Relationship between Lung Biopersistence and Biological Effects of Man-made Vitreous Fibers after Chronic Inhalation in Rats

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This article describes the relationship between fiber biopersistence and the chronic toxicity of different chemical compositions of man-made vitreous fibers (MMVF) in the lung. Rats were exposed in "nose-only" inhalation chambers, 6 hr/day, 5 days/week, for 24 months to aerosol concentrations of 30 mg/m^3 containing comparable fiber numbers and similar dimensions of fibrous glass (FG) or refractory ceramic fiber (RCF). Interim sacrifices were performed periodically to monitor fiber number and dimensions in the lung and the progression of pulmonary alterations. At each interim sacrifice, three to six recovery animals were removed from each exposure group and held until two years to determine the biopersistence of fibers after different exposure times. Fibers were recovered from the ashed lungs, counted, and measured using optical and scanning electron microscopy (SEM). Fiber chemistry was assessed in 91-week recovery lungs using energy dispersive spectroscopy (EDS) analysis. RCF induced lung fibrosis and an elevation in lung tumors and pleural mesotheliomas. FG exposure resulted in no lung fibrosis, no statistically significant increase in the lung tumor incidence, and no mesotheliomas. After two years of continuous exposure, the number of World Health Organization fibers per milligram dry lung recovered from RCF and FG exposed lungs was comparable. EDS analysis of recovery lungs showed that most of the alkalis and alkaline earths had leached from the FG fibers over time. A slight change in RCF chemistry was observed. These findings indicate that the change in the chemical composition of fibers may be an important determinant of the chronic toxicity of MMVFs. — Environ Health Perspect 102(Suppl 5):133–137 (1994).

Key words: biopersistence, MMVF, fibrous glass, RCF, lung burden, chemical durability, WHO fiber lung ashing

Introduction

There is a logical concern about the safety of any material that has the potential to release particles or vapors into the human environment that can be inhaled. Because fibrous materials can fall into this category, many studies have been and continue to be conducted to evaluate the possible health hazards of such materials. The present studies are part of a series of investigations into the chronic inhalation effects of two major categories of man-made vitreous fibers (MMVF).

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MMVFs are amorphous fibrous inorganic substances made primarily from rock, clay, slag, or glass. The three major classes of MMVF are refractory ceramic fibers (RCF), fibrous glass, and rock- or slagwool. Products from all three classes contain respirable fractions of fibers. RCFs are of particular value in high temperature, industrial applications. A variety of RCF types is produced by altering the proportions of alumina and silica with other refractory oxides. Fibrous glass makes up the largest category of the MMVFs, followed by rock- and slagwool. Fibrous glass and rock- slagwool are used typically in ambient to mid-temperature range insulation applications and building products.

RCF toxicity has been evaluated in three previous chronic inhalation studies, one using rats (*1*), one using rats and hamsters (*2*), and the third using hamsters (*3,4*). One abdominal mesothelioma was observed in a rat in the first study (*1*), and a pleural mesothelioma in a hamster in the second (*2*). The two studies differed sharply in the incidences of pulmonary tumors. In the third study, RCF 1 (kaolin) induced mesotheliomas and pulmonary fibrosis in the lungs of 42% of the hamsters (*3,4*).

A variety of fibrous glass compositions also has been evaluated in animal inhalation models (*2,5–11*). None of these studies identified a significant increase in either fibrosis or neoplasms following glass fiber inhalation in spite of FG lung burdens in excess of several hundred thousand fibers/mg dry lung tissue (f/mg DL). While none of these studies is completely adequate for health risk classification, considered as a group they provide strong evidence for a lack of significant adverse effects following glass fiber inhalation.

Recently, a new fiber aerosol generation and "nose-only" exposure system became available (*12*). This exposure system, coupled with the use of specially size selected fibers, resulted in a greater lung fiber burden than had been achieved following inhalation of a similar number of airborne fibers in a previous study (*2*). Additionally, this new system allowed exposure of larger groups of animals (up to 140 rats) and thus made possible periodic sacrifice for evaluation of potential pathological changes, while still providing adequate numbers of animals for evaluation of potential tumor formation.

The availability of this new technology led to the design and conduct of a chronic

Table 1. Fiber aerosol characteristics.

