Biopersistences of Man-made Vitreous Fibers and Crocidolite Fibers in Rat Lungs Following Short-term Exposures

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Biopersistence of commercial man-made vitreous fibers (MMVF) and crocidolite were studied in Fischer 344 rats. MMVF used were size-selected to be rat-respirable, and rats were exposed nose-only 6 h/day for 5 days to gravimetric concentrations (30 mg/m³) of two fiber glass compositions—a rockwool, and a slagwool—or to 10 mg/m³ of long-fibered crocidolite, or to filtered air. Animals were sacrificed at 1 hr, 1, 5, 31, 90, 180, 270, 365, and 545 days after exposure stopped. Fibers were recovered from digested lung tissue to determine changes in concentrations (fibers/mg dry lung) and fiber retentions (expressed as percent of day 1 retention [PR]) for selected dimension categories. One-day average concentrations of lung-retained MMVF and crocidolite fibers, of diameter ≥0.5 µm or >20 µm in length, were nearly equal, permitting direct comparisons between MMVF and crocidolite. At 270 days average PR for MMVF ≥0.5 µm in diameter were from 3 to 6 ± 2% and 27 ± 9% for crocidolite. For fibers >20 µm, PR were 1 to 4 ± 4% for MMVF and 37 ± 20% for crocidolite. At 545 days, MMVF >20 µm in length were at background level while concentration of crocidolite fibers >20 µm in length remained at 2000 ± 400 f/mg DL (dry lung), or 38 ± 9% of day-1 retention. These results suggest strongly that MMVF dissolved or fractured in vivo whereas crocidolite fibers did not change. — Environ Health Perspect 102(Suppl 1):139–143 (1994)

Key words: man-made vitreous fiber, MMVF, crocidolite, asbestos, inhalation, rat, respirable, biopersistence, durability

Introduction

Studies of animal exposure to airborne fibrous materials (e.g., crocidolite fibers) have shown that certain factors largely control tissue response in terms of fibrogenesis or malignant transformation. First, fibers must be respirable, i.e., short and thin enough to reach the distal parts of the pulmonary region (1,2). Second, durable fibers of specific diameters and lengths ("Stanton" fibers) retained in the pulmonary region are needed to produce an adverse tissue response. Those fibers must be "biopersistent," so that specific dimensions are maintained at the cellular level for long enough (e.g., years) to attain the cumulative dose necessary to induce pathogenic effects (3,4). Moreover, the durable fibers should be longer than 17 µm to have the greatest effect, because such fibers cannot be phagocytized by the alveolar macrophages and would therefore be the most biopersistent (5–7). Focus on the fate of these fibers in the lung would also add to our understanding of in vivo dissolution.

This study compared the biopersistences in rat lung of respirable fractions of some commercial MMVF, with the biopersistence of respirable crocidolite fibers under the same conditions. Parameters studied included changes in concentrations (f/mg dry lung tissue) and dimensions (percent of day 1 lung retention, for selected lengths and diameters) of fibers

![Figure 1](https://example.com/figure1.png)

Figure 1. Geometric mean lengths of crocidolite and MMVF fibers, in original stock, after lofting and after recovery from rat lungs, 1 hr, 1, 5, 31, and 90 days postexposure. The lofted fiber diameters are calculated from seven aerosol samples per fiber type, and the diameters of lung-retained fibers are calculated from numbers of fibers recovered from five lungs per fiber type for each sacrifice period.
Table 1. Chemical composition of bulk fiber samples by wt.-%.

|         | SiO₂ | Al₂O₃ | CaO | MgO | Fe²⁺ | Fe³⁺ | Na₂O | K₂O | TiO₂ | S | B₂O₃ |
|---------|------|-------|-----|-----|------|------|------|-----|------|---|------|
| Croc   | 50.9 | 0.7   | 3.4 | 14.7| 21.4 | 5.6  | 0.1  | 0.1 |      |   |      |
| # 10   | 57.5 | 5.1   | 7.5 | 4.1 | 0.1  | 15.0 | 1.1  | 0.1 | 8.8  |   |      |
| # 11   | 62.4 | 3.9   | 7.5 | 2.8 | 0.3  | 15.5 | 1.3  | 0.3 | 4.5  |   |      |
| # 21   | 46.2 | 13.0  | 16.9| 9.3 | 4.6  | 1.8  | 2.6  | 1.3 | 3.0  | 0.2|      |
| # 22   | 38.4 | 10.6  | 38.0| 9.9 | 0.3  | 0.4  | 0.5  | 0.5 | 1.8  |   |      |

