Pleural Dosimetry and Pathobiological Responses in Rats and Hamsters Exposed Subchronically to MMVF 10a Fiberglass

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Interspecies differences in pulmonary and pleural responses to the inhalation of natural mineral and synthetic vitreous fibers have been observed in chronic and subchronic studies. However, the reasons for these differences are not clearly understood. There are also fiber-specific differences in the outcome of chronic inhalation exposure to natural mineral and synthetic vitreous fibers. Whether these differences are dependent upon the ability of these fibers to translocate to the pleural space is unknown. The present study was conducted to compare retained fiber burdens and selected pathological responses in the pleural compartments of rats and hamsters following subchronic inhalation of MMVF 10a fiberglass, a fiber negative for tumorigenesis or fibrosis in chronic studies. Fischer 344 rats and Syrian golden hamsters were exposed for 4 or 12 weeks by nose-only inhalation at nominal aerosol mass concentrations of 45 mg/m³ (610 WHO fibers/cc). Pulmonary fiber burdens and pulmonary inflammatory responses were greater in rats than in hamsters. The total number of fibers in the lung was approximately three orders of magnitude greater than in the pleural compartment. Pleural burdens in the hamster (160 fibers/cm² surface area) were significantly greater than burdens in similarly exposed rats (60 fibers/cm² surface area) following 12 weeks of exposure. With time postexposure, pleural burdens decreased in hamsters but were essentially unchanged in rats. Pleural inflammatory responses in both species were minimal. In rats, pleural inflammation was characterized by increased numbers of macrophages and increases in mesothelial cell replication during the period of fiber exposure. In contrast, hamsters had increased numbers of macrophages and lymphocytes, and mesothelial-cell replication indices were elevated on the parietal pleura of the costal wall and diaphragm, with some of these responses persisting through 12 weeks of postexposure recovery. Taken together, the results suggest that differences among rodent species in pleural responses to inhaled fibers are due to a delivered dose of fibers and to the biological responses to the presence of the fibers.

Key Words: vitreous fibers; mineral fibers; tumorigenesis; lung; pleural compartment; inflammatory response.

Man-made vitreous fibers (MMVF) comprise a diverse group of fibrous materials with compositional differences that are primarily due to the starting materials used to produce them. Three major classes of these fibers are produced and used in residential and commercial applications. The largest of these classes, fibrous glass, has found widespread use in thermal and acoustical insulation and filtration. Research using rodents exposed by inhalation has demonstrated that the MMVF group contains fibers with a broad range of pathogenic potential (Hesterberg and Hart, 2001). Various epidemiological (Doll, 1986; Lippman, 1990) and animal studies (Hesterberg et al., 1993, 1999; Marsh et al., 2001a,b; McConnell et al., 1999) have been conducted to assess the possible detrimental health effects resulting from the inhalation of fibrous glass. The large database of research on health effects resulting from inhalation of fibrous glass supports the conclusion that fiber respirability, length, and biopersistence in the lung are the primary determinants of toxicity for these materials.

The sequelae of natural mineral fiber exposure in humans have been well documented and comprise a variety of lung and pleural responses, including inflammation, fibrosis, and cancer. Similarly, lung and pleural responses of rodents to inhaled fibers include a generalized inflammation as an immediate consequence, and, with prolonged exposure, fibrosis and cancer of the lung and pleura may develop. It has been suggested that effects in the pleural compartment may be mediated through soluble factors elaborated in the lung rather than by translocated fibers (Adamson et al., 1993). Although this may be true for effects on pleural mesothelial cells adjacent to the alveoli, the physical presence of fibers appears to be necessary for the observed biological pleural responses at sites distant from the lung surface. This conclusion is supported by the translocation of inhaled fibers to the pleural space of humans (Sebastien et al., 1980) and rodents (Gelzelechter et al., 1996a), the temporal separation of lung and pleural inflammatory responses following fiber inhalation, and by the induction of inflammation, fibrosis, and mesothelioma following intracavitary injection of fibers (Davis et al., 1991).

