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# CHRONIC INHALATION STUDIES OF MAN-MADE VITREOUS FIBRES: CHARACTERIZATION OF FIBRES IN THE EXPOSURE AEROSOL AND LUNGS

Thomas W. Hesterberg, William C. Miiller, Philippe Thevenaz* and Robert Anderson

Mountain Technical Center, Schuller International, Inc., Littleton, CO 80127, U.S.A.; and *Research and Consulting Company, Geneva, Switzerland

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**Abstract**—Inhalation studies were conducted to determine the chronic biological effects in rodents of respirable fractions of different man-made vitreous fibres (MMVFs), including refractory ceramic fibre (RCF), fibrous glass, rock (stone) wool and slag wool. Animals were exposed nose-only, 6 h per day, 5 days per week, for 18 months (hamsters) or 24 months (rats). Exposure to 10 mg $m^{-3}$ of crocidolite or chrysotile asbestos induced pulmonary fibrosis, lung tumours and mesothelioma in rats, thus validating the inhalation model with known human carcinogenic fibres. Exposure of rats to 30 mg $m^{-3}$ of refractory ceramic fibres (RCF) also resulted in pulmonary fibrosis as well as significant increases in lung tumours and mesothelioma. In hamsters, 30 mg $m^{-3}$ of RCF induced a 41% incidence of mesotheliomas. Exposure of rats to 30 mg $m^{-3}$ of fibre glasses (MMVF 10 or 11) or of slag wool (MMVF 22) was associated with an inflammatory response, but no mesotheliomas or significant increase in the lung tumours were observed. Rock wool (stone wool: MMVF 21) at the same exposure level resulted in minimal lung fibrosis, but no mesotheliomas or significant increase in the lung tumours were observed. Fibre numbers (WHO fibres) and dimensions in the aerosols and lungs of exposed animals were comparable in this series of inhalation studies. Differences in lung fibre burdens and lung clearance rates could not explain the differences observed in the toxicologic effects of the MMVFs. These findings indicate that dose, dimension and durability may not be the only determinants of fibre toxicity. Chemical composition and the surface physico-chemical properties of the fibres may also play an important role.

## INTRODUCTION

Man-made vitreous fibres (MMVFs) are a class of materials which have found many applications in both residential and industrial settings. MMVFs are fibrous inorganic substances that are made primarily from rock, clay, slag or glass. Sometimes referred to as man-made mineral fibres (MMMFs), the major classes of MMVF are refractory ceramic fibres (RCFs), fibrous glass, rock (stone) wool and slag wool.

RCF, the smallest category of MMVF, represents only about 1–2% of the world production. It is made by melting $Al_2O_3$ and $SiO_2$ in about equal amounts, or by melting kaolin clay, and then 'spinning' or 'blowing' this molten material into fibres. Most RCF is used as high-temperature furnace insulation. World production of RCF in 1990 was about 80 million lbs. Fibrous glass is the largest category of the MMVFs and is used in insulation, air handling, filtration and sound absorption. The thermal, acoustical and fire resistant properties of these products have led to their widespread use in a variety of residential and commercial applications. Production of fibrous glass in North America in 1989 was approximately 1.8 million tons. Slag wool and rock wool are composed primarily of calcium, magnesium, aluminium and silica. Since 1975, most slag wool has been produced from the waste slag that resulted from the reduction

of iron ore to iron. Rock wool fibres are made from basaltic rocks with additives such as limestone or dolomite. Slag wool and rock wool are used in residential and commercial low- and high-temperature insulation and in acoustical ceiling tiles and wall panels. In North America about 75% of slag wool production is used in acoustical ceiling tile manufacture.

Animal studies have been conducted to assess the potential biological effects of MMVFs. This research has been reviewed by the International Agency for Research on Cancer (IARC, 1988), the International Programme on Chemical Safety (IPCS, 1988), the World Health Organization (WHO, 1984), and the U.S. Environmental Protection Agency (Vu, 1988). These reviews are consistent in the judgement that chronic inhalation studies of airborne fibres provide the best model for assessing the potential risk to man (McClellan *et al.*, 1992).

This paper summarizes and compares the results from a recent series of chronic inhalation studies that evaluated the toxic effects of each of the major MMVF categories: fibrous glass, RCF, rock wool and slag wool. Its purpose is to examine the characteristics of the fibres in the exposure aerosols and in the lungs of the exposed animals, in order to understand more fully the critical fibre characterstics that are responsible for the differences in toxicities induced by the different MMVFs. Since RCF was the only MMVF type that induced lung tumours and this effect only occurred at the highest dose (30 mg $m^{-3}$), this paper focuses on comparing fibre characteristics only at this highest dose. A more detailed comparison of the fibre characteristics at the lower doses will be addressed in another paper.

