ENVIRONMENTAL RESEARCH 31, 32–53 (1983)

Characterization of Three Types of Chrysotile Asbestos after Aerosolization

KENT E. PINKERTON,*,[1] ARNOLD R. BRODY,† DANIEL A. MCLAURIN,‡ BERNARD ADKINS, JR.,§ ROBERT W. O'CONNOR,§ PHILIP C. PRATT,* AND JAMES D. CRAPO*

*Departments of Pathology and Medicine, Duke University and Durham Veterans Administration Medical Center, Durham, North Carolina 27705; †Laboratory of Pulmonary Function and Toxicology, National Institute of Environmental Health Sciences, ‡Becton–Dickinson Research Center, and §Northrop Services Inc., Research Triangle Park, North Carolina 27711

Received December 22, 1981

Jeffrey Mine and Coalinga Mine chrysotile, two asbestos samples prepared for experimental research by the National Institute of Environmental Health Sciences, and the UICC B chrysotile reference sample have been characterized in the aerosolized state using gravimetric measurements, light microscopy, scanning electron microscopy, and x-ray energy spectrometry. These methods revealed (1) a greater "respirable" mass fraction in the Jeffrey and UICC B preparations compared to the Coalinga sample, (2) for fibers greater than 5 μm in length and less than 3 μm in diameter, Jeffrey Mine chrysotile contained a significantly greater fraction of fibers longer than 40 μm in length compared to the UICC B or Coalinga Mine chrysotiles, and (3) Jeffrey and UICC B chrysotile contained no fibers or fiber clusters which exceeded 2 μm in diameter while Coalinga chrysotile contained numerous fibers and fiber clusters which were greater than 2 μm in diameter. The characterization of these chrysotile preparations in the aerosolized state, in particular the Coalinga Mine chrysotile, demonstrated different fiber length and fiber width distributions when compared with previous characterizations of samples that had been dispersed in a liquid medium by ultrasonification. These observations emphasize the importance of determining the size distribution of fibers in the aerosolized state for inhalation studies and the size distribution of fibers in a liquid suspension for oral ingestion, instillation, or injection studies. Because of differences in length–width distributions, each of the studied chrysotile preparations would be expected to have different patterns of deposition in the alveolar regions of the lung after an inhalation exposure.

INTRODUCTION

There is increasing evidence that asbestos-induced pulmonary fibrosis and neoplasia are due to the physical and chemical characteristics of the inhaled fibers (Wagner, 1965; Seaton, 1975; Stanton and Layard, 1978; Pott, 1978). This makes it important to use well-characterized fiber preparations in experimental research to more clearly identify those factors leading to lung injury. The purpose of this paper is to describe two new samples of chrysotile which have been prepared for experimental research by the National Institute of Environmental Health Sciences (NIEHS). A third chrysotile, UICC B, has also been characterized in this paper.

[1] To whom correspondence and reprint requests should be addressed: Box 3177, Duke University Medical Center, Durham, N.C. 27710.

0013-9351/83 $3.00

Chrysotile represents over 90% of the world's asbestos production and is ubiquitous in our environment. The properties of fire retardance, chemical inertness, tensile strength, and flexibility make chrysotile important in numerous applications such as insulation, ceiling tiles, brake linings, and cement products. These utilizations of asbestos enhance the frequency of inhalation of chrysotile by people not directly involved with the mining or processing of this material. Chrysotile is a member of the serpentine family of minerals, whereas all other asbestos minerals belong to the amphibole family (Sinclair, 1959). Mechanical agitation of chrysotile can lead to disruption of the fiber at weak points along the fiber. This disruption can cause the fiber to "open up" into its fibrillar subunits, creating new fibers of smaller diameter and/or shorter length, fibers with splayed ends, fibers with an uneven diameter along the length, or combinations of the above (Assuncao and Corn, 1975). These features make chrysotile more complex to analyze in terms of fiber size and number than the amphibole types of asbestos in which fibers are basically straight and uniform in diameter.

The characterization of a chrysotile preparation in terms of particle and fiber size distribution is influenced by a number of factors. These include (1) the state in which the chrysotile is found, i.e., in bulk, in suspension, or in an aerosol, (2) the method of sample collection, i.e., on a slide or filter, (3) the manner in which the collected sample is prepared for examination, i.e., by ultrasonification, transfer or direct preparation of the filter or slide, (4) the instrument used to measure the particles and fibers in the sample, i.e., the optical microscope, transmission electron microscope, or scanning electron microscope, and (5) the criteria used for defining fibers, i.e., a minimum fiber length, a 3 to 1 aspect ratio, or characteristics of fiber morphology.

Characterization of chrysotile in the aerosolized state has been done for the UICC A and B reference samples (Timbrell, 1970a; Beckett, 1973). Two new research samples of chrysotile have been prepared in bulk (approximately 1000 pounds of each) and are available for experimental research through the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. These new preparations (Jeffrey and Coalinga chrysotile) have been characterized for elemental composition, mineral composition, particle surface area and density, and thermal properties using optical emission spectrography, x-ray diffraction, thermogravimetry, pycnometry, and a number of petrographic microscopic techniques (Campbell *et al.*, 1980). Particle size analysis has been done with samples dispersed in a liquid medium in which measurements of fiber length and diameter were made (Wylie, 1979; Campbell *et al.*, 1980; Siegrist and Wylie, 1980). Particle size analysis of these preparations in the aerosolized state was not done. The purpose of this study is to characterize in the aerosolized state the Jeffrey and Coalinga chrysotile preparations along with the UICC B reference sample. To characterize each preparation, gravimetric dust measurements, optical microscopy, scanning electron microscopy (SEM), and x-ray energy spectrometry were used. Information obtained using these techniques has helped identify characteristics of these asbestos preparations which may influence their deposition pattern when inhaled as an aerosol and which could potentially contribute to their ability to cause pulmonary injury.

MATERIALS AND METHODS

Fiber Preparations

Chrysotile samples were obtained from the following locations: the Coalinga Mine in California (Union Carbide), the Jeffrey Mine in Quebec, Canada (Johns–Manville), and the Canadian reference sample prepared by the International Union Against Cancer (UICC B). A brief history for each preparation is given.

Coalinga Mine fiber. Identified as COF-25, this unique form of chrysotile is obtained from the New Idria serpentinite mass located in the Diablo range in California. The deposit is unusual in that the fibers are randomly oriented as a mat, rather than as parallel fibers running in veins, and the mining process is done with bulldozers. The deposit is almost pure chrysotile. The fibers are short in length and are not of spinning grade. Because of the absence of long fibers in this chrysotile, a great deal of interest has been generated in using this chrysotile as a "short–range fiber" preparation.

Preparation of this short fiber material for experimental research was done in the following manner. The ore from the mine was first screened to remove contaminating rocks. From this point the material was processed in water as a slurry of 1% solids and 99% water. The slurry was passed through a grinder and a magnetic separator three times to open the fiber bundles and to remove any iron-containing minerals. Between each grinding the slurry was passed through a particle size separator (hydroclone) under pressure. The aqueous slurry was rotated inside the hydroclone forming a vortex. Heavy particles escape from the hydroclone through a side port located near the bottom of the hydroclone. These coarse particles were reground and put through the hydroclone again. The light particles leave the hydroclone through a side port located near the top of the vortex. These particles were fed into a series of hydroclones in which the top exit ports (overflow port) are progressively smaller to allow for the collection of finer and finer chrysotile fibers. The final hydroclone port had an external diameter of 25 mm from which the chrysotile preparation was collected. (For industrial purposes the final hydroclone port is usually six inches (personal communication, Asbestos Group, Union Carbide, Niagara Falls, N.Y.).)

Langer *et al.* (1978) described in detail a chrysotile sample also obtained from the Coalinga Mine deposit, referred to as Calidria RG-144. The differences between COF-25 and RG-144 are a result of the differences in the processing of the raw material. COF-25 has a finer particle size than RG-144. This finer particle size is a result of differences in the pressure, vortex characteristics, and overflow port diameters used in the hydroclone. COF-25 is not derived from RG-144 by further grinding or pellet milling.