Rats and hamsters demonstrate a differential response to chronic inhalation of the very durable refractory ceramic fiber RCF-1 (Mast et al., 1994). Rats had a significant increase in lung tumors and a low incidence of mesothelioma, whereas hamsters had a high incidence of mesothelioma but no significant increase in lung tumors. Hamsters also developed a high...
incidence of pleural fibrosis and mesothelioma, but not cancer of the lung, following the inhalation of amosite, an amphibole asbestos fiber (McConnell et al., 1999), whereas rats developed tumors at both sites (Davis et al., 1986). The basis for this species difference in response remains unclear, but the possibility that it is partly based on dosimetry considerations has been explored. Our previous work has shown that subchronic inhalation of RCF-1 fibers by rats and hamsters leads to biological responses in the lung that are similar in kind (cell proliferation, inflammation, and fibrosis) and magnitude, whereas pleural responses were greater in hamsters than in rats. Analysis of the fibers translocating to the pleural space showed that total fiber burden adjusted for surface area was similar in rats and hamsters but that there was a significant accumulation of fibers greater than 5 μm long in the hamster (Gelzleichter et al., 1999).

MMVF 10a is a fibrous glass preparation that is a size-selected fraction, with slightly thinner and longer fiber dimensions, of the material used in a chronic inhalation study with rats. In that study, pulmonary fiber burdens were examined and the fiber dimensions characterized. Chronic inhalation of MMVF 10 did not result in either pulmonary fibrogenic or carcinogenic outcomes in rats (Hesterberg et al., 1993). The pulmonary responses of rats were also studied, following subchronic inhalation of MMVF 10. Results from this study demonstrated that pulmonary inflammation, increased lung cell proliferation, and decrements in lung clearance of particles in rats exposed to MMVF 10 (Hesterberg et al., 1996a). More recent studies by the same group entailed the chronic and subchronic inhalation exposure of hamsters to MMVF 10a and the examination of both lung and pleural biological responses. As in the study with rats, MMVF10a-exposed hamsters showed no pulmonary fibrotic or neoplastic responses to the inhalation of these fibers but did exhibit pulmonary inflammation, nonlinear lung burdens, and pleural mesothelial-cell proliferation (Hesterberg et al., 1999; McConnell et al., 1999).

In the present study we chose MMVF10a to study because it is a soluble fiber that doesn’t lead to fibroproliferative changes but is known to induce pulmonary and pleural inflammation in high-dose exposure scenarios. It should not be forgotten that inflammation of lung and pleura is an important fiber-induced disease state. Our interest has centered on the differences in pleural responses between rat and hamster, and, to date, the rat pleural response to a low-toxicity fiberglass fiber has not been characterized. Moreover, it is of interest to determine whether a component of the pleural toxicity of a fiber is in part determined by the ability to translocate to the pleural compartment. We undertook the present study to determine whether the number and size of inhaled MMVF 10a fibers translocating to the pleural compartment are determinants of the pleural responses in rats and hamsters and to compare the pleural responses in these species.

<table>
<thead>
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<th>Table 1: Time Points and Analyses</th>
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<td>Analysis</td>
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<tr>
<td>Lung and pleural fiber burden</td>
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<td>Cell proliferation, lung and</td>
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<td>pleural lavage, histopathology</td>
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*The number of animals of each species utilized for the indicated analysis.

MATERIALS AND METHODS

Animals. The animals used were male CDF (F344)/CrBr Fischer rats weighing approximately 250–275 g (Charles River Breeding Laboratories, Inc., Raleigh, NC) and male Lk: LVG (SYR) BR Syrian golden hamsters weighing approximately 140–150 g (Charles River Breeding Laboratories, Inc., Montreal, Canada). All animals were negative for antibody to murine mycoplasmal and viral respiratory disease. Animals were housed in an AAA-LAC-accredited barrier facility in polystyrene cages on direct-contact cellulose bedding and supplied NIH07 cereal-based diet and water ad libitum. Animals were individually identified by implanted microchip transponder (Biomedic, Inc., NJ) in the subcutis and were randomly assigned to exposure groups. Room temperature was maintained at 60–65°F and humidity at 40–60% throughout the exposure and postexposure periods. All animals were quarantined for a minimum of 10 days prior to exposure.

