

REMOVAL AND DETECTION OF LIQUID BORNE
ASBESTOS FIBERS WITH NUCLEPORE MEMBRANES

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ABSTRACT

The recent publication in the Federal Register with regard to "Asbestos Particles in Food and Drugs" prompted a study of the unique characteristics of Nuclepore membranes for use in detection and removal of liquid-borne asbestos fibers. It was found that the Nuclepore membrane provides an ideal substrate for collection and viewing of asbestos fibers in the transmission or scanning electron microscope. Retention studies indicated that the 0.4 μ m pore size offers virtually complete retention of all chrysotile asbestos fibers while the 0.2 μ m and 0.1 μ m pore sizes provide absolute retention. Increasing challenge levels resulted in decreased fiber passage presumably due to fiber matting. The effect was most pronounced at challenge levels above 10⁸ fibers/cm².

REMOVAL AND DETECTION OF LIQUID BORNE ASBESTOS FIBERS WITH NUCLEPORE MEMBRANES

I. Introduction

A. Asbestos Structure and Usage

Asbestos is a unique material which has found a variety of applications ranging from asbestos cement products to filtration media. It has been called "the magic mineral", "the mineral of unparalleled properties", "the mineral of a thousand uses".¹

The mineral can be divided into six types (see Table I)². All types occur in long crystals which are so fine as to be fibrous in structure. They vary in chemical composition containing magnesium, ferrous or ferric iron, calcium, or sodium cations in various proportions with the silicate anion. All are incombustible and can be separated by mechanical or chemical means into fibers of various lengths and diameters. The six generally recognized varieties include the fibrous form of serpentine, known as chrysotile, and five minerals of the amphibole group.

Chrysotile, or white asbestos, is by far the most common variety, accounting for over 90% of the world's usage. Its positive surface charge, softness and flexibility, low refractive index and poor acid resistance, set it apart from the other varieties of asbestos. With individual fiber diameters down to 0.30 microns (300 Angstroms) it represents the smallest commonly-known fiber. The usable surface area, as measured by the nitrogen adsorption method, is from two to five times greater than any other known asbestos. The high surface area and positive surface charge make it ideal for a depth filtration media with exceptional dirt loading capacity.

Chrysotile's crystal structure is similar to that of the kaolinite minerals consisting of alternate layers of tetrahedrally coordinated silica and octahedrally coordinated magnesium hydroxide joined in a composite sheet. The outer surface of the fibril is essentially magnesium hydroxide in the brucite form. Figure 1³ is a representation of the structure showing the hydroxyl outer surface of the fibril. The resultant cationic charge on the surface enhances its affinity for electronegative particulates (in filtration) and for many resin systems (reinforced plastics). Pauling⁴, long before the layer structure of chrysotile was recognized, predicted that a magnesium analog of the kaolinite minerals would have to exist as curved sheets because of a mismatch in the repeat distances of the individual layers (8.9 Å for the silica layers versus 9.4 Å for brucite). Pauling's prediction has been verified by x-ray diffraction studies and by electron microscopy showing closed cylinders of

Table I

Comparative properties of asbestos minerals (2)						
Property	Chrysotile	Anthophyllite	Crocidolite	Actinolite	Tremolite	Amosite
Composition	$Mg_3Si_2O_5(OH)_4$	$Mg_7Si_8O_{22}(OH)_2$	$Na_2Fe_3Si_3O_{10}(OH)_2$	$Ca_2Fe_3Si_3O_{10}(OH)_2$	$Mg_5Ca_2Si_8O_{22}(OH)_2$	$(Fe, Mg)_7Si_8O_{22}(OH)_2$
Crystal habit	Fine, easily separable fibers	Prismatic crystals and fibers	Long brittle fibers	Prismatic crystals and fibers	Prismatic crystals and fibers	Prismatic crystals and fibers
Veining	Cross-, slip-, & massive-fiber	Slip- & massive-fiber	Cross-fiber	Slip- & massive-fiber	Slip- & massive-fiber	Cross-fiber
Color	White, gray, green	Gray-white	Blue	Green	White, gray-white, green	Ash gray or brown
Luster	Silky	Vitreous to pearly	Silky to dull	Silky	Silky	Vitreous to pearly
Texture	Soft to harsh, also silky	Harsh	Soft to harsh	Harsh	Harsh	Harsh
Flexibility	Very good	Poor	Good	Poor	Poor	Good
Hardness	2.5-4	5.5-6	4	6	5.5	5.5-6
Fiber diameter, A	200-400	600-900	600-900	600-900	600-900	600-900
Tensile strength, p.s.i.	300,000-800,000	<4000	400,000-600,000	<1000	1000-8000	100,000-200,000
Modulus of elasticity, p.s.i.	23×10^6	—	27×10^6	—	—	24×10^6
Specific heat, Btu/lb./° F.	0.266	0.210	0.201	0.217	0.212	0.193
Specific gravity	2.4-2.6	2.8-3.1	3.2-3.3	3.0-3.2	2.9-3.2	3.1-3.3
Refractive index (mean)	1.51-1.55	1.61	1.7	1.63	1.61	1.64
pH	9.5-10.3	9.4	9.1	9.5	9.5	9.1
Electric charge in aqueous slurry	Positive	Negative	Negative	Negative	Negative	Negative
Resistance to acids	Poor	Very good	Good	Fair	Good	Good

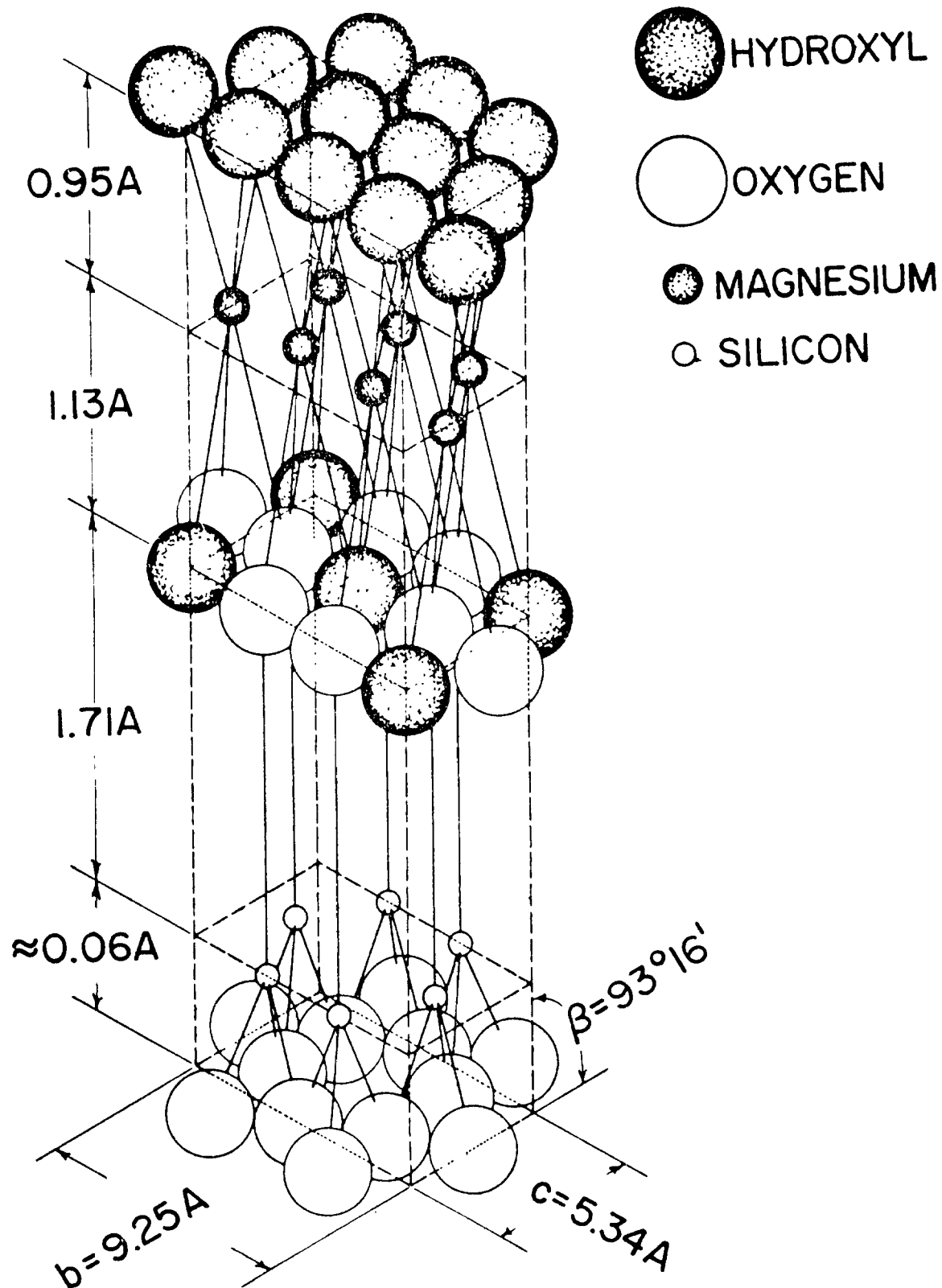


FIGURE I: Chrysotile structure (after Whittaker). ³

extreme length-to-diameter ratios up to 1000:1. (See Figure 2). Recent high resolution electron micrographs taken in Japan, have shown the average outside diameter of the individual chrysotile "fibril" to be about .03 micron (250Å) and its inside diameter to be about 140Å.²

In addition to use as a filtration media and as a reinforcement for plastics (including cold-molded, thermoplastic, and thermosetting plastics) chrysotile is used in the manufacture of safety clothing, curtains, lagging cloth, woven brake linings, clutch facings, asbestos cement, millboard, roofing felts, pipe covering, fine quality electrical papers, insulating papers, asbestos-latex flooring felt, vinyl floor tile, paint, roof coating, caulks, sealants, sprayed insulation, asphalt paving and curbing, and many other products.

Crocidolite, or blue asbestos, accounts for 3.5% of the total asbestos useage¹ with principle use in the production of asbestos cement sheets and pressure pipes.

Amosite represents 2.5% of the total asbestos usage primarily in the insulation field and in asbestos cement.

Anthophyllite, because of its low strength (see Table I) finds limited usage in products in which reinforcement is required such as asbestos cement, floor tile, etc. It is, however, used as a filler in the rubber and plastic industries and in various adhesives and cements.

Tremolite and actinolite are the least important of the asbestos minerals from an economic standpoint due to insufficient fiber strength. They find limited utilization as filter media and as inexpensive fillers.

B. Use of Asbestos as a Liquid Filtration Media

As mentioned above, chrysotile fibers represent one of the most effective fibrous depth filtration mediums commercially available.

It is, of course, recognized that fibers with high length to diameter ratios (L/D) provide a large effective collision radius and a large area for adsorption of small particles thereby enhancing the "dirt loading" capability of the filtration media. Chrysotile has an exceptionally high L/D ratio and adsorption area (50-60 m²/gm-BET nitrogen value)⁶. However, glass fibers with similar geometry and kieselguhr with high BET adsorption areas are not as effective as chrysotile fibers.⁷

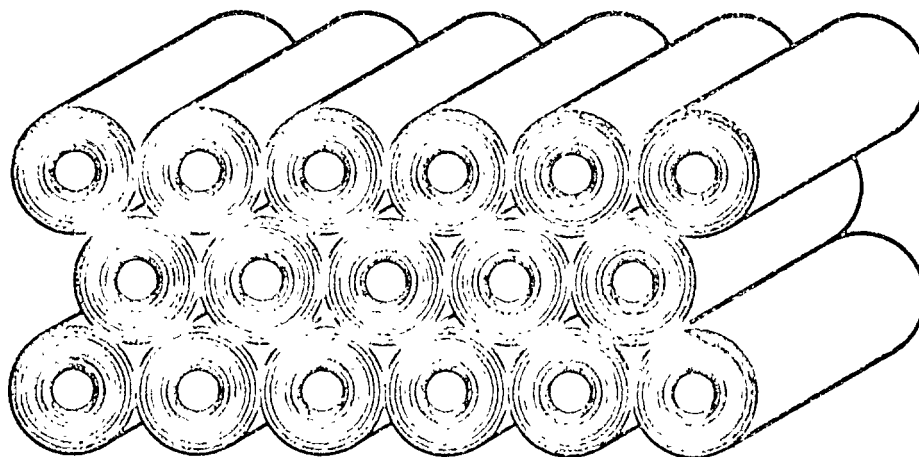


FIGURE 2
IDEALLIZED PACKING CONFIGURATION
CHRYSTILE FIBRILS

The explanation appears to be in chrysotile's positive zeta-potential. Because of chrysotile's magnesium hydroxide surface layer, in aqueous solution, the fibril adsorbs positive ions-notably H^+ . Even in the range of pH 10-12, the surface displays a positive charge.⁵ Because many particulates are electronegative at neutral pH (organics particularly), chrysotile is an effective "getter". (It is interesting to note that adsorption can also occur with cationic species like methylene blue dye⁶ under favorable conditions.) The importance of the OH groups for effective filtration is demonstrated by the report that a severe acid treatment, leaving only the silica residue, resulted in a loss of filtration efficiency.⁷

As a result, chrysotile asbestos pads have found increasing use in the food and pharmaceutical industries. Filtration of beverages (such as beer and wine), liquid sugar, and parenteral solutions are notable. The fibers have been felted into sheets with cellulose and glass to provide pre-formed, pre-cut filter media for plate and frame or tank-type filters. Although such pads do not provide absolute filtration, they are nevertheless effective in removing yeast along with other microorganisms and particulates.

