Biopersistence of Man-Made Vitreous Fibers and Crocidolite Asbestos in the Rat Lung Following Inhalation

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This study investigated possible relationships between fiber biopersistence in the lung and previously observed differences in pulmonary toxicity between asbestos and man-made vitreous fibers (MMVF) following inhalation exposure. Fischer 344/N rats were exposed nose only, 6 hr/day for 5 days to 30 mg/m³ MMVF (two fiberglass compositions, rock wool, or slag wool) or to 10 mg/m³ crocidolite asbestos. At eight time points up to 1 year postexposure, lung fiber burdens were analyzed for number/lung and bivariate dimensions using scanning electron microscopy (SEM) and for chemical composition using SEM energy dispersive spectroscopy. After 365 days, >95% of long (>20 μm) MMVFs had disappeared from the lung compared to only 17% of long crocidolite fibers. Longer MMVF disappered more rapidly than short MMVFs, suggesting that long fibers were dissolving or breaking. Mean diameters and lengths of the MMVFs decreased with time, while the mean diameter of crocidolite remained unchanged and its mean length showed an apparent increase, probably related to macrophage-mediated clearance of short fibers. Leaching of oxides occurred in the fibrous glasses and slag wool and correlated with morphological changes in the fibers over time. No chemical or morphological changes were observed in crocidolite fibers. These changes in MMVF number, chemistry, and morphology over time in lung tissue compared to crocidolite asbestos demonstrate the relatively low biopersistence of some MMVFs in the lung and may explain why these MMVFs are not tumorigenic in rats, even after chronic exposure at high concentrations.

Man-made vitreous fibers (MMVFs) are inorganic amorphous fibers made primarily from glass, rock, clay, slag, or pure oxide raw materials. Glass wool, rock wool, and slag wool fall under the major classification of insulation wool and are used as residential and commercial thermal and acoustic insulations. These fibers are also incorporated in other building products such as pipe insulation and ceiling tile. Human respirable size fractions make up varying percentages of all MMVFs and depend on the nature of the product of which they are a part.

Since the discovery of the toxic nature of asbestos, an ongoing concern has existed regarding the safety of all materials which contain respirable fibers. A series of long-term inhalation studies was conducted to determine the chronic biological effects in rodents of respirable fractions of different MMVFs, including fibrous glass, rock (stone) wool, and slag wool (Hesterberg et al., 1993; McConnell et al., 1994; Mast et al., 1995; reviewed in Hesterberg et al., 1995). Exposure to 10 mg/m³ of crocidolite or chrysotile asbestos induced early pulmonary fibrosis, lung tumors, and mesothelioma in rats, thus validating the inhalation model with known human carcinogenic fibers. Exposure of rats to 30 mg/m³ of fiberglass (MMVF 10 or 11) or slag wool (MMVF 22) was associated with an inflammatory response, but no lung fibrosis, mesotheliomas, or significant increase in the lung tumors were observed. Rock wool (stone wool, MMVF 21) at the same exposure level resulted in minimal lung fibrosis late in the study, but no mesotheliomas or significant increase in lung tumors were observed.

The present study was initiated to gain a better understanding of the role of biopersistence in pulmonary toxicity of fibers. Biopersistence of four different compositions of commercial MMVFs was compared to that of crocidolite asbestos. Inhalation was chosen as the method of exposure because that is the human exposure route. Furthermore, inhalation ensures uniform distribution of fibers in the deep lung. The four MMVF study fibers were size separated from commercial insulation wools to have approximate arithmetic mean dimensions of 1 × 20 μm. These dimensions were chosen to simulate workplace exposures (Hesterberg and Hart, 1994; Cherrie et al., 1986; Esman, 1984) and to maximize deposition in the deep lung of rats (Morgan et al., 1980). The crocidolite asbestos was also size separated to

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TABLE 1
Aerosol Fiber Dimensions and Concentrations