Jeffrey Mine fiber. Identified as Plastibest-20, this form of chrysotile is a grade 4 asbestos used by the plastics industry. The fibers run in parallel bundles, oriented crosswise in veins in serpentine rock and for this project were purified by roller milling and air separation using standard industrial techniques. The final fiber preparation by volume is greater than 96% chrysotile (Campbell *et al.*, 1980). Preparation of the material for experimental research was accomplished by passing the material through a hurricane pulverizer three times to open fiber bundles.

UICC B chrysotile reference sample. This preparation is a grade 4 chrysotile (Timbrell *et al.*, 1968) obtained from eight different Canadian chrysotile mines and mixed according to the proportional production of each mine during the year of 1950. The approval of this preparation was made in 1966 by the Union Internationale Contre Cancer (UICC) to standardize asbestos samples used in experimental research. The literature is replete with information regarding the physical and chemical makeup of this chrysotile preparation (Timbrell *et al.*, 1968; Timbrell, 1970a, b; Rendall, 1970, 1980; Morgan and Cralley, 1973; Beckett, 1973). We have reexamined UICC B chrysotile in the aerosolized state with a twofold purpose: (1) to compare our results for the UICC B fiber size distribution with those found in prior studies in the literature, and (2) to correlate the size distribution of the Coalinga and Jeffrey Mine fibers to the UICC chrysotile fiber preparations available for experimental inhalation research. No manipulation of these chrysotile preparations was done prior to aerosolizing the fibers.

Fiber Aerosolization

A modified Timbrell generator (Timbrell, 1968) was used to create a dust cloud within a 5-m^3-stainless steel-exposure chamber. Each asbestos preparation was hand compressed with a plunger in a 2.8 × 9.0-cm cylinder to form a plug. This was mechanically advanced into the pathway of a blade rotating 1500 rpm to create an aerosol of fibers within the dispersing bowl of the generator. A copper tube connecting the dispersing bowl to the exposure chamber facilitated the passage of aerosolized fibers into the exposure chamber. The concentration of the asbestos dust cloud was regulated by adjustment of the airflow through the exposure chamber. This resulted in the flow of air being maintained between 200 to 400 liters per minute.

Total Dust Mass Concentration

Once the exposure chamber had stabilized (normally after 1 hr of dust generation), a gravimetric measurement of the dust concentration within the exposure chamber was made. This was accomplished by sampling 100 liters of chamber air drawn through a 0.8-μm Nucleopore filter housed within a Gelman filter holder. The sample time was 10 min at a flowrate of 10.0 liters per minute.

Respirable Mass Concentration

The respirable mass concentration in the exposure chamber for each chrysotile preparation was measured using two different instruments: a Casella sampler and a Cascade impactor. The term "respirable mass concentration" is arbitrary in that it was decided by totally different parameters for each apparatus used. Using the Casella sampler, a gravimetric estimate of the respiratory mass concentration was made by collecting fibers and particles with an equivalent aerodynamic diameter of 7.1 μm or less on a glass fiber filter. Fibers and particles larger than this equivalent diameter were prevented from depositing on the filter by a multichannel horizontal elutriator. Each sample was collected over a 6-hr period at a flowrate of 2.5 liters per minute with the Casella sampler placed inside the exposure chamber.

Using the Cascade impactor, the respirable mass concentration was defined to

include filters on which the majority (>50%) of fibers collected were less than 10 μm in length. This was determined by examination of the filter by optical microscopy. This respirable mass was obtained by using precutter stages in the Cascade impactor to eliminate the longer and thicker fibers and by adjusting the flowrate to obtain a fiber size distribution (cut point) of 10 μm or less in length.

The volume of air sampled through the Cascade impactor was 200 liters. A total sample mass was also collected on the same day by sampling 0.2 m^3 of chamber air at a flowrate of 10.5 liters per minute for 19 min 3 sec using the same setup minus the Cascade impactor. All gravimetric measurements were expressed in mg/m^3. Gravimetric measurements of the respirable mass collected in the Cascade impactor were done for the Jeffrey and Coalinga Mine preparations only, because of the known similarity between Jeffrey and UICC B chrysotile.

Collection of Fiber Samples

The same apparatus used to collect samples for dust concentration measurements was also used to collect fiber samples. Samples to be analyzed by light microscopy were collected on Millipore-type AAWP membrane filters at a flowrate of 0.1 liters per minute for 3 to 5 min depending upon the chamber concentration of the chrysotile preparation. Before use, the filters were treated in a 0.1% solution of Hyamin 2389 and dried at room temperature overnight to prevent static charging of the filter. Samples analyzed by scanning electron microscopy were collected on a 0.2-μm Nucleopore filter for 2 sec and 5 sec at a flowrate of 10 liters per minute.

Preparation of Filters for Examination

Light microscopy. After sampling, the filter was placed on a 25 × 75-mm glass microscope slide, sample side down. The filter was cleared by holding the slide over an evaporating flask of boiling acetone. After clearing, a drop of glycerol-triacetate (Permount) was placed on the dissolved filter and a cover slip applied.

Electron microscopy. Filters were secured to the polished side of a Gough planchet with carbon paint and were gold coated. The thickness of the gold coat was 100 to 150 nm.

Fiber Characterization

Light microscopy. A Beckett G22 graticule was used. At 500×, the magnification used for sizing and counting, the graticule was a square, 100 μm on each side. The lattice on the graticule had a spacing of 5 μm in one direction and 3 μm in the perpendicular direction. The following rules were used for fiber characterization: All fibers counted had at least a 3 to 1 length-to-diameter ratio (aspect ratio). Only fibers 5 μm in length or longer were counted. A fiber bundle which met the 3 to 1 aspect ratio requirement was counted as a fiber. Bundles with a diameter greater than 3 μm were not counted. Only fibers whose midpoint was located within the graticule were counted. At least 200 fibers were counted from 20 to 100 random graticule fields for each filter sample. The characterization of each chrysotile preparation by light microscopy was based upon measurements from filters collected on a daily basis (5 days/week for 12 months).

Electron microscopy. The magnification used for particle sizing and counting

was 10,000. To facilitate measurement of long fibers and fiber width, magnifications ranging from 1200 to 18,000× were used. Counting and sizing of particles were done directly on the electron microscope screen. Only particles whose midpoint fell within the area of the viewing screen were analyzed. This procedure essentially reduced all particles to points, thus causing every particle to have an equal probability of being counted. To assure accuracy in measurement, a standard was made consisting of latex beads 1.099 μm in diameter on a 0.2-μm Nucleopore filter. The filter and beads were gold coated and used to verify the magnification prior to each counting session. The area of the filter analyzed consisted of bands randomly selected across portions of the filter. A field was randomly selected at 10,000 magnification and then moved to the right. Several bands were counted per filter and a range of 4 to 8 filters was analyzed for each chrysotile preparation.

The following three categories were used to characterize the material on the filters for each of the chrysotile preparations:

(1) Fiber: (a) At least a 3 to 1 aspect ratio was required. (b) Generally, a fiber was cylinder-shaped with a uniform diameter. However, a slight separation of the fibrils along any section of the fiber or at the ends was permissible. (c) The diameter of the fiber was measured at the widest portion of the fiber whether it occurred at the end or at some point along the fiber. (d) Smaller fibrils solidly attached to the major body of the fiber constituted a portion of the fiber. It was not counted as a separate fiber. The diameter of the fiber would be measured at this point if it constituted the widest portion of the fiber.

(2) Fiber cluster: (a) A fiber mass with at least a 3 to 1 aspect ratio was required. (b) It was composed of small fibrils usually oriented in the same direction, but partially separated. Separation of fibrils may occur at any point along the length of the bundle or cluster. (c) The diameter of the cluster was measured at its greatest combined width along the fiber mass. A fiber cluster usually has a more variable width than a "fiber."

(3) Nonfibrous particle: (a) Any particle with less than a 3 to 1 aspect ratio. (b) The longest dimension was measured as well as the greatest perpendicular measurement.

Fiber "flocs" or clumps were also occasionally present on the filter consisting of tangled fibers and fiber clusters. These were not analyzed because their complexity prevented accurate separation into individual fibers and fiber clusters.

Representative fibers, clusters, and nonfibrous particles were analyzed for elemental composition using x-ray energy spectrometry for each chrysotile preparation. Each particle was analyzed for the presence of an element by a scoring system ranging from 0 to 4+. At 10,000×, 50 nonfibrous particles whose midpoints fell within the viewing area of a randomly selected band across the filter were analyzed. In addition, the magnesium-to-silicon ratio was determined for all particles containing these two elements.

