Chronic Inhalation Study of Fiber Glass and Amosite Asbestos in Hamsters: Twelve-month Preliminary Results

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The effects of chronic inhalation of glass fibers and amosite asbestos are currently under study in hamsters. The study includes 18 months of inhalation exposure followed by lifetime recovery. Syrian golden hamsters are exposed, nose only, for 6 hr/day, 5 days/week to size-selected test fibers: MMVF10a (Schuller 901 insulation glass); MMVF33 (Schuller 475 durable glass); amosite asbestos (three doses); or to filtered air (controls). Here we report interim results on airborne fiber characterization, lung fiber burden, and pathology (preliminary) through 12 months. Aerosolized test fibers averaged 15 to 20 μm in length and 0.5 to 1 μm in diameter. Target aerosol concentrations of World Health Organization (WHO) fibers (longer than 5 μm) were 250 fibers/cc for MMVF10a and MMVF33, and 25, 125, or 250 fibers/cc for amosite. WHO fiber lung burdens showed time-dependent and (for amosite) dose-dependent increases. After a 12-month exposure, lung burdens of fibers longer than 20 μm were greatest with amosite high and mid doses, similar for low-dose amosite and MMVF33, and smaller for MMVF10a. Biological responses of animals exposed for 12 months to MMVF10a were limited to nonspecific pulmonary inflammation. However, exposures to MMVF33 and each of three doses of amosite were associated with lung fibrosis and possible mesotheliomas (1 with MMVF33 and 2, 3, and 1 with amosite low, mid, and high doses, respectively). Pulmonary and pleural changes associated with amosite were qualitatively and quantitatively more severe than those associated with MMVF33. As of the 12-month time point, this study demonstrates that two different fiber glass compositions with similar fiber dimensions but different durabilities can have distinctly different effects on the hamster lung and pleura after inhalation exposure. (Preliminary tumor data through 18 months of exposure and 6 weeks of postexposure recovery became available as this manuscript went to press: No tumors were observed in the control or MMVF10a groups, and no additional tumors were observed in the MMVF33 group; however, a number of additional mesotheliomas were observed in the amosite groups.) — Environ Health Perspect 105(Suppl 5):1223–1229 (1997)

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Introduction

Synthetic vitreous fibers (SVF) are a class of inorganic fibrous materials made primarily from rock, clay, slag, or glass. The three major classes of SVF are fiber glass, rock/slag wool, and refractory ceramic fibers (RCF). Recently, a series of chronic inhalation studies was conducted to evaluate the biological effects in rodents of fibers from each of the major classes of SVF and of two forms of asbestos. Results from these and previous studies suggest that the rat is a useful laboratory model for assessing both fibrotic and tumorigenic properties of airborne fibers (1–3). The maximum SVF exposure concentration used in these studies was 30 mg/m3 air (approximately 200–300 fibers/cc longer than 5 μm). In the rat, this concentration of RCF was associated with a significant increase in lung and pleural tumors, but similar concentrations of insulation fiber glass or rock/slag wool were not associated with an increase in tumors. Lower RCF concentrations did not induce fibrosis or elevate tumor incidence in rats.

The hamster model was also used in a previous study in this series, in which the inhalation effects of a refractory ceramic fiber (RCF1) and chrysotile asbestos were evaluated (2). While both RCF1 (at 30 mg/m3, 215 fibers/cc longer than 5 μm) and chrysotile (at 10 mg/m3, 3000 fibers/cc longer than 5 μm) induced fibrosis, neither fiber induced any lung tumors. However, RCF1, but not chrysotile, induced pleural mesotheliomas [in 42 of 112 hamsters (4)].

In the present hamster inhalation study, hamsters are exposed to SVF at an average aerosol concentration of 30 to 37 mg/m3 (approximately 250 fibers/cc longer than 5 μm). This concentration was chosen as the maximum exposure based on results from a previous 13-week range-finder study that estimated that this exposure would be the maximum tolerated dose for hamsters (5). The present paper reports interim results on airborne fiber characterization, lung fiber burden, and pathology (preliminary) through 52 weeks of exposure. A later paper will present findings from other assays and from the remaining time points.