<table>
<thead>
<tr>
<th>Test fiber</th>
<th>Diameter (μm)</th>
<th>Length (μm)</th>
<th>Diameter (μm)</th>
<th>Length (μm)</th>
<th>mg/m³ Air (SD)</th>
<th>Fibers/cc by length category (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMVF 10</td>
<td>1.3 (0.7)</td>
<td>15 (15)</td>
<td>1.1 (1.2)</td>
<td>11 (1.9)</td>
<td>32 (3.4)</td>
<td>420 (15) 348 (12) 203 (25) 81 (21)</td>
</tr>
<tr>
<td>MMVF 11</td>
<td>1.0 (0.7)</td>
<td>17 (17)</td>
<td>0.7 (2.1)</td>
<td>12 (2.0)</td>
<td>29 (3.8)</td>
<td>329 (51) 252 (32) 174 (24) 84 (7)</td>
</tr>
<tr>
<td>MMVF 21</td>
<td>1.1 (0.6)</td>
<td>27 (29)</td>
<td>0.9 (2.0)</td>
<td>16 (2.3)</td>
<td>31 (6.3)</td>
<td>276 (35) 228 (28) 181 (23) 119 (13)</td>
</tr>
<tr>
<td>MMVF 22</td>
<td>1.0 (0.5)</td>
<td>22 (23)</td>
<td>0.9 (1.8)</td>
<td>15 (2.2)</td>
<td>30 (3.2)</td>
<td>330 (44) 283 (24) 209 (21) 123 (17)</td>
</tr>
<tr>
<td>Crocidolite</td>
<td>0.3 (0.1)</td>
<td>5.7 (5.3)</td>
<td>0.3 (1.6)</td>
<td>4.2 (2.1)</td>
<td>11 (5.5)</td>
<td>6928 (2418) 2568 (899) 935 (366) 287 (234)</td>
</tr>
</tbody>
</table>

Note. n = 7 for mg/m³; n = 7 for total fibers; n = 4 for all others.

increase the fraction of fibers with lengths greater than 5 μm, i.e., fibers meeting the World Health Organization definition for respirable fibers (‘‘WHO fibers’’; WHO, 1985). The MMVF target aerosol concentration of 30 mg/m³ was chosen because significant lung deposition of fibers had been achieved at this concentration in previous inhalation studies (Hesterberg et al., 1993). The crocidolite aerosol target concentration of 10 mg/m³ was chosen to be similar to the aerosol level used in a previous chronic inhalation study (McConnell et al., 1994).

The parameters studied included the number of fibers per cm³ of aerosol, the number of fibers per lung, fiber dimensions (length and diameter), surface characteristics, and chemistry of fibers retained in the lungs at various time points up to 1 year after the termination of the inhalation exposure.

FIG. 1. Bivariate histogram showing the frequency of paired diameter and length measurements of fibers in (A) MMVF 21 aerosol and (B) MMVF 21 recovered from lungs after 1 day of recovery.

METHODS AND MATERIALS

Fiber characteristics. The four MMVF test fibers used in this study were derived from insulation wools: Schuller 901 glass wool (MMVF 10), CertainTeed B glass wool (MMVF 11), Rockwool International rock (stone) wool (MMVF 21), and USG Interiors slag wool (MMVF 22). MMVF 10 and 21 were supplied without oil or binder. MMVF 11 was supplied with oil on the surface, most of which was removed by ethyl ether extraction after size separation. MMVF 22 was supplied with an oil treatment on the surface of the fiber which was not removed after size separation. Size separation of study fibers from bulk insulation was accomplished by aqueous suspension techniques at Mountain Technical Center (MTC; Littleton, CO). The mass of each size-separated study fiber was <5% of the original mass of the insulation material from which it was derived. Crocidolite, obtained from the National Institute of Environmental Health Sciences (NIEHS), originally had geometric mean dimensions of 0.2 × 3 μm. To make the NIEHS crocidolite more comparable to the length of the MMVF s, this natural mineral fiber was size separated at MTC to eliminate the shorter fibers and increase the geometric mean length to approximately 7 μm.
were determined once during pretest and twice each day during the 5-day exposure period. In addition, to assure the uniformity of exposure during each 6-hr exposure period, the fiber concentrations were monitored continuously using a RAS (GCA Corp.) light scattering monitor. Each aerosol was sampled twice during the first 2 days of exposure and once per day during the last 3 days of the 5-day exposure period to determine fiber concentrations. Clarified slides were made from the MMVF aerosol sample filters for counting by phase-contrast optical microscopy using WHO Monograph 4 counting rules. Crocidolite aerosol samples were counted using scanning electron microscopy (SEM) at 5000× using WHO Monograph 4 rules with the exception that all fibers with aspect ratio greater than 3 were counted. Crocidolite fibers meeting the WHO fiber definition of aspect ratio of ≥3:1, length >5 μm, and diameter <3 μm were calculated from the count of all fibers with aspect ratio >3 and the diameter and length distribution data. Details of sampling and counting were described previously (Hesterberg et al., 1993).

Each day during the exposure period a sample of each fiber aerosol was captured on a filter for determination of bivariate distribution. Fibers captured on these filters were deposited onto 0.2-μm pore size Nuclepore membranes (Nuclepore Corp., Pleasanton, CA) and prepared for SEM analysis. The bivariate distribution was determined according to the method outlined in WHO Monograph 4 for measuring airborne man-made mineral fibers (WHO, 1985). Diameter and lengths were measured at 2000–5000× in a minimum of 20 fields or 100 fibers on either a JEOL T 300 SEM or JEOL 840 SEM equipped with a Videoplan Image Analysis System. Magnification was adjusted for length measurements so that no lengths were truncated.