RESULTS

Dust Concentration Measurements

The total mass concentration and corresponding respirable mass concentration for each chrysotile preparation are given in Table 1 using both the Casella sampler

TABLE 1
GRAVIMETRIC MEASUREMENTS FOR EACH CHRYSOTILE PREPARATION IN THE EXPOSURE CHAMBER

Preparation	(A) Chamber dust mass concentration (mg/m^3)[a]	(B) Respirable concentration (mg/m^3)[a]	Ratio B/A
		Casella sampler	
Jeffrey	11.36 ± 2.18	9.90 ± 1.63	0.871
UICC B	10.99 ± 2.11	8.32 ± 1.75	0.757
Coalinga	7.76 ± 1.46	3.28 ± 0.83	0.423
		Cascade impactor	
Jeffrey	12.29 ± 3.33[b]	2.62 ± 0.81[b]	0.213
Coalinga	15.63 ± 2.41[c]	0.80[c]	0.051

[a] All data are means ± SD, $n = 240$ for each chrysotile preparation.
[b] For Jeffrey all data are means ± SD, $n = 12$.
[c] For Coalinga the chamber mass concentration is based on three samples and the respirable concentration is a single sample.

and the Cascade impactor. When comparing the ratio of the respirable mass concentration to the total dust concentration found in the exposure chamber, the Jeffrey Mine chrysotile and UICC B chrysotile have a relatively high percentage of respirable material based upon the Casella sampler measurements (87% for the Jeffrey chrysotile and 76% for the UICC B chrysotile). The ratio of the respirable mass concentration to the total mass concentration is significantly lower for Coalinga Mine chrysotile (42%). Using the Cascade impactor a substantially smaller fraction of the total dust concentration was found to be respirable. For the Jeffrey fiber 21% of the total dust concentration was respirable and 5% of the total dust concentration was respirable for the Coalinga fiber.

Fiber Characterization—Light Microscopy

Table 2 lists the percentage of fibers found for each given length interval by light microscopy for each of the three chrysotile preparations. Fibers greater than 100 μm in length and less than 3 μm in diameter were found in each preparation. The percentage of fibers present from 5 to 30 μm in length was similar for all three aerosolized preparations with greater than 70% of all counted fibers falling within this length interval. For the fiber-length intervals of 40–50, 50–100, and greater than 100 μm, the percentage of fibers contained within each of these length intervals was significantly greater ($P < 0.05$) for the Jeffrey Mine preparation than for either the UICC B preparation or the Coalinga Mine preparation.

Fiber Characterization—SEM

The length, width, and aspect ratios for the combined fibers and fiber clusters are illustrated in Figs. 1–3. Figures 1A–D illustrate fiber length characteristics. Those fibers or fiber clusters having a diameter greater than 0.6 μm are shown by the crosshatched portions in these figures. This cutoff was chosen because fibers

OPTICA

Fiber size (μm)
5–10
10–20
20–30
30–40
40–50
50–100
>100

[a] All data are me
sample = 1000–20
[b] Fibers with a d
[c] $P < 0.05$ when
[d] $P < 0.05$ when
[e] $P < 0.05$ when

greater than 0.6
do the fibers w
the length distr
chrysotile usin
length distribut
of fibers and fil
similar to that c
in Coalinga Mi
fibers exceedin
For both the
combined fiber
2% of the com
ess than 5 μm
he Jeffrey and
he combined fi
than 10 μm.
The log distr
Jeffrey Mine cl
ibers or fiber c
ibers and fiber
preparation.
The log aspe
the majority of
(Figs. 3A and
fibers exceedin
fiber clusters e
Tables 3–5 c

TABLE 2

OPTICAL MICROSCOPY FIBER CHARACTERIZATION: PERCENTAGE (%) OF ALL FIBERS >5 μm IN EACH SIZE CLASS[a,b]

Fiber size (μm)	Coalinga	UICC B	Jeffrey
5–10	32.9 ± 7.4	31.0 ± 6.8	28.5 ± 6.5*
10–20	28.8 ± 2.7	27.9 ± 2.3	25.3 ± 2.7*#
20–30	17.1 ± 3.4	17.6 ± 3.5	17.2 ± 3.0
30–40	10.2 ± 2.3	11.0 ± 2.5	11.6 ± 2.5*
40–50	6.0 ± 1.8	6.8 ± 1.7†	8.2 ± 1.8*#
50–100	3.3 ± 1.3	3.9 ± 1.5	5.7 ± 1.9*#
>100	1.5 ± 1.1	1.9 ± 0.8	3.6 ± 1.7*#

[a] All data are means ± SD, $n = 52$ for each chrysotile preparation. Number of fibers counted per sample = 1000–2000.

[b] Fibers with a diameter greater than 3 μm were not included in this study.

* $P < 0.05$ when comparing Jeffrey to Coalinga using Duncan's multiple comparison test.

† $P < 0.05$ when comparing UICC B to Coalinga using Duncan's multiple comparison test.

# $P < 0.05$ when comparing Jeffrey to UICC B using Duncan's multiple comparison test.

greater than 0.6 μm have a substantially smaller probability of being respired than do the fibers with smaller diameters (Pooley and Clark, 1979). Figure 1A shows the length distribution for combined fibers and fiber clusters of Jeffrey Mine chrysotile using a normal numerical distribution plot. Figure 1B illustrates the length distribution for Jeffrey chrysotile using a log scale. The length distribution of fibers and fiber clusters for UICC B chrysotile, illustrated in Fig. 1C, is very similar to that of Jeffrey Mine chrysotile. Combined fiber and fiber cluster length in Coalinga Mine chrysotile, seen in Fig. 1D, shows a distribution with many fibers exceeding 30 μm in length.

For both the Jeffrey and the UICC B chrysotile, approximately 75% of the combined fibers and fiber clusters were less than 5 μm in length, while less than 52% of the combined fibers and fiber clusters from the Coalinga chrysotile were less than 5 μm in length. At least 92% of the combined fibers and fiber clusters in the Jeffrey and UICC B aerosols were less than 10 μm in length, but only 66% of the combined fibers and fiber clusters in the Coalinga chrysotile aerosol were less than 10 μm.

The log distribution for combined fiber and fiber cluster width is similar for Jeffrey Mine chrysotile and UICC B chrysotile as seen in Figs. 2A and 2B. Few fibers or fiber clusters exceed 0.6 μm in diameter. In contrast, (Fig. 2C) numerous fibers and fiber clusters exceed 0.6 μm in width in the Coalinga Mine chrysotile preparation.

The log aspect ratio for the Jeffrey Mine chrysotile and UICC B chrysotile show the majority of the fibers and fiber clusters having an aspect ratio less than 100:1 (Figs. 3A and 3B). However, both the Jeffrey and UICC B chrysotile have some fibers exceeding this aspect ratio. The Coalinga Mine chrysotile had no fibers or fiber clusters exceeding an aspect ratio of 100:1 (Fig. 3C).

Tables 3–5 contain a detailed description of the length distribution of fibers and

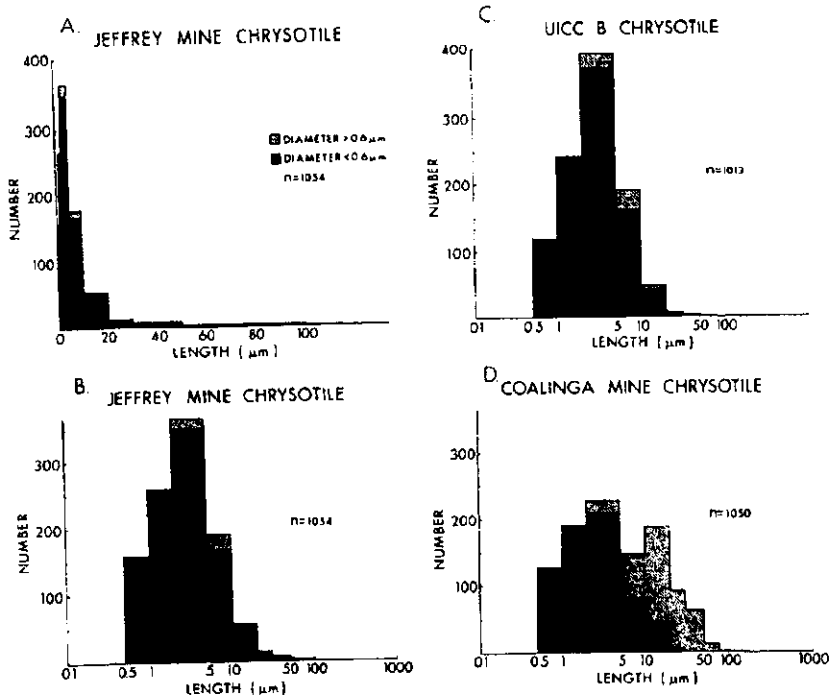

FIG. 1. Frequency distribution of length for combined fibers and fiber clusters in each of the aerosolized chrysotile preparations. (A) Jeffrey Mine chrysotile plotted on a linear scale for length. (B–D) Chrysotile preparations plotted on a log scale for length.

fiber clusters, each as a separate category. The fibers and fiber clusters are expressed as numbers counted per length interval, the percentage of the total found in each length interval, the cumulative percentage, and the mean diameter of fibers or fiber clusters in each length interval. The fibers and fiber clusters for each preparation are also expressed in terms of their respective aspect ratios in Tables 3–5.