Materials and Methods

Experimental Design

Syrian golden hamsters (125–140 males per exposure group, 13–15 weeks of age at onset of exposure [Charles River Laboratories, Quebec, Canada]) are exposed to test fiber aerosol or to filtered air in nose-only inhalation chambers, 6 hr/day, 5 days/week, for 18 months. The original protocol designates five time points (after 3, 6, 12, and 18 months of exposure and after a postexposure recovery period to be terminated at 10–20% survival) at which hamsters from each of the six exposure groups are to be euthanized to evaluate pulmonary and...
pleural responses and lung burden. Here we report interim findings through 12 months of exposure.

Because of increased mortality (see "Results"), the planned 26-week (6-month) sacrifice was not conducted; however, lung burdens and pathology were analyzed in five animals per exposure group that died spontaneously or were euthanized in extremis after 26 ±1 week of exposure.

Test Fibers

The present study evaluates the chronic inhalation effects of three fiber compositions, each of which was size-selected to be similar to the dimensions of fibers in workplace air (6) and to rodent respirable (Table 1). MMVF10a was derived from Schuller 901, which is representative of commercial insulation glass and is comparable in composition to the MMVF10 test fiber used in previous rat inhalation studies (1). MMVF33 was derived from Schuller 475 glass, one of the more durable glass fibers used commercially. Because 475 glass is used in high efficiency air filtration products (HEPA filters), it has been engineered to have greater environmental durability, including resistance to high humidity, than insulation glass. The 475 glass differs from 901 glass in composition as well as in manufacturing process: 475 glass contains zinc and barium for greater durability, and it is manufactured using a flame attenuation process. The 901 insulation wool from which MMVF10a was derived is not flame attenuated and does not include zinc or barium in its formulation.

Amosite asbestos was included in this study because it is a known human carcinogen and because a test fiber was available that is longer and thicker than most other asbestos test fibers. After size-selection for the longer fibers, the amosite test fiber dimensions were comparable to the dimensions of the SVF (Table 1). A primary goal was to have dimensions for the three test fibers be as similar to each other as possible to achieve comparable lung dosing and thus focus on any possible pathogenic differences due to fiber composition or surface reactivity.

Fiber Aerosol Exposure

The study included an air control group (exposed to filtered air) and five fiber exposure groups: MMVF10a and MMVF33 (both at target concentrations of 250 World Health Organization (WHO) fibers/cc) and three doses of amosite asbestos (25, 125, and 250 WHO fibers/cc, respectively) (Table 1). WHO fibers are defined as having a length/diameter ratio ≥ 3, diameter <3 μm and length > 5 μm (7). Nose-only inhalation exposure was conducted according to the method of Bernstein et al. (8).

Techniques for nondestructive aerosolization and aerosol monitoring have been described previously (9).

Aerosol samples were collected on filters placed in animal exposure ports for 5 hr. Fiber mass concentrations (mg/m³) were determined for each fiber aerosol once per exposure day throughout the study. The concentrations of fibers per cubic centimeter and their bivariate dimensions were determined at least once every 2 weeks using scanning electron microscopy described by Hesterberg et al. (10). Counting procedures were conducted according to WHO Monograph 4 counting rules (7) modified for use with electron microscopy.

Lung Burden Analysis

To allow upper airway clearance, animals were euthanized for histopathological and lung burden analyses 48 hr after the last exposure. Thus, the measured lung fiber burdens are assumed to reflect fibers that were essentially deposited in the alveolar region. At necropsy, the left lung (without the bronchi) of each animal was removed, stored frozen, dried, ashed by a low temperature method, and suspended in water. The lung fibers were recovered from the suspension by filtration and analyzed using electron microscopy according to procedures described by Hesterberg et al. (10).