### Lung burden analysis

Groups of four or five randomly selected rats from each exposure group were killed at 1 hr and 1, 5, 31, 91, 182, 266, and 365 days after termination of the inhalation exposure. Lungs were removed, weighed, frozen, and transferred to Mountain Technical Center for lung burden analysis. Lungs were thawed, the infracardiac lobe of each lung was removed, and the remainder of the lung tissue was refrozen. The infracardiac lobe was dispersed in distilled water and captured onto a Nuclepore filter for SEM stub preparation. The fiber recovery procedure was validated as follows: A known mass of MMVF fiber was injected into an excised rat lung; the mass of fibers recovered from the lung by the above process was not significantly different from the mass injected. The lung burden (fibers/lung) was calculated based on the number of fibers with an aspect ratio >3 found in 200 fields at 5000× on the SEM using WHO Monograph 4 rules for counting and measuring of fibers. Fiber diameter and length were determined using the same criteria as measurements made on the aerosol samples. Lung fiber disappearance half-times (days required for half of the Day 1 fibers to clear from the lungs) were calculated from an exponential curve for the MMVFs and a logarithmic curve for crocidolite.

### Scanning electron micrographs

Scanning electron micrographs were made of fibers recovered from the lungs of animals terminated after 91 and 182 days for comparison to the morphology of fibers from their respective aerosols. A portion of the remaining filters from each exposed group from the 1 hr and 91-, 266-, and 365-day terminations was carbon coated for energy dispersive spectroscopy (EDS) analysis using a Tracer Northern 5500 analysis unit. The first 15–20 fibers having diameters between 0.5 and 1.0 μm and lengths between 5 and 15 μm encountered during examination were selected as representing

### TABLE 2

<table>
<thead>
<tr>
<th>Fiber</th>
<th>&lt;5</th>
<th>&gt;5 WHO</th>
<th>&gt;10</th>
<th>&gt;20</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMVF10</td>
<td>1.7 ± 0.5</td>
<td>2.8 ± 0.4</td>
<td>1.0 ± 0.2</td>
<td>0.14 ± 0.09</td>
</tr>
<tr>
<td>MMVF11</td>
<td>3.0 ± 0.5</td>
<td>5.6 ± 1.2</td>
<td>3.1 ± 0.7</td>
<td>0.97 ± 0.33</td>
</tr>
<tr>
<td>MMVF21</td>
<td>0.7 ± 0.1</td>
<td>1.8 ± 0.4</td>
<td>1.1 ± 0.3</td>
<td>0.55 ± 0.21</td>
</tr>
<tr>
<td>MMVF22</td>
<td>2.4 ± 0.5</td>
<td>3.4 ± 0.6</td>
<td>1.9 ± 0.6</td>
<td>0.40 ± 0.10</td>
</tr>
<tr>
<td>Crocidolite</td>
<td>69.8 ± 13.7</td>
<td>29.8 ± 7.1</td>
<td>7.8 ± 4.2</td>
<td>0.95 ± 1.00</td>
</tr>
</tbody>
</table>

#### Fiber aerosol exposure

The test system consisted of 432 male Fischer 344/N rats (Charles River Breeding Laboratories, Raleigh, NC) randomly divided into six groups each having 72 animals. Animals were exposed to the aerosols of filtered air for 6 hr per day for 5 consecutive days in nose-only inhalation chambers to 30 mg/m³ MMVF or to 10 mg/m³ of crocidolite asbestos. The fiber aerosols were produced using the Research and Consulting Company, Geneva (RCC) fiber aerosol generation system described previously (Hesterberg et al., 1993; Bernstein et al., 1994). When not being exposed, the rats were housed individually in polycarbonate cages containing hardwood bedding in Hazelton 2000 chambers (LAB Products) in rooms operated under negative pressure (−20 mm H₂O) with 20 air changes/hr. Temperature was maintained at 22 ± 3°C, with a relative humidity of 30–70%, on a 12-hr light–dark cycle. The animals were fed pelleted standard Kliba 343 rat maintenance diet (Klingenthaluegel AE, 4303 Kaiserstaug, Switzerland) and filtered freshwater was supplied in individual bottles ad libitum during the nonexposure period.