A comparison of Tables 3–5 demonstrates that the mean diameter of fibers or fiber clusters for any given length interval is similar for the Jeffrey and UICC B preparations. There was no fiber or fiber cluster measured in either the Jeffrey or UICC B preparation that exceeded 2 μm in diameter. The aspect ratio increased for both fibers and fiber clusters with increasing length in the Jeffrey and UICC B preparations. The Coalinga preparation has a similar mean fiber and fiber cluster diameter below the 5-μm length interval. However, for fibers and fiber clusters longer than 5 μm in length, the mean diameter in the Coalinga preparation is significantly greater for both fibers and fiber clusters compared to the Jeffrey and UICC B preparations. In general, as the fiber or fiber cluster length increased in the Coalinga preparation, the width also increased. The presence of "thick" fibers

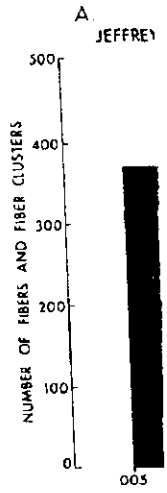

FIG. 2. Frequ
aerosolized chrys

and "thick" f
aspect ratios
The results
correlate clos
criteria for fib

FIG. 3. Freq
aerosolized chr

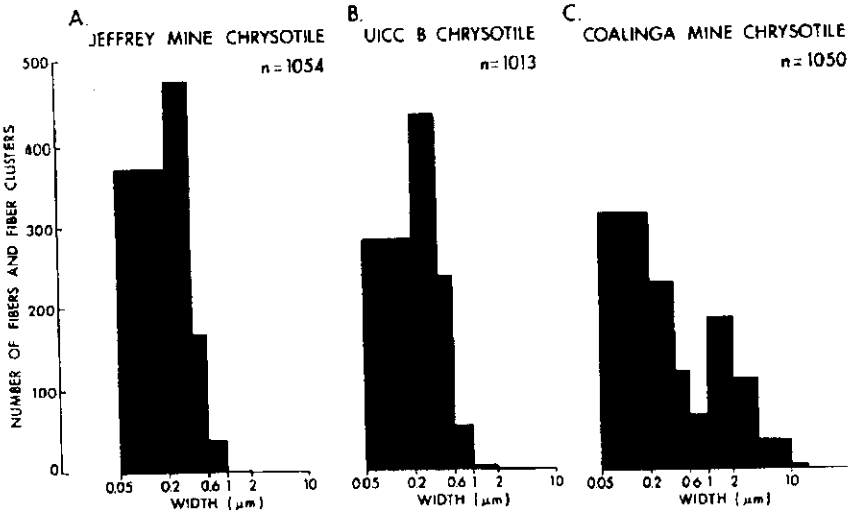

FIG. 2. Frequency distribution of log width for combined fibers and fiber clusters in each aerosolized chrysotile preparation.

and "thick" fiber clusters in the Coalinga preparation is reflected in the smaller aspect ratios which in any length interval seldom exceeded 60:1.

The results of fiber counting by optical microscopy cannot be expected to correlate closely with those obtained by scanning electron microscopy. The criteria for fiber counting by optical microscopy includes all fibers less than 3 μm

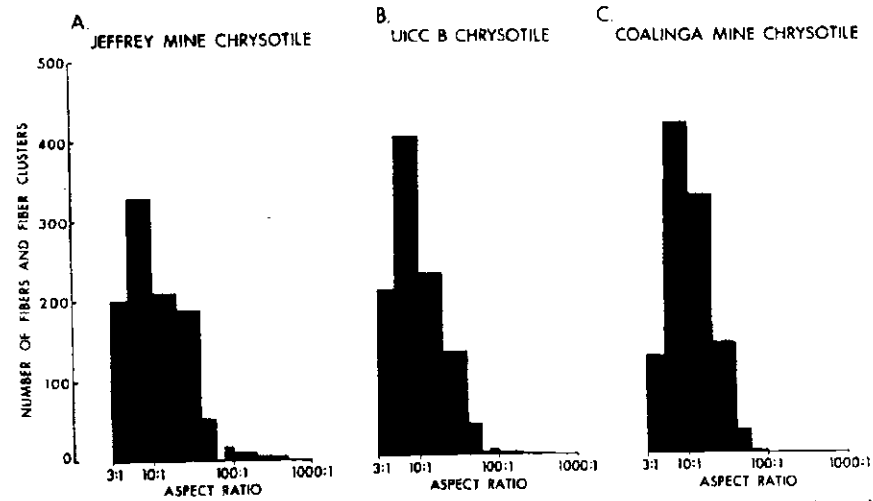

FIG. 3. Frequency distribution of aspect ratio for combined fibers and fiber clusters in each aerosolized chrysotile preparation. (n = 1054, 1013, and 1050, respectively.)

TABLE 3

PARTICLE SIZE DISTRIBUTION OF JEFFREY MINE CHRYSOTILE IN THE AEROSOLIZED STATE

	Length interval (μm)								
	0–0.99	1–1.99	2–4.99	5–9.99	10–19.9	20–29.9	30–49.9	50–99.9	>100
Fiber, n = 767									
Number	137	205	250	110	42	13	5	4	1
Percentage of total	17.9	26.7	32.6	14.3	5.5	1.7	0.7	0.6	0.1
Cumulative percentage	17.9	44.6	77.2	91.5	97.0	98.7	99.3	99.9	100
Mean diameter	0.20	0.22	0.28	0.29	0.31	0.48	0.30	0.50	0.50
Percentage of fibers by aspect ratio									
3:1–4.99:1	100	3	2	0	0	0	0	0	0
5:1–9.99:1	0	97	5	0	0	0	0	0	0
10:1–19.9:1	0	0	56	10	0	0	0	0	0
20:1–39.9:1	0	0	37	68	17	8	0	0	0
40:1–59.9:1	0	0	0	22	67	0	20	0	0
60:1–79.9:1	0	0	0	0	0	0	0	0	0
80:1–99.9:1	0	0	0	0	17	69	20	0	0
100:1–199:1	0	0	0	0	0	23	60	50	0
200:1–499:1	0	0	0	0	0	0	0	50	100
>500:1	0	0	0	0	0	0	0	0	0
Fiber cluster, n = 287									
Number	21	62	117	68	14	1	2	2	0
Percentage of total	7.3	21.6	40.8	23.7	4.9	0.3	0.7	0.6	0.0
Cumulative percentage	7.3	28.9	69.7	93.4	98.3	98.6	99.3	100	100
Mean diameter	0.30	0.34	0.44	0.48	0.46	0.8	0.4	0.6	—
Percentage of fiber clusters by aspect ratio									
3:1–4.99:1	100	100	11	1	0	0	0	0	0
5:1–9.99:1	0	0	89	15	0	0	0	0	0
10:1–19.9:1	0	0	0	84	14	0	0	0	0
20:1–39.9:1	0	0	0	0	86	100	0	0	0
40:1–59.9:1	0	0	0	0	0	0	0	0	0
60:1–79.9:1	0	0	0	0	0	0	0	0	0
80:1–99.9:1	0	0	0	0	0	0	0	0	0
100:1–199:1	0	0	0	0	0	0	100	100	0
200:1–499:1	0	0	0	0	0	0	0	0	0
>500:1	0	0	0	0	0	0	0	0	0
Nonfibrous particle, n = 174									
Number	105	47	22	0	0	0	0	0	0