Pathology

Hamsters were observed daily for clinical signs, morbidity, and mortality throughout the study. Euthanasia was performed using pentobarbital and exsanguination as described by McConnell et al. (11). Each hamster was necropsied upon death. The lungs were removed, weighed, and examined under a dissecting microscope. The right lung and the diaphragm were prepared for histopathological examination according to the method of McConnell et al. (2) and graded for inflammatory change, fibrosis, and neoplasms by the study pathologist (11). This paper focuses on the pathology observed at three time points (13, 26, and 52 weeks) and provides preliminary results of the study pathologist. At the termination of the study, a panel of pathologists will independently review the pulmonary and pleural lesions. After discussions with this panel, the study pathologist will then determine the final diagnoses.

Results

Test Aerosols

Because of differences in fiber dimensions and in specific gravity between the three test fibers, the mass concentration targets for MMVF10a (30 mg/m³), MMVF33 (37 mg/m³), and high dose amosite (7.5 mg/m³) were set at different levels in an attempt to achieve similar aerosol concentrations of WHO fibers (fibers longer than 5 μm). The WHO fiber concentration

Table 1. Fiber concentrations and dimensions, averages through 12 months.

| Test fibers | Insulation glass | Durable glass | Amosite asbestos |
|-------------|-----------------|---------------|-----------------|
| Mass, mg/m³ | 30 ± 2          | 37 ± 2        | 0.8 ± 0.2       |
| WHO fibers/cc | 323 ± 57      | 283 ± 42      | 33 ± 23         |
| Fiber dimensions | 151 ± 22     | 106 ± 20      | 9 ± 8           |

*Arithmetic mean ± SD. *Geometric mean ± the mean of the SD. *WHO respirable fibers (fibers having an aspect ratio ≥ 3 and length > 5 μm, as defined by the WHO. *Fibers longer than 20 μm.
of MMVF10a (323 WHO fibers/cc) was significantly greater than that of either MMVF33 (283 WHO fibers/cc) or amosite high dose (255 WHO fibers/cc), but the latter two were not significantly different (Table 1). The concentration of fibers per cubic centimeter longer than 20 μm for MMVF10a (151 fibers/cc > 20 μm) was also significantly greater than that of MMVF33 (106 fibers/cc > 20 μm), which was significantly greater than that of amosite high dose (67 fibers/cc > 20 μm) (Table 1).

**Mortality**

An increased mortality rate occurred in all exposure groups during weeks 17 to 26 due to an infectious disease diagnosed as wet tail, a common disease of hamsters. Because mortality rates during this time were high in air controls and in unexposed sentinel hamsters, the mortality was judged to be unrelated to fiber exposure. Tetracycline was administered to all animals at two different time points (400 mg tetracycline/liter of drinking water for 17 days and 13 days, respectively). After the second tetracycline treatment the diet was also modified to include higher roughage content (from 5.2–7% with compensatory decrease in carbohydrates). After the 26-week time point, mortality rates returned to levels similar to those of previous hamster studies.

**Body and Lung Weights**

Average body weights of the five fiber exposure groups did not differ significantly from sham-exposed controls. After 13 and 52 weeks of inhalation, lung weights for the mid and high doses of amosite were significantly elevated compared to the air controls (Figure 1). In contrast, lung weights for the other fiber exposure groups were similar to those of air controls.

**Lung Fiber Burden**

Several lung burden findings are noteworthy:

*a* The average dimensions of MMVF10a and MMVF33 fibers in the lung are smaller than in the aerosols, which suggested that the larger fibers of the aerosol were not able to penetrate to the lower lung (Table 1). However, amosite lung fiber dimensions are similar to aerosol dimensions, probably because the vast majority of amosite aerosol fibers were in the respirable range.  