The MMVFs used in this series of studies were prepared to be respirable by rats and to have dimensions comparable to those of fibres found in the workplace air (Hesterberg and Hart, 1995). Fibres were pre-selected for their size, and the actual size distributions of the aerosols used for fibre exposure were verified. Further, it was essential to document that fibre preparation, handling and aerosolization did not alter the physical–chemical characteristics of the fibre, since these are critical determinants of fibre toxicity. The methodology and results from these individual studies have been described in detail elsewhere (Hesterberg *et al.*, 1993; Bunn *et al.*, 1993; Mast *et al.*, 1995; McConnell *et al.*, 1994, 1995).

## MATERIALS AND METHODS

### *Fibres*

Five respirable-size test fibres were prepared from MMVF products for these studies: a kaolin-based refractory ceramic fibre (RCF 1), two fibrous glass compositions (MMVF 10 and 11), a rock wool (MMVF 21) and a slag wool (MMVF 22) (see Table 1). In order to simulate fibre size distributions found in workplace air, these test fibres were size-selected from commercial fibre products by a water based separation technique that produces fractions of fine, respirable fibres. Positive control animals were exposed to intermediate length National Institute of Environmental Health Sciences (NIEHS) chrysotile asbestos (Hesterberg *et al.*, 1993) or to size-selected crocidolite asbestos (McConnell *et al.*, 1995). These two asbestos types were included in the studies to validate the animal model. Because the average dimensions of these two asbestos types were very different from those of the MMVFs it is not appropriate to compare them with the MMVFs on a fibre-by-fibre basis.

Table 1. Respirable test fibres that were size-selected from man-made mineral fibres (MMVF) used in a recent series of rodent inhalation studies conducted at Research and Consulting Company (RCC), Geneva, Switzerland

| | |
|---|---|
| RCF 1 | Refractory ceramic fibre |
| MMVF 10 | Fibreglass |
| MMVF 11 | Fibreglass |
| MMVF 21 | Rock wool (stone wool) |
| MMVF 22 | Slag wool |

*Experimental design*

This series of studies was initiated in mid-1988 at Research and Consulting Company in Geneva, Switzerland. The time-lines of these studies, with their various phases of exposure, recovery and analysis, are shown in Fig. 1. Six-week-old male Fischer 344/N rats and Syrian golden hamsters were obtained from Charles River Laboratories. Hamsters were exposed to RCF or chrysotile asbestos for 18 months. In similar studies, rats were exposed for 24 months to RCF, fibrous glass, rock wool, slag wool or crocidolite asbestos. A schematic showing the exposure and recovery phases as well as the disposition of animals during the rat inhalation studies is shown in Fig. 2. There were 140 animals in each of the MMVF-exposed and negative control (exposed to filtered air only) groups. Groups of three or six randomly selected animals from each exposure group were killed at 3, 6, 12, 18 and (rats only) 24 months to follow the progression of histopathological changes and to determine lung fibre burdens. An additional six 'recovery' animals were removed from each exposure group at 3, 6, 12 and (rats only) 18 months and held without further treatment until the end of the exposure period, when they were killed to assess progression or regression of lung lesions and lung retention and clearance of fibres after cessation of exposure. Following the exposure period, the remaining animals were held for lifetime observation, i.e. until

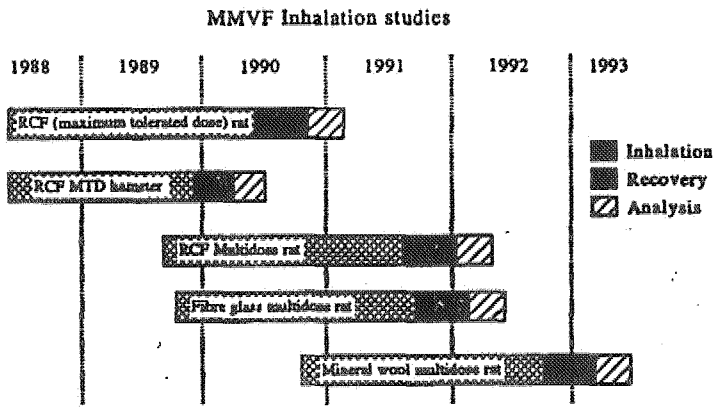


Fig. 1. Time lines for the series of studies begun in mid-1988 to assess the chronic inhalation effects of the three major categories of MMVF: fibre glass, refractory ceramic fibre (RCF) and mineral wool. Rats were exposed for 24 months and held without further exposure (Recovery) until they reached about 20% survival when all remaining animals were killed. Hamsters were exposed (RCF only) for 18 months and also held without further exposure until 20% survival.