Exposures. Rats and hamsters were exposed to an aerosol of MMVF10a (obtained from the North American Insulation Manufacturers Association, NAIMA) generated as previously described (Gelzleichter et al., 1996a), in open-end, nose-only exposure tubes (Battelle Memorial Institute, WA). Fiber concentrations were continuously monitored using light scatter (RAM, Monitoring Instruments for the Environment, Inc., Billerica, MA). The nominal target mass concentration was 45 mg/m³. Actual fiber mass values were determined from samples captured on open-faced, 0.2 μm polycarbonate filters (Gelman Sciences, Ann Arbor, MI). Table 1 shows the time points and analyses performed. Animals were exposed for 4 h on 5 consecutive days each week for a maximum of 12 consecutive weeks. Air-control and fiber-exposed groups of animals were killed following 4, or 12 weeks of exposure or after a recovery period of 12 weeks. A group of cage-control animals was also sacrificed at the beginning of the experiment to provide background fiber levels.

Fiber size distributions. Pulmonary and pleural fiber burdens were determined after 0, 4, and 12 weeks of exposure (these animals were euthanized immediately after the final exposure) and 12 weeks postexposure. Characterization of lung and pleural fiber burdens and fiber aerosols were as previously described (Bermudez, 1994; Gelzleichter et al., 1999). Briefly, intrapleural injection of a 41°C, 2% solution of low-melting-point agarose (BRL, Gaithersburg, MD), following euthanasia and exsanguination, was used to trap fibers in the pleura and pleural space. Injection volumes for the pleural cavity were approximately 8–11 ml for rats and 4–5 ml for hamsters. Filling of the pleural cavity with agarose was followed by inflation of the lungs with 3 or 2 ml (rat and hamster, respectively) of agarose, after which the animals were chilled on ice for 30 min to allow the agarose solutions to harden into a solid cast. The cast was carefully separated from the lung and thoracic surfaces and frozen at −20°C in a clean glass tube. The lungs were also removed and frozen in a clean glass tube at −20°C. Frozen lung and pleural samples were freeze-dried, ashed in an oxygen plasma (Anatech, Ltd., Springfield, VA, model LTA–504), resuspended in 5 ml doubly-filtered nanopure water, and filtered onto 0.1-μm pore size polycarbonate filters (Corning Costar Corporation, Separations Division, Cambridge, MA). Filters were gold-coated (Hummer V, Anatech, Ltd.,
Springfield, VA) and visualized with a scanning electron microscope (JEOL Model JSM 840A Scanning Microscope, Tokyo, Japan). The volume of suspension filtered and the proportion of filter surface examined were less for the lung than for the pleural samples, due to the presence of many more fibers. Analyses of fiber sizes were as previously described (Gelzleichter et al., 1996b). Fiber lengths were determined at magnifications of ×2000 or ×500. Fiber diameters were determined exclusively at a magnification of ×2000. The limit of detection for lung ranged from approximately 3.3 × 10^3 to 4.3 × 10^3 fibers in the whole lung, and for the pleural cavity, from 23 to 141 total fibers.

**BrdU labeling.** Animals to be used for cellular and biochemical analyses were surgically implanted with BrdU-filled (10 mg/ml, Sigma Chemical Co., St. Louis, MO) mini-osmotic pumps (5 μl/h, Alza Corp., Palo Alto, CA) at the end of 4 or 12 weeks of exposure or 12 weeks of recovery. For all time points, these animals were euthanized and lavaged (see below) 6 days after the exposure/recovery interval, inclusive of 3 days labeling.