Unfortunately, "sluff-off" from these pads adds some contamination to the filtrate.

C. Asbestos Health Hazard

Recently, the Food and Drug Administration was petitioned to publish in the Federal Register⁸, a proposed regulation:

"Food or drugs that have come into contact with filters made wholly or partially of asbestos may reasonably be expected to become contaminated with asbestos particles which may be injurious to health when ingested. Accordingly, any food or food additive, drug or drug component produced, manufactured, processed or prepared using a fiber made wholly or partially of asbestos shall be deemed to be adulterated in violation of section 402(a) of the Act."

The Federal Register pointed out that asbestos inhalation has been recognized for sometime to be an occupational hazard for workers in asbestos mines or factories. The asbestos is inhaled and lodges in lungs causing the development of a fibrotic disease known as "asbestosis". It is reported that there is a 5 to 7 fold increase in lung cancer in asbestos workers and that 7% of deaths of asbestos workers are caused by pleural and peritoneal mesothelioma.

More recently, it has been discovered that pulmonary fibrosis and a variety of neoplasms can occur with much less exposure, such as that experienced by those living in the household of an asbestos worker or merely living within 800 yards of an asbestos plant.⁹

On the other hand, the evidence for possible hazard from ingestion of asbestos particles is contradictory. There is some evidence of increased incidence of gastrointestinal cancer among asbestos workers.¹⁰

A number of workers have shown in animal tests that asbestos fibers can penetrate through the gut into the blood stream and accumulate in various tissues.¹¹ However, there is also evidence that the bloodstream can clear itself of these fibers.¹¹

It has been demonstrated that parenteral administration of asbestos may lead to wide dissemination of the fibers causing local malignant tumors, mesotheliomas of the pleura and peritoneum, similar to those occurring after inhalation of the fibers.^{9, 12}

Evidence such as the above, prompted the National Institute of Environmental Health Sciences and the Environmental Protection Agency to sponsor an international conference on the biological effects of ingested asbestos in Durham, North Carolina in November 1973. Attended by 130 scientists and public health officials from 12 countries, the conference emphasized the limited amount of information available on the effects of low-level, prolonged exposure. The drawbacks of making inferences from animal models were repeatedly pointed out by speakers at the conference.

It should also be realized that a health hazard may exist with other fibers besides asbestos. Tests conducted by Dr. Mearl F. Stanton at the U.S. National Institutes of Health (National Cancer Institute) with conventional asbestos fibers, fine glass and sapphire fibers, compared these fibers with coarser fibers and powders of the same materials.¹³ Fine fibers (1.5 to 5 microns in diameter and less than 80 microns long) appeared to cause a high incidence of pleural cancers much more readily than the coarser materials. These tests suggest that fiber geometry is more important than chemical composition.

D. Removal and Detection of Asbestos Fibers with Membrane Filters

Since asbestos is found in many municipal drinking water supplies (10^8 fibers/liter in some cases) and is used in virtually all pipes carrying drinking water, it may be impossible to eliminate asbestos by simply prohibiting its use as a filtration media in food and drug plants. Thus, the FDA commissioner has suggested that a "non-asbestos-containing" or "non-fiber-releasing" filter such as a membrane

filter be used as a final filter prior to bottling to reduce the asbestos concentration.

The effectiveness of membrane filters in removing asbestos fibers has not been thoroughly investigated. Recently, Manalan and Reti¹⁴ presented a paper on the effectiveness of a Millipore cellulose ester membrane filter in retaining asbestos fibers dispersed in water. A summary of their results is presented in Table II. Unfortunately, Manalan and Reti were unable to reduce the background count downstream of the membrane to zero. The background count was about 10^3 fibers/liter.

This necessitated the assumption that fiber passage occurred if, and only if, a concomitant increase in the downstream fiber count resulted when the number of fibers added upstream of the membrane increased. Naturally, passage cannot be disproven on this basis. Nevertheless, two conclusions were drawn from this work:

- 1) Passage of some fibers can occur in cellulose ester membrane filters having an equivalent pore diameter greater than $0.45\mu\text{m}$,
- 2) Retention on $1.2\mu\text{m}$ pore size cellulose ester membrane filters is increased by small amounts of beer or wine (from 95.8% to 99.8%, and 99.7%, respectively).

Any investigation of membrane retention or passage of fibers is plagued by the sensitivity of the technique used to detect and count fibers downstream of the membrane. With microorganisms, detection and counting is simplified since the microorganism can be cultured and developed into a large colony visible in a low-power optical microscope or even with the naked eye. With non-living, inorganic fibers of the dimensions specified (down to 0.03 microns in diameter with various lengths), one must resort to the electron microscope. Even with magnifications of 10,000 to 30,000X, the fibers are often hard to detect, depending on the texture of the support.

This paper will describe a detection technique using Nuclepore membranes which we believe greatly facilitates detection and counting of asbestos fibers. The technique has been used in determining the retentivity of various pore size Nuclepore membranes (from 0.1 to $1.0\mu\text{m}$) for chrysotile asbestos fibers-which data are presented at the end of the paper.

TABLE II
Retention of Asbestos Fibers
With Millipore Cellulose Ester Membranes¹⁴

<u>Pore Size</u>	<u>Filter Type</u>	<u>Fiber Challenge</u>	<u>Minimum % retained</u>	<u>% Passed</u>
1.2 μ m	RAWP	1,000,000	98.28	1.72
1.2 μ m	RAWP	100,000,000	96.56	3.44
0.8 μ m	AAWP	1,000,000	98.62	1.38
0.8 μ m	AAWP	100,000,000	99.93	.07
0.65 μ m	DAWP	1,000,000	99.93	.07
0.65 μ m	DAWP	10,000,000	99.11	.89
0.45 μ m	HAWP	100,000,000	99.993	.007
0.22 μ m	GSWP	100,000,000	99.997	.003

II. Methodology for Detection and Counting of Asbestos Fibers

A. Microscopic Analysis of Membrane Collected Materials

The key to reliable detection and counting of asbestos fibers is the selection of an appropriate substrate for collection and viewing of the fibers with an electron microscope. In the last five years, a new membrane filter has evolved which makes possible ideal collection, preparation, and viewing of particulates--whether from aerosols or liquid media.

Membrane filters have pore sizes ranging between 300Å to 12 microns and may be broadly categorized into two major types according to pore configuration. Figure 3 is a scanning electron photomicrograph (SEM) of the surface of a cellulose ester membrane filter typical of the first type--having a homogenous, sponge-like pore network. Figure 4 is an SEM of a Nuclepore membrane filter which is unique because of its sieve-like straight through cylindrical pores.

Although the pores in the cellulose ester membrane look considerably larger than those in the Nuclepore filter, their retention is equivalent because of pore constrictions and tortuosity in the depth of these membranes.

The Nuclepore membrane has a lower porosity (10% compared to 80% for the cellulosic membrane) but is only one-twelfth as thick, resulting in higher or equivalent flow rates due to less resistance to flow. This is important since air and liquid samples are passed through the membrane to obtain relatively uniform distributions of particulates over the surface. However, the high porosity (and consequent high surface area) of the sponge-like membranes are associated with a higher dirt loading capacity permitting collection of larger quantities of particulates before plugging.

Although both membranes are widely used for particulate and aerosol analysis, the Nuclepore membrane has been recognized¹⁵⁻¹⁸ to possess some unique advantages for particulate sample collection, preparation, and analysis.¹⁹ We have endeavored to exploit some of these advantages in the collection and detection of liquid-borne asbestos fibers.

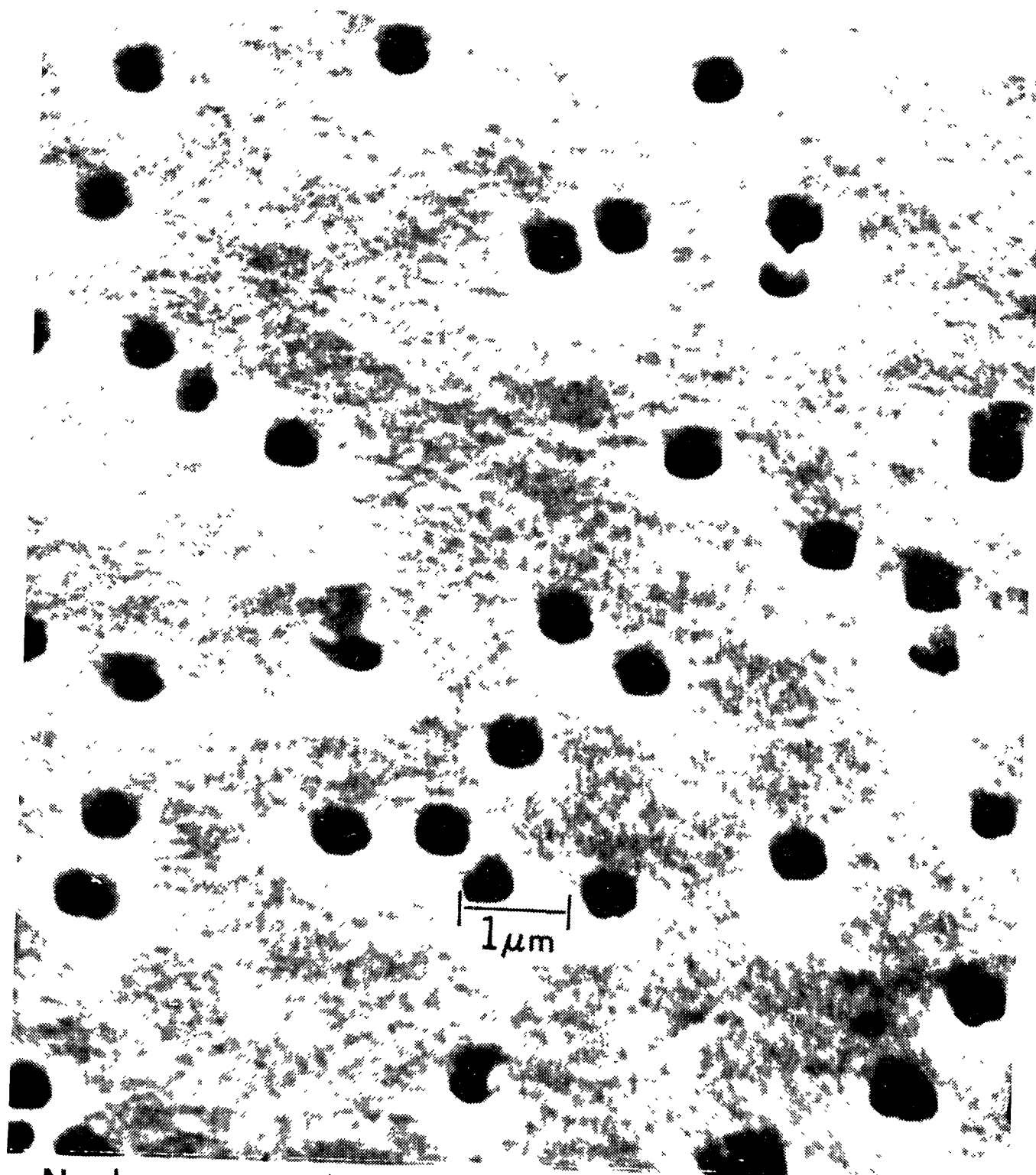
1. Sample Collection

The uniform pore size distribution across the filter face allows for uniform particle deposition.¹⁷



Cellulose membrane filter, 0.45 micron pore size

FIGURE 3



Nuclepore membrane filter, 0.4 micron pore size*

*Scanning electron micrograph

FIGURE 4

2. Sample Preparation

- a. Surface replication for transmission electron microscopy (TEM) is easy. Silicon monoxide replicas of the surface usually enclose and "extract" the collected particles for subsequent examination. The membrane is readily dissolved in chloroform or methylene chloride. (For an example of a TEM of asbestos on Nuclepore, see Figure 5).
- b. Coating with gold for scanning electron microscopy (SEM) is easily done to obtain uniform metal coatings.^{17, 18}

3. Sample Analysis

- a. The membrane is extremely smooth ($<0.3\mu\text{m}$ peak-to-valley distance), particularly on the shiny side (one side is dull). Since particles larger than the pore rating are retained on the surface rather than embedded in the pore network, all particulates will be in the same focal plane and will appear with greater contrast against the smooth, lawn-like background.^{15, 17, 18}
- b. The uniform pore diameter affords a coarse internal size calibration.¹⁸
- c. Neutron activation analysis, x-ray analysis, transmission infrared and emission spectroscopy, and atomic absorption spectroscopy analysis of particulates is facilitated due to the low trace element background in the membrane (ash weight is one-third that of a typical cellulosic membrane.)