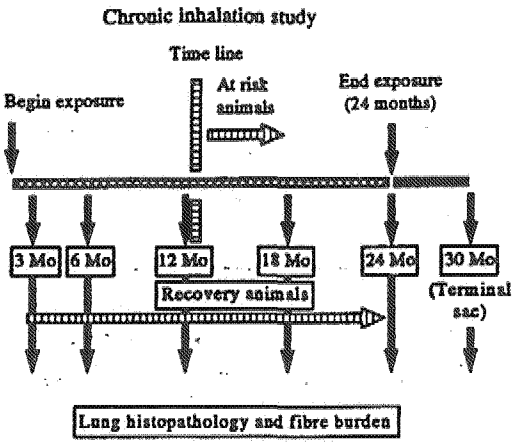


Fig. 2. Schematic of protocol for exposure and recovery groups in a typical chronic inhalation study in rats. Groups of 140 rats per dose group were exposed nose-only, 6 h per day, 5 days per week for 24 months. Periodically (3, 6, 12, 18 and 24 months) rats were killed and their lungs examined microscopically for pathological effects; the accessory lobe was frozen for fibre lung burden determination. Also at these time points (except 24 months), six rats per group were removed from exposure and held until 24 months for assessment of reversal of lung histological changes and for determination of fibre disappearance from the lung. All remaining animals were killed at about 20% survival, approximately 30 months.

about 20% survival. This observation period was chosen because it has been shown in previous studies with asbestos that the induction of mesothelioma does not occur until late in the animal's lifetime (Wagner *et al.*, 1974). For calculating per cent tumour incidence, only animals that were exposed to fibres for at least 1 year were considered at risk for induction of neoplasms as this was the earliest time point a neoplastic finding was observed in this series of studies.

*Fibre aerosol exposure*

Animals were exposed by nose-only inhalation to the different MMVF types according to the method of Sachsse *et al.* (1976). Advanced techniques of fibre preparation and sizing, non-destructive aerosolization and fibre measurement, as described previously (Hesterberg *et al.*, 1991; Bernstein *et al.*, 1994), were used. Aerosols of the test fibres were produced using the Research and Consulting Company, Geneva (RCC) fibre aerosol generation system (Bernstein *et al.*, 1994), which produces large numbers of unbroken fibres with lower levels of non-fibrous dust than in the aerosols used in previous studies. Temperature, relative humidity and oxygen concentration were monitored continuously. The animals were confined separately in tubes which were positioned radially around the flow-past, nose-only exposure chamber (Cannon *et al.*, 1983). This unique system provides positive pressure laminar flow to each animal individually, and unlike conventional nose-only exposure systems each animal is supplied fresh aerosol and the air exhaled by one animal does not reach any other animal in the chamber.

Rodents were exposed in these chambers for 6 h per day, 5 days per week, for either 24 months (rats) or 18 months (hamsters): the maximum dose was 30 mg $m^{-3}$ of each type of man-made fibre. Negative control animals were exposed similarly to filtered air. Positive controls were exposed to 10 mg $m^{-3}$ either of crocidolite or of chrysotile

asbestos. Fibre size distributions were determined on a quarterly basis using scanning electron microscopy. The numbers of fibres per $cm^3$ were determined using the World Health Organization (WHO) criteria (WHO, 1985). Details of the fibre aerosol measurement techniques have been described elsewhere (Hesterberg *et al.*, 1993).

*Aerosol monitoring and characterization*

Aerosol samples were collected on Millipore membrane filters at the animal exposure port. To determine the number of fibres $cm^{-3}$ and eliminate isokinetic sampling bias sampling was isoaxial, directly from the output of one of the laminar flow feed tubes. Filters were then placed between glass slides and clarified for counting. Fibre numbers were determined using a Bausch and Lomb Balpan Phase Contrast microscope at a magnification of $\times 400$. WHO Monograph 4 counting rules were applied for counting the number of WHO fibres $cm^{-3}$ (WHO, 1985).