**Pulmonary and pleural free cells.** Six animals, per time point, from each group were anesthetized with pentobarbital and exsanguinated. Bronchoalveolar (BAL) and pleural (PL) lavages were performed using sterile Dulbecco’s phosphate-buffered solution (PBS) (Gibco Laboratories, Grand Island, NY) as previously described (Gelzleichter et al., 1996b). Rat and hamster lungs were lavaged twice with 5 and 4 ml, respectively. Pleurae were lavaged twice with 4 (rat) and 3 ml (hamster) PBS. For both pleural and lung lavages, lavage samples were pooled and centrifuged at 200 × g for 10 min at 4°C. Supernatants were separated from the cell pellets and kept on ice. The cell pellets were resuspended in Ham’s F12 media (Gibco) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT). Cell densities in these suspensions were determined using a Coulter particle counter (Coulter ZM, Coulter Electronics, Marietta, GA). Differential cell counts were performed on cytopsin preparations stained with Wright Geimsa Diff-Quik (Leukostat, Fisher Diagnostics) as previously described (Gelzleichter et al., 1996b).

**Mesothelial cell proliferation.** Following lavage, the lungs and thoracic cavity were fixed in situ with 10% phosphate-buffered formaldehyde. After 24 h the tissues were rinsed and stored in 70% ethanol. Sections of the left intracostal parietal wall, diaphragm, and left lung were cut and embedded in paraffin. Paraffin sections were subsequently cut and stained by established immunohistochemical methods for incorporated BrdU. The labeling indices (LI) for mesothelial cells lining the surface of the lung (visceral), thoracic wall (costal), and the diaphragm in the pleural compartment were determined by counting BrdU-labeled and unlabeled mesothelial cells and expressing the result as a percentage of the total cells counted. The number of cells available for counting in individual cross sections varied, but in all cases, all of the cells present on the section were enumerated. On average, at least 500 cells were counted per section.

**Biochemical assays.** Bronchoalveolar lavage fluid (BALF) and pleural lavage fluid (PLF) supernatants were immediately analyzed for lactate dehydrogenase (LDH), N-acetylglucosaminidase (NAG), total protein (TP), and alkaline phosphatase (BALF only), using a COBAS FARA II autoanalyzer (Roche Diagnostic Systems, Inc., Montclair, NJ).

**Statistical methods.** Statistical analysis was performed as previously described (Gelzleichter et al., 1999). Fiber length and diameter were assumed to follow a bivariate lognormal distribution (Cheng, 1986; Moss et al., 1992; Siegrist et al., 1980; WHO, 1988). Size distributions were described by the means and variances of the natural logarithms of fiber length and diameter and the correlation between ln (length) and ln (diameter). With the data collected from 6 animals per group, estimates were made of the 5 parameters of the bivariate distribution: the geometric mean length (GML), the geometric standard deviation of length (GSD [L]), the geometric mean diameter (GMD), the geometric standard deviation of diameter (GSD [D]), and the correlation between ln (length) and ln (diameter) (tau). Small numbers of objects were recovered for analysis in some of the animals, and therefore comparisons between species and groups were made using the total estimated number of fibers in each animal fitting in either 4 or 2 length intervals (1 < L ≤ 3, 3 < L ≤ 5, 5 < L ≤ 8, L > 8 or L ≥ 5, L > 5). There were insufficient numbers of fibers, particularly from the pleura, to create diameter classes. Standard counting rules (WHO, 1988) were applied for weighting and scaling up the total count represented by each observed fiber. For each length interval, the mean value ± standard error of the mean (SEM) is reported (n = 6). The weighted fiber counts were then scaled up on a per-lung or per-pleura basis and normalized by the surface area. Lung surface areas for the hamster (2800 cm²) and rat (6600 cm²) were obtained from the literature (Sahebjami, 1992; Valberg et al., 1992). Pleural surface area for the rat (24.5 cm²) was determined empirically (R. R. Mercer, personal communication). The pleural surface area for the hamster (14.1 cm²) was estimated by calculating the surface area of a sphere with a volume equivalent to the volume (5 ml) of agarose necessary to fill the pleural space. For cellular and biochemical assays, all results are expressed as mean values ± 1 SD. Significant differences between groups were determined by a Student’s t-test; p values of less than 0.05 were considered statistically significant.