B. Transmission Electron Microscopy

Workers at the National Center for Atmospheric Research have published techniques for preparing replicas of particulates on the Nuclepore membrane surface for transmission electron microscopy.¹⁵ The filter was mounted with cellophane tape on a microscope slide. Silicon monoxide was vacuum evaporated to a thickness of about $1000\text{\AA}$ at vertical incidence, followed by a thin ($<200\text{\AA}$) coating of chromium at 15° . The membrane was then gently freed from the slide, and a 5mm square excised with clean scissors. The square was then placed SiO side down on an electron microscopy grid which, in turn, was placed upon a small square of polyurethane foam about 5mm thick in a glass petri dish. Chloroform was poured into the dish and the cover was placed on it for 7 minutes (as shown below).

REPLICATION TECHNIQUE

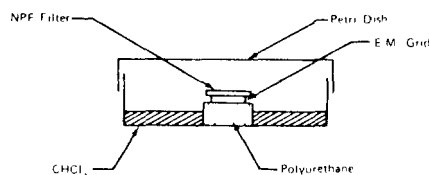




FIGURE 5
CHRYSOTILE ASBESTOS PARTICLES ON
1.0 μ M PORE SIZE NUCLEPORE MEMBRANE
20,000 X TEM (20)

The wick action of the chloroform through the polyurethane foam and through the pores of the SiO replica of the filter surface dissolved the membrane leaving the replica on the grid with fibers on top of the SiO substrate. Chloroform-insoluble particles were trapped in the replica, while soluble species left their impressions. Figure 6 is an example of a TEM prepared with this technique showing Amosite asbestos fibers on a 3 μ m pore size Nuclepore membrane.²¹

Murchio and coworkers²² have also expressed a preference for transmission electron microscopy (TEM) over scanning electron microscopy (SEM) in detecting and counting asbestos fibers due to the superior resolution obtained with TEM. Murchio's technique was similar to that used by Frank, et al¹⁵ at NCAR. The specimen was, however, left in the petri dish for 4-6 hours, rather than 7 minutes, to insure that all of the membrane dissolved.

C. Scanning Electron Microscopy

The measurement of asbestos fiber diameters is greatly facilitated by TEM due to its superior resolution. However, for general membrane counts where fiber numbers are more important than dimensions, we believe SEM to be equally reliable and more convenient.

The membrane sample is easily mounted on a brass stub with conductive silver paint and coated with a 300 $\text{\AA}$ thick gold film in a matter of minutes. The scanning electron microscope facilitates rapid scanning so that a larger area may be counted.

Figure 7 is an example of an SEM showing chrysotile asbestos fibers on the surface. It will be noted that individual fibrils (300-500 $\text{\AA}$ in diameter) and fibril bundles (up to 2000 $\text{\AA}$ diameter) are easily seen. The 0.2 μ m pores provide a convenient built-in calibration (2000 $\text{\AA}$) for estimating fiber diameter.

For the current study, a relatively pure sample of chrysotile asbestos was obtained. For more contaminated samples, asbestos fibers can be distinguished from other fibers and particulates by looking at the fractured ends. Gross and coworkers²³ point out that asbestos fibers may be readily distinguished from non-asbestos fibers by recognizing that asbestos fibers are composed of compactly opposed finer fibrils, whereas non-asbestos fibers are homogeneous units. Because of this difference in structure, the fractured ends of asbestos fibers are irregular and resemble a bundle of fragile sticks which have been broken transversely. (Different fibers of the bundle break at different points.) This irregular profile with multiple step-like projections and depressions

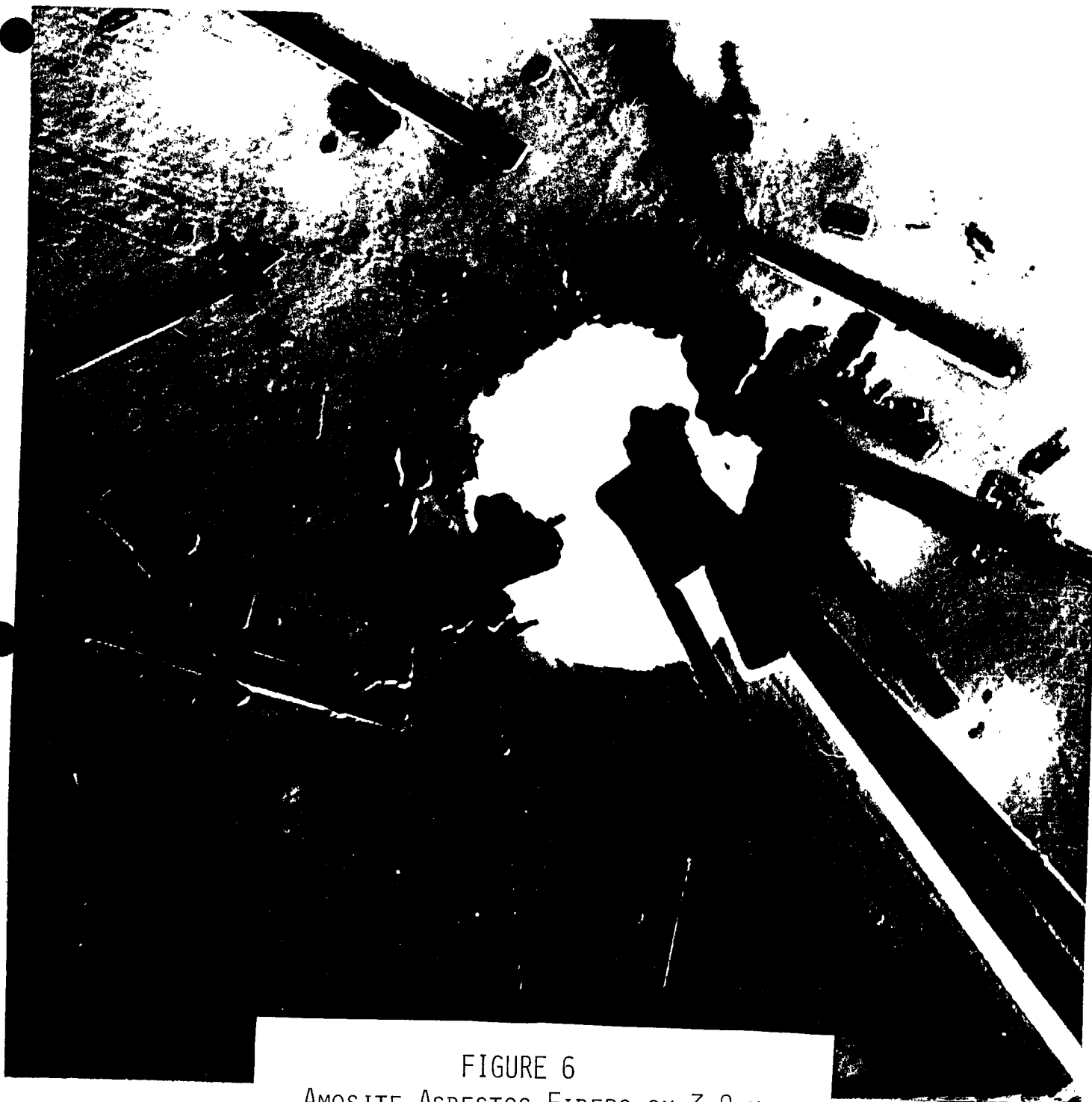


FIGURE 6
AMOSITE ASBESTOS FIBERS ON 3.0 μ M
PORE SIZE NUCLEPORE MEMBRANE
22,000 X TEM (21)



FIGURE 7
CHRYSTILE ASBESTOS FIBERS COLLECTED
ON 0.2 μ M PORE SIZE NUCLEPORE MEMBRANE
10,000 X SEM

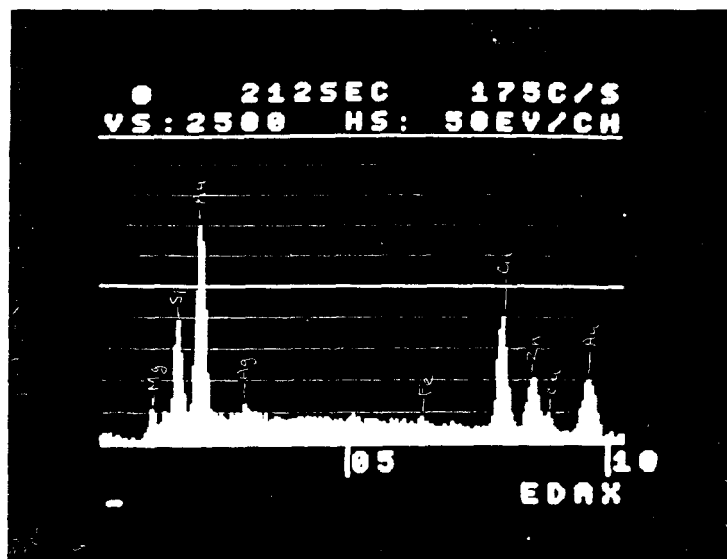


FIGURE 3

X-RAY FLUORESCENCE SPECTROGRAM OF
CHRYSTILE ASBESTOS FIBERS ON A
NUCLEPORE MEMBRANE MOUNTED ON A
BRASS STUB WITH GOLD COATING FOR SEM

is not seen with non-asbestos fibers which are generally homogeneous in character and exhibit uninterrupted linear profiles at the fractured ends. In addition, scanning electron microscopy (surface view) shows the longitudinal, parallel delineation of individual asbestos fibrils, whereas a sample of a non-asbestos fiber is devoid of such longitudinal lines. These characteristics are evident in the asbestos fibers shown in the SEM of Figure 7. Fractured ends are also evident in the TEMs of Figures 5 and 6.

Doubtful cases can be resolved with the use of electron diffraction, microprobe techniques, or x-ray fluorescence spectrometry. The latter was used in conjunction with the SEM to look at the fibers of Figure 7; the results are shown in Figure 8. The spectrogram registers peaks of all elements present having an atomic number greater than 8--Mg and Si from the chrysotile asbestos; Au from the gold coating, Ag from the silver paint; Cu and Zn from the brass stub mount; and Fe from magnetic contamination.

Estimates of the total number of fibers collected on a membrane can be made by counting the number of fibers on a statistically appropriate number of fields in the SEM--as discussed below. This in turn can be directly related to the volume of the original liquid suspension and the concentration of fibers in that suspension.

III. Retention of Asbestos Fibers on Nuclepore Membranes

The following will summarize the experimental procedures and data acquired in a study of the retention of chrysotile asbestos fibers on six different pore size Nuclepore membranes.

Chrysotile asbestos was selected for these studies for several reasons:

- 1) It is by far the most common variety of asbestos--accounting for over 90% of the world's usage.
- 2) It is the fiber of choice in asbestos filtration pads for use in the food and pharmaceutical industries and therefore represents the type most likely to contaminate these products.
- 3) With individual fibril diameters down to 300 Angstroms, it represents the smallest commonly known fiber and should therefore, present the membrane with its severest challenge.

A. Experimental Procedure

1. Preparation of Asbestos Test Suspensions

The chrysotile asbestos used in this study was Union Carbide's RG-144 which is a highly refined, purified fiber containing more than 98% chrysotile fiber and less than 0.7% magnetite. The RG-144 is also unique because it is said to contain a higher concentration of fully liberated asbestos fibrils than any other chrysotile product. Thus, if these claims are correct, it should represent a severe challenge for the Nuclepore membranes.