During pre-exposure trials and once every 3 months thereafter, samples of the MMVF aerosols were captured on filters for determination of fibre length and diameter. Until analysis these samples were stored in glass bottles containing approximately 100 ml distilled water and 8 mg of sodium azide. To retain all fibres the filter surface was washed into the bottle, then filters were ashed and the ash was added back to the fibre suspension in the bottle. The suspensions were diluted to 250 ml with distilled water and homogenized by sonification. Aliquots were filtered onto membrances for examination either by electron microscopy (for measurement of fibre diameters) or by phase-contrast optical microscopy (for measuremet of fibre lengths). Dimensions were determined according to the methods for measuring airborne man-made mineral fibres outlined in WHO Monograph 4 (WHO, 1985). Diameters were measured at $\times 5000$ either on a JEOL T 300 SEM or on a JEOL 840 SEM equipped with a Videoplan Image Analysis System.

*Lung pathology*

The lungs and attached mediastinal lymph nodes were removed *in toto* and weighed. The right accessory lobe was tied off, removed, weighed, frozen and stored at $-20°C$ for lung burden analysis (see next section). The remaining lung lobes were then inflated to a pressure of 30 cm $H_2O$ with Karnovsky's fixative for 2 h. The lungs were examined under a dissecting microscope prior to fixation. Uniform sections of the left lung and of the right diaphragmatic lobe were embedded in paraffin, cut at a thickness of 4 mm and replicate sections were routinely stained with hematoxylin and eosin (H&E) and with Masson–Goldner's trichome stain for collagen staining. In addition, sections were made from all grossly visible lesions from that and other portions of the lung. Proliferative lesions of the pulmonary parenchyma were designated as bronchoalveolar hyperplasia (BAH), pulmonary adenoma or adenocarcinoma. Other types of lesions, including those in the pleura were noted where appropriate.

*Lung burden analysis*

Immediately after necropsy, the infracardiac (accessory) lobe of each animal's lung was frozen and later analysed for lung fibre burden using methods described by Hesterberg *et al.* (1993). Briefly, lung tissue was thawed, rapidly dehydrated with acetone and ashed using a low-temperature process. Fibres recovered were dispersed in distilled water, filtered onto membranes and examined using scanning electron

microscopy. The number, dimensions and other characteristics of the lung fibres were determined.

## RESULTS

### *Histopathology of rats*

Chrysotile and crocidolite asbestos (10 mg $m^{-3}$) induced pulmonary interstitial fibrosis in rats as early as 3 months after the inhalation exposure was initiated. The exposure to crocidolite had to be stopped at 10 months because of excess mortality of the exposed animals. These animals were held without further crocidolite exposure until the end of the study and no further excess in mortality was observed. Each asbestos type induced a single mesothelioma and significant increases in lung tumours in rats (Table 2), thus validating the rat inhalation model for these disease end-points.

After 3 months of exposure all five types of MMVF produced an inflammatory response, including increased macrophage levels in the alveoli, bronchiolization of the alveolar ducts, and microgranuloma formation in the terminal airways. After 6 months of fibre exposure only RCF 1 induced minimal pulmonary fibrosis (Table 3). This type of fibre also induced two mesotheliomas and a significant increase in lung tumours. Although rock wool (MMVF 21) produced minimal lung fibrosis after 18 months of exposure, no mesotheliomas were induced and no significant increase in lung tumours was observed. The two fibre glass compositions (MMVFs 10 and 11) and the slag wool (MMVF 22) produced neither pulmonary fibrosis nor mesotheliomas and no significant increase in lung tumours compared to the unexposed control animals.

Table 2. Rats were exposed to 10 mg $m^{-3}$ of NIEHS asbestos, either chrysotile or size-selected long-fibre crocidolite. Exposure regime was the same as for man-made vitreous fibres, 6 h per day, 5 day per week. Chrysotile exposure continued for 2 years but crocidolite exposure was stopped at 10 months owing to elevated mortality

| Fibre group | WHO fibres $cm^{-1}$ ($\times 10^4$) | Lung fibrosis | Lung tumours | Mesotheliomas |
|---|---|---|---|---|
| Air control | 0 | — | 1–3% | 0 |
| Chrysotile | 1.1 ± 1.1 | + | 13 (18.9%) | 1 (1.4%) |
| Crocidolite | 0.16 ± 0.1 | + | 15 (14.2%) | 1 (0.9%) |