Target, mg/m³	Average, mg/m³	Fiber no, WHO F/cm³	Mean, diameter	Mean, length
RCF1				
30	29.1 ± 5.2	187 ± 53	0.82 ± 1.89[a] 0.98 ± 0.61[b]	15.9 ± 2.4[a] 22.3 ± 17.0[b]
MMVF 10				
30	29.1 ± 5.2	232 ± 56	1.27 ± 1.81[a] 1.43 ± 0.75[b]	13.1 ± 2.0[a] 16.8 ± 12.8[b]
MMVF 11				
30	28.9 ± 4.5	246 ± 76	0.68 ± 2.10[a] 0.90 ± 0.73[b]	13.7 ± 2.2[a] 18.3 ± 14.6[b]

Abbreviations: RCF1, Refractory ceramic fiber (Kaolin); MMVF, man-made vitreous fiber; MMVF 10, 11, are respirable fibrous glass from insulation. [a]Geometric mean. [b]Arithmetic mean.

multidose inhalation study of a variety of MMVFs. The present study reports the chronic biological effects of two different types of MMVF (RCF and FG) and relates these chronic effects to differences in the biopersistence of the two fiber types in the lung.

Materials and Methods

Fibers

A kaolin-based refractory ceramic fiber (RCF 1) and two fibrous glass compositions typical of those used for building insulation (MMVF 10 and 11) were presized so that they would be comparable to the dimensions of fibers found in workplace air and also be rat respirable. Positive control animals were exposed to intermediate length NIEHS chrysotile asbestos (Jeffrey Mine, Asbestos, Quebec).

Fiber Aerosol Exposure

Groups of 140 rats were exposed in nose-only inhalation chambers, 6 hr/day, 5 days/week, for 24 months to the 30 mg/m³ gravimetric concentrations of the test fibers. Negative control rats were exposed similarly to filtered air. Positive controls (*80*) were exposed to 10 mg/m³ of chrysotile asbestos. Aerosol concentrations were monitored at the level of each animal's nose for both fiber mass (mg/m³) and fiber number (fibers/cm³). Fiber size distributions of the aerosols were determined on a quarterly basis using scanning electron and optical microscopy (Table 1).

Animals

Weanling Fischer 344 male rats, (Charles River Breeding Laboratories, Kingston, NY) were randomly distributed into the exposure groups. After the 24-month exposure, the animals were held for lifetime observation (until approximately 20% survival), and were then sacrificed using intraperitoneal injections of sodium pentobarbital and examined.

Pathology

Three to six rats were randomly selected from each exposure group and killed at 3, 6, 12, 18, and 24 months. In addition three to six rats were taken off exposure at 3, 6, 12, and 18 months and allowed to recover until the 24-month sacrifice. Lungs were removed *in toto*, sectioned and stained with hematoxylin and eosin (H&E) and Masson's trichrome stain for collagen deposition. Histopathology of the lungs was examined and each lung was given a Wagner Pathology Grading Score in accordance with the guidelines presented at the World Heath Organization (WHO) conference (*8*). A lung of normal appearance was given a Wagner score of 1, while lungs showing increased cellularity, e.g., macrophage response, bronchiolization and inflammation were given scores of 2 and 3. These cellular changes are reversible. A Wagner score of 4 or greater, however, indicates that interstitial fibrosis, an irreversible change, has occurred in the lung.

Lung Burden Analysis

Immediately after necropsy, the right accessory lobe of each animal's lung was ligated, removed, and frozen for later analysis of lung fiber burden. To recover fibers from the lung, the tissue was rapidly dehydrated with acetone, dried to constant weight, and ashed using a low-temperature process. Recovered fibers were dispersed in distilled water, filtered onto membranes, and examined by optical and scanning electron microscopy (SEM). Number, dimensions and other physical characteristics of the inhaled lung fibers were determined using counting rules described in the WHO monograph (*13*). A WHO fiber is defined as having length/diameter (L/D) ratio of ≥3, a diameter <3 μm and a length >5 μm.