TABLE 4

PARTICLE SIZE DISTRIBUTION OF UICC B CHRYSOTILE IN THE AEROSOLIZED STATE

	Length interval (μm)								
	0–0.99	1–1.99	2–4.99	5–9.99	10–19.9	20–29.9	30–49.9	50–99.9	>100
Fiber, n = 610									
Number	104	181	211	75	26	8	3	1	1
Percentage of total	17.0	29.7	34.6	12.3	4.3	1.3	0.5	0.2	0.2
Cumulative percentage	17.0	46.7	81.3	93.6	97.8	99.1	99.6	99.8	100
Mean diameter	0.19	0.23	0.30	0.34	0.34	0.38	0.36	0.30	0.80
Percentage of fibers by aspect ratio									
[illegible]	100	7	1	0	0	0	0	0	0

80:1–99.9:1	0	0	0	0	0	0	100	100	0
100:1–199:1	0	0	0	0	0	0	0	0	0
200:1–499:1	0	0	0	0	0	0	0	0	0
>500:1	0	0	0	0	0	0	0	0	0
Nonfibrous particle, n = 174									
Number	105	47	22	0	0	0	0	0	0

TABLE 4

PARTICLE SIZE DISTRIBUTION OF UICC B CHRYSOTILE IN THE AEROSOLIZED STATE

	Length interval (μm)								
	0–0.99	1–1.99	2–4.99	5–9.99	10–19.9	20–29.9	30–49.9	50–99.9	>100
Fiber, n = 610									
Number	104	181	211	75	26	8	3	1	1
Percentage of total	17.0	29.7	34.6	12.3	4.3	1.3	0.5	0.2	0.2
Cumulative percentage	17.0	46.7	81.3	93.6	97.8	99.1	99.6	99.8	100
Mean diameter	0.19	0.23	0.30	0.34	0.34	0.38	0.36	0.30	0.80
Percentage of fibers by aspect ratio									
3:1–4.99:1	100	2	1	0	0	0	0	0	0
5:1–9.99:1	0	98	14	3	0	0	0	0	0
10:1–19.9:1	0	0	53	25	0	0	0	0	0
20:1–39.9:1	0	0	31	49	23	0	0	0	0
40:1–59.9:1	0	0	0	23	73	38	0	0	0
60:1–79.9:1	0	0	0	0	0	0	0	0	0
80:1–99.9:1	0	0	0	0	4	63	33	0	0
100:1–199:1	0	0	0	0	0	0	67	0	100
200:1–499:1	0	0	0	0	0	0	0	100	0
>500:1	0	0	0	0	0	0	0	0	0
Fiber cluster, n = 403									
Number	16	62	181	115	23	5	1	0	0
Percentage of total	4.0	15.4	44.9	28.5	5.7	1.2	0.2	0.0	0.0
Cumulative percentage	4.0	19.4	64.3	92.8	98.5	99.8	100	100	100
Mean diameter	0.30	0.30	0.44	0.51	0.41	0.67	0.40	—	—
Percentage of fiber clusters by aspect ratio									
3:1–4.99:1	100	100	9	1	0	0	0	0	0
5:1–9.99:1	0	0	91	21	0	0	0	0	0
10:1–19.9:1	0	0	0	78	26	0	0	0	0
20:1–39.9:1	0	0	0	0	74	60	0	0	0
40:1–59.9:1	0	0	0	0	0	0	0	0	0
60:1–79.9:1	0	0	0	0	0	40	0	0	0
80:1–99.9:1	0	0	0	0	0	0	0	0	0
100:1–199:1	0	0	0	0	0	0	100	0	0
200:1–499:1	0	0	0	0	0	0	0	0	0
>500:1	0	0	0	0	0	0	0	0	0
Nonfibrous particle, n = 138									
Number	105	27	6	0	0	0	0	0	0

TABLE 5
PARTICLE SIZE DISTRIBUTION OF COALINGA MINE CHRYSOTILE IN THE AEROSOLIZED STATE

	Length interval (μm)									
	0–.99	1–1.99	2–2.99	3–4.99	5–9.99	10–19.9	20–29.9	30–49.9	50–99.9	>100
Fiber, n = 818										
Number	125	188	112	93	110	117	48	20	5	0
Percentage of total	15.3	23.0	13.7	11.4	13.4	14.3	5.9	2.4	0.6	0.0
Cumulative percentage	15.3	38.3	52.0	63.3	76.8	91.1	96.9	99.4	100	100
Mean diameter	0.15	0.20	0.26	0.39	0.71	1.08	2.15	2.36	2.92	—
Percentage of fibers by aspect ratio										
3:1–4.99:1	52	3	0	5	11	3	6	5	0	—
5:1–9.99:1	48	75	60	27	13	25	19	5	0	—
10:1–19.9:1	0	22	20	48	45	26	40	35	40	—
20:1–39.9:1	0	0	11	16	25	33	23	45	20	—
40:1–59.9:1	0	0	0	3	5	12	10	5	20	—
60:1–79.9:1	0	0	0	0	1	0	0	0	0	—
80:1–99.9:1	0	0	0	0	0	1	2	5	20	—
100:1–199:1	0	0	0	0	0	0	0	0	0	—
200:1–499:1	0	0	0	0	0	0	0	0	0	—
>500:1	0	0	0	0	0	0	0	0	0	—
Fiber cluster, n = 232										
Number	1	3	4	19	37	72	44	42	9	1
Percentage of total	0.4	1.3	1.7	8.2	15.9	31.0	19.0	18.1	3.8	0.4
Cumulative percentage	0.4	1.7	3.4	11.6	27.6	58.6	77.6	95.7	99.6	100
Mean diameter	0.30	0.30	0.40	0.59	0.95	1.79	2.42	3.32	6.17	5.5
Percent of fiber clusters by aspect ratio										
3:1–4.99:1	100	100	100	16	22	13	2	2	11	0
5:1–9.99:1	0	0	0	26	22	42	25	26	11	0
10:1–19.9:1	0	0	0	58	56	31	50	38	67	0
20:1–39.9:1	0	0	0	0	0	8	23	33	11	100
40:1–59.9:1	0	0	0	0	0	7	0	0	0	0
60:1–79.9:1	0	0	0	0	0	0	0	0	0	0
80:1–99.9:1	0	0	0	0	0	0	0	0	0	0
100:1–199:1	0	0	0	0	0	0	0	0	0	0
200:1–499:1	0	0	0	0	0	0	0	0	0	0
>500:1	0	0	0	0	0	0	0	0	0	0
Nonfibrous particle, n = 224										
Number	143	46	16	12	7	0	0	0	0	0

in diameter and greater thai
counting by scanning electr
eters so long as they possess
of the fiber–fiber clusters i
counted by scanning electro
microscopy. For the aeros
clusters would not have be
fiber–fiber cluster number e
fiber–fiber cluster number

Nonfibrous Particle Analys

The elements detected in t
chrysotile preparation are sl
analyzed in the Jeffrey and
ence of magnesium and silic
fiber clusters analyzed in all
not a means of identification
to represent fragments of cl
same Mg-to-Si ratio as chry
the UICC B reference sam
particles with low magnesi
Jeffrey Mine aerosolized sa

Two of the chrysotile p
characterized in this study
state. UICC B chrysotile h
and Beckett (1973) in the a
Mine samples into the spec
mental inhalation research,
present study to the work
aerosolization studies of Ti
manner of fiber collection
analysis of samples, the stu
ble to the present study (Tal
tion of the fibers falling int
Beckett and Timbrell. This
and/or counting techniques.
UICC B chrysotile are near
fiber lengths. Beckett (197
only fibers longer than 5 μ
expressed in a similar fashi
tionally fewer long fibers th
to differences in the manne
collected.

The Jeffrey Mine and Co

in diameter and greater than 5 μm in length. The criteria for fiber–fiber cluster counting by scanning electron microscopy includes fibers of all lengths and diameters so long as they possess at least a 3 to 1 aspect ratio. By these standards, 75% of the fiber–fiber clusters in the aerosolized Jeffrey and UICC B preparations counted by scanning electron microscopy would not have been counted by optical microscopy. For the aerosolized Coalinga preparation, 60% of the fiber–fiber clusters would not have been counted by optical microscopy (10% of the total fiber–fiber cluster number exceeded the 3-μm-diameter limit and 50% of the total fiber–fiber cluster number was less than 5 μm in length).