Analyses: SiO₂, Al₂O₃, CaO, MgO by gravimetric and/or AA, Fe²⁺, TiO₂ by ICP, Na₂O, K₂O by AA; B₂O₃ by mannitol titration, FeO by ferrous iron titration with potassium dichromate, using a diphenylamine sulfonate indicator. Fibers 10, 11 and 22 not analyzed for FeO due to low Fe content. Sulfur as SO₂ by LECO CS-344 furnace. Fiber 10 fluoride content = 0.8% by selective ion electrode analysis. MnO content of crocidolite = 0.1%, of Fiber 21, 0.2% and of Fiber 22, 0.7%, by ICP. All percent values rounded to the nearest 0.1%.

Figure 2. Geometric mean diameters of crocidolite and MMVF fibers, in original stock, after lofting and after recovery from rat lungs, 1 hr, 1, 5, 31, and 90 days postexposure. The lofted fiber diameters are calculated from seven aerosol samples per fiber type, and the diameters of lung-retained fibers are calculated from numbers of fibers recovered from five lungs per fiber type for each sacrifice period.

Table 2. Fiber concentration (f/mg DL x 1000) and percent of day 1 retention for crocidolite in rat lungs from 1 hr to 545 days by selected dimension categories.a

| Length (µm) | 1 day | 5 days | 31 days | 90 days | 180 days | 270 days | 365 days | 545 days |
|-------------|-------|--------|---------|---------|----------|----------|----------|----------|
| <5 µm       | 390 (79) | 238 (24) | 137 (24) | 112 (16) | 68 (9) | 65 (11) | 86 (38) | 67 (24) |
| % Retention | 100 (20) | 78 (19) | 35 (6) | 29 (4) | 17 (3) | 17 (2) | 22 (10) | 17 (6) |
| >5 µm       | 168 (47) | 114 (29) | 100 (31) | 76 (12) | 46 (8) | 43 (12) | 56 (12) | 56 (6) |
| % Retention | 100 (29) | 71 (49) | 59 (16) | 45 (7) | 27 (6) | 25 (4) | 35 (7) | 33 (3) |
| >10 µm      | 44 (24) | 30 (7) | 22 (5) | 16 (7) | 12 (6) | 9 (2) | 17 (5) | 13 (1) |
| % Retention | 100 (47) | 71 (17) | 52 (13) | 39 (16) | 29 (14) | 22 (4) | 40 (12) | 30 (2) |
| >20 µm      | 5.3 (5.9) | 3.2 (1.8) | 4.0 (2.8) | 2.2 (1.7) | 1.1 (0.8) | 2.0 (1.0) | 2.8 (1.7) | 2 (0.4) |
| % Retention | 100 (111) | 60 (17) | 77 (53) | 43 (33) | 21 (16) | 37 (20) | 53 (32) | 38 (9) |

Diameter (µm)
| Concentration | % Retention | % Retention | % Retention | % Retention | % Retention |
|---------------|-------------|-------------|-------------|-------------|-------------|
| <0.5 µm       | 533 (14) | 331 (9) | 224 (7) | 178 (2) | 105 (2.0) | 102 (2.0) | 136 (5) | 117 (24) |
| >0.5 µm       | 23.3 (8.5) | 25 (7.9) | 13.1 (6) | 8.0 (2.2) | 8.8 (2.6) | 6.4 (2.1) | 7.1 (5) | 4.3 (0.7) |
| >11 µm        | 100 (36) | 107 (34) | 56 (28) | 34 (10) | 38 (11) | 27 (9) | 30 (26) | 18 (3.0) |

Air controls
| Concentration | % Retention | % Retention | % Retention |
|---------------|-------------|-------------|-------------|
| >5 µm Length  | 0.8 | 0.07 | 0.1 | 0.4 | NIL | 0.07 | NIL | 0.05 |
| >20 µm Length | ND | NIL | NIL | NIL | ND | NIL | ND | NIL |
| >0.5 µm Diameter | 0.1 | NIL | 0.05 | NIL | NIL | 0.05 | NIL | ND |

Abbreviations: [ ], one standard deviation; ND, not detected; NIL, average < 50 f/mg DL. a Values are average of fiber concentrations from five lungs per fiber type for each time period.