Fiber Chemistry

A portion of the fibers recovered from the lungs of animals sacrificed at 104 weeks but exposed for only 13 weeks and held without further exposure for 91 weeks (recovery animals) was filtered onto separate membranes and carbon coated. These fibers and stock fibers were analyzed by energy dispersive spectroscopy (EDS) using SEM for comparative chemical composition.

Table 2. Inhalation toxicity of kaolin-based refractory ceramic fiber (RCF1) and fibrous glasses (MMVF 10, 11).

Fiber group	Aerosol conc. fibers/cc		Lung burden fibers/mg (X 10⁴)		Wagner score	Total		
	WHO	> 10 μm	WHO	> 10 μm	104 week	lung tumors[a]	Mesotheliomas[a]	Chemical changes[b]
Air control 1[c]	ND	ND	<0.03	ND	1.0	2(1.6%)	0	—
Air control 2[c]	ND	ND	<0.03	ND	1.0	4(3.3%)	0	—
Chrysotile	11,000	4000	280	7.6	4.0	13(18.9%)	1(1.4%)	None
RCF1	187	156	28	14	4.0	16(13.0%)	2(1.6%)	Minor
MMVF 10	232	166	29	11	3.0	7(5.9%)	0	Major[d]
MMVF 11	246	153	50	17	2.5	3(2.7%)	0	Major

ND, not determined. [a]Tumor finding at end of study. [b]Based on comparative analysis of fibers from stock and 13-week recovery animals. [c]Air Control 1 were negative control animals used for the RCF1 study; Air Control 2 were negative control animals for the MMVF 10 and 11 study. [d]Based on results of MMVF 10 fibers recovered from lung of rats exposed for 5 days and held without further exposure for 6 months (*14,15*).

Results

Histopathology

Refractory Ceramic Fiber Study. At the 3-month sacrifice, the RCF 1 treatment group showed an increase in lung cellularity (pulmonary change grades 3.3 on the Wagner Scale). At the 6-month sacrifice, the lungs of animals exposed to RCF 1 had progressed to minimal fibrosis (Wagner grade 4). The lung pathology scores did not change appreciably from the 6- to 24-month sacrifices and remained the same up to the terminal sacrifice of animals held without further exposure for 6 months after cessation of inhalation treatment. In addition to lung fibrosis, a significant increase in lung tumors (13%) and the two pleural mesotheliomas (1.6%) were observed in these animals (Table 2).

Chrysotile Asbestos Control. Animals exposed to chrysotile asbestos (10 mg/m^3) at the 3-month timepoint were scored as grade 4, indicating lung fibrosis, and this grade persisted in the chrysotile group for the rest of the exposure and recovery period. In addition to pulmonary fibrosis, incidence of lung tumors was 18.9%, and one mesothelioma (1.4%) was observed.

Fibrous Glass Study. The first evidence (3 months) of fiber-induced microscopic changes in the lung consisted of an influx of pulmonary macrophages. Occasional microgranulomas were noted along the walls of the alveolar duct in the two groups exposed to fibrous glass (MMVF 10 and 11) along with a minimal amount of alveolar bronchiolization. These groups were scored as grade 3.0 (Table 2).

After 6 months exposure to fibrous glass there was a slight increase both in the macrophage response and in the number of microgranulomas. Bronchiolization was also noted. Fibers were present in many macrophages and within the interstitium. Short fibers also were noted in macrophages within the peribronchial lymphoid sheaths. The average Wagner grade remained at 3.0.

At 12 months the macrophage and microgranuloma responses were slightly more intense than at 6 months. At 18 and 24 months the pulmonary changes were comparable to those observed at 12 months. The Wagner grade remained at 3.0 between 12 months, and 24 months when the exposure stopped.

There was no evidence of treatment-related interstitial lung fibrosis at any time in the FG exposed animals. Neither were there any mesotheliomas, nor was there a statistically significant elevation of lung tumor incidence compared to the negative controls (Table 2).