It has been estimated that there are about 1×10^{14} colloidal fibrils per gram of RG-144 asbestos. Since the "stock" suspension was required to have a concentration of $1-5 \times 10^{10}$ fibrils per ml, 0.3 gram was suspended in one liter of ultrapure water using a blender to yield a theoretical concentration of 3×10^{10} fibrils per ml. The resulting suspension was allowed to settle overnight and the uppermost 100 ml withdrawn by pipette. This 100 ml was labeled "stock" suspension. Serial dilutions were then made from the "stock" suspension; each dilution was 100 fold.

The "stock" suspension and its various dilutions were then filtered through a $0.1 \mu\text{m}$ pore size Nuclepore membrane to determine the concentrations of the suspensions. The results were as follows:

STOCK	$1.29 \times 10^{10}/\text{ml}$
A	$1.29 \times 10^8/\text{ml}$
B	$1.29 \times 10^6/\text{ml}$
C	$1.29 \times 10^4/\text{ml}$
D	$1.29 \times 10^2/\text{ml}$

The stock solution had 43% of the theoretical concentration which is not unreasonable when 24 hour settling is taken into account.

Figure 9 is a typical SEM field of the membrane surface used to collect the fibers in suspension "A". One ml of the suspension was diluted with ultrapure water and washed through a 47mm $0.1 \mu\text{m}$ pore size Nuclepore membrane, with a "capture" area of 9.6cm^2 . One SEM field at $10,000\times$ had an area of $9.71 \times 10^{-7}\text{cm}^2$. The average number of fibers found in one SEM field was 13. Therefore, the concentration of "A" suspension was

$$13 \left(\frac{9.6}{9.71 \times 10^{-7}} \right) = 1.29 \times 10^8 \text{ fibers/ml}$$

2. Background Counts

In view of the difficulties encountered by Manalan and Reti (of Millipore) in reducing background counts to zero (clean water showed 10^3 fibers/liter), exceptional precautions were taken to eliminate background counts in this study.

Fortunately, we had access to water from Nuclepore's ultrapure water loop. This is a recirculating loop utilizing reverse osmosis, ion exchange, UV sterilization, and final filtration with an $0.2 \mu\text{m}$ pore size Nuclepore pleated cartridge.

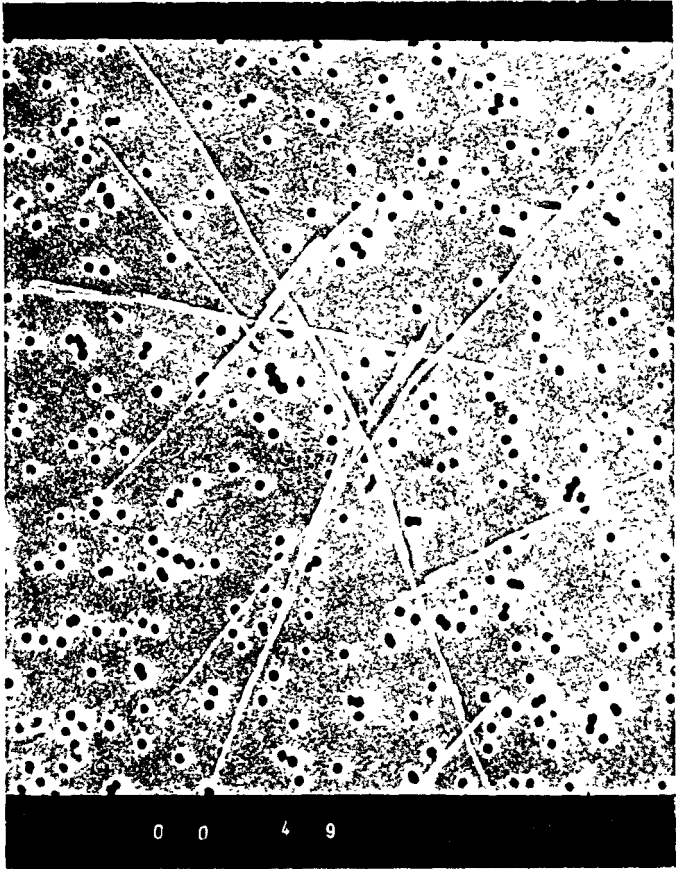


FIGURE 9
CHRYSOTILE ASBESTOS FIBERS FROM
SUSPENSION "A" COLLECTED ON $0.1\mu\text{M}$
NUCLEPORE MEMBRANE, 10,000 X SEM

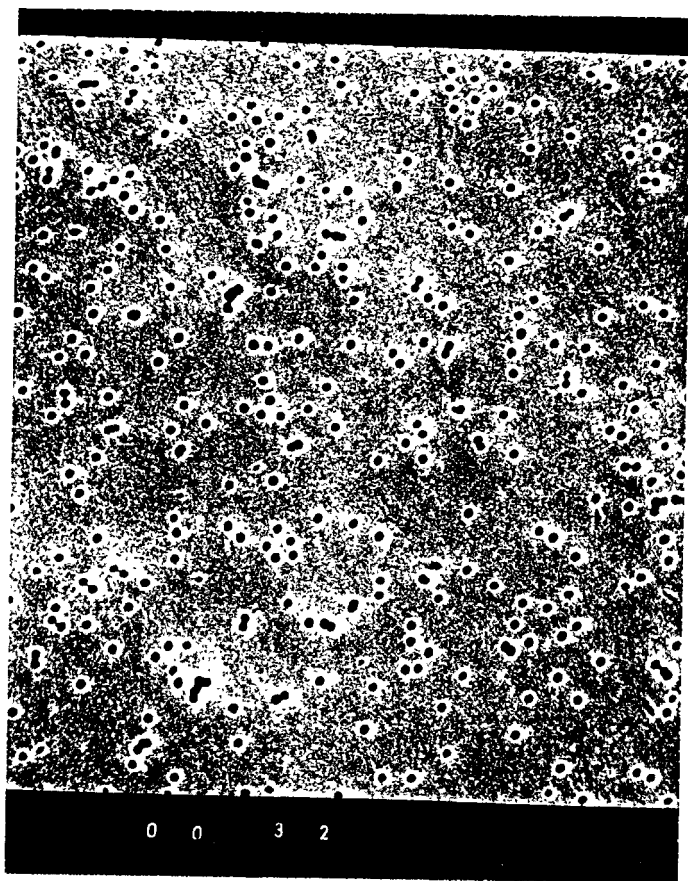
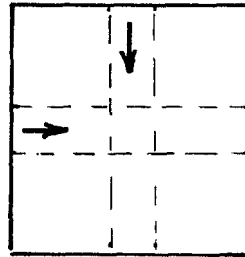


FIGURE 10
BLANK 0.1 μ M NUCLEPORE MEMBRANE
AFTER PASSING 1 LITER OF ULTRAPURE
WATER THROUGH, 10,000 X SEM

Blanks were run passing 1 liter samples of this ultrapure water through the test hardware described below (washed in ultrapure water), collecting on an 0.1 μ m Nuclepore membrane, and scanning with the electron microscope. Because of the exceptional cleanliness of the membrane in these scans (see Figure 10), 860 fields were scanned at 10,000X (each having an area of 10⁻⁶cm²) on each SEM mount (a square measuring 0.43cm x 0.43cm). The scans were made in the form of a cross, as shown below.



SEM mounts were made from different positions on the membrane filter to check against mal-distribution of contamination.

NO asbestos fibers were discovered in the course of these scans. One organic fiber (lint, perhaps) was detected on one of the "blanks"--probably due to contamination from the atmosphere while transferring the membrane sample from its holder to the SEM mounting stub.

The reliable zero background count as determined above, greatly facilitated interpretation of results.

3. Challenge Tests

The determination of retention of asbestos fibers by the six pore sizes of Nuclepore membranes tested was accomplished by a two stage filtration: 1) the challenge suspension was passed through the test membrane in a 47mm Nuclepore Swin-Lok polycarbonate holder; 2) the filtrate from this holder (which was potentially contaminated with fibers which slipped through the pores of the test filter) was then passed through a 47mm, or 13mm, 0.1 μ m Nuclepore membrane held in a Hydrosol (funnel type) stainless steel filter holder.

The collection filter (0.1 μ m) was removed from its holder and one or more sections cut out for mounting on a brass SEM stub, as described previously. For samples having low counts of fibers on the membrane surface, three cuts were made (center, mid-radius and edge) to check for mal-distribution of particles in the Hydrosol type of holder.

Two standard challenges were selected for use in testing all pore sizes from $0.1\mu\text{m}$ to $1.0\mu\text{m}$ - 1.29×10^{10} and 1.29×10^{11} fibers. This was a compromise between the desire to insure that every pore in the membrane was challenged and the fear of significant fiber "matting" which would increase the apparent retention of fibers by the membrane. Of course, if fibers are capable of passing through the pores of a specific membrane, a certain number will pass through before a mat can form. In other words, a membrane which shows absolute retention for asbestos cannot be said to do so because of fiber matting.

4. Fiber Counting Procedures

Fiber counting was usually done at 10,000X. Three counting procedures were used depending on the number of fibers found on the $0.1\mu\text{m}$ collection filter.

- 1) If the number of fibers on the membrane ranged between 50-150 fibers per field (10^{-6}cm^2), two fields were selected at random and counted. This amounted to $2 \times 10^{-5}\%$ of the total membrane surface.
- 2) If the number of fibers ranged between 1-50 fibers per field, 10 fields were selected at random and counted. This amounted to roughly $10^{-4}\%$ of the membrane surface.
- 3) If the number of fibers per field was less than one, a large number of scans were made across the entire SEM mount in "cross" fashion as discussed above (860 fields were scanned per SEM mount). The results from 2-6 SEM mounts were then averaged. This amounted to roughly .02 to .05% of the membrane surface when 47mm discs were used. Smaller area membranes (13mm) were also used to concentrate the fibers in a smaller area (0.6cm^2 as opposed to 9.6cm^2). Only one SEM mount could be obtained from these 13mm membranes. In this case, a total of 860 fields were scanned, representing 0.14% of the total collection surface.

B. Experimental Results

1. Retention as a Function of Pore Size

Table III is a summary of the retention data obtained on Nuclepore membranes with pore sizes ranging between 0.1 and $1.0\mu\text{m}$. The total number of fibers passing the membrane and the percentage of the challenge which passed are calculated.

TABLE III
RETENTION OF CHRYSOTILE ASBESTOS FIBERS
ON NUCLEPORE MEMBRANES AS A FUNCTION OF PORE SIZE

Membrane Pore Size (Microns)	Challenge (Fibers)	No. of Fields Scanned	Total Fibers Passed ¹	Maximum Fibers Passed ²	Percent Passed (%)	Maximum Percent Passed ² (%)
0.10	1.29×10^{10}	2580	0	-	0	-
0.10	1.29×10^{11}	2580	0	-	0	-
0.20	1.29×10^{10}	1720	0	-	0	-
0.20	1.29×10^{11}	1720	0	-	0	-
0.40	1.29×10^{10}	5160	2.42×10^4	3.53×10^4	1.88×10^{-4}	2.74×10^{-4}
23 0.40	1.29×10^{11}	3440	2.79×10^3	7.38×10^3	2.16×10^{-6}	5.72×10^{-6}
0.60	1.29×10^{10}	10	4.13×10^7	6.14×10^7	0.320	0.476
0.60	1.29×10^{11}	9	4.43×10^7	5.85×10^7	0.0343	0.0453
0.80	1.29×10^{10}	2	7.42×10^8	10.54×10^8	5.75	8.17
0.80	1.29×10^{11}	2	6.92×10^8	10.05×10^8	0.536	0.779
1.00	1.29×10^{10}	2	1.44×10^9	1.78×10^9	11.16	13.80
1.00	1.29×10^{11}	2	1.24×10^9	1.55×10^9	0.961	1.20

1. Total calculated from area factor, total fibers passed = fibers counted ($\frac{\text{membrane area}}{\text{area scanned}}$)
 2. 95% confidence range calculated from one-sided t test.