*b* The dimensions of the three test fibers in the lung were more similar to each other than the dimensions in the aerosols (Table 1). In contrast to the aerosol fiber dimensions, the average lung fiber dimensions for the three test fibers did not differ significantly from each other (geometric mean dimensions after 13 weeks of exposure).  

*c* Lung burden data showed both time- and dose-dependence. The number of WHO fibers per lung for MMVF10a and MMVF33 was time dependent from 26 to 52 weeks of inhalation (but not from 13–26 weeks) (Figure 2A). WHO fiber burdens for amosite were both time- and dose-dependent with the exception that the mid and low doses did not differ statistically from each other at the 13-week time point.  

*d* WHO fiber lung burdens were similar for the two fiber glasses and low-dose amosite and for RCF1 (Figure 2) [RCF1 data is from a previous study (2)].  

*e* The lung burdens of fibers longer than 20 μm for amosite also tended to be dose- and time-dependent; in contrast, the number of long glass fibers/lung tended either to decrease (MMVF10a) or remain the same (MMVF33) from 13 to 52 weeks (Figure 2B). In contrast to the fiber glasses, the long fiber lung burden of RCF1 (2) showed time-dependent increases through 52 weeks of exposure (Figure 2B).  

*f* Lung fiber retention in the amosite high-dose group was greater than in the SVF groups even though aerosol concentrations were comparable. At 52 weeks, the WHO fiber lung burden for amosite high dose was 8-fold greater than for MMVF10a and 5-fold greater than for MMVF33 (Figure 2B).  

**Histopathology**

**Air Controls.** Air controls showed no significant macroscopic pulmonary changes. Microscopically, a few pulmonary macrophages were noted scattered randomly throughout the parenchyma. There was no progression in severity during the course of the study. No changes were observed in the pleura at any time point.

**MMVF10a (323 WHO Fibers/cc).** Pulmonary changes seen at 3 months were limited to a slight to mild excess in the number of pulmonary macrophages and the presence of microgranulomas and a few multinucleated giant cells concentrated
Table 2. Severity of changes in hamster lungs and pleura: average scores.a,b

| Fiber        | Exposure time, weeks | Macrophage infiltration | Alveolar bronchiolization | Microgranuloma | Giant cells | Interstitial fibrosis | Wagner grade | Pleura |
|--------------|----------------------|-------------------------|---------------------------|----------------|-------------|----------------------|--------------|--------|
| Air controls | 13                   | 0                       | 0                         | 0              | 0           | 1                    | 0            | 0      |
|              | 26                   | 0                       | 0                         | 0              | 0           | 1                    | 0            | 0      |
|              | 52                   | 0                       | 0                         | 0              | 0           | 1                    | 0            | 0      |
| MMVF10a      | 13                   | 2.0                     | 0                         | 0.6            | 0.4         | 0                    | 2.0          | 0      |
|              | 26                   | 1.0                     | 0                         | 0.4            | 0.2         | 0                    | 2.0          | 0      |
|              | 52                   | 0.6                     | 0.5                       | 1.3            | 0           | 0                    | 2.3          | 0      |
| MMVF33       | 13                   | 2.0                     | 0.8                       | 1.6            | 2.0         | 0                    | 2.6          | 0      |
|              | 26                   | 2.0                     | 0.4                       | 2.0            | 0           | 0                    | 3.1          | 0.2    |
|              | 52                   | 2.3                     | 2.0                       | 2.3            | 0.8         | 4.0                  | 2.0          | 0      |
| Amosite, low | 13                   | 2.4                     | 1.4                       | 2.0            | 2.0         | 0                    | 3.2          | 0      |
|              | 26                   | 2.4                     | 1.6                       | 2.6            | 2.0         | 0                    | 4.0          | 0.8    |
|              | 52                   | 2.8                     | 2.3                       | 3.0            | 2.0         | 0.8                  | 4.0          | 1.5    |
| Amosite, mid | 13                   | 3.0                     | 2.2                       | 3.2            | 2.0         | 0                    | 3.6          | 0.6    |
|              | 26                   | 3.0                     | 2.6                       | 3.0            | 4.0         | 0.8                  | 4.1          | 1.6    |
|              | 52                   | 3.8                     | 3.0                       | 4.0            | 4.0         | 2.3                  | 5.3          | 1.8    |
| Amosite, high| 13                   | 3.0                     | 2.8                       | 3.6            | 2.0         | 0                    | 3.8          | 1.6    |
|              | 26                   | 3.2                     | 3.2                       | 3.4            | 2.8         | 1.6                  | 4.3          | 0.6    |
|              | 52                   | 3.8                     | 2.3                       | 4.0            | 3.0         | 2.3                  | 6.0          | 2.8    |