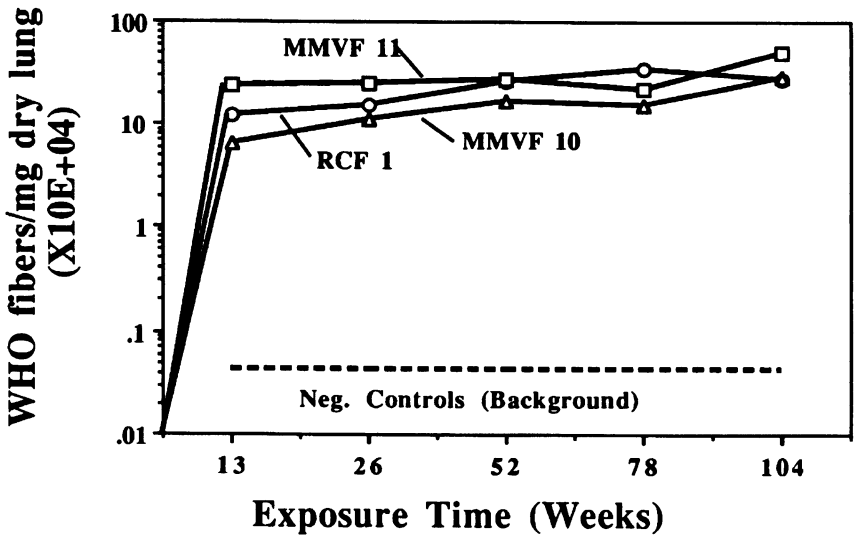

Figure 1. Lung burdens, expressed in WHO fibers/mg dry lung tissue, in rats exposed continuously to 30 mg/m^3 of RCF 1, MMVF 10, or MMVF 11. A WHO fiber is defined as having length:diameter ratio of ≥3, a diameter <3 µm and a length ≥5 µm.

Lung Burden Analysis

RCF Study. The RCF lung fiber burden data is expressed as the number of WHO fibers per mg dry lung weight (Figure 1). In the RCF 1 treatment group the lung burden increased to 3.6 ± 1.0 × 10^5 fibers/mg at 78 weeks of exposure, then leveled off at 2.8 ± 0.6 × 10^5 fibers/mg. There was an 87% reduction in the number of WHO fibers/mg from animals sacrificed immediately after 13 weeks exposure to RCF 1, compared to lung burdens of animals exposed for 13 weeks and allowed to recover for 91 weeks (Figure 2).

FG Study. The FG lung fiber burden data is expressed as WHO fibers/mg dry lung weight (Figure 1). In the MMVF 11

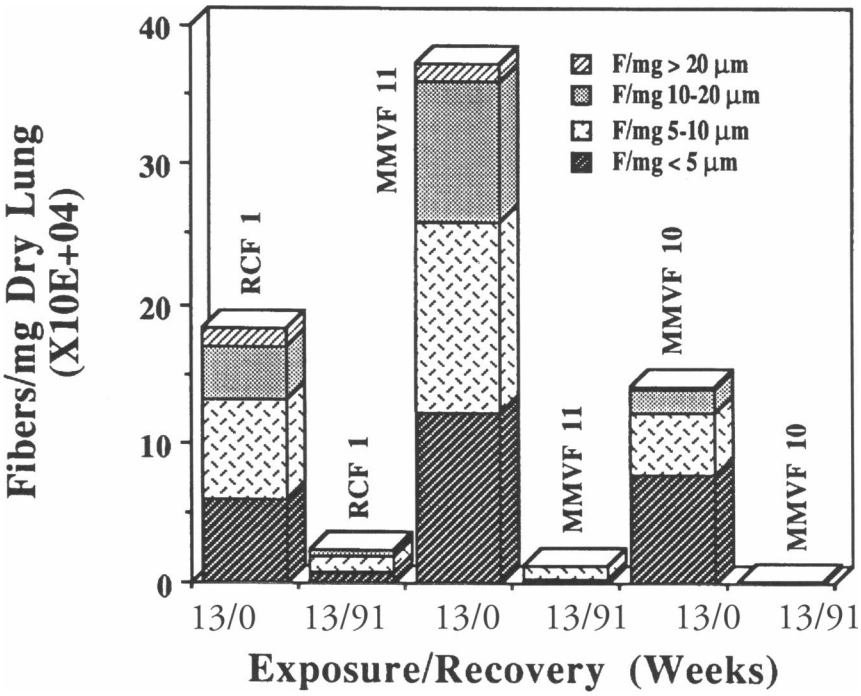

Figure 2. Lung burdens (number of WHO fibers/mg dry lung tissue) from animals sacrificed immediately after 13 weeks exposure to RCF 1, MMVF 10, or MMVF 11 compared to lung burdens of animals exposed for 13 weeks and allowed to recover for 91 weeks.