#### Aerosol monitoring and characterization

Fiber mass concentrations were determined once during pretest and twice each day during the 5-day exposure period. In addition, to assure the uniformity of exposure during each 6-hr exposure period, the fiber concentrations were monitored continuously using a RAS (GCA Corp.) light scattering monitor. Each aerosol was sampled twice during the first 2 days of exposure and once per day during the last 3 days of the 5-day exposure period to determine fiber concentrations. Clarified slides were made from the MMVF aerosol sample filters for counting by phase-contrast optical microscopy using WHO Monograph 4 counting rules. Crocidolite aerosol samples were counted using scanning electron microscopy (SEM) at 5000× using WHO Monograph 4 rules with the exception that all fibers with aspect ratio greater than 3 were counted. Crocidolite fibers meeting the WHO fiber definition of aspect ratio of ≥3:1, length >5 μm, and diameter <3 μm were calculated from the count of all fibers with aspect ratio >3 and the diameter and length distribution data. Details of sampling and counting were described previously (Hesterberg et al., 1993).

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### Scanning electron micrographs

Scanning electron micrographs were made of fibers recovered from the lungs of animals terminated after 91 and 182 days for comparison to the morphology of fibers from their respective aerosols. A portion of the remaining filters from each exposed group from the 1 hr and 91-, 266-, and 365-day terminations was carbon coated for energy dispersive spectroscopy (EDS) analysis using a Tracer Northern 5500 analysis unit. The first 15–20 fibers having diameters between 0.5 and 1.0 μm and lengths between 5 and 15 μm encountered during examination were selected as representing
Changes in the number of fibers/lung (as fraction of Day 1) in various fiber length categories of (≤5, >5, >10, and >20 μm) during recovery of rats exposed for 5 days to (A) MMVF 10, (B) MMVF 11, (C) MMVF 21, (D) MMVF 22, or (E) crocidolite asbestos.

RESULTS

Aerosol Characterization

Mass concentrations (mg of fibers/m³ of air) of each of the fiber aerosols were close to target: MMVF aerosols ranged from 29 to 32 mg/m³, while the asbestos aerosol was 11 mg/m³. Aerosol fibers dimensions were similar to those of the respective stock fibers prior to aerosolization (data not shown). Arithmetic mean diameter (AMD) and length (AML) of the aerosolized MMVFs varied somewhat from the target dimensions of 1 × 20 μm, with AMD ranging from 1.0 to 1.3 μm and AML from 15 to 27 μm. Geometric mean diameter (GMD) and length (GML) for the MMVF aerosols had smaller ranges: GMD ranged from 0.9 to 1.1 μm and GML ranged from 11 to 16 μm (Table 1). Crocidolite asbestos aerosol fibers were shorter and thinner than the MMVFs, with arithmetic mean dimensions of 0.3 × 5.7 μm. The mean diameter of the MMVF 10 aerosol was greater than that of the other three MMVFs. The mean lengths of the MMVFs 21 and 22 aerosols were greater than the mean lengths of MMVFs 10 and 11. Due to its smaller diameter, crocidolite total fibers/cm³ values were more than 10-fold greater than the MMVFs, but this difference decreased with increasing fiber length (Table 1).

Lung Burden Characterization

Figures 1A and 1B show surface plots of the bivariate distribution of MMVF 21 in the aerosol averaged over the 5-day exposure (Fig. 1A) and in the lung 1 day after the exposure was stopped (Fig. 1B). The lung fiber distribution is much narrower than the aerosol distribution, indicating that the lung has selected the thinner and shorter fiber sub-population. This shift in size distribution from aerosol to lung fibers is also typical of the other three MMVFs. The change in size distribution from the crocidolite aerosol to the lung was negligible (data not shown).