aScoring system for all but Wagner: 0 = normal; 1 = minimal; 2 = mild; 3 = moderate; 4 = marked; 5 = widespread and severe. Scores for five animals/time point/exposure were averaged. bWagner grades: 1 = normal; 2 = minimal cellular change (macrophage response); 3 = mild cellular change (macrophages and bronchiolization); 4 = minimal fibrosis (restricted to bronchoalveolar junctions); 5 = mild fibrosis (interlobular linking); 6 = moderate fibrosis (early consolidation); 7 = severe fibrosis (marked consolidation); 8 = very severe fibrosis (complete obstruction of airways) [McConnell et al. (16)]. n = 4–5 hamsters.

Near the bronchoalveolar junctions (Table 2). There was no progression in severity from 3 to 6 months, but after 12 months of exposure, the number of macrophages had increased and clusters of macrophages appeared. These macrophage clusters appear to be a response to high dosing; in previous studies, high doses of nonfibrous particles induced lung overload (12). Many fibers were observed, most of which were within these macrophages and microgranulomas. Overall, the pulmonary changes in most of the hamsters was consistent with Wagner grade 2 (Table 2, footnote b). No treatment-related changes were found in the pleura through 12 months.

MMVF33 (283 WHO Fibers/cc). The only macroscopic abnormalities noted at the scheduled time points were at 12 months: a 2-mm diameter white focus on the lung surface of one hamster and variable sized fine translucent granular patches on the surface of the rib cages (only discernible with a dissecting microscope) of several of the hamsters. One hamster that died spontaneously at 7.5 months showed variably sized white flat foci on the surface of the lung, rib cage, and diaphragm, which were subsequently diagnosed as mesothelioma.

The primary microscopic lung change at 3 months was a slight to mild excess of macrophages randomly scattered throughout the parenchyma but concentrated in the bronchoalveolar junctions (Table 2). Microgranulomas and occasional multinucleated giant cells were also seen at the bronchoalveolar junctions. Many fibers and fiber fragments were found within the inflammatory lesions and free in the alveoli. A slight amount of alveolar bronchiolization (change from flat to cuboidal cells) was noted in the alveoli adjacent to the terminal bronchiole. The overall severity at 3 months was consistent with Wagner grade 2 to 3 (Table 2). The inflammatory changes progressed in severity by 6 months of exposure and were accompanied by the presence of collagen (fibrosis) in the proximal portion of occasional alveolar ducts and were classified as Wagner grade 4. The lesions had further progressed by 12 months when most of the alveolar ducts and the terminal bronchioles showed evidence of mild interstitial fibrosis, but the lesions were still classified at Wagner grade 4 because no interlobular linking was observed.

Pleural changes were observed at 6 months; all hamsters exhibited a slight amount of collagen deposition appearing as small focal accumulations in the pleura just subjacent to the mesothelial covering. The mesothelial cells overlying these foci at times showed change to a spherical (normally flat) appearance that was interpreted as hypertrophy. By 12 months, the lesions had progressed to include microvillus-like hyperplasia of the pleura at the edge of the lobes.