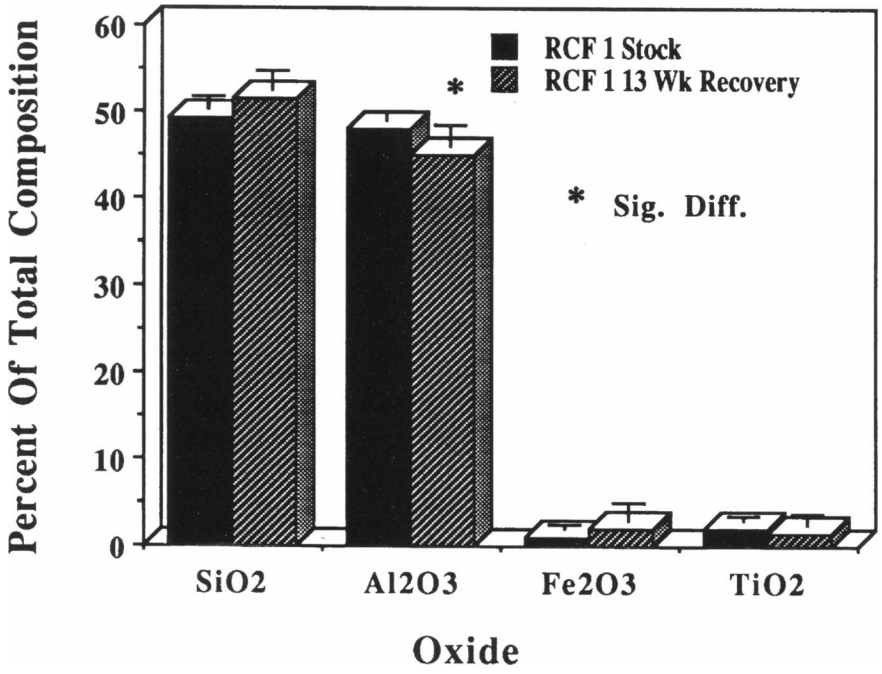

Figure 3. Chemistry of stock RCF 1 fibers compared to RCF 1 fibers recovered from the lungs of animals exposed for 13 weeks and allowed to recover for 91 weeks.

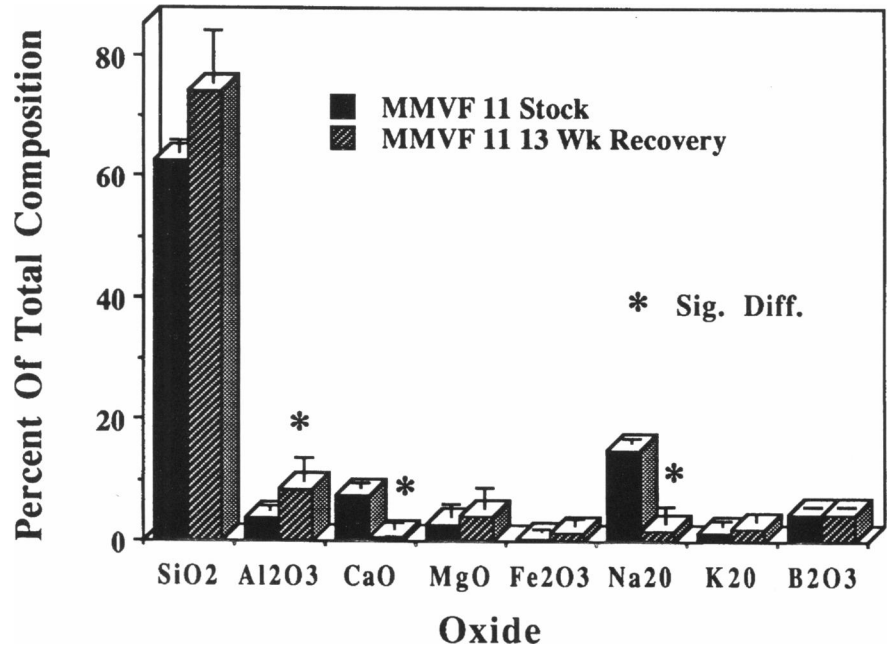

Figure 4. Chemistry of stock MMVF 11 fibers (fibrous glass) compared to MMVF 11 fibers recovered from the lungs of animals exposed for 13 weeks and allowed to recover for 91 weeks.