Table 3. Rat lung pathology induced by 30 mg $m^{-3}$ of size-selected man-made vitreous fibres (MMVF): kaolin-based refractory ceramic fibre (RCF 1), fibrous glasses (MMVF 10 and MMVF 11) or mineral wools (MMVF 21 and MMVF 22)

| Fibre group | Exposure WHO fibres $cm^{-3}$ (30 mg $m^{-3}$) | Lung fibrosis | Lung tumours | Mesotheliomas |
|---|---|---|---|---|
| Air control | 0 | — | 1–3% | 0 |
| RCF 1 | 187 ± 53 | + | 16 (13.0%)* | 2 (1.6%) |
| MMVF 10 | 232 ± 56 | — | 7 (5.9%) | 0 |
| MMVF 11 | 246 ± 76 | — | 3 (2.7%) | 0 |
| MMVF 21 | 243 ± 67 | + | 5 (4.4%) | 0 |
| MMVF 22 | 213 ± 62 | — | 3 (2.6%) | 0 |

*Tumour incidence significantly elevated above air controls ($P < 0.05$).

*Histopathology of hamsters*

In hamsters, chrysotile asbestos induced pulmonary fibrosis, but no mesotheliomas or lung tumours (Table 4). In contrast, RCF 1 induced a 41% incidence of mesotheliomas in addition to pulmonary fibrosis. As with chrysotile, RCF 1 did not induce lung tumours in the hamster. The results of this study are reported in detail elsewhere (McConnell *et al.*, 1994).

*Aerosol fibre characterization*

The average WHO respirable fibre $cm^{-3}$ levels that resulted from exposure to 30 mg $m^{-3}$ of each MMVF type are shown in Table 3. The WHO (World Health Organization) criteria define a respirable fibre as having an aspect ratio of $\geq 3:1$, a length $> 5$ $\mu$m, and a diameter $< 3$ $\mu$m. The length distributions of the MMVF types in the aerosols were comparable, with the possible exception of MMVF 10, which appeared to have more fibres $cm^{-3}$ in the shorter length categories as shown in Fig. 3(a). The diameter distributions of the MMVF types in the aerosols were also comparable, although MMVF 10 appeared to have slightly more thick fibres than the other MMVF types [Fig. 3(b)]. It was shown in a previous publication (Hesterberg *et al.*, 1993) that because the size separation procedure used in preparing RCF 1 was different from that used to prepare the other MMVFs, there were from 5- to 10-fold more non-fibrous particulates in the RCF aerosols than in the aerosols of the other MMVFs.

*Lung fibre burden analyses, rats*

The dimensions of fibres recovered from the lungs of animals after 13 weeks of exposure to 30 mg $m^{-3}$ of the different MMVFs are shown in Fig. 4. The length distributions of fibres recovered from lungs [Fig. 4(a)] are much closer to one another than those found in the aerosols [Fig. 3(a)]. Many of the fibres longer than 20 $\mu$m were not found in the lung, either because they did not reach the pulmonary region or because they were breaking in the lung. The diameter distributions of all MMVFs in the lung were also closer to one another [Fig. 4(b)], and many of the large diameter aerosol fibres ($> 1.5$ $\mu$m diameter) were not found in the pulmonary region of the lung.

Quantification of the number of WHO fibres in the lung at different times during the exposure phase of the study shows that lung fibre levels of the different MMVF types were comparable [Fig. 5(a)]. This was also true when the number of fibres in the lung $> 10$ $\mu$m in length were compared at different times during the exposure phase of the study [Fig. 5(b)]. However, the number of lung fibres $> 20$ $\mu$m in length were higher at the later time points for RCF 1 and MMVF 21 (rock wool) than for the other two fibre types [Fig. 5(c)].

Table 4. Hamster lung pathology induced by 30 mg $m^{-3}$ size-selected kaolin-based refractory ceramic fibre (RCF 1) or 10 mg $m^{-3}$ of chrysotile asbestos

| Fibre group | Exposure WHO fibres $cm^{-3}$ | Lung fibrosis | Lung tumours | Mesotheliomas |
|---|---|---|---|---|
| Air control | 0 | — | 0 | 0 |
| RCF 1 | $215 \pm 56$ | + | 0 | 42 (41%) |
| Chrysotile | $3000 \pm 1400$ | + | 0 | 0 |