Amosite Asbestos (33 WHO Fibers/cc). Three macroscopic abnormalities appeared in this group after 12 months of exposure: failure of the lungs to collapse normally when the thorax was opened, pleural thickening, and irregular lung margins. Microscopically, at 3 months a moderate number of neutrophils (not seen with SVF), macrophages, clumps of macrophages, many well-defined microgranulomas (some containing fibers) and a few multinucleated giant cells were found, primarily in the area of the bronchoalveolar junctions (Wagner grade 3, Table 2). In most of the hamsters exposed for 3 months, a minimal to slight amount of bronchiolization was observed, as well as minor collagen deposition in the walls of the alveoli subjacent to microgranulomas. After 6 months of exposure the lesions had progressed in severity. The inflammatory response was much more intense, and pulmonary fibrosis (found in all of the animals), while slight, affected nearly all of the bronchioles and extended peripherally along the alveolar duct and adjacent alveoli (Wagner grade 4). Fibers, some quite long (>40 μm), were numerous within the lesions and occasionally penetrated the pleura. Occasional fibers had a knobby appearance comparable to classic asbestos bodies. The lesions had further progressed by 12 months and were more severe than with MMVF33, although they were still interpreted as Wagner grade 4.
Table 3. Pleural mesotheliomas in hamsters during 12 months of inhalation exposure.

| Test fibers | No. at risk | Hyperplasia-borderline mesotheliomas* | Early to advanced mesotheliomas | Incidence, % |
|-------------|-------------|---------------------------------------|---------------------------------|--------------|
| Air controls | 63          | 0                                     | 0                               | –            |
| MMVF10a     | 63          | 0                                     | 0                               | –            |
| MMVF33      | 50          | 0                                     | 1                               | 2.0          |
| Amosite     | Low         | 42                                    | 2                               | 4.8          |
|             | Mid         | 43                                    | 1                               | 7.0          |
|             | High        | 56                                    | 1                               | 1.8          |

*Tentatively diagnosed as early mesotheliomas. Final diagnoses to be made by a panel of pathologists at termination of study approximately April 1997.

Slight pleural collagen deposition similar to that described with MMVF33 was observed as early as 3 months. By 6 months it had progressed in severity and was accompanied by mesothelial hypertrophy and hyperplasia. By 12 months pleural fibrosis had become more diffuse and thicker, and mesothelial hypertrophy and hyperplasia were common features. Similar, but somewhat more prominent, changes were observed in the pleura lining the rib cage and diaphragm. Two hamsters showed mesothelial hyperplasia with dysplastic changes that were given the preliminary diagnosis of early, borderline mesotheliomas (Table 3).

**Amosite Asbestos (157 WHO Fibers/cc).**

Macroscopic abnormalities were observed in this group after 6 months of exposure: lungs failed to collapse normally when the thorax was opened, and a few small grayish-white foci were scattered on the surface of the lungs of some hamsters. After 12 months these macroscopic abnormalities were more severe and were noted in all hamsters. Microscopically, the lung lesions were qualitatively similar to those of the low-dose amosite group but more widespread and severe. Although the lesion was certainly more severe than with 25 fibers/cc, by definition it was still consistent with a Wagner grade 4, which is a limitation of this grading system. At 6 months of exposure, the overall lesion had progressed in severity, although it was still in the Wagner grade 4 category. The fibrotic lesion had extended peripherally along the alveolar duct and into the adjacent alveoli, almost to the level of the pleura. Numerous fibers could be found in macrophages, granulomas, and giant cells at this time. At 12 months the fibroinflammatory lesions were much more apparent than at 6 months. Fibers and asbestos bodies were abundant within the lesions. In addition, interstitial fibrosis had progressed to the point of interlobular linking, which is the essential feature for classifying the overall lesion as Wagner grade 5.