There is, of course, some uncertainty associated with missing some fibers which should have been counted. Therefore, the question was asked, "what value represents the maximum number which could have passed the membrane with only a 5% chance that the number is higher still?" This 95% confidence limit was calculated from a "one-sided t test".

$$N_{\max,95\%} = \bar{N} + t s(\bar{N})$$

where $N_{\max,95\%}$ = the maximum number of fibers passing the membrane within 95% confidence limits
 $\bar{N}$ = the average number of fibers passing the membrane
 t = the difference between the mean of a sample and the true mean of the population from which the sample was drawn, divided by the estimated standard deviation of the mean - determined from standard statistical tables
 $s(\bar{N})$ = the estimated standard deviation of the mean. This is estimated by dividing the standard deviation of the counts by the square root of the number of counts

The results of applying the ttest with a 95% confidence level are shown in Table III as the "maximum fibers passed" and the "maximum percent of challenge passed".

Figures 11 through 16 are photomicrographs of the 0.1 μ m collection membranes used to collect asbestos fibers in the filtrates from 1.0, 0.8, 0.6, and 0.4 μ m pore size membranes at the challenge levels of Table III. It will be noted that stray contamination with a diameter of 0.5 μ m is evident from the filtrates of the 1.0 μ m filter (see Figure 11). There is a progression towards shorter fiber lengths with the smaller pore size filtrates. The lengths are however, often 2 to 3 times larger than pore size through which they passed. The number of fibers appearing in a given field decreases with decreasing pore size; it markedly decreases as the pore size decreases from 0.6 to 0.4 μ m (compare Figures 15 and 16). With the 0.4 μ m pore size, no field on the collection filter contained more than one fiber and most had zero. Figure 16 (0.4 μ m filtrate) shows a contaminant particle (not asbestos) having an 0.2 μ m diameter; this would not have been counted.

Figures 17 and 18 plot the percentage of the challenge passing the Nuclepore membrane as a function of pore size on semilog and linear graph paper. Figure 18 shows that the 0.4 μ m membrane removes virtually all of the asbestos. However, Table III and Figure 17 show that only the 0.2 and 0.1 μ m pore sizes show truly absolute retention. (This was further checked by running an additional filtrate through a 13mm membrane and scanning 860 fields. Again, no fibers could be found.)

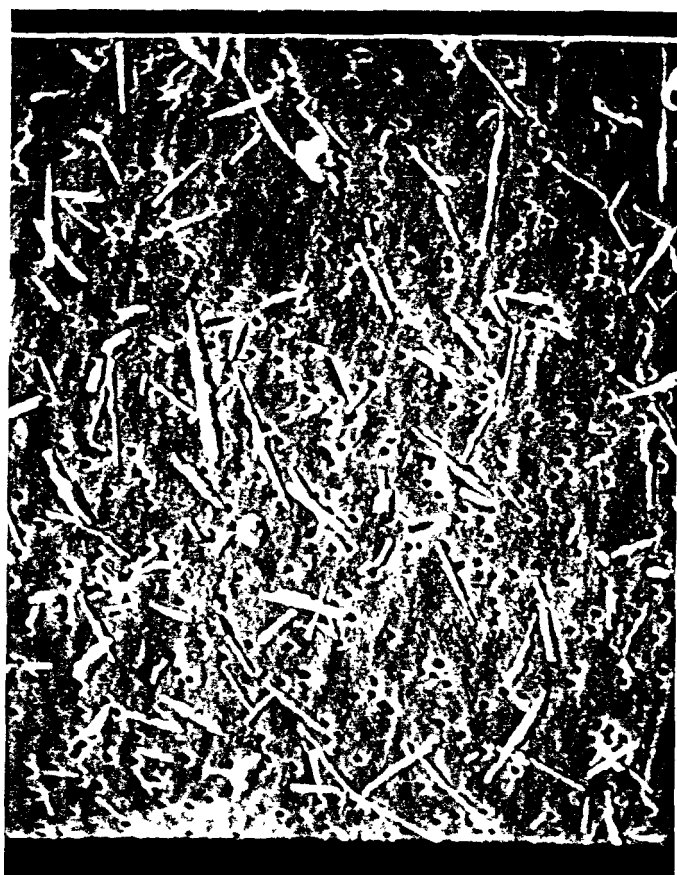


FIGURE 11
CHRYSOTILE ASBESTOS FIBERS IN
FILTRATE FROM 1.0 μ M NUCLEPORE
MEMBRANE-CHALLENGED WITH
1.29 $\times 10^{10}$ FIBERS, 10,000 X (45 $^{\circ}$ ANGLE) SEM
(NOTE 0.5 μ M STRAY CONTAMINATION)

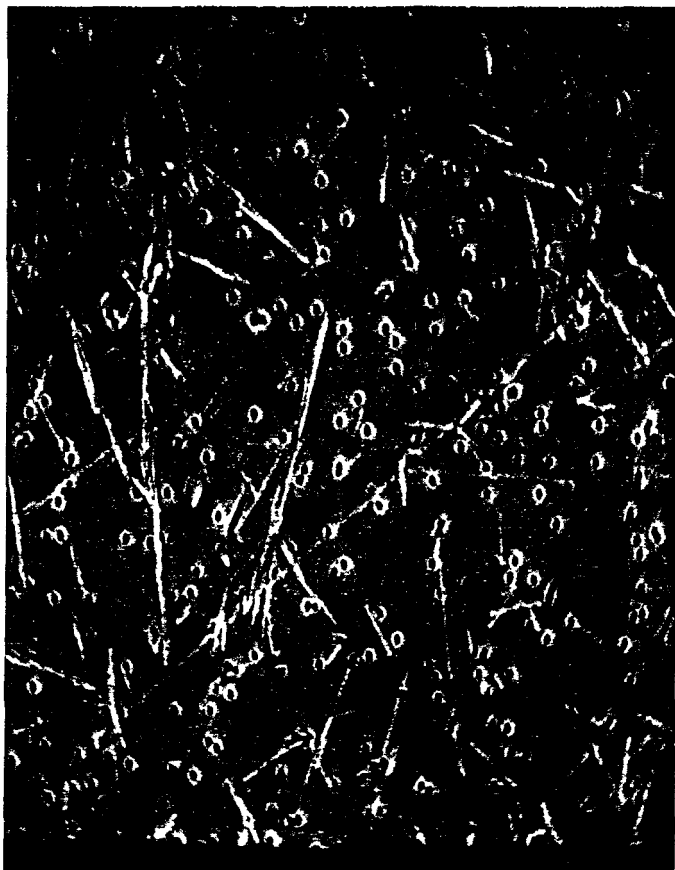


FIGURE 12
CHRYSOTILE ASBESTOS FIBERS IN
FILTRATE FROM 1.0 μ M NUCLEPORE
MEMBRANE-CHALLENGED WITH
 1.29×10^{11} FIBERS, 10,000 X SEM

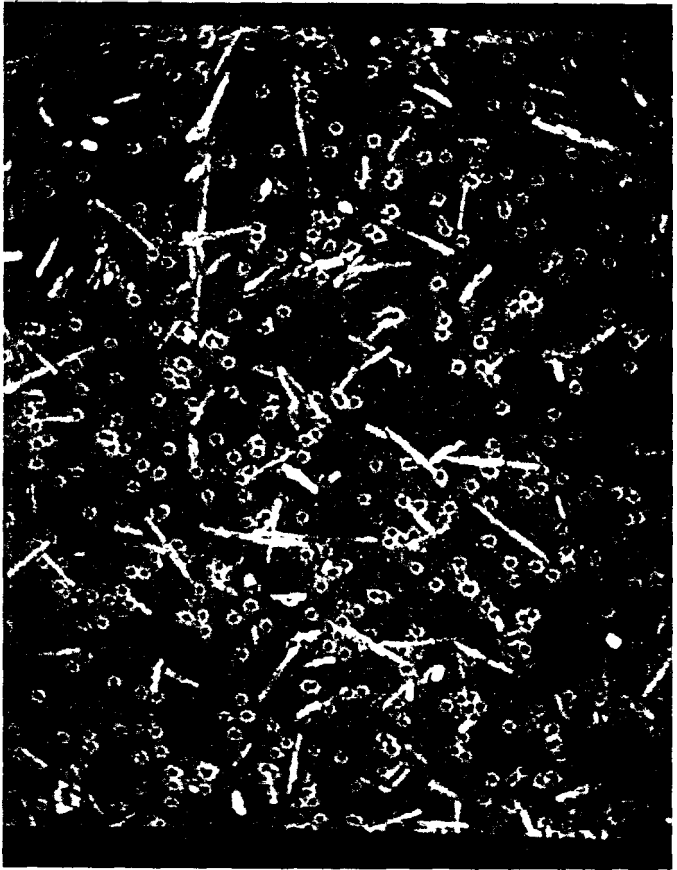


FIGURE 13
CHRYSTILE ASBESTOS FIBERS IN
FILTRATE FROM $0.8\mu\text{M}$ NUCLEPORE
MEMBRANE-CHALLENGED WITH
 1.29×10^{10} FIBERS, 10,000 X SEM



FIGURE 14
CHRYSOTILE ASBESTOS FIBERS IN
FILTRATE FROM 0.8 μ M NUCLEPORE
MEMBRANE-CHALLENGED WITH
1.29x10¹¹ FIBERS, 10,000 X SEM

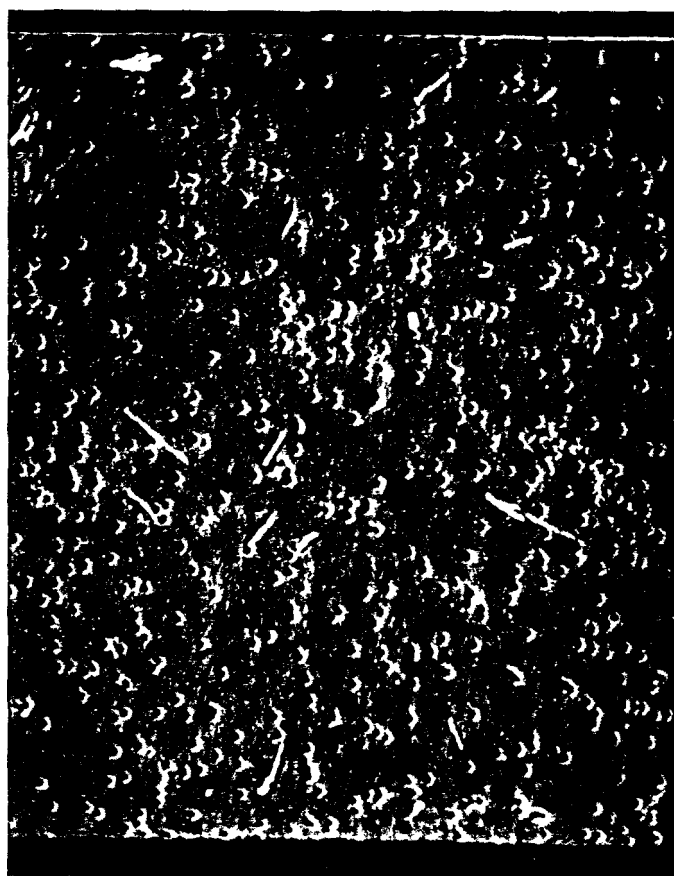


FIGURE 15
CHRYSOTILE ASBESTOS FIBERS IN
FILTRATE FROM 0.6 μ M NUCLEPORE
MEMBRANE-CHALLENGED WITH
1.29 $\times 10^{11}$ FIBERS, 10,000 X SEM