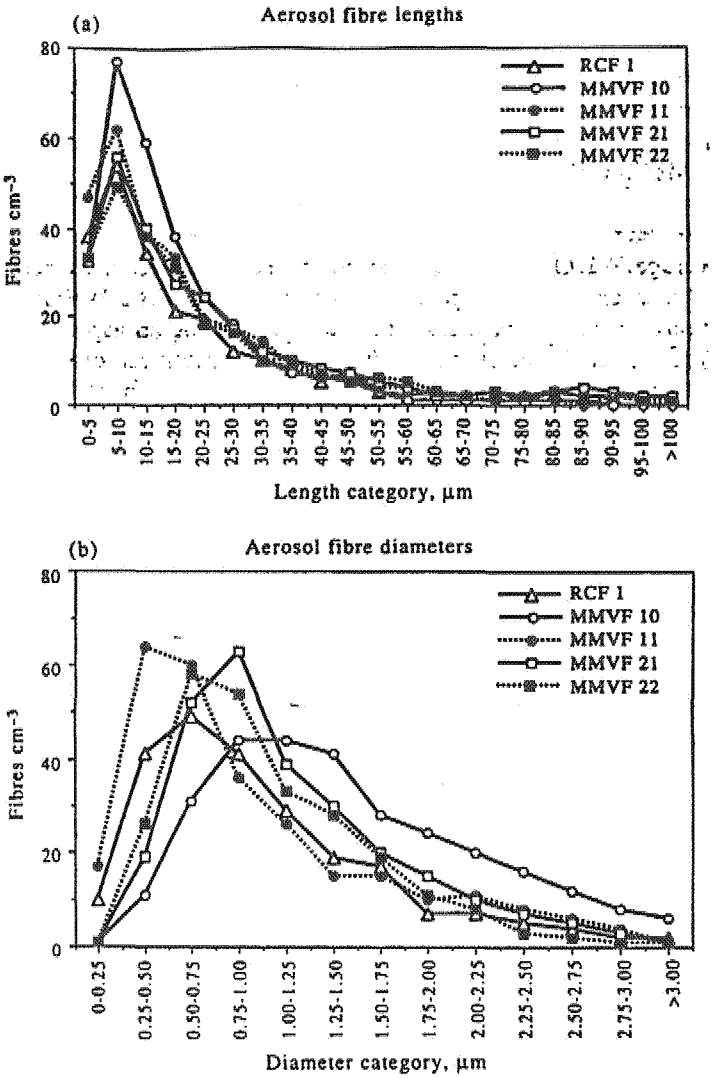


Fig. 3. (a) Length distributions and (b) diameter distributions of fibres in the exposure aerosols of the five different MMVFs in the chronic rat inhalation studies. Fibres were collected on filters at animal nose-only exposure ports. Fibre lengths were determined using phase-contrast optical microscopy, while fibre diameters were determined using scanning electron microscopy.

'Recovery animals' were removed from inhalation treatment at each time point (13, 26, 52 and 78 weeks) and held without further treatment until the end of the 2 years, thus providing groups of animals representing a range of recovery periods of from 13 to 78 weeks. Figure 6 shows the lung burdens and clearance of WHO fibres in rats exposed to 30 mg $m^{-3}$ of fibre glass (MMVF 11). Animals that were killed soon after 78, 52, 26 or 13 weeks' exposure (solid bars) tended to maintain a fairly constant lung burden whereas those permitted to recover (striped bars) demonstrated a recovery-time-dependent decrease in lung fibre burden. The per cent of WHO fibres, fibres