By 3 months, collagen deposition in the pleura was evident in most hamsters. After 6 months it was more extensive in all hamsters and was accompanied by mesothelial hypertrophy and hyperplasia. By 12 months, the pleural fibrosis had become thicker and more diffuse and mesothelial hypertrophy and hyperplasia were common features. Again, these lesions were more prominent in the pleura lining the rib cage and diaphragm, where a fine granular appearance was observed at necropsy. Occasional multinucleated giant cells, comparable to those observed in the lung, were found in hyperplastic lymphoid tissue at the costophrenic junction. In two hamsters, small mesotheliomas of the papillary type were noted. Additionally, one hamster had mesothelial hyperplasia with dysplastic changes characteristic of early mesothelioma.

**Amosite Asbestos (255 WHO Fibers/cc).**

Macroscopically and microscopically, the lesions in this group were similar to those described for the low- and mid-dose amosite groups through 3 months. (Wagner grade 4). By 6 months, the pulmonary lesions had progressed in severity and appeared comparable to those in the mid-dose group. The most apparent difference was that interstitial fibrosis had progressed to a fibrotic linking of the lobules in a majority of the hamsters, which placed the lesion in Wagner grade 5 category. After 6 and 12 months of exposure, the lungs failed to collapse and appeared even more stiff than in the mid-dose group. Focal (2–6 mm diameter) reddish solid areas resembling liver (hepatization) were seen in all of the lungs. Microscopically, the fibroinflammatory lesion had progressed further and the lungs were severely compromised, even more so than in the mid-dose group. Fibers, as well as asbestos bodies, were again commonly observed within the lesions. Areas of consolidation were a prominent feature in most lobes (Wagner grade 6). Pleural collagen deposition was seen in all hamsters after 3 months and was advanced and accompanied by moderate mesothelial hypertrophy by 6 months. By 12 months the pleural fibrosis had progressed and mesothelial hypertrophy and hyperplasia were common features. In some cases the fibrotic lesion was thicker than the diaphragm itself and was just as prominent in the rib cage. One hamster had mesothelial hyperplasia with dysplastic changes that are characteristic of early mesothelioma (Table 3).

**Preliminary Results through the End of the Study**

Preliminary tumor data through the end of the study (18 months of exposure and 6 weeks of postexposure recovery) were received just before this manuscript went to press. No additional tumors were observed in any of the air controls or fiber glass-exposed hamsters; however, a number of additional mesotheliomas were observed in the amosite-exposed groups.

**Discussion**

Even though exposure concentrations and dimensions were comparable for the two fiber glasses and high-dose amosite, later lung burdens differed considerably, especially for fibers longer than 20 µm. For example, the long-fiber lung burden for MMVF33 was significantly greater than for MMVF10a (by an order of magnitude) even though the long-fiber aerosol concentration for MMVF33 was 30% less than for MMVF10a. High-dose amosite (67 fibers/cc > 20 µm) resulted in a 50-fold greater lung burden than MMVF10a (aerosol concentration of 150 fibers/cc > 20 µm). Differences in lung burdens could result from differences in lung deposition, differences in lung biopersistence, or both. To determine whether lung depositions were similar for each of the fibers administered at similar aerosol concentrations, a brief lung deposition test was conducted toward the end of the 18-month exposure period. Hamsters were exposed to test aerosols for 1 day (6 hr), held for an additional day, then sacrificed. The data, which became available just before this manuscript went to press, indicate that lung depositions were very similar; the numbers of fibers longer than 20 µm/lung (x10⁵) were 1.6 ± 0.6 (MMVF10a), 2.2 ± 0.6
(MMVF33), and 2.0±0.5 (amosite high dose). These data suggest that the observed differences in the 12-month lung burdens of the three fibers were related to differences in biopersistence rather than in lung deposition.