Nonfibrous Particle Analysis

The elements detected in the 50 randomly selected nonfibrous particles for each chrysotile preparation are shown in Table 6. One half of the nonfibrous particles analyzed in the Jeffrey and UICC B preparation samples demonstrated the presence of magnesium and silicon only. The magnesium-to-silicon ratio for fibers and fiber clusters analyzed in all three preparations ranged from 0.62 to 1.34. Although not a means of identification, particles with a Mg-to-Si ratio in this range are likely to represent fragments of chrysotile. The percentage of particles possessing the same Mg-to-Si ratio as chrysotile is 40% in the Jeffrey Mine preparation, 52% in the UICC B reference sample, and 20% in the Coalinga Mine preparation. The particles with low magnesium-to-silicon ratios of 0.30 to 0.40, found only in the Jeffrey Mine aerosolized sample, may represent talc particles.

DISCUSSION

Two of the chrysotile preparations designed for experimental research and characterized in this study have not been previously studied in the aerosolized state. UICC B chrysotile has been studied extensively by both Timbrell (1970a) and Beckett (1973) in the aerosolized state. To fit the Coalinga Mine and Jeffrey Mine samples into the spectrum of chrysotile preparations available for experimental inhalation research, UICC B chrysotile was used as a reference to link the present study to the work carried out by other investigators, in particular, the aerosolization studies of Timbrell and Beckett. Although differences exist in the manner of fiber collection from the dust chambers and in the preparation and analysis of samples, the studies of Timbrell and Beckett on UICC B are comparable to the present study (Table 7). By light microscopy we found a greater proportion of the fibers falling into longer fiber length intervals than did the studies of Beckett and Timbrell. This difference may reflect differences in fiber preparation and/or counting techniques. By electron microscopy, the results of this study for UICC B chrysotile are nearly identical to those of Timbrell for the distribution of fiber lengths. Beckett (1973) used SEM to determine fiber length and evaluated only fibers longer than 5 μm. When our present results were recalculated and expressed in a similar fashion (Table 7), we found a similar pattern, but proportionally fewer long fibers than did Beckett. Some of these differences may be due to differences in the manner in which the aerosols were generated and the samples collected.

The Jeffrey Mine and Coalinga Mine chrysotiles have been elegantly analyzed

TABLE 6
ELEMENTAL COMPOSITION OF THE NONFIBROUS PARTICLES IN EACH CHRYSOTILE PREPARATION[a]

Elements detected	Percentage occurrence		
	Jeffrey	UICC B	Coalinga
NaMgAlSiCa	2	—	—
NaAl	—	—	6
NaAlSiKCaFe	2	—	—
Mg	2	2	4
MgAl	4	—	—
MgAlSi	14	30	10
MgAlSiKFe	2	—	—
MgAlSiCaFe	—	2	—
MgAlSiCrFe	—	—	2
MgAlSiFe	6	—	2
MgSi ratio	50	54	20
Mg to Si = 0.3–0.4	(6)	(—)	(—)
Mg to Si = 0.6–1.1	(40)	(52)	(20)
Mg to Si = 2.0–10	(4)	(2)	(—)
MgSiCa	—	2	—
MgSiFe	—	—	6
MgK	—	2	—
Al	2	4	14
AlSi	6	—	4
AlSiCa	2	—	—
AlSiK	—	—	2
AlSiCr	—	—	4
AlSiFe	—	—	2
AlK	2	—	—
AlKCa	—	—	2
AlCr	2	—	14[b]
SiCr	—	—	2
CaCr	—	—	2
Cr	—	—	4[b]
Fe	2	—	—
None	2	4	—

[a] 50 random nonfibrous particles were analyzed for each chrysotile preparation.
[b] May represent a contaminant from the rotary blade used to aerosolize the preparation.

by others using the sample preparation technique of fiber dispersion in a liquid medium (Wylie, 1979; Campbell *et al.*, 1980; Siegrist and Wylie, 1980). This type of analysis is satisfactory for studies in which the preparations are to be ingested or injected in suspension. However, these studies do not provide an adequate characterization of the asbestos preparations for inhalation studies since the process of aerosolization is generally less efficient in fiber dispersion. A case in point is the Coalinga Mine chrysotile preparation. This asbestos preparation was characterized by Campbell *et al.* (1980) using fiber samples which were dispersed in a liquid medium by ultrasonification for 10 min. The samples were analyzed by transmission electron microscopy. They found that 2.1% of the total chrysotile

TABLE 7

FIBER LENGTH DISTRIBUTION—OPTICAL MICROSCOPY

Preparation	Dispersion Method	Study	Percentage longer than stated length (μm) 4	5	10	20	40
UICC B	Aerosol	Present		100	68.2	40.5	12.6
UICC B	Aerosol	Beckett[a]		100	32	11	5
UICC B	Aerosol	Timbrell[b]	100		22.3	11	1.7
UICC B	Alcohol	Timbrell[b]		100	46.9	21.5	7.0
UICC B	Celloidin	Timbrell[b]		100	53.6	16.9	3.8

FIBER LENGTH DISTRIBUTION—ELECTRON MICROSCOPY

Asbestos-dispersion method	Instrument	Study	Percentage longer than stated length (μm) 0.2	1	2	5	10	20
UICC B aerosol	TEM	Timbrell[b]	100	73.6	54.4	27.0	9.6	3.2
UICC B aerosol	SEM	Present	100	88.2	64.2	25.5	6.7	1.9
JEFFREY aerosol	SEM	Present	100	85.0	50.7	24.9	8.0	2.7
			5	10	20	30	50	60
UICC B aerosol	SEM	Beckett[a]	100	45	12	6		1
UICC B aerosol	SEM	Present	100	26.4	7.4	2.3	0.8	
JEFFREY aerosol	SEM	Present	100	32.1	10.8	5.5	2.7	

[a] From Figure 2a in Beckett, 1975.
[b] Modified from Tables 6, 7, or 8 in Timbrell, 1970a.

particles analyzed were greater than 10 μm in length and had a mean diameter of 0.42 μm. In the present study using aerosolized samples drawn directly upon Nucleopore filters and subsequently gold coated, it was found that 34.0% of the combined fibers and fiber clusters or 28.1% of all particles (fibers, fiber clusters, and nonfibrous particles) in the Coalinga preparation were greater than 10 μm in length and had a mean diameter of 2.92 μm. In a subsequent study by Siegrist and Wylie (1980), the Coalinga preparation (referred to as short-range chrysotile) was characterized for particle size distribution by both transmission and scanning electron microscopy. The samples were prepared by hand swirling in distilled water and dishwashing liquid (for SEM analysis) and by ultrasonification in water for 10 min (for TEM analysis). The results demonstrated that by both SEM and TEM more than 90–95% of the particles were less than 10 μm in length and more than 95% of the particles were less than 1 μm in diameter. In our study using aerosolized samples 71.9% of the total number of particles were less than 10 μm in length and 68% of the total number of particles were less than 1 μm in diameter.

