fibers relative to the AED, 3.5 μm appears to be the expected upper limit of long asbestos fibers that can reach pulmonary air spaces (31). The coarser particles are deposited in the ciliated portion of the respiratory tract—down to the terminal bronchioles, removed by ciliary action, and eventually either swallowed or spat out (29). Observations on the histological distribution of the dusts in the lungs of rats have shown that dusts tend to accumulate in the alveoli arising from the respiratory bronchioles (28). An evaluation of fiber deposition by Timbrell (31) suggests that sedimentation and inertial precipitation operate throughout the respiratory system to deposit particles in the nose, at the bifurcation of the respiratory tract, and on the walls of the bronchi and bronchioles. The distribution of particles that proceed to the pulmonary air spaces is composed primarily of the finer particles. However, many of these finer particles are eliminated from the respiratory system without being deposited, presumably because of their ability to follow the laminar air stream within the respiratory air passages. The deposition and retention study conducted on rats by Wagner and Skidmore (28) revealed that the elimination rate of chrysotile is three times greater than that of amosite and crocidolite, which suggests that the reduced fibrigenicity of chrysotile is the consequence of the greater elimination of this mineral. The reason for the difference in elimination is not understood, but it is proposed here that the finer size distribution of chrysotile fibers relative to amphibole fibers is responsible for this behavior.

APPENDIX B

DATA CORRECTIONS

The distributions of fiber lengths used here have been corrected for finite frame size. When photographs are taken of a sample, some fibers will inevitably extend beyond the edge of the frame. The lengths of fibers only partially in the field are not known, and the problem becomes more severe as the magnification is increased. If the magnification were so great that any fiber would overlap an edge, count statistics per frame would be very poor, and no information about fiber lengths would be obtainable. In the other extreme, if the magnification were so low that the longest fibers were very much smaller than the frame size, no corrections would be needed, although resolving power would be adversely affected. In order to have both sufficient resolving power and meaningful length distribution data, the data have been corrected to eliminate the effects of finite frame sizes. This was accomplished by first assuming that, on the average, each fiber extending beyond the edge of the frame was halfway in and halfway beyond the frame. Statistics were then collected on the number and length of fibers per frame entirely within the field and on the number and length of fibers only partially within the frame. The distributions corrected by the above assumptions were combined with the distribution for totally observed fibers; in this way, distributions for all fibers, even those partially observed, were obtained. An effective area of the photographic frame was then corrected to include all fibers, including those that extended beyond the frame. In calculation of the extended area, it was assumed that the fiber concentration for totally observed fibers using less than the total frame area was identical to the fiber concentration for all fibers. These corrections converted data at all magnifications to a common base. (Methods of counting only totally observed fibers do not provide correction for different magnifications.)

When the above corrections of data were made, it was determined that the actual magnification of any individual photograph differed by as much as 10 percent from the nominal magnification of that series. Thus, superimposed on the data is an uncertainty in magnification that increases the probable error of each individual measurement. Unquestionably, this uncertainty is responsible for the displacement of the chi-squared plot in Figure 3 and increases the standard deviation calculated from results of individual photographs.

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16. ABSTRACT The report gives results of an evaluation of the transmission electron microscope (TEM) and the scanning electron microscope (SEM) as potential tools for fine particle asbestos fiber counting for process control in the asbestos industry. The study defined the capabilities and limitations of the instruments in applications where asbestos specificity is not necessarily required, and where analysis cost must be minimal. The study showed that the microscopes are equally capable of counting all fibers in the full particle size distribution; but, for reasons of agglomeration and confusion with the filter texture, each microscope can observe only 75% of the distribution. In contrast, present standard light microscopy methods observe only the coarser 10% of the distribution, without resolving the fine fibers. Optimum asbestos fiber counting was done at 15,000 times magnification and at fiber concentrations on the filter between 40,000 and 80,000 fibers per sq mm. The minimum number of fibers counted to obtain high statistical confidence was 200 fibers per datum point. Standard techniques for filter sample preparation were found to have no effect for either instrument. Ashing of filters to remove non-asbestos fibers was responsible for 85% asbestos fiber loss.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Air Pollution	Microscopes	Air Pollution Control	13B
Industrial Processes	Electron Microscopes	Stationary Sources	13H
Asbestos		Fine Particulate	11E
Fibers	Transmission	Standard Light	
Measurement	Scanning		14B
Particles			

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