The three fiber types in the present study induced a range of pathogenicities that paralleled differences in lung burdens of fibers longer than 20 µm (Table 2; Figure 2B). As in previous studies, the initial response induced by each of the fiber exposures appeared at the level of the bronchoalveolar junction, the area of highest fiber deposition, and consisted of an influx of macrophages, similar to that induced by most inhaled foreign particulates, followed by microgranulomas. In the MMVF10a hamsters, the lung response did not progress further through 12 months of exposure. However, in the MMVF33 animals, the lesions did progress with time/exposure, with more intense inflammation, interstitial fibrosis, pleural collagen deposition, mesothelial hypertrophy, and hyperplasia, and a single mesothelioma. The pulmonary and pleural lesions in the amosite-exposed hamsters were more severe and differed qualitatively from those associated with MMVF33. Previous studies in hamsters of RCF at approximately equal exposures (200–300 WHO fibers/cc) and with chrysotile asbestos at much higher exposures (3000 WHO fibers/cc) failed to show lesions as severe as those induced by amosite, even after 20 months of exposure (2). The present study suggests that in the hamster, long-fiber amosite asbestos is not only more toxic than the two fiber glasses, but also more toxic than chrysotile asbestos. Average aerosol concentrations of fibers/cc longer than 20 µm were comparable for amosite and chrysotile (67 and 77, respectively).

Differences in long-fiber lung burdens between MMVF10a and MMVF33 and between the fiber glasses and amosite were apparently related to differences in fiber biopersistence and not to differences in lung deposition. Fibers that are relatively soluble or leachable could either dissolve in the lung or break into shorter segments, allowing more rapid removal from the lung by clearance mechanisms. Differences in the biopersistence of long fibers in the lung would, in turn, be reflected in the quality and severity of the pathological responses of the lung and pleura to the fibers. However, as mentioned above, without data on initial lung deposition, it could also be argued that long-fiber deposition efficiency could have been greater for amosite because of its smaller diameters compared to the two glass test fibers.

As for the differences in pathogenicity between amosite asbestos and chrysotile asbestos, there are at least three possible explanations. First, there were very few chrysotile fibers longer than 20 µm, and longer fibers are thought to be more toxic than shorter fibers (13). In contrast, 25% of the amosite aerosol fibers were greater than 20 µm. Second, chrysotile is more soluble and therefore less persistent in the lung than amphibole types of asbestos, although chrysotile is several orders of magnitude more durable than fiberglass (14). Finally, chrysotile is a relatively soft serpentine (curly) fiber, while amosite, an amphibole, is a straight (needlleike) fiber. It could be that this latter feature allowed more of the long amosite fibers to reach the pleura than was possible for chrysotile, and this could explain the differential pleural response.

The pathogenesis associated with 475 glass in the present study does not agree with five previous rodent inhalation studies of this composition, all of which reported no fibrosis or increase in tumor incidence after 1 or 2 years exposure to 3 to 10 mg/m³ (15–19). The lack of reported effects could be explained by the facts that, in all but the Smith et al. study (19), the 475 fibers were relatively short and the test system was rats; furthermore, the first three studies used whole-body exposure rather than nose only. Smith et al. (19) exposed hamsters for up to 2 years to 5 mg/m³ (530 fibers longer than 10 µm/cc), compared with the present study that exposed hamsters to 283 fibers longer than 5 µm and 106 fibers longer than 20 µm. There is no obvious explanation for the differences in the effects of 475 glass in the two studies. Two possibilities are offered: a) Although the Smith study aerosol had more fibers/cc longer than 5 µm, it may not have had as many fibers/cc longer than 20 µm as the present study—the report by Smith et al. (1987) does not provide these data. b) Lung burden data were not as thoroughly reported in the Smith study, so it is not known whether target tissue dosing of longer fibers was as high in the Smith study as in the present study.

In conclusion, it is clear from these and other studies that not all types of asbestos have the same pathogenic potential. It is equally clear that not all SVF or even all glass fibers have the same biological reactivity after inhalation exposure. The fact that strikingly different pathogenicities were induced by each of the three fiber compositions, even when aerosols were comparable in fiber dimensions and concentrations, points to the importance of fiber biopersistence and surface reactivity.

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