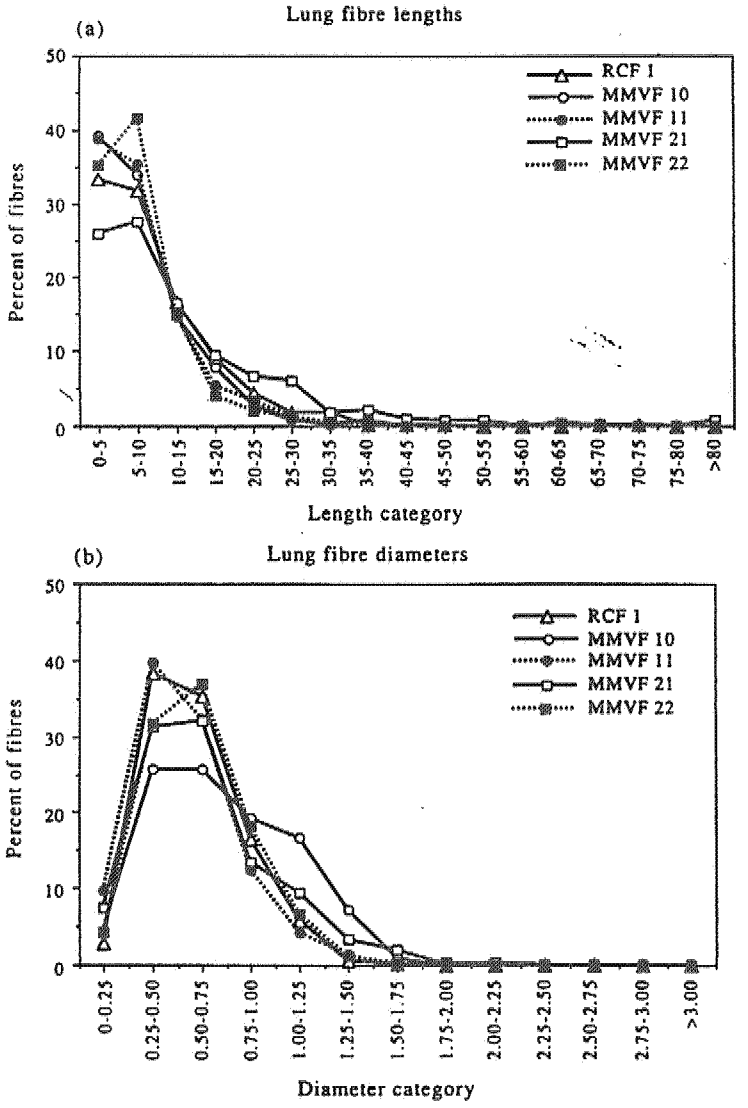


Fig. 4. (a) Length distributions and (b) diameter distributions of fibres from the lungs of rats exposed for 13 weeks to the five different MMVFs in the chronic inhalation studies. To permit clearance of the upper airways, rats were killed a minimum of 24 h after the exposure was stopped; the right accessory lobe was frozen and later low-temperature ashed for fibre recovery. Fibre lengths were determined using phase contrast optical microscopy, while fibre diameters were determined using scanning electron microscopy.

$>10$ $\mu$m in length and fibres $>20$ $\mu$m in length retained in the lung over time for each MMVF type is shown in Fig. 7. For WHO fibres and fibres $>10$ $\mu$m in length, the two fibre glass compositions (MMVFs 10 and 11) appear to be cleared more slowly from the lung than RCF 1. For these two length categories, rock wool and slag wool were cleared most rapidly from the lung. When the lung clearance of fibres $>20$ $\mu$m was examined, a different picture emerged [Fig. 7(c)]: (i) the long fibres ($>20$ $\mu$m) of all

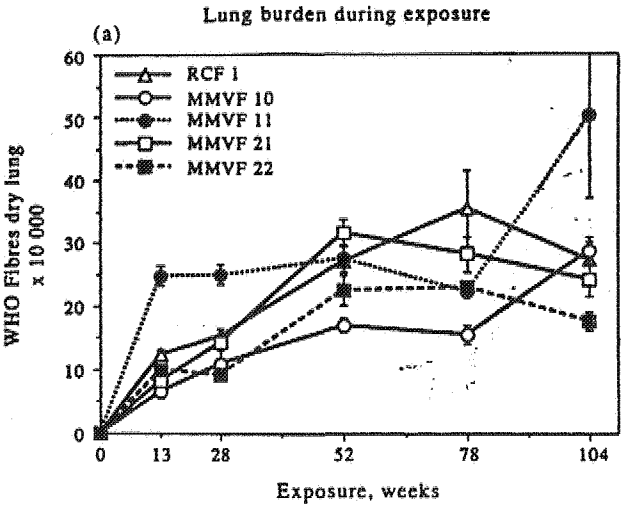


Fig. 5 (a).

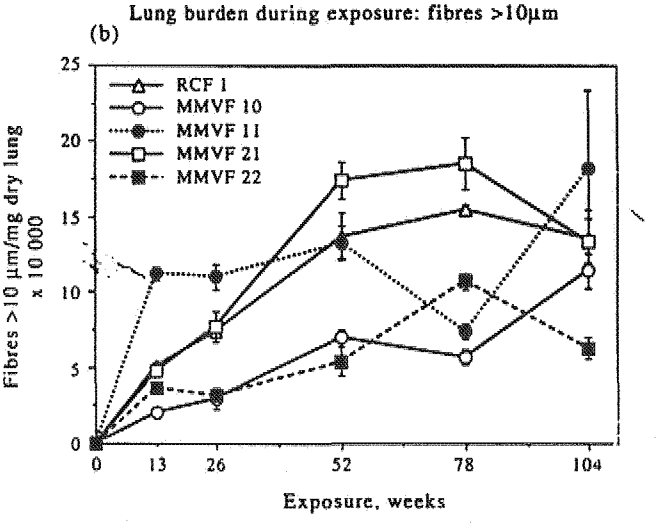


Fig. 5 (b).