**RESULTS**

**Characterization of MMVF 10a Aerosol**

The aerosol mass concentration averaged 44 ± 1.6 mg/m³. From the bivariate size analysis of ln (length) and ln (diameter), the GML was 12.5 μm with a GSD (L) of 2.5, the GMD was 0.93 μm with a GSD (D) of 1.6, and a tau of 0.52. The average fiber number concentration (all lengths) was 761 fibers/cc, while the average number of WHO fibers (aspect ratio ≥ 3, length ≥ 5 μm) was 610 fibers/cc. About 13% of the total aerosol by number were nonfibrous particles having a ln-normal, bivariate-size distribution of 1.5 μm, 1.7, 0.8 μm, 0.6, and 0.84 (GML, GSD (L), GMD, GSD (D), and tau) based on 3753 particles and fibers sized.

**Analyses of Pulmonary and Pleural Fiber Burdens**

Samples were collected after 0, 4, and 12 weeks of exposure and 12 weeks postexposure for the characterization of lung and pleural fiber burdens. Fibers were present in very low numbers (less than 1% of the number in exposed animals) in the lungs of control animals, presumably resulting from environmental contaminants (Table 2). The fibers recovered from the lungs of rats and hamsters were shorter (GML 6.9 μm and 6.9 μm versus 12.5 μm) and thinner (GMD 0.71 μm and 0.68 μm versus 0.93 μm) than fibers of the aerosol. The total number of fibers per lung was calculated based on the fibers counted and, when averaged over the three time points, were eightfold greater in rats (hamsters; 6.4 × 10⁵, rats; 50.1 × 10⁵). When these data were normalized on a surface area basis, the lung burdens in rats (7.6 × 10⁷/cm²) remained significantly greater (p < 0.05) than those of hamsters (2.3 × 10⁷/cm²). The ratio of short (< 5 μm) to long (> 5 μm) fibers in the rat lung remained relatively constant over time at approximately 1:2, with a decrease in the total number of fibers with time post-exposure to 56% of peak values (Table 2). Similarly, the ratio of short to long fibers in the hamster lung was approximately 1:2, but there was a shift to shorter, thinner fibers with time.
Control ranges for the number of cells recovered from the rat lung ranged from 5.6–6.6 × 10^5, 1.5–9.8 × 10^3, and 1.9–7.6 × 10^3 for macrophages, neutrophils, and lymphocytes, respectively. In the control animals, the macrophages were at least 97% of the recovered cells. There was a mild inflammatory response in the lungs of the rats characterized by a significant increase in the number of neutrophils and increases in biochemical indicators of toxicity. The number of neutrophils increased with exposure to values that were significantly elevated over concurrent controls (Fig. 1). The percentage of the cells identified as neutrophils rose to a maximum of 15% of the cells and subsequently declined, after 12 weeks of recovery, to 3%. Lymphocytes also increased significantly during the exposure period (Fig. 1) but returned to control values by 12-weeks postexposure. Control values for the biochemical markers in rat BALF ranged from 24 to 29 units/l, 2.3–2.9 units/l, 92–134 μg/ml, and 54–64 units/l for LDH, NAG, TP, and alkaline phosphatase, respectively. All of the biochemical markers examined in rat BALF were elevated after 4 weeks of exposure (Fig. 2). LDH and alkaline phosphatase levels remained elevated and unchanged through 12 weeks of recovery, whereas TP decreased with time postexposure but remained significantly greater than concurrent controls.

As in rats, macrophages accounted for the majority of the cells recovered from control animals and ranged from 87 to 96% of the total. Control ranges for the number of cells recovered from the hamster lung were 6.0 to 9.4 × 10^5, 1.5 to 3.8 × 10^5, and 4.4 to 5.9 × 10^3 for macrophages, neutrophils, and lymphocytes, respectively. An increase in the number of neutrophils was noted in the lungs of fiber-exposed hamsters, but this was significant only at the end of the exposure period and was of a lesser magnitude, relative to concurrent controls, than was observed in the lungs of rats (Fig. 1). Significant increases in the population of lymphocytes were seen in hamsters following 4 and 12 weeks of exposure. Control values for the biochemical markers in hamster BALF ranged from 22 to 22 units/l, 0.8 to 1.1 units/l, 96 to 153 μg/ml, and 5.1 to 8.2 units/l for LDH, NAG, TP, and alkaline phosphatase, respectively. There were no significant increases in the biochemical markers of toxicity in the BALF of hamsters exposed to MMVF10a.