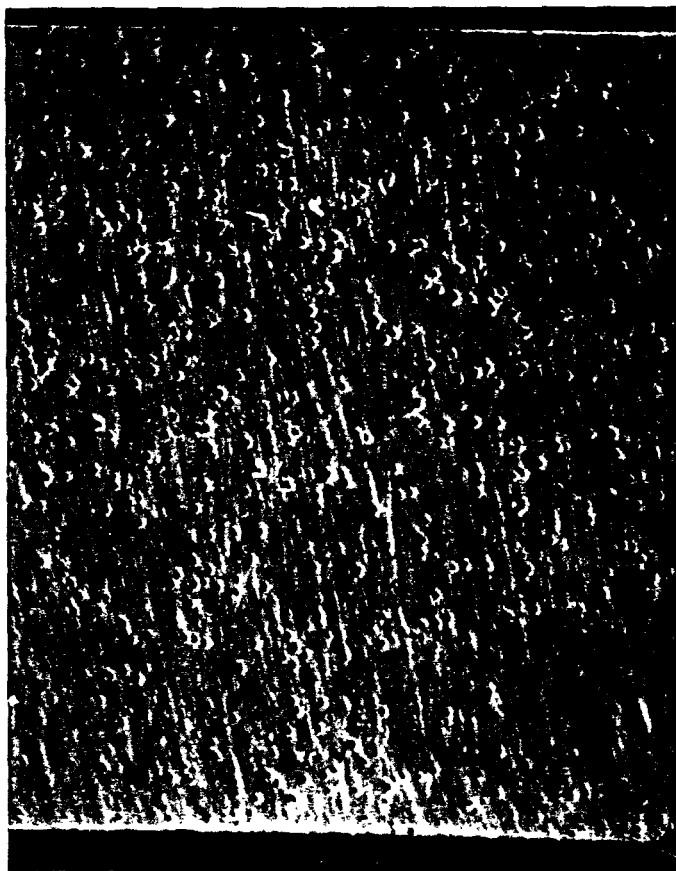
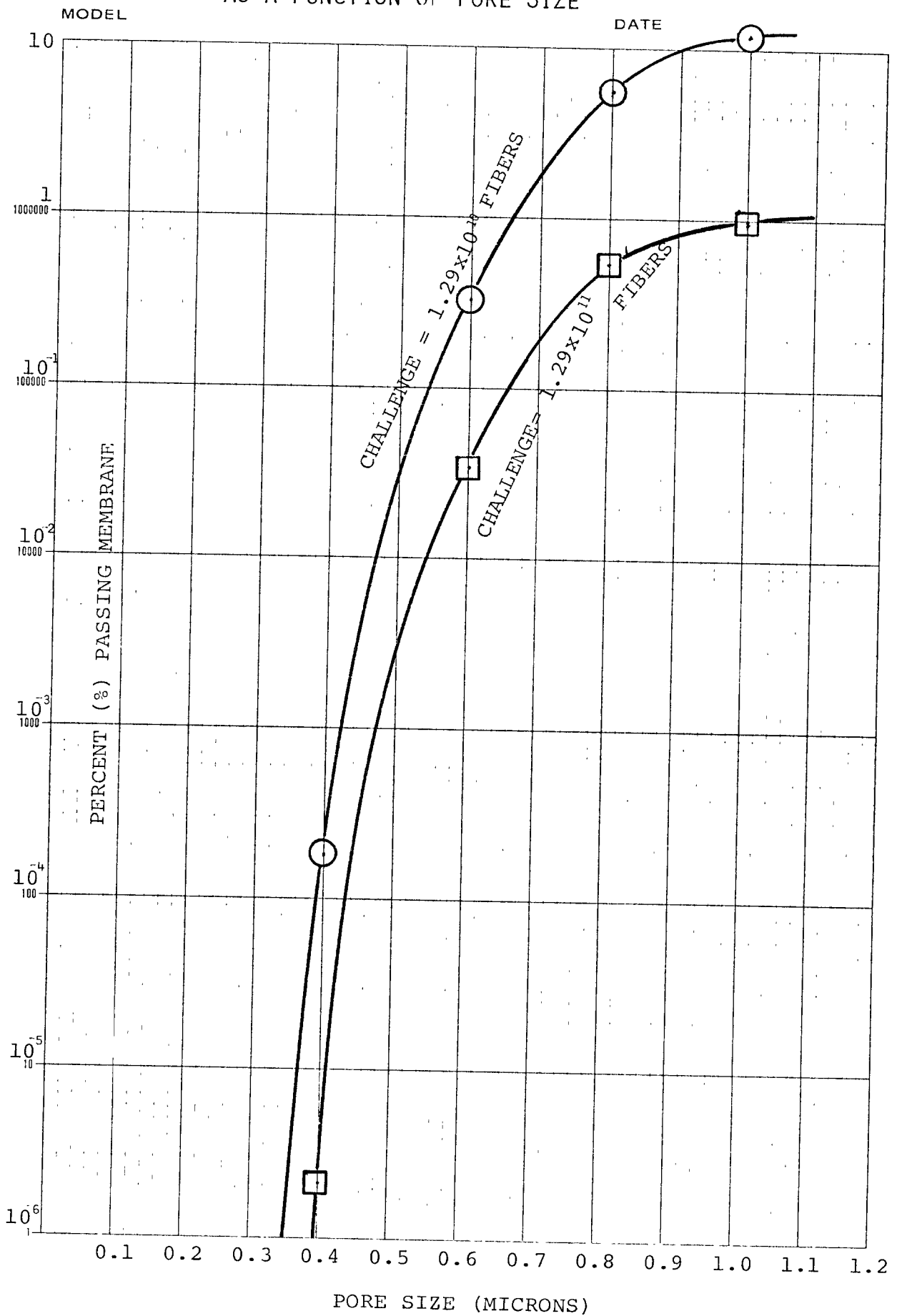
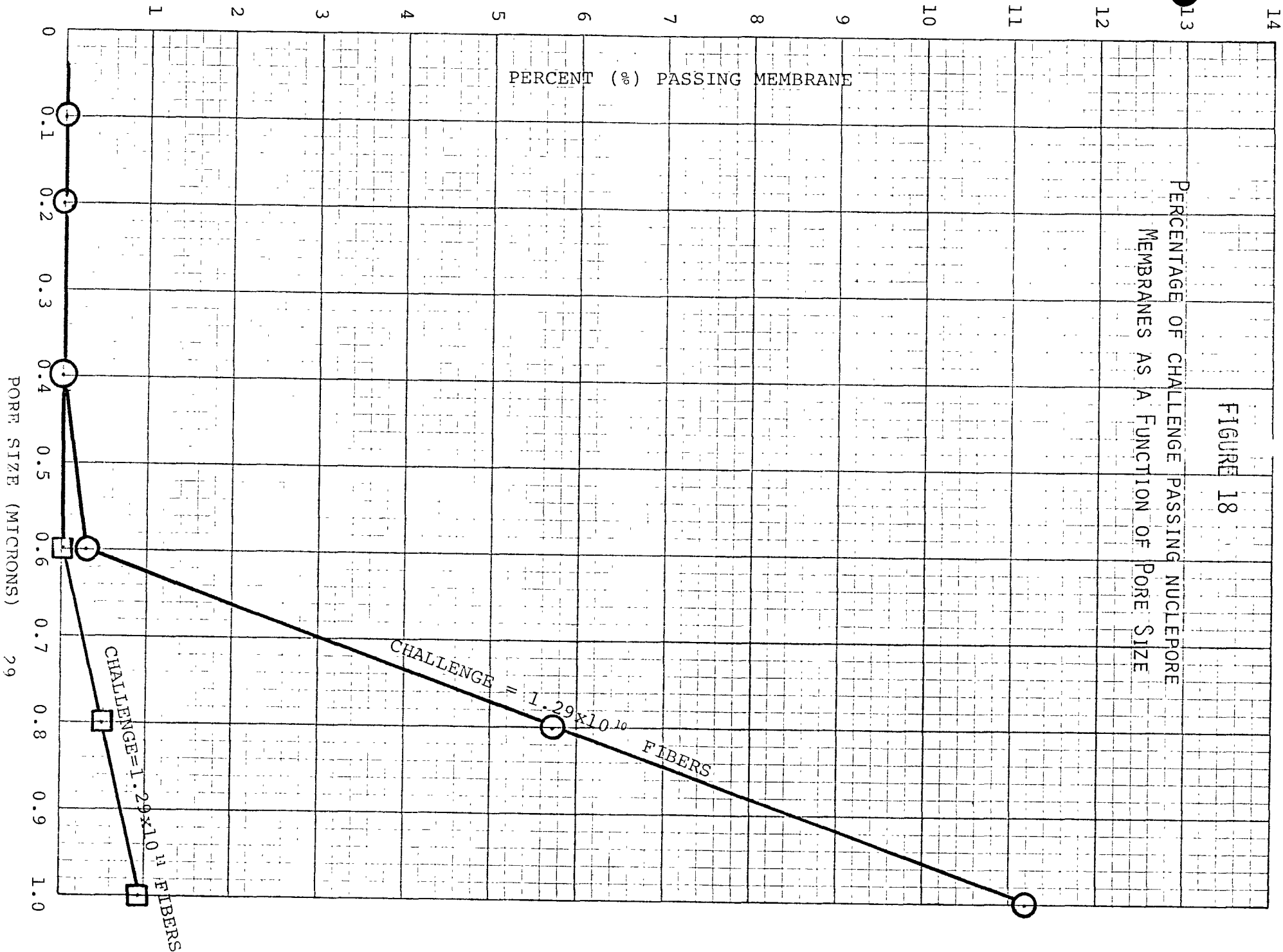


FIGURE 16
CHRYSOTILE ASBESTOS FIBERS IN
FILTRATE FROM $0.4\mu\text{M}$ NUCLEPORE
MEMBRANE-CHALLENGED WITH
 1.29×10^{10} FIBERS, $10,000 \times$ SEM
(NOTE $0.2\mu\text{M}$ STRAY CONTAMINANT)

FIGURE 17

PERCENTAGE OF CHALLENGE PASSING NUCLEPORE MEMBRANES
AS A FUNCTION OF PORE SIZE





The difference in percent retention (or passage) for the two different challenge levels is believed to reflect the phenomenon of fiber matting which is increased at the higher challenge resulting in reduced passage of fibers.

Figure 19 is a plot of the equivalent data from the work of Manalan and Reti¹⁴ with Millipore membranes. The reason for the cross-over of the two challenge curves is unknown. It has been suggested that the non-zero background counts may explain the scatter. However, a finite background (1000 fibers/liter) should only lend uncertainty to low fiber count data whereas the most difficult data point (on Figure 19) to explain is the higher percentage passage with a higher challenge level at the 1.2 μ m pore size where large fiber counts were obtained.

Photomicrographs were also made of the test membrane surface to better ascertain the degree of matting. Figures 20-27 are a collection of SEMs taken at 10,000 and 3,000X showing the total matte build-up on the surface. There is considerable mal-distribution of fibers on the membrane surface with free areas for passage in some cases, and dense entanglement in others. As expected, the fibers in these photomicrographs are considerably longer than those detected in the filtrates (Figures 11-16).

2. Retention as a Function of Challenge Level

Since the effect of fiber matting at the challenge levels of 10^{10} and 10^{11} fibers was shown to be significant, a study of the effect of lower challenge levels was run on the 0.4 μ m pore size membrane. The 0.4 μ m pore size was selected since it represented the cut-off point between absolute retention and detectable fiber passage.

Table IV indicates the total fibers passed and the percentage passed as a function of challenge level down to the 1.29×10^6 fiber level. Again, the one-sided t test was used to calculate maximum fiber passage at the 95% confidence level.

Figure 28 plots the percent of challenge passing an 0.4 μ m Nuclepore membrane as a function of challenge level. As expected, the retention is enhanced by higher challenge levels. The percentage passed at the 10^6 challenge level is not plotted since only one fiber found in 1720 fields makes the difference between 0 and 0.4% passed.