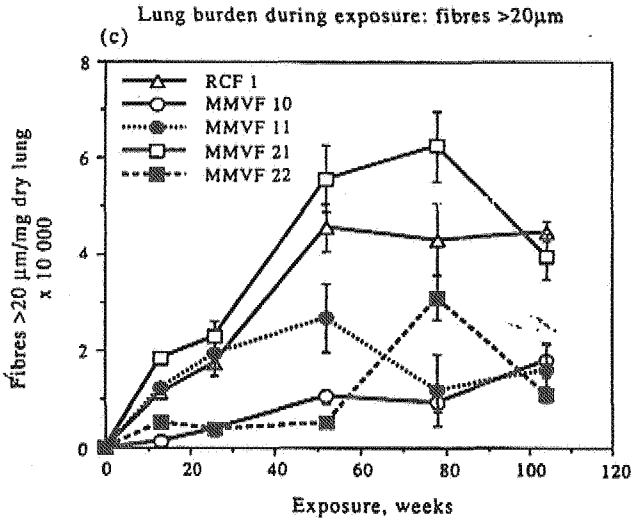


Fig. 5. Lung burdens of (a) WHO fibres, (b) fibres > 10 μm in length and (c) fibres > 20 μm in length per mg lung tissue from the lungs of rats continuously exposed to 30 mg $m^{-3}$ of the five different MMVFs. Rats were killed at least 24 h after the exposure was stopped, the right accessory lobe was frozen and later low-temperature ashed for fibre recovery.

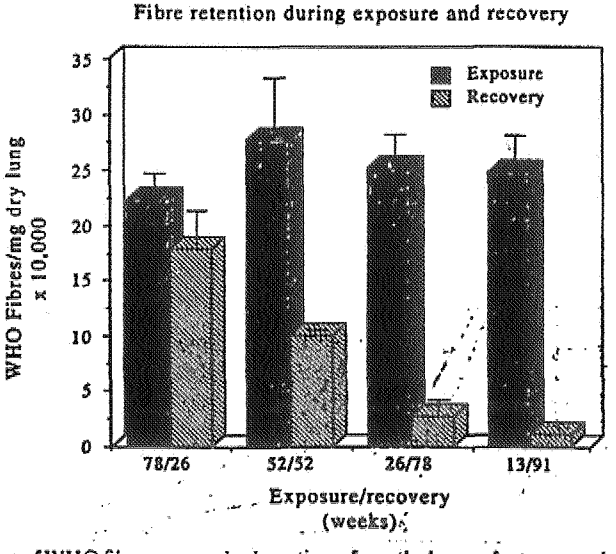


Fig. 6. Lung burdens of WHO fibres per mg dry lung tissue from the lungs of rats exposed to 30 mg $m^{-3}$ fibre (MMVF 11). Solid bars indicate lung burdens of animals continuously exposed. To permit upper airway clearance rats were killed at least 24 h after the exposure was stopped. Striped bars indicate lung burdens of animals removed from exposure for various recovery periods. The right accessory lobe was frozen and later low-temperature ashed for fibre recovery.

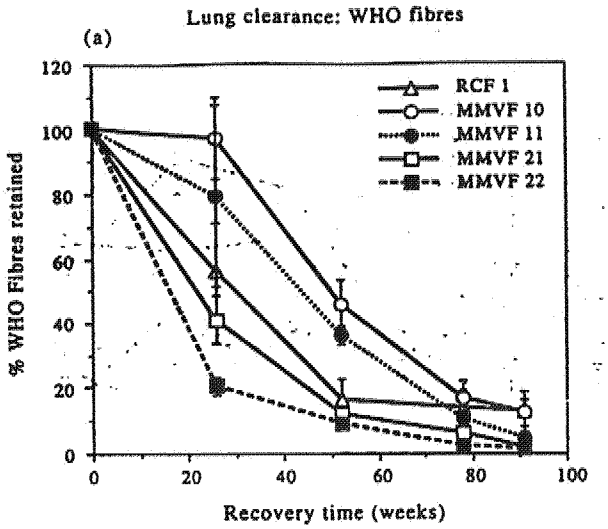
Lung clearance: WHO fibres
(a)
% WHO Fibres retained
Recovery time (weeks)
RCF 1
MMVF 10
MMVF 11
MMVF 21
MMVF 22
0
20
40
60
80
100
120

Fig. 7 (a).