Ranges for the number of cells recovered from the pleural space of control rats were 5.0 to 6.8 × 10^5, 0.3 to 2.3 × 10^4, and 2.9 to 10.8 × 10^4 for macrophages, neutrophils, and lymphocytes, respectively. Similar to the lung, the majority of

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**Table 2**

<table>
<thead>
<tr>
<th>Week</th>
<th>Length category (μm)</th>
<th>Fibers per cm² lung surface(10^9)</th>
<th>Fibers per cm² pleural surface(10^9)</th>
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</thead>
<tbody>
<tr>
<td>0^d</td>
<td>1 &lt; L ≤ 3</td>
<td>4.5 ± 1.8</td>
<td>5.9 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>3 &lt; L ≤ 5</td>
<td>8.0 ± 1.9</td>
<td>13.0 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>5 &lt; L ≤ 8</td>
<td>8.9 ± 2.3</td>
<td>14.3 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>8 &gt; L</td>
<td>15.6 ± 4.9</td>
<td>33.0 ± 6.7</td>
</tr>
<tr>
<td>12</td>
<td>1 &lt; L ≤ 3</td>
<td>2.4 ± 0.3</td>
<td>8.5 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>3 &lt; L ≤ 5</td>
<td>7.4 ± 0.9</td>
<td>25.4 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>5 &lt; L ≤ 8</td>
<td>7.3 ± 0.7</td>
<td>25.7 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>8 &gt; L</td>
<td>11.8 ± 1.7</td>
<td>44.0 ± 7.7</td>
</tr>
<tr>
<td>12/12^d</td>
<td>1 &lt; L ≤ 3</td>
<td>0.7 ± 0.4</td>
<td>7.0 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>3 &lt; L ≤ 5</td>
<td>0.9 ± 0.2</td>
<td>14.6 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>5 &lt; L ≤ 8</td>
<td>0.7 ± 0.2</td>
<td>16.3 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>8 &gt; L</td>
<td>0.3 ± 0.1</td>
<td>20.2 ± 2.0</td>
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</table>

Note. 0 ± 0 values are a result of rounding.

^aMean count ± SEM, n = 6.

^bUnexposed cage control animals.

^cNo fibers found in this class.

^d12 weeks of exposure followed by 12 weeks of recovery.

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postexposure (approximate short-to-long ratio of 1:1) and the total number of fibers decreased to approximately 10% of peak values (Table 2).

The total number of fibers in the pleural compartment of rats and hamsters was at least three orders of magnitude lower than the number in the lungs. Although present, fibers in the pleural samples of control animals were few (1 to 2 per sample) and were probably environmental contaminants (Table 2). In fiber-exposed animals, the numbers of fibers counted and sized from the pleural space were quite low (an average of approximately 20 fibers per sample), and therefore the pooled values for 6 animals were used to characterize the fiber burden in the pleural compartment. Estimation of the distribution parameters from the aggregate fibers sized (379 and 367 for hamsters and rats, respectively) showed that the fibers in the pleural space of hamsters (GML, 10.3 ± 1.9; GMD, 0.67 ± 1.5) and rats (GML, 8.3 ± 1.6; GMD, 0.63 ± 1.5) were shorter and thinner than those in the aerosol but were longer than, and of the same diameter as those found in the lungs. Of the fibers recovered from the pleural compartment of either species, 85–90% were of lengths greater than 5 μm (Table 2). Average total fiber burdens in the rat pleural compartment (1.2 × 10^3) were approximately the same as those of hamsters (1.4 × 10^3). Normalization of the pleural burdens to the surface area of the pleural space resulted in statistically higher fiber burdens (p < 0.05) in hamsters for fibers longer than 5 μm after 12 weeks of exposure. Pleural burdens in the rat did not decrease with time after the end of the exposure period, whereas pleural burdens in hamsters had decreased to approximately 38% of the pleural burden at the end of exposure (Table 2).
the cells recovered were macrophages but comprised a smaller fraction (76-80%). Pleural cellular responses in rats consisted of increased numbers of macrophages following 4 and 12 weeks of exposure (Fig. 1) and mast cells after 12 weeks of recovery (data not shown). Control values for the biochemical markers in rat PLF ranged from 28 to 46 units/l, 0.7 to 0.8 units/l, and 210 to 330 μg/ml for LDH, NAG, and TP, respectively. Of the biochemical markers of toxicity, only LDH was elevated in the rat PLF and only after 12 weeks of exposure to MMVF10a.