After 1 day of recovery, crocidolite-exposed lungs contained more fibers in ≤20 μm-length categories than MMVF-exposed lungs (Table 2). This is consistent with the much thinner diameter and higher fibers/cm³ in the crocidolite aerosol. Figures 2A–2E illustrate the differential clearance of fibers with respect to length. Disappearance of all length categories of MMVFs 10, 11, and 22 fit an exponential decay curve fairly well; \( r^2 = 0.91–0.98 \) (Figs. 2A, 2B, and 2D). The disappearance data for the >10 and >20 μm MMVF 21 fibers also fit this curve well; \( r^2 = 0.98–0.99 \).
The disappearance of MMVF 21 fibers >5 \( \mu \text{m} \) and <5 \( \mu \text{m} \) in length did not fit the exponential curve as well, perhaps due to the significant increase in fibers <5 \( \mu \text{m} \) in length seen at 5, 31, and 91 days (Fig. 2E). The best fit that could be obtained for the crocidolite retention data was logarithmic rather than exponential suggesting a different mechanism of disappearance of this fiber characterized by a more rapid initial disappearance of fibers which slowed considerably after about 90 days. Due to the scatter in the crocidolite data at longer time points, even the fit to the logarithmic model for fibers longer than 10 and 20 \( \mu \text{m} \) was relatively poor. After 365 days of recovery, most of the MMVF 21 fibers had disappeared from the lung, whereas a significant number of crocidolite fibers still remained in the lung—nearly 83% of crocidolite lung fibers \( \geq 20 \mu \text{m} \) in length persisted through the year of recovery (Table 2).
The pattern of fiber disappearance was similar in all the MMVF-exposed rat lungs: The longer fibers disappeared from the lung more rapidly than the shorter fibers (Figs. 2A–2D; Table 2). The reverse was true for crocidolite: Crocidolite fibers >5 μm disappeared much more slowly than crocidolite fibers <5 μm and MMVPFs >5 μm (Fig. 2E; Table 2). Fiber disappearance half-times (Table 2) for the MMVPFs increased as fiber length decreased. In contrast, disappearance half-times for crocidolite increased with increasing fiber length. The percentage of MMVF fibers remaining in the lung after 365 days was greater for the shorter fibers than for the longer fibers (Table 2). Percentages of crocidolite fibers remaining in the lung after 365 days were much higher than MMVPFs in all length categories and percentage retained increased with fiber length. It is interesting to note that an increase in the number of short fibers (<5 μm in length) was observed at early time points for MMVPFs 21 and 22 (Figs. 2C and 2D).

A few fibers were observed in the lungs of 14 of the 38 control animals. Due to the large multiplication factors...
involved, a value of $1-3 \times 10^4$ fibers/lung can be obtained from finding only one fiber in 200 SEM fields at 5000× magnification in the analysis of a single lung. Thus, the background level could represent fibers deposited on membranes as a result of inadvertent fiber contamination during sample preparation and not fibers actually in the control animal’s lungs. In two cases, noted in Table 2, fiber/lung levels did not significantly differ from background for those length categories.

Figures 3A and 3B demonstrate the changes in geometric mean dimensions of lung fibers over time. Although the average diameters and lengths of all MMVFIs decreased with time in the lung, the average diameter of crocidolite remained unchanged and its average length appeared to increase.

Chemical and Morphological Changes in Lung Fibers

Table 3 compares the oxide content of fibers from the aerosol to the content of fibers recovered from the lung 1 hr and 91 and 365 days after termination of exposure. MMVFIs 10, 11, and 22 all showed significant decreases in alkali (Na, O) and alkaline earth oxides (MgO and/or CaO) over time, while MMVF 21 and crocidolite showed no change in chemical composition (Table 3). With the more soluble MMVFIs (MMVFIs 10 and 22), losses of alkalies and alkaline earth oxides in the lung environment were observed as early as 1 hr after exposure terminated. The MMVF 21 fibers shown in Fig. 4A are typical of the morphology of the other MMVF aerosolized fibers. Scanning electron micrographs of MMVF fibers found in the lungs after 182 days of recovery (Figs. 4B–4E) show changes in the surface morphology of the MMVFIs with time in the lung. No changes in the morphology of crocidolite were observed (Fig. 4F).

DISCUSSION

Lung Deposition and Fiber Dimension

The data from the present study support a conclusion that fiber dimension, especially diameter, plays a major role in lung deposition. Most of the fibers deposited in the tracheobronchial airways are assumed to clear within the first day after cessation of exposure (Lippmann, 1990). Therefore, fibers recovered from the lung 1 day after exposure was terminated are assumed to be those deposited beyond the terminal bronchioles. As seen in Figs. 1A and 1B, the rat deep lung preferentially selected shorter and thinner MMVFIs fibers from the aerosol (diameters less than 1.2 μm and lengths less than 50 μm). In contrast to the MMVFIs, the bivariate distribution of crocidolite in the lung was similar to that of the aerosol, presumably because the aerosolized crocidolite was already relatively short and thin.

The role of fiber dimension in pulmonary deposition has been demonstrated previously by others (Morgan et al., 1980; Bernstein et al., 1995). Morgan et al. (1980) demonstrated that alveolar deposition of fibers drops off rapidly with increasing diameter, which could explain why greater lung burdens of the thin MMVF 11 were observed on Day 1 (Table 2). Day-1 WHO fibers/lung values for MMVF 11 agree well with the findings of Bernstein et al. (1995) who studied this same fiber. The present lung burden data also agree well with the results of Musselman et al. (1994) who reported univariate data from this same exposure.