Materials and Methods

Commercial fibers were Fiber 10, a glass-wool (Manville Corp.), Fiber 11, a glass-wool (Certainteed Corp.), Fiber 21, a rockwool ( Roxul International), and Fiber 22, a slagwool (USG Interiors). Table 1 presents the chemical compositions of these bulk fibers.

From each MMVF bulk product, the fraction selected had fibers of average dimensions of approximately 1 µm diameter and 15 µm length. Fibers of such dimensions are found in workplace aerosols. The fibers obtained accounted for 0.25 to 5 wt-% of the bulk product, depending on the initial diameter distribution of the MMVF. Crocidolite [obtained from the National Institute of Environmental Health Sciences (NIEHS)], also was size-selected to obtain a fraction of an average length of 7 µm.

Groups of Fischer 344 rats were exposed "nose-only" for 6 hr/day for 5 days to a gravimetric concentration of 30 mg/m³ for each MMVF. A group of rats was exposed to 10 mg/m³ of crocidolite fibers, and another group breathed filtered air. Fiber aerosols were lofted using a generation system designed not to break, grind, or contaminate the fibers. Aerosol concentrations for fiber mass were monitored by gravimetric sampling. Fiber concentration levels (fibers/cm³) were determined by phase contrast microscopy (<400 magnification) for total and WHO fiber numbers (8), and by optical and scanning electron microscopy (SEM) for length retained in lung tissues for various periods of time.
were sacrificed and the remaining lung tissues were preserved for future reference and study. Lobes from five rats from each exposed group and from the controls were dehydrated and dried to constant weight to obtain the initial dry weight of tissue for determination of fiber concentrations. Tissues were low-temperature ashed, the fibers were recovered, and numbers of fibers, and their diameters and lengths were determined using SEM and optical microscopy. Concentrations of fibers per mg of dry lung tissue (μg/mL) were calculated, as were concentrations of fibers and the percent-ages of day 1 retention (or PR) for selected diameter and length categories for each sacrifice period.

Results

Geometric mean diameters and lengths of lofted fibers and fibers recovered from lungs are shown in Tables 1 and 2. The average diameters and lengths of crocidolite fibers recovered from the rat lungs at various retention times differed little, whereas the MMVF showed a decrease in both geometric mean diameter and length with time retained in the animals’ lungs.

Fibers recovered from the 1-hr sacrifice represent the deposition in both the conducting airway and alveolar regions of the lung, since particle clearance from the conducting airways is assumed to be complete within 24 hr after stopping exposure (9). Results were reported as average f/mL DL for selected dimension categories (Tables 2, 3) and as PR for each sacrifice period (Tables 2, 4).

For crocidolite fibers ≥5 μm in length, the average fiber concentrations from the day 1 sacrifice were 168,000 ± 47,000 and for 31, 100,000 ± 31,000 f/mL DL. The average decreased to 58,000 ± 12,000 at day 365 sacrifice and 56,000 ± 6000 at day 545 (Table 2).

Long crocidolite fiber retention tended to be prolonged compared to retention of crocidolite particles (i.e., <5 μm) as indicated by higher retention values for the longer fibers beginning after 31 days. Percent retentions of fibers ≥10 μm did not differ significantly from the PRs of fibers ≥5 μm. PRs of fibers ≥20 μm in length tended to be higher than those for fibers ≥5 μm at 365 and 545 days. For crocidolite fibers ≥20 μm in length, average concentrations were roughly equal between days 1 and 31 (first day, 5300; 31st day, 4000 f/mL DL), decreased to 1100 ± 800 f/mL DL at 180 days, and tended to remain constant thereafter (2000 ± 1000 f/mL DL, Table 2).

Table 3 presents average concentrations of MMVF retained from day 1 to day 545. For MMVF ≥5 μm in length, the average fiber concentrations from day 1 sacrifices ranged from 11,500 f/mL DL to 37,600 f/mL DL. PRs of MMVF ≥5 μm at day 31 ranged from 42 ± 36% to 87 ± 196% and were about equal by day 180 (Table 4).  