Ranges for the number of cells recovered from the pleural space of control hamsters were 2.5 to 3.4 × 10⁶, 2.2 to 5.3 × 10⁴, and 5.6 to 6.4 × 10⁵ for macrophages, neutrophils, and lymphocytes, respectively. Macrophages accounted for at least 89% of the cells recovered from the pleural space of controls. Pleural macrophages were increased in fiber-exposed hamsters but (unlike rats) remained significantly elevated at the end of the 12-week recovery period. Similarly, lymphocytes were increased after 4 weeks of exposure and were still significantly greater than concurrent controls after 12 weeks of recovery. Mast cell numbers were significantly greater than controls following 4 weeks of exposure but returned to control values by 12 weeks of exposure (data not shown). Control values for the biochemical markers in hamster PLF ranged from 37 to 59 units/l, 0.7 to 0.8 units/l, and 196 to 275 μg/ml for LDH, NAG, and TP, respectively. There were no significant increases in the
biochemical markers of toxicity in PLF of hamsters exposed to MMVF10a.

**BrdU Labeling of Pleural Mesothelium**

DNA replication in mesothelial cells was detected as the incorporation of BrdU and quantified for three distinct pleural sites: left lung visceral, left costal wall, and diaphragm. The only site in rats where there was significantly increased DNA synthesis was at the diaphragm, and this was limited to animals that had been exposed to fibers for 4 weeks (Fig. 3). Significantly increased DNA synthesis was found in hamsters at the costal wall after 4 weeks of fiber exposure and at the diaphragm after 12 weeks of fiber exposure (Fig. 3).

**DISCUSSION**

The objective of the present study was to characterize and compare the pleural-MMVF 10a dose and the pleural response of species having substantial differences in the long-term pleural effects following chronic exposure to fibers. Multiple chronic inhalation studies have examined the effects of various manmade and natural fibers in rats and hamsters (reviewed in (Hesterberg and Hart, 2001)), and the general observation can be made that rats are more sensitive to the induction of pulmonary tumors whereas hamsters are more sensitive to the induction of mesothelioma (Maxim et al., 2001). Exposure of rats to RCF-1 (Mast et al., 1995), or amosite (Davis et al., 1986) resulted in significant incidences of lung tumors (13 and 38%) but low incidences of mesothelioma (1.6 and 5%) and fibroproliferative disease. In contrast, exposure of hamsters to RCF-1 (McConnell et al., 1995), or amosite (McConnell et al., 1999) resulted in no lung tumors and greatly increased incidences of mesothelioma (20 and 41%) and pleural fibrosis. Long-term inhalation of fiberglass preparation, MMVF 10a, by hamsters (McConnell et al., 1999) did not result in fibrosis, mesothelioma, nor lung tumors. Similarly, chronic inhalation of MMVF 10 (slightly shorter and thicker fiber dimensions than those of MMVF 10a) by rats did not result in the induction of fibrosis or mesotheliomas (Hesterberg et al., 1993). A slight, but not statistically significant, increase in the incidence of lung tumors in the highest MMVF 10 dose group (5.9%) over concurrent controls (3.3%) was observed in the same study.