Lung Clearance and Fiber Dimension

MMVFIs vs crocidolite. Crocidolite was more biopersistent than the MMVFIs in the rat lung. After 365 days postexposure, WHO fiber lung burdens in each MMVF group had decreased to 0—11% of Day-1 levels (Table 2). In sharp contrast, crocidolite WHO fibers/lung dropped to 55% of Day 1 after 365 days. In comparing the relative pulmonary clearance of crocidolite to that of the MMVFIs, two factors must be considered: (1) The number of WHO fibers/lung on Day 1 for crocidolite was an order of magnitude higher than that for MMVF; and (2) most of the crocidolite fibers deposited in the lung were significantly shorter than the MMVFIs and may therefore have been cleared more efficiently (Morgan et al., 1978; Bellman et al., 1986; Roggli et al., 1987; Coin et al., 1992). However, for long fibers (>20 μm) a direct comparison between MMVF 11 and crocidolite can be made due to the number of fibers/lung on Day 1 (Day-1 lung burden of fibers >20 μm was $9.7 \times 10^6$ for MMVF 11 and $9.5 \times 10^6$ for crocidolite). At 365 days only 0.2% of the long MMVF 11 fibers present on Day 1 remained in the lung, while 83% of the long crocidolite still remained.

Fiber length. There was a clear downward trend in the mean length of MMVFIs with increasing time in the lung; the greatest decrease in length occurred within the first 5 days (Fig. 3B). The reduction in mean length for all MMVFIs is consistent with the more rapid disappearance of fibers in the >20- and >10-μm categories compared to WHO (>5 μm) fibers. The reduction in mean length of MMVFIs in the lung could be the result of extracellular dissolution or breakage to shorter fibers followed by macrophage clearance, or a combination of these mechanisms. MMVF 22 exhibited the most rapid and the greatest reduction in length. Considerable reduction in mean length also occurred in MMVF 21, which exhibited the least chemical change of the MMVFIs. The length reduction in the two mineral wool fibers could be explained by breakage of long fibers at sites of exposure to intracellular low pH environments of macrophages. Both Bellman et al. (1994) and Christensen et al. (1994) showed similar fiber compositions to be more soluble both in vivo and in vitro than glass wools. Previous studies

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FIG. 3. Changes in (A) average geometric mean diameter and (B) average geometric mean length of fibers isolated from lung tissue after different recovery times.

(Morgan et al., 1978; Bernstein et al., 1980; Roggli et al., 1987; Coin et al., 1992) have shown that shorter fibers are cleared by macrophages more rapidly than long fibers. Hammond (1984) saw an increase in the number of short refractory ceramic fibers after 30 days of recovery; however, it is unlikely that dissolution of the longer fibers of this durable composition was the cause of this observation. Bernstein et al. (1995) reported a relatively constant reduction in length of three MMVF s including MMVF 11 and attributed this observation to fiber breakage. In contrast to the MMVFs, the average length of crocidolite increased between 5 and 31 days (Fig. 3B), which might be explained by selective macrophage clearance of short crocidolite fibers without breakage or dissolution of long fibers. Similar findings were reported for crocidolite by Roggli et al. (1987). The biological translocation of shorter crocidolite fibers would be the predominant, if not only, mechanism responsible for the disappearance of this extremely durable fiber from the lung.

Fiber diameter and durability. Whether the reduction in diameter seen in the MMVFs is the result of fiber dissolution or selective clearance is not known. However, the data for MMVF 22 suggest that chemical durability was at least partly responsible: MMVF 22, the least chemically durable fiber based on the EDS analysis, showed the most rapid and the greatest reduction in diameter (Fig. 3A). This finding is in contrast to the observations of Bellman et al. (1987) who reported that the diameter and length of intratracheally instilled glass fibers (with the exception of E glass and rock-
Changes in Fiber Chemistry and Morphology

MMVF10 and 22 showed significant losses of both alkali and alkaline earth oxides as early as 1 hr after the end of the 5-day exposure period (Table 3). Hammad et al. (1988) reported a slower loss of CaO and MgO in a slag wool of a slightly different composition; however, that slag wool fiber itself disappeared as rapidly as the slag wool in this study. Significant reductions in Na₂O, CaO, and MgO were seen in MMVF 10 by 91 days of recovery (Table 3), suggesting that the starting composition plays an important role in mediating the change in fiber chemistry over time in the lung. No compositional changes were observed in MMVF 21 or crocidolite through 365 days (Table 3). Because the diameter of MMVF 21 decreased with time in the lung, it might be argued that this fiber was dissolving congruently, with little or no leaching of alkali or alkaline earth oxides. Chemical durability in vivo has been related to the lack of leachable alkalis and alkaline earths by Hammad et al. (1988), but in the present study, even though MMVF 21 had higher levels of these oxides than the other MMVF, it still showed no evidence of leaching.