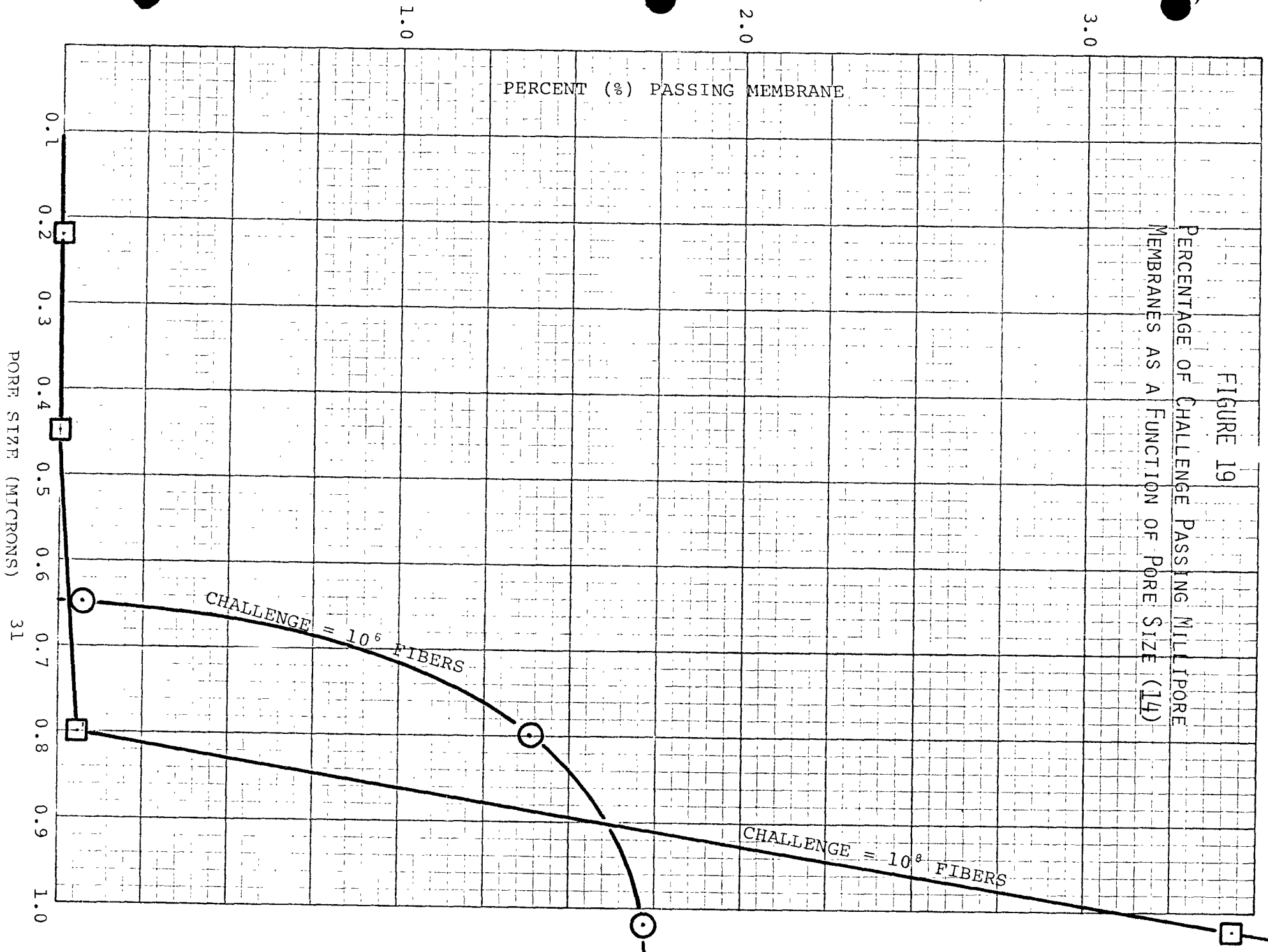


TABLE IV
RETENTION OF CHRYSOTILE ASBESTOS FIBERS
ON 0.4 μ m NUCLEPORE AS A FUNCTION OF CHALLENGE LEVEL

<u>Challenge (Fibers)</u>	<u>No. of Fields Scanned</u>	<u>Total Fibers Passed¹</u>	<u>Maximum Fibers Passed²</u>	<u>Percent Passed (%)</u>	<u>Maximum Percent Passed² (%)</u>
1.29x10 ⁶	1720	0	-	0	-
1.29x10 ⁷	1720	2.24x10 ⁴	4.07x10 ⁴	0.174	0.316
1.29x10 ⁸	1720	4.46x10 ⁴	7.07x10 ⁴	0.0346	0.0548
1.29x10 ⁹	1720	1.11x10 ⁴	2.41x10 ⁴	8.6x10 ⁻⁴	1.87x10 ⁻³
1.29x10 ¹⁰	5160	2.42x10 ⁴	3.53x10 ⁴	1.88x10 ⁻⁴	2.74x10 ⁻⁴
1.29x10 ¹¹	3440	2.79x10 ³	7.38x10 ³	2.16x10 ⁻⁶	5.72x10 ⁻⁶

1. Total calculated from area factor, total fibers passed = fibers counted ($\frac{\text{membrane area}}{\text{area canned}}$)
2. 95% confidence range calculated from one-sided t test.

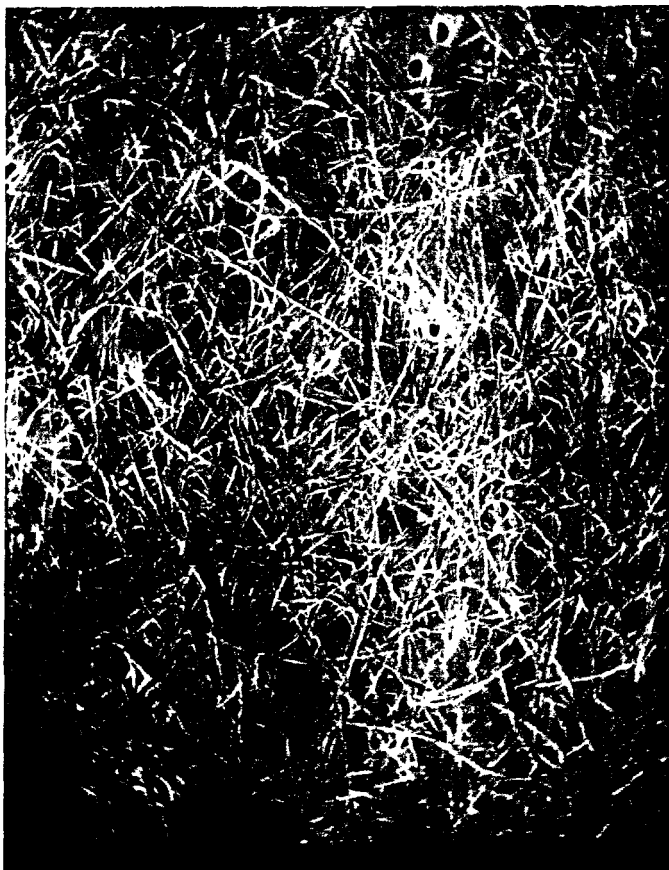


FIGURE 20
CHRYSOTILE ASBESTOS FIBERS RETAINED
ON $1.0\mu\text{M}$ NUCLEPORE MEMBRANE
(1.29×10^{10} FIBERS DISTRIBUTED ON
 9.6cm^2) 3,000 X SEM



FIGURE 21

CHRYSOTILE ASBESTOS FIBERS RETAINED
ON 1.0 μ M NUCLEPORE MEMBRANE
(1.29×10^{10} FIBERS DISTRIBUTED ON
9.6cm²) 10,000 X SEM

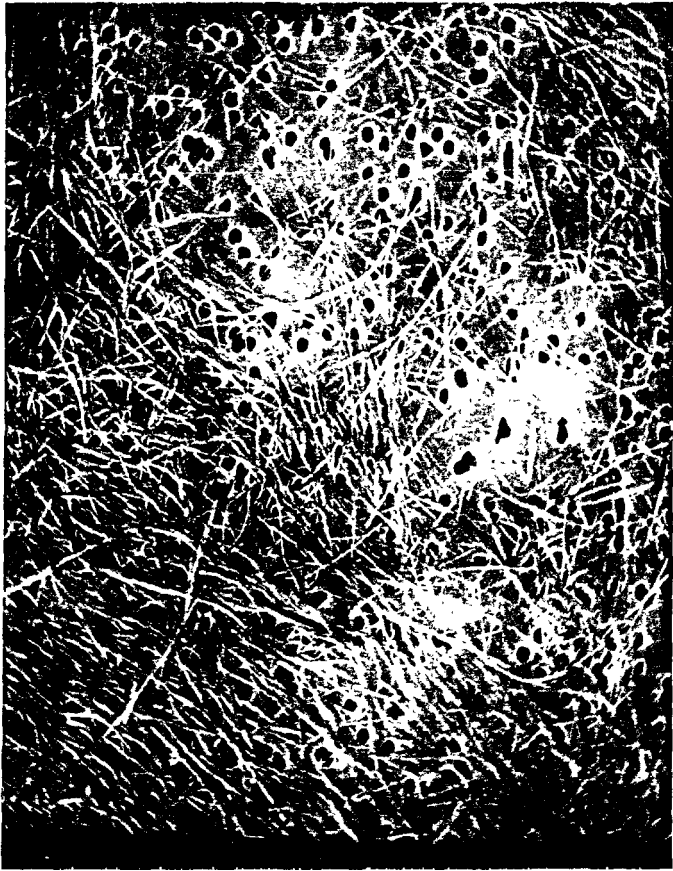


FIGURE 22
CHRYSTILE ASBESTOS FIBERS RETAINED
ON 0.8 μ M NUCLEPORE MEMBRANE
(1.29×10^{10} FIBERS DISTRIBUTED ON
9.6cm²) 3,000 X SEM



FIGURE 23

CHRYSOTILE ASBESTOS FIBERS RETAINED
ON 0.3 μ M NUCLEPORE MEMBRANE
(1.29×10^{10} FIBERS DISTRIBUTED
ON 9.6cm²) 10,000 X SEM





FIGURE 24

CHRYSOTILE ASBESTOS FIBERS RETAINED
ON 0.6 μ M NUCLEPORE MEMBRANE
(1.29×10^{11} FIBERS DISTRIBUTED ON
9.6cm²) 10,000 X SEM

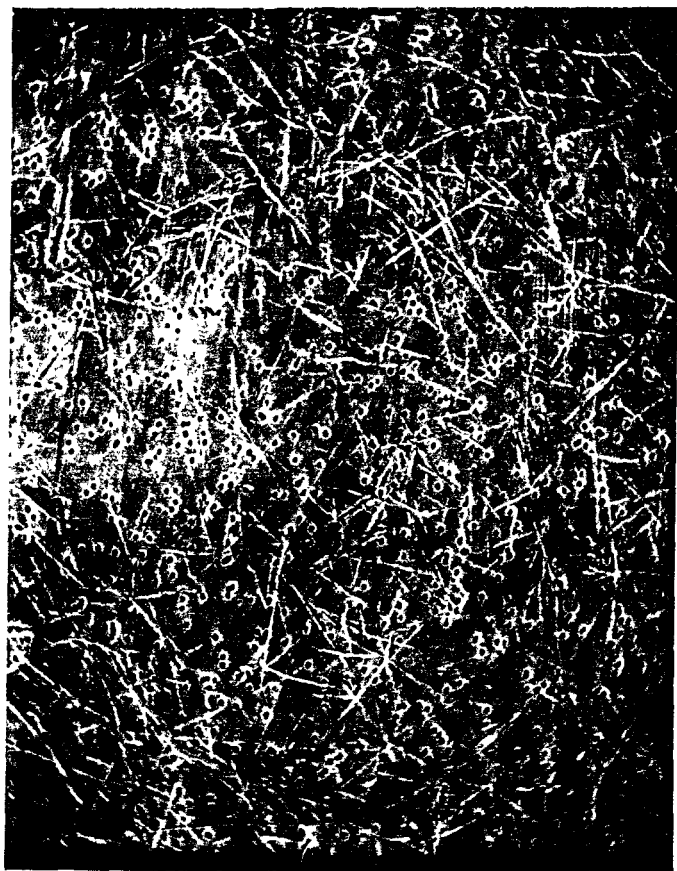


FIGURE 25
CHRYOTILE ASBESTOS FIBERS RETAINED
ON 0.4 μ m NUCLEPORE MEMBRANE
(1.29×10^{10} FIBERS DISTRIBUTED
ON 9.6cm²) 3,000 x SEM

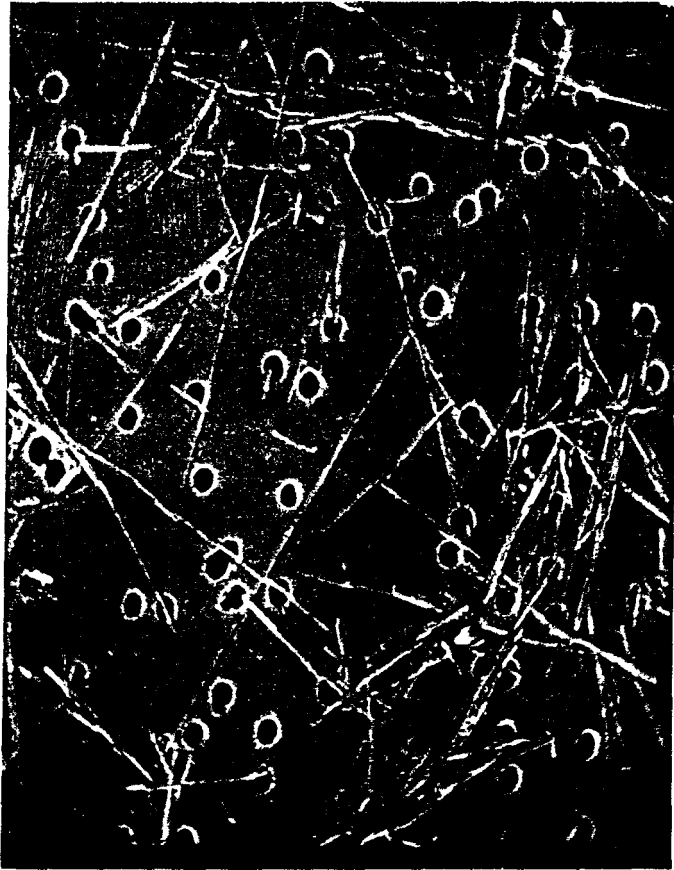


FIGURE 26
CHRYSTILE ASBESTOS FIBERS RETAINED
ON 0.4 μ M NUCLEPORE MEMBRANE
(1.29×10^{10} FIBERS DISTRIBUTED ON
9.6cm²) 10,000 X SEM