### Table 3. Fiber concentrations in f/mL DL×1000 for MMVF retained in rat lungs from 1 hour to 545 days by selected dimension categories.  

| Dimension | 1 day | 5 days | 31 days | 90 days | 180 days | 270 days | 365 days | 545 days |
|-----------|-------|--------|---------|---------|----------|----------|----------|----------|
| Diameter |       |        |         |         |          |          |          |          |
| <1 μm     |       |        |         |         |          |          |          |          |
| ≥1 μm     |       |        |         |         |          |          |          |          |
| ≥2 μm     |       |        |         |         |          |          |          |          |
| ≥5 μm     |       |        |         |         |          |          |          |          |
| ≥10 μm    |       |        |         |         |          |          |          |          |
| ≥20 μm    |       |        |         |         |          |          |          |          |

### Abbreviations:  

1. standard deviation from mean; NIL, average <50 f/mL DL; ND, not detected.  
2. Values are average of fiber concentrations from five lungs per fiber type for each time period.
Retention of MMVF particles <5 μm in length generally exceeded the corresponding PR’s of MMVF ≥5 μm long. Likewise, fibers <0.5 μm in diameter had consistently higher PRs than fibers ≥0.5 μm in diameter at each corresponding sacrifice point (Table 4).

Day 1 concentrations of fibers >20 μm in length ranged from 1500 ± 1700 fibers/mg DL (Fiber 10) to 4700 ± 600 fibers/mg DL (Fiber 11, Table 3). The PRs for Fiber 21 were higher than the average PR for the other MMVF both at 31 and 90 days; but by day 180 the PR had decreased to values close to those of the other MMVF. At 545 days, the PRs for MMVF > 20 μm in length were near background level.

### Discussion

Movement of a particle in the pulmonary region is principally mediated by the alveolar macrophage that engulfs the particle and migrates either towards the bronchial lumen or into subpleural, peraseptal, perivascular, or peribronchial locations or into bronchial lymphatics and onward to lymphoid nodules (7,9). Clearance by this mechanism is slow, taking months or longer. The average diameter of an alveolar macrophage is 7.5 μm in rats and 8.5 μm in humans (11). Macrophage-mediated clearance is less effective for fibers ≥10 μm in length than for fibers between 5 μm and 10 μm in length (7,12). Analyses of anthophyllite fibers in lung tissues from Finnish workers suggested that the critical length for mechanical clearance of a fiber from the lung is 17 μm (13). Hence, macrophage clearance is most probably ineffective for fibers > 20 μm in length, both in rat and human lungs, and study of this fraction should permit more direct observation of in vivo fiber dissolution.

In this study approximately equal concentrations of MMVF and crocidolite fibers longer than 20 μm were retained in the pulmonary region of lungs of animals one day after exposure had ceased (Tables 2, 3) but after 180 days 90 to 100% of the long MMVF had been cleared, while no significant differences in percent clearance (PC) values were observed for long crocidolite fibers from 31 days on, and at 545 days, the PC for these fibers was about 60%.* Furthermore, results from the 1-day sacrifices indicated that roughly the same concentrations of MMVF and crocidolite fibers ≥0.5 μm in diameter (Tables 2,3) were retained in the pulmonary regions of exposed animals. Within 180 days, approximately 90 to 95% of MMVF ≥0.5 μm in diameter had cleared from the lungs, and by 270 days this had increased to approximately 95 to 99%. In contrast, no significant differences were observed in the clearance of crocidolite fibers ≥0.5 μm in diameter from day 90 on, with values remaining approximately 70 to 75%.

Comparison of MMVF clearances is difficult because the day 1 deposition for each fiber type differed in size distribution. However, clearance of MMVF 21 > 20 μm appeared to be slower up to 90 days compared to the other MMVFs. A previous study suggested that rockwool with an iron content similar to that of MMVF 21 appeared to be more persistent than glass fibers in rat lungs (12). Further study of the MMVF size distributions is needed to better understand these observations.