The morphology of the fibers recovered from the lungs after 180 days correlates with the rate and degree of leaching observed in the respective MMVF. An apparent increase in diameter at

### Table 3

**Chemical Composition of Aerosol and Lung Fibers by Energy Dispersive Spectroscopy**

<table>
<thead>
<tr>
<th>Sample</th>
<th>SiO₂</th>
<th>Al₂O₃</th>
<th>Fe₂O₃</th>
<th>MgO</th>
<th>CaO</th>
<th>Na₂O</th>
<th>K₂O</th>
<th>TiO₂</th>
<th>SO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerosol</td>
<td>57 ± 0.8</td>
<td>5.4 ± 1.1</td>
<td>0.1 ± 0.2</td>
<td>4.2 ± 0.5</td>
<td>7.2 ± 0.3</td>
<td>14.1 ± 0.4</td>
<td>1.1 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hr</td>
<td>63 ± 5.2</td>
<td>8.1 ± 1.1</td>
<td>0.4 ± 0.5</td>
<td>2.7 ± 1.7</td>
<td>3.5 ± 1.7</td>
<td>7.1 ± 3.9</td>
<td>2.6 ± 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>91 Days</td>
<td>72 ± 1.7</td>
<td>8.8 ± 1.5</td>
<td>0.2 ± 0.5</td>
<td>0.1 ± 0.4</td>
<td>0.2 ± 0.2</td>
<td>0.8 ± 0.7</td>
<td>4.4 ± 0.9</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>365 days</td>
<td>68 ± 5.8</td>
<td>12 ± 2.9</td>
<td>0.4 ± 0.7</td>
<td>2.2 ± 2.0</td>
<td>1.0 ± 3.2</td>
<td>1.1 ± 1.3</td>
<td>2.2 ± 0.8</td>
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<tr>
<td>MMVF11</td>
<td>63 ± 2.0</td>
<td>3.8 ± 0.6</td>
<td>0.4 ± 0.4</td>
<td>2.8 ± 1.9</td>
<td>7.4 ± 0.9</td>
<td>15 ± 0.6</td>
<td>1.5 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hr</td>
<td>61 ± 3.0</td>
<td>3.9 ± 1.3</td>
<td>0.5 ± 0.6</td>
<td>2.9 ± 1.6</td>
<td>6.9 ± 0.8</td>
<td>13 ± 2.7</td>
<td>6.2 ± 3.3</td>
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<td></td>
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<tr>
<td>91 Days</td>
<td>71 ± 3.4</td>
<td>4.7 ± 0.9</td>
<td>0.6 ± 0.5</td>
<td>1.9 ± 1.0</td>
<td>3.9 ± 1.0</td>
<td>7.0 ± 2.2</td>
<td>4.4 ± 1.4</td>
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<tr>
<td>365 days</td>
<td>71 ± 11.6</td>
<td>10 ± 5.0</td>
<td>1.0 ± 0.9</td>
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<td>5.2 ± 7.5</td>
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<tr>
<td>MMVF22</td>
<td>47 ± 1.5</td>
<td>13 ± 0.9</td>
<td>7.1 ± 0.4</td>
<td>9.6 ± 1.2</td>
<td>17 ± 1.4</td>
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<td>1.3 ± 0.1</td>
<td>3.1 ± 0.5</td>
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<tr>
<td>1 hr</td>
<td>47 ± 1.4</td>
<td>14 ± 1.1</td>
<td>6.9 ± 0.8</td>
<td>9.6 ± 1.2</td>
<td>16 ± 1.1</td>
<td>2.5 ± 1.1</td>
<td>1.3 ± 0.2</td>
<td>3.1 ± 0.5</td>
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<tr>
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<td>14 ± 0.8</td>
<td>6.6 ± 0.5</td>
<td>9.7 ± 1.8</td>
<td>15 ± 1.3</td>
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<td>1.2 ± 0.2</td>
<td>3.0 ± 0.5</td>
<td>NA</td>
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<tr>
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<td>47 ± 2.8</td>
<td>14 ± 1.8</td>
<td>6.2 ± 1.4</td>
<td>11 ± 2.0</td>
<td>14 ± 1.2</td>
<td>3.6 ± 1.4</td>
<td>1.2 ± 0.8</td>
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<tr>
<td>Crocidolite</td>
<td>38 ± 1.4</td>
<td>11 ± 0.6</td>
<td>1.1 ± 2.0</td>
<td>9.3 ± 2.0</td>
<td>37.7 ± 2.5</td>
<td>0.4 ± 0.4</td>
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<td>1.8 ± 0.4</td>
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<tr>
<td>1 hr</td>
<td>60 ± 13</td>
<td>17 ± 3.9</td>
<td>0.6 ± 1.2</td>
<td>3.2 ± 4.6</td>
<td>9.6 ± 15</td>
<td>0.7 ± 0.8</td>
<td>6.6 ± 3.6</td>
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</tr>
<tr>
<td>91 Days</td>
<td>72 ± 3.7</td>
<td>17 ± 1.6</td>
<td>1.0 ± 1.3</td>
<td>0.3 ± 0.8</td>
<td>0.7 ± 0.6</td>
<td>0.8 ± 1.3</td>
<td>5.9 ± 1.0</td>
<td>0.6 ± 0.6</td>
<td>2.2 ± 1.2</td>
</tr>
<tr>
<td>365 days</td>
<td>68 ± 9.3</td>
<td>17 ± 6.9</td>
<td>1.9 ± 2.4</td>
<td>2.7 ± 2.2</td>
<td>0.5 ± 0.6</td>
<td>1.7 ± 1.1</td>
<td>1.9 ± 1.5</td>
<td>1.9 ± 2.4</td>
<td>4.9 ± 4.0</td>
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</table>