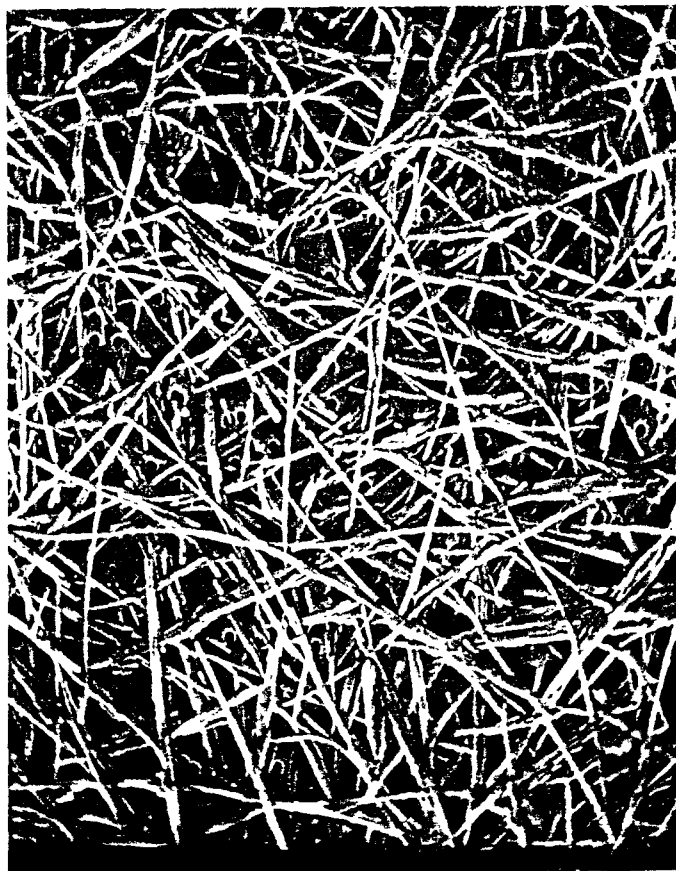


FIGURE 27

CHRYSOTILE ASBESTOS FIBERS RETAINED
ON 0.2 μ m NUCLEPORE MEMBRANE
(1.29×10^{10} FIBERS DISTRIBUTED ON
9.6cm²) 10,000 X SEM

FIGURE 28
PERCENT OF CHALLENGE PASSING AN $0.4\mu\text{m}$
NUCLEPORE MEMBRANE AS A FUNCTION OF CHALLENGE LEVEL

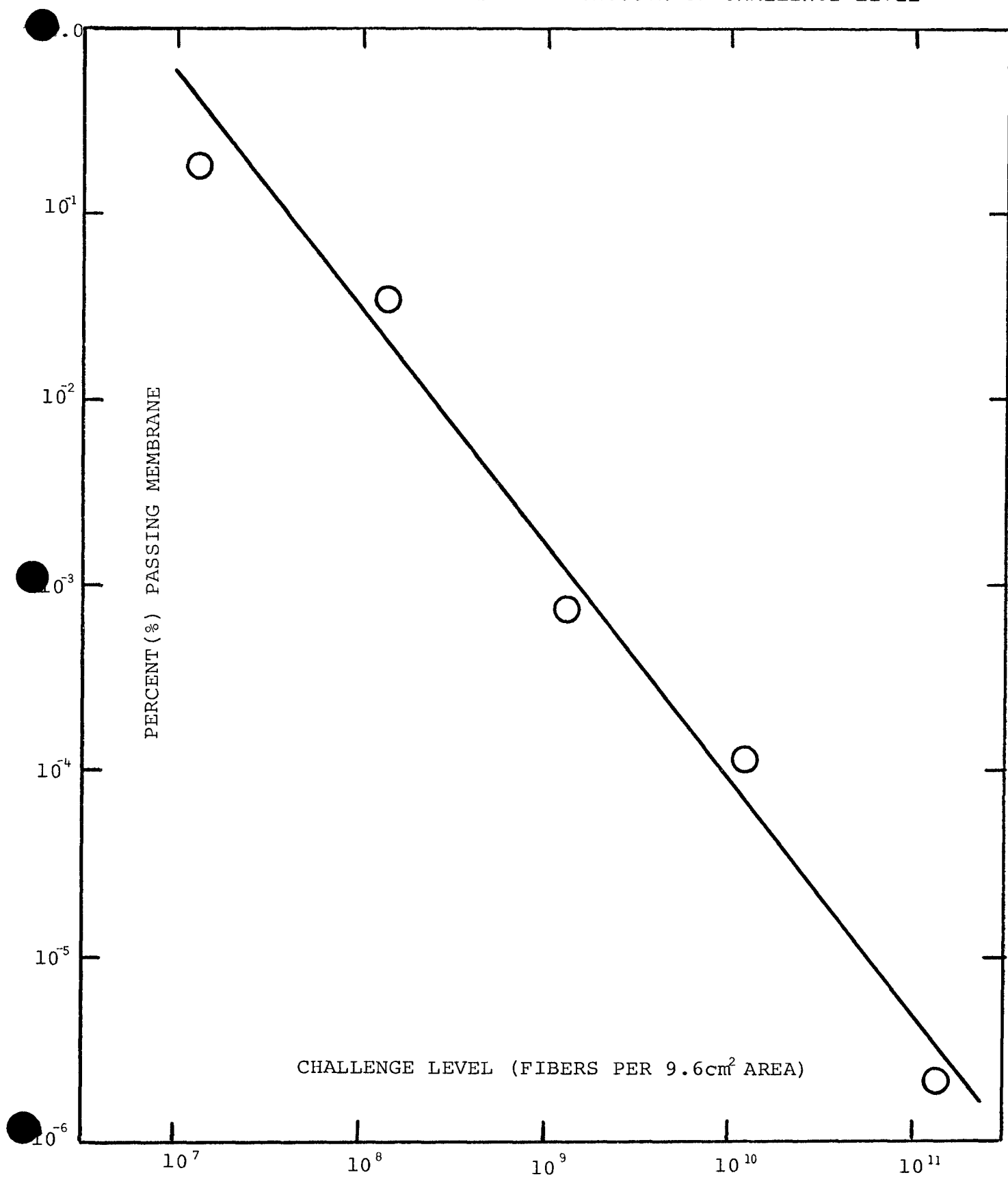
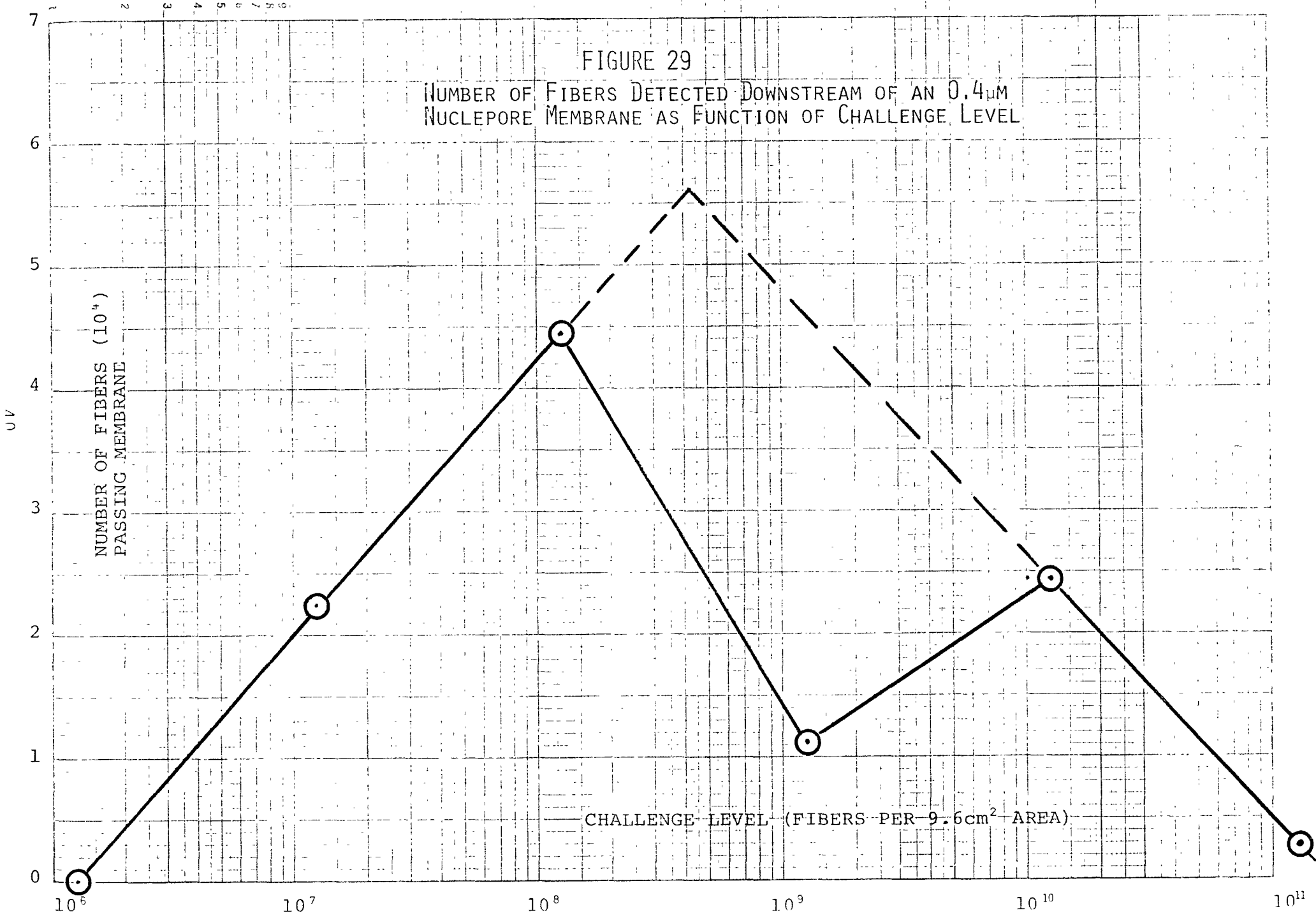


Figure 29 plots the number of fibers detected downstream as a function of challenge level. The number of fibers increases with increasing challenge until levels of 10^8 - 10^9 are attained and thereafter declines. Figure 29 can also be looked upon as a plot of the number of fibers passing as a function of throughput volume. Every ml passing through the filter adds to the membrane's challenge. As the volumetric throughput increases, the fiber build-up on the membrane increases. The plot indicates that when enough volume has passed through the membrane to lay down 10^8 - 10^9 fibers, significant matting begins to form and further passage is retarded. Thus, the number of fibers detected downstream would be expected to level off at a constant-maximum value. The fact that the number declines indicates that at the higher challenge levels, the concentration is high enough to result in some entanglement of fibers prior to deposition on the membrane surface. This may explain the mal-distribution of fibers seen in Figures 20-27.

IV. Conclusion

- A. The Nuclepore membrane greatly facilitates detection and counting of asbestos fibers by virtue of its extremely smooth, flat surface against which the asbestos fibers are easily seen and counted.
- B. Scanning electron microscopy appears to offer some convenience over transmission electron microscopy in sample preparation, asbestos fiber identification, and in rapid counting scans.
- C. The $0.1\mu\text{m}$ and $0.2\mu\text{m}$ pore size Nuclepore membranes appear to offer absolute retention of chrysotile asbestos fibers.
- D. The $0.4\mu\text{m}$ pore size Nuclepore membrane appears to offer virtual retention of all chrysotile asbestos fibers with maximum penetration rated at 0.3%.
- E. Increasing challenge levels result in decreased fiber passage presumably due to fiber matting.
- F. Significant matting appeared to occur at challenge levels above 10^8 fibers/cm².

FIGURE 29
NUMBER OF FIBERS DETECTED DOWNSTREAM OF AN $0.4\mu\text{m}$
NUCLEPORE MEMBRANE AS FUNCTION OF CHALLENGE LEVEL



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