Lifetime animal inhalation studies of MMVF21 and 22 and crocidolite fibers were completed at Research and Consulting Company (RCC), Geneva in 1993 (14). Sixteen of 106 (14.1%) crocidolite-exposed animals had primary lung neoplasms compared to 2 of 126 (1.6%; p<0.01) unexposed control animals. One mesothelioma was observed in a rat from the crocidolite group. Fibrosis was observed in the crocidolite exposure group in all lungs examined after 3 months of exposure.

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**Table 4. Percentages of day 1 retentions in rat lungs for MMVF from 1 to 545 days.**

| MMVF | 1 day | 5 days | 31 days | 90 days | 180 days | 270 days | 365 days | 545 days |
|------|-------|--------|---------|---------|----------|----------|----------|----------|
| 10    | Length |
| <0.5μm | 100 (52) | 100 (23) | 96 (23) | 86 (33) | 86 (36) | 84 (32) | 80 (34) | 76 (34) |
| >0.5μm | 100 (116) | 99 (39) | 90 (40) | 83 (36) | 75 (36) | 67 (36) | 60 (40) | 55 (46) |
| Diameter |
| <0.5μm | 100 (100) | 100 (23) | 96 (23) | 86 (33) | 86 (36) | 84 (32) | 80 (34) | 76 (34) |
| >0.5μm | 100 (116) | 99 (39) | 90 (40) | 83 (36) | 75 (36) | 67 (36) | 60 (40) | 55 (46) |

**Abbreviations:** (1) one standard deviation from mean; ND, not detected. *Day 1 retention assumed to represent 100% retention in the pulmonary region. Values calculated from an average fiber concentration of 5 lungs per fiber type for each time period.**
No mesothelioma was found in animals exposed to MMVF21 or 22. For animals exposed to MMVF21, lung tumors were observed in all dose groups (5 of 114 rats, low (3 mg/m³) dose; 5 of 115, mid (16 mg/m³) dose; and 5 of 114, high (30 mg/m³) dose). The numbers of tumors were not statistically significant from the number reported for the unexposed control group. At 12 months minimal fibrosis was observed in some of the lungs from animals exposed to MMVF21 at the high dose level. At 18 months minimal (mid dose) and mild (high dose) fibrosis were observed in all lungs examined. No significant increase in severity of fibrosis was noted at the end of exposure (24 months) nor at the end of the study (28 months) among rats exposed to MMVF21 aerosols.

For animals exposed to MMVF22, lung tumors were observed in the low dose (5 of 116 animals) and high dose (3 of 115 animals) groups. The numbers of tumors were not statistically significant. No fibrosis was observed in the lungs of rats exposed to MMVF22 at any point during the study. Lifetime studies with MMVF10 and 11 were completed in 1992; neither fibrosis nor significant numbers of tumors were observed in the lungs of the exposed animals (15).

Conclusion

Results from this study indicated that, of the long crocidolite fibers retained in the pulmonary region at day 1, approximately 40% remained through 545 days. Little, if any, dissolution or breakage had occurred in crocidolite fibers, since the percentage of fibers of diameter <0.5 μm or ≥0.5 μm was relatively constant from day 90 on. In contrast, the nearly complete disappearances of MMVF fibers ≥0.5 μm in diameter and >20 μm in length within 270 days post-exposure strongly suggested dissolution. Moreover, these disappearances were accompanied by increases in the percentages observed for fibers <0.5 μm in diameter and <5 μm in length, suggesting that the thicker and longer fibers had dissolved or broken with time, "enriching" the thinner and shorter fractions. In addition, none, or very few MMVF fibers ≥20 μm in length were detected in the lung tissues from exposed animals at 545 days, whereas for crocidolite, fiber concentration at 545 days remained at 2000 ± 400 f/mg DL.

The results for the MMVF are consistent with those from analyses of dust content of lung tissues from 145 deceased MMVF workers (17). Only 26% of the lung tissues contained any MMVF, and that was almost all siliceous in nature and in low, average concentration of <200 f/mg DL.

Finally, the methodology employed in this study followed closely that used by Hammad et al. (17,18), and the present results for the slag wool fiber (MMVF22) appear to be similar to their results for slag-wool. This suggests that the methodology gives reproducible results and may be useful for selecting fiber types for lifetime inhalation studies.

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