The differences between these two methods of sample preparation and analysis

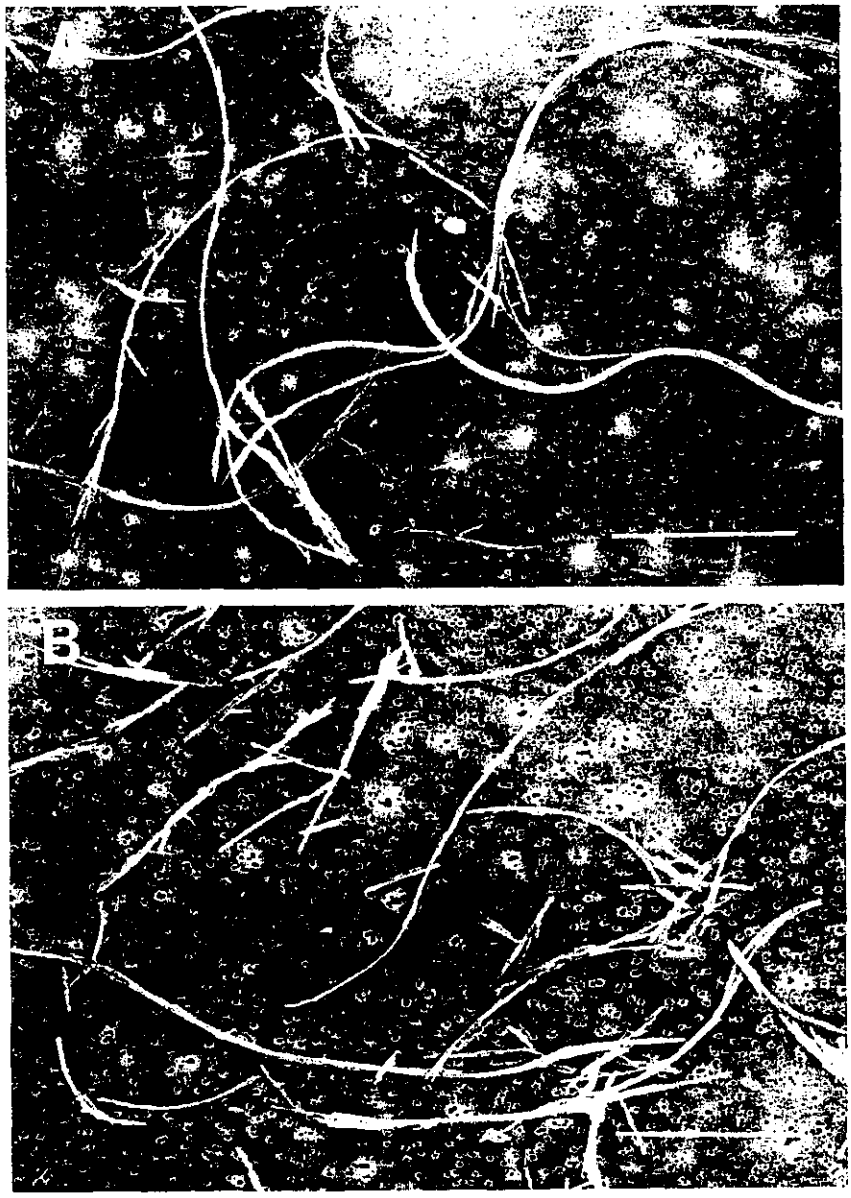

FIG. 4. (A) Aerosolized Jeffrey Mine chrysotile collected on a 0.2-μm Nucleopore filter and gold coated. Bar = 10 μm. (B) Aerosolized UICC B chrysotile. Bar = 10 μm. (C) Aerosolized Coalinga Mine chrysotile. Bar = 10 μm. Examples of a fiber (short arrow) and a fiber cluster (long arrow) are indicated in this micrograph. (D) Higher magnification of an aerosolized Coalinga Mine chrysotile fiber cluster demonstrating the fibrillar subunit composition. Bar = 1 μm.

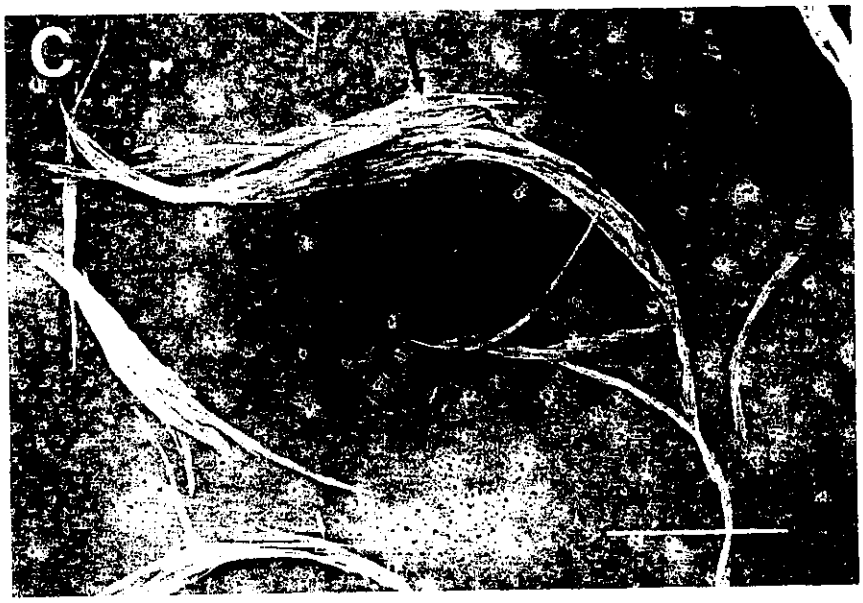

FIG. 4—*Continued.*

ıpore filter and gold
erosolized Coalinga
ster (long arrow) are
Mine chrysotile fiber

suggest that either ultrasonification breaks down fiber bundles into smaller bundles and fibrils or that fibers tend to cluster in the aerosolized state. Other investigators have shown that ultrasonification can result in the breakdown of chrysotile into smaller fibers (Spurny *et al.*, 1980). In addition, clustering of fibers caused by the aerosolization does not seem likely since the Jeffrey and UICC B chrysotile preparations collected in the same manner as the Coalinga chrysotile have distinctly different particle size distributions from that seen in the aerosolized Coalinga preparation. The illustration of each fiber preparation on the Nucleopore filters (Fig. 4) also show a distinct fiber morphology for the Coalinga chrysotile compared to the Jeffrey and UICC B chrysotile.

Some of the differences between each of the chrysotile preparations were also reflected by the gravimetric measurements taken of each chrysotile aerosol in the exposure chambers. Comparisons made between each chrysotile preparation of the ratio of respirable mass concentration to total mass concentration demonstrate a distinct difference between the Coalinga chrysotile and the other two preparations of Jeffrey and UICC B chrysotile. It would appear that compared to the Jeffrey and UICC B fibers the Coalinga preparation contains a greater proportion of particles which are captured in the elutriator system of the Casella sampler and in the precutter stages of the Cascade impactor. These fibers and particles are captured because of their greater mass.

Using scanning electron microscopy we found that the range of diameters of fibers and fiber clusters was greater for the Coalinga preparation than for the Jeffrey and UICC B preparations in the aerosolized state. The trend of increasing diameter with increasing length was found only in the Coalinga preparation. Fibers and fiber clusters greater than 10 μm in length with diameters exceeding 2 μm were present in the aerosolized Coalinga preparation while in the Jeffrey and UICC B preparations no fiber or fiber cluster of any length exceeded 2 μm in diameter.

The characterization of chrysotile in the aerosolized state in terms of fiber length and fiber diameter is paramount in understanding the nature of any preparation used for inhalation studies. Although the physical characteristics of a fiber may not be the only factor in causing lung injury, fiber diameter and, to a lesser degree, fiber length play key roles in the potential for a fiber to reach the alveolar regions of the lung where injury as a result of asbestos inhalation appears to be more severe.

The potential for each of these chrysotile preparations to cause lung injury by inhalation can be best assessed by a review of what is known about the physical characteristics a fiber must possess in order to reach the alveolar portions of the lung. Spherical particles below a certain diameter (approximately 3–4 μm) can reach the alveolar airspaces of the lung (Lippman and Albert, 1969). Larger particles are eliminated by deposition in the upper airways primarily through sedimentation and impaction as a result of their greater mass. Timbrell, Harris and Fraser have examined the more complex aerodynamic properties of fibers by comparing the deposition pattern of fibers to those of artificial spheres (Timbrell, 1965). In general they found that fibers have a similar deposition pattern to that of spheres of three to four times greater diameter. This would suggest that fibers

much greater than 1 μm in diameter would not be likely to reach the alveolar airspaces although the curvature of the fiber should also be taken into consideration (Timbrell, 1970b). Additional studies have shown that an equivalent fiber diameter of 0.5 to 2.0 μm results in deposition within respiratory bronchioles and proximal alveolar ducts, while fibers of smaller equivalent diameters result in maximal deposition in the more distal portions of the lung (Harris and Fraser, 1976). Pooley and Clark (1979) measured the diameter of chrysotile fibers recovered from lung specimens after tissue digestion. They found that only 0.06% of the fibers had a diameter exceeding 0.5 μm. Although it is possible that these chrysotile fibers have fragmented longitudinally while *in vivo* (Suzuki and Churg, 1969), these results suggest that few fibers with a diameter greater than 0.5 μm reach the alveolar regions of the lung. If this limit in fiber diameter is true for alveolar lung deposition and the length of the fiber plays the major role in fibrogenesis and cell injury, the potential for the three asbestos preparations reported in this study to cause lung injury by inhalation will be different. Only the Jeffrey and UICC B chrysotile preparations have fibers which are less than 0.6 μm in diameter when length is greater than 30 μm. The Coalinga Mine chrysotile does not possess this property; instead, with increasing fiber length, fiber diameter also increases. Most fibers longer than 20 μm in length in the aerosolized Coalinga preparations are also greater than 0.6 μm in diameter. Thus, in an inhalation study, more fibers of greater length distribution would be deposited in the alveolar region of the lungs for the Jeffrey and UICC B chrysotile preparations than for the Coalinga chrysotile preparation.