**Note.** Boldface numbers: Significant reduction compared to value in aerosol composition according to Student’s *t* test at *p* < 0.05. Aerosol, *n* = 6; all others, *n* = 15. NA, no analyses conducted for these oxides.

*a* Aerosol compared with 1 hr, 91 days, and 365 days postexposure.

Wool) increased or remained constant through 730 days of postexposure recovery. Bellman et al. explained the increase in fiber dimensions during the first 6 months of recovery as the result of more rapid clearance of shorter fibers, which also tended to be the finer diameter fibers. Also in contrast to the present study, Morgan and Holmes (1984) reported no change in median diameter of intratracheally instilled rock wool. Yamato et al. (1994) demonstrated that the mean diameter of refractory ceramic fibers administered by inhalation decreased with recovery time. Bernstein et al. (1995) reported no change in the diameter of MMVF 11 and two other MMVF through 6 months of recovery following a 5-day inhalation exposure. This lack of change in diameter was attributed to buildup of a leached layer on the fiber surface, which, in turn, may have contributed to fiber breakage and reduction in length with time. Bellman et al. (1987) also reported no change in crocidolite diameter after 2 years in the lung, which agrees with the present work.
the ends of many MMVF 10 and 11 fibers was observed by SEM in addition to considerable etching of the surface (Fig. 4). This observation of greater diameters at the glass fiber ends is in contrast to the sharp ends of rock wool fibers recovered from lung tissue by Morgan and Holmes (1984) but in agreement with observations of Bellman et al. (1987) for glass fibers. Based
on the SEM examination, very little morphological change was observed in the MMVF 21 fibers, while severe degradation of the MMVF 22 fibers was apparent (Fig. 4). There was no evidence of morphological change in crocidolite, consistent with the lack of chemical compositional change in this fiber.

### Relationship of Biopersistence to Lung Toxicity

It is clear from this work and the work of others that glass, slag wool, and rock wool fibers disappear from the lung more rapidly and completely than crocidolite asbestos. Ex-
tracellular and/or intracellular dissolution leading to breakage appears to have been responsible for the more rapid disappearance of the longer MMVF fibers compared to the shorter fibers. The relatively large changes that occurred in MMVF lung burdens (decrease in fibers/lung and alterations in composition and morphology) compared to little or no changes in crocidolite lung burden could provide a mechanistic basis to explain the differences in lung pathology ob-
served in the previously conducted series of chronic inhalation studies using these same fibers. MMVF 10, 11, and 22 produced no fibrosis, no mesotheliomas, and no significant increase in lung tumors (Hesterberg et al., 1993; McConnell et al., 1994). Although MMVF 21 produced some minimal fibrosis after 18 months of inhalation exposure, it was not associated with mesotheliomas or a significant increase in lung tumors (McConnell et al., 1994). In contrast, crocidolite asbestos, which showed no tendency toward chemical change and only a minor loss of long fibers during 365 recovery days in the present study, was previously shown to produce lung fibrosis after only 3 months, mesotheliomas, and a significant increase in lung tumors in rats (Wagner et al., 1974; McConnell et al., 1994). Thus, low pulmonary biopersistence could explain why some MMVF s are not tumorigenic in rats, even after chronic exposure at high concentrations. In conclusion, the present study demonstrates that greater biopersistence in the lung is associated with a greater potential to cause lung disease.

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REFERENCES