Lung burdens in the present study differed between rats and hamsters. After 12 weeks of exposure, total lung burdens in rats were approximately eight-fold greater than in hamster. These differences have been observed in other studies with inhaled fibers (Gelzleichter et al., 1999; Hesterberg et al., 1993, 1999) and are probably due to the inherent species differences in lung size and surface area, physiological parameters (e.g., min volume), and the ability to clear fibers. Attained lung burdens in hamsters in the present study, after adjusting for total exposure hours, are in good agreement with those found in a study with the same species and aerosol mass concentration of MMVF 10.1, a preparation with a slightly higher fluorine content than MMVF 10a, but with approximately the same dissolution rate in vitro (Hesterberg et al., 1999). The species difference in the ability to remove fibers from the lung is evident upon examination of lung fiber burdens following 12 weeks of postexposure recovery. Hamster lung burdens decreased approximately 90% in the 12 weeks of recovery, whereas lung burdens in rats only decreased approximately 44%. The ability of hamsters to efficiently clear particles from the lung has been previously noted (Bermudez et al., 2002; Creutzenberg et al., 1998), as have differences in how alveolar macrophages handle fibers (Dorger et al., 2000).

Although pulmonary inflammation was observed in rats and hamsters, the character of the responses differed. Rats had significant increases in neutrophils and a concomitant increase in biochemical markers of toxicity that remained elevated through the end of the recovery period. Hamsters, on the other hand, had a mild pulmonary inflammatory response limited to...
increased neutrophils after 12 weeks of exposure, and decreased alkaline phosphatase levels after 4 weeks of exposure. In the case of the rats, but not hamsters, the mean neutrophil response correlated ($r^2 = 0.9792$) with the measured lung fiber burdens. The extent of the pulmonary inflammatory response observed in the present study is in good agreement with the work of Hesterberg et al. (1999), where hamsters were exposed to MMVF 10.1 fibers for 13 weeks. In comparison with the pulmonary response elicited by the inhalation of RCF-1 fibers, the response seen here was of a much lesser magnitude. Neutrophils in the lung, following exposure for 90 days to RCF-1, comprised approximately 50% (unpublished data) of the recovered cells, whereas approximately 12% were neutrophils following MMVF 10a inhalation. This difference is probably due to the greater lung fiber burdens observed in rats and hamsters exposed to the more durable RCF-1 fibers.

We (Gelzleichter et al., 1999), and others (Sebastien et al., 1980; Suzuki et al., 1991), have previously demonstrated that fibers translocate from the lung to the pleural space, but that these fibers are relatively few in number. This observation
holds for MMVF 10a in the present study, where the total number of fibers in the pleural compartment was at least a thousand times lower than in the lung. A shift in the distribution of fiber length toward longer fibers was noted in the pleural compartment, relative to the lung, such that the majority of fibers were of lengths greater than 5 μm. This may be a result of more rapid clearance of “short” fibers in the lung, as has been observed in rats exposed to crocidolite (Hesterberg et al., 1996b). Burdens in the pleural compartment increased with time of exposure in both species. The measured burdens are approximately proportional to those measured by Hesterberg and colleagues (Hesterberg et al., 1999) in hamsters exposed for 78 weeks to MMVF 10a, demonstrating the continued accumulation of fibers with exposure. Pleural burdens in the hamster only differed from rats after 12 weeks of exposure and in the accumulation of fibers greater than 5 μm. We previously noted this apparent accumulation of longer fibers in the hamster as compared to the rat (Gelzleichter et al., 1999). After 12 weeks of recovery, pleural fiber burdens equalized between rats and hamsters and probably reflect the rapidly declining pleural reaction were limited to increased macrophage numbers and lymphocyte numbers through the end of the postexposure phase of the experiment. Hamsters had increased macrophage and lymphocyte numbers through the end of the postexposure recovery period and cell replication of costal and diaphragmatic mesothelial cells. Taken together, it is evident that the species differences observed following the subchronic inhalation of MMVF 10a are due not only to retained fiber burdens and the physical properties of the fiber, but on differences in the kinetics of fiber translocation, macrophage function, mesothelial cell reactivity, and other biological differences.

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