At this point one may ask the question, what constitutes a short–range fiber preparation? We have seen that the Coalinga preparation originally thought to be a short-fiber preparation contains numerous long fibers in the aerosolized state. The answer to this question should be based on the potential of a fiber to reach the alveolar regions of lung. In other words, is the fiber respirable? The work of investigators who have studied the aerodynamic properties of fibers (Timbrell, 1965, 1973) and the physical dimensions of respired fibers (Pooley and Clark, 1979), would suggest that the Coalinga chrysotile in the aerosolized state represents a short-fiber preparation since only fibers less than 30 μm in length are likely to be respirable. In contrast, the Jeffrey and UICC B chrysotile preparations contain fibers greater than 30 μm in length possessing a diameter which would permit penetration of the fiber into the alveolar regions of the lung. Based on these observations, the Coalinga Mine chrysotile preparation can be considered to represent a "shorter" fiber preparation in the aerosolized state.

In summary, the three chrysotile preparations characterized in the aerosolized state in this study demonstrated distinct properties. Gravimetric measurements of each chrysotile preparation revealed that both the Jeffrey and UICC B preparations have a significantly greater portion of the total chamber dust concentration which is respirable compared to the Coalinga preparation. By light microscopy it was found that for fibers greater than 5 μm in length, the Jeffrey preparation in the aerosolized state has a significantly greater fraction of fibers exceeding 40 μm in length than does UICC B chrysotile or Coalinga chrysotile. Finally, by scanning electron microscopy it was found that the Jeffrey and UICC B preparations pos-

sess fibers and fiber clusters which cross a large length range (the longest measured was 150 μm), but none which exceeded 2 μm in diameter. The Coalinga preparation also possessed fibers and fiber clusters across a similar length range, but many exceeded 2 μm in diameter. In terms of respirability, the Jeffrey and UICC B aerosolized fibers constitute a mixed short-range and long-range fiber preparation, while the Coalinga aerosolized fibers represent a somewhat short-fiber preparation in which very long respirable fibers are not present. In applying the above fiber characterization studies to experimental research it is essential to remember that further manipulation of these chrysotile preparations by grinding (Langer *et al.*, 1978) or by fiber separation techniques may alter the fiber size distribution from that presented in this paper.

ACKNOWLEDGMENTS

This work was supported in part by NIEHS Contracts NO1-ES-0-0004 and NO1-4-21-64-BCD.

REFERENCES

Assuncao, J., and Corn, M. (1975). The effects of milling on diameters and lengths of fibrous glass and chrysotile asbestos fibers. *Amer. Ind. Hyg. Assoc. J.* 36, 811–819.

Beckett, S. T. (1973). The evaluation of airborne asbestos fibres using a scanning electron microscope. *Ann. Occup. Hyg.* 16, 405–408.

Campbell, W. J., Huggins, C. W., and Wylie, A. G. (1980). "Chemical and Physical Characterization of Amosite, Chrysotile, and Nonfibrous Tremolite for Oral Ingestion Studies by the National Institute of Environmental Health Sciences." U.S. Bur. Mines Rep. Invest. 8452.

Harris, R. L., and Fraser, D. A. (1976). A model for deposition of fibers in the human respiratory system. *Amer. Ind. Hyg. Assoc. J.* 37, 73.

Langer, A. M., Wolff, M. S., Rohl, A. N., and Selikoff, I. F. (1978). Variation of properties of chrysotile asbestos subjected to milling. *J. Toxicol. Environ. Health* 4, 173–188.

Lippman, M., and Albert, R. E. (1969). The effect of particle size on the regional deposition of inhaled aerosols in the human respiratory tract. *Amer. Ind. Hyg. Assoc. J.* 30, 257–275.

Morgan, A., and Cralley, L. J. (1973). Chemical characteristics of asbestos and associated trace elements. *In* "Biological Effects of Asbestos" (J. C. Wagner, Ed.), pp. 113–118. IARC Scientific Publication, Lyon.

Pooley, F. D., and Clark, N. (1979). Fiber dimensions and aspect ratio of crocidolite, chrysotile and amosite particles detected in lung tissue specimens. *Ann. N.Y. Acad. Sci.* 330, 711–716.

Pott, F. (1978). Some aspects on the dosimetry of the carcinogenic potency of asbestos and other fibrous dusts. *Staub–Reinhalt Luft* 38, 486–490.

Rendall, R. E. G. (1970). The data sheets on the chemical and physical properties of the UICC standard reference samples. *In* "Pneumoconiosis: Proceedings of the International Conference, Johannesburg, 1969" (H. A. Shapiro, Ed.), pp. 23–27. Oxford Univ. Press, London.

Rendall, R. E. G. (1980). Physical and chemical characteristics of UICC reference samples. *In* "Biological Effects of Mineral Fibres, (J. C. Wagner, Ed.), Vol. 1, pp. 87–96. IARC Scientific Publication No. 30; IARC, Lyon.

Seaton, A. (1975). Asbestosis. *In* "Occupational Lung Diseases" (Morgan, C. Keith, and A. Seaton, Eds.). Saunders, Philadelphia.

Siegrist, H. G., and Wylie, A. G. (1980). Characterizing and discriminating the shape of asbestos particles. *Environ. Res.* 23, 348–361.

Sinclair, W. E. (1959). "Asbestos—Its Origin, Production, and Utilization." Mining publication, London.

Spurny, K. R., Opiela, H., and Weiss, G. (1980). On the milling and ultrasonic treatment of fibres for biological and analytical applications. *In* "Biological Effects of Mineral Fibres" (J. C. Wagner, Ed.), pp. 931–936. IARC Scientific Publication No. 30, IARC, Lyon.

Stanton, M. F., and Layard, M. (1978). "The Carcinogenicity of Fibrous Minerals. Workshop on Asbestos: Definitions and Measurement Methods," pp. 143–151. NBS Special Publication 506.

Suzuki, M. D., and Churg, J. (1969). Structure and development of the asbestos body. *Amer. J. Pathol.* **55**, 79–107.

Timbrell, V. (1965). The inhalation of fibrous dusts. *Ann. N.Y. Acad. Sci.* **132**, 255–273.

Timbrell, V. (1968). A simple dispenser for generating dust clouds from standard reference samples of asbestos. *Ann. Occup. Hyg.* **11**, 273–281.

Timbrell, V. (1970a). Characteristics of the International Union Against Cancer standard reference samples of asbestos. *In* "Pneumoconiosis: Proceedings of the International Conference, Johannesburg, 1969" (H. A. Shapiro, Ed.), pp. 28–36. Oxford Univ. Press, London.

Timbrell, V. (1970b). The inhalation of fibers. *In* "Pneumoconiosis: Proceedings of the International Conference, Johannesburg, 1969" (H. A. Shapiro, Ed.), pp. 3–9. Oxford Univ. Press, London.

Timbrell, V. (1973). Physical factors as etiological mechanisms. *In* "Biological Effects of Asbestos" (J. C. Wagner, Ed.), pp. 295–303. IARC Scientific Publication, Lyon.

Timbrell, V., Gibson, J. C., and Webster, I. (1968). UICC standard reference samples of asbestos. *Int. J. Cancer* **3**, 406–408.

Wagner, J. C. (1965). The sequelae of exposure to asbestos dust. *Ann. N.Y. Acad. Sci.* **132**, 691.

Wylie, A. G. (1979). Fiber length and aspect ratio of some selected asbestos samples. *Ann. N.Y. Acad. Sci.* **330**, 605–610.