treatment group, the lung burden reached $2.5 \pm 0.3 \times 10^5$ in 13 weeks of exposure, increasing to $5.0 \pm 2.9 \times 10^5$ fibers/mg at 24 months. In the MMVF 10 treatment group the lung burden steadily increased from $6.6 \pm 1.8 \times 10^4$ after 13 weeks of exposure to $2.9 \pm 0.6 \times 10^5$ at 24 months. Between 13 weeks of exposure and 91 weeks of recovery, the WHO fiber/mg value dropped 95% in the MMVF 11 treatment group and 88% in the MMVF 10 treatment group (Figure 2).

Fiber Chemistry

RCF Study. The chemistry of RCF 1 stock fibers was compared to that of fibers recovered from the lungs of animals exposed for 13 weeks and allowed to recover for 91 weeks (Figure 3). There was a small but significant change in the Al_2O_3 content in the RCF 1 fiber as a result of 91 weeks residence in the lung.

FG Study. The chemistry of MMVF 11 stock fibers was compared to that of fibers recovered from the lungs of animals exposed for 13 weeks and allowed to recover for 91 weeks (Figure 4). Nearly total loss of CaO and Na_2O occurred during the 91 weeks of residence in the lung. The apparent increases in SiO_2 and Al_2O_3 are artifacts related to the decreases in other fiber components and the expression of the data in relative percentages. The chemistry of fibers recovered from MMVF 10 exposed lungs has not yet been analyzed. However, significant changes in fiber chemistry were observed in MMVF 10 fibers recovered from rat lungs exposed for 5 days and held without further exposure for 6 months (*14,15*). This suggests that both fibrous glass compositions are undergoing significant chemical alteration in the lung environment.

Discussion

In the present study, RCF induced pulmonary fibrosis and significant increases in lung tumors in a rodent chronic inhalation model. Pleural mesothelioma formation was also associated with RCF exposure. Details of the RCF study have been reported elsewhere (*3,4,16,17*). A comparable lung burden of the glass fibers produced neither pulmonary fibrosis nor a significant increase in lung tumors. The only exposure-related finding in the FG-exposed animals was an increase in mild cellularity in the lungs that did not appear to progress after 6 months of exposure. These cellular changes are reversible and are similar to the effects observed after inhalation of an inert dust. The FG study has been reported in more detail elsewhere (*18*).

In the present study, lung fiber levels increased comparably in the FG and RCF exposed animals. Since lung levels during continuous fiber exposure are determined by the balance between the deposition of fibers in the lung and their disappearance from the lung, and since the deposition of similarly sized fibers is assumed to be comparable, the disappearance of the two fiber types from the lung could also be assumed to be comparable. The finding that during a recovery period of 91 weeks following 13 weeks of exposure the disappearance of FG from the lung was similar to that of RCF confirms this assumption.

Previous short-term fiber exposure studies indicated that the biopersistence of fibers may be an important factor in their toxicity to the lung (*19–21*). Those studies showed that different fibers had different rates of disappearance (e.g., by dissolution and clearance), as well as different leaching rates. The present chronic inhalation study showed that the rates of disappearance of RCF and FG over 91 weeks were similar. However, the amount of chemical leaching of the fibers within the lung was very different for the two fiber types. This suggests that fibers that are more readily altered chemically within the lung may be less biologically active. Further analyses of the data are necessary before the importance of changes in chemistry, fiber number, or morphology can be related to the observed differences in biological activity of different MMVF types.

In conclusion, these findings demonstrate that this rodent inhalation model provides a sound basis to identify the potential hazards of fibrous materials in man. Further, these results suggest that the toxicologic potential of an MMVF may be dependent upon its chemical composition. Finally, these results indicate that some compositions of MMVFs that are not toxic to experimental animals may represent no significant hazard for fibrotic or neoplastic disease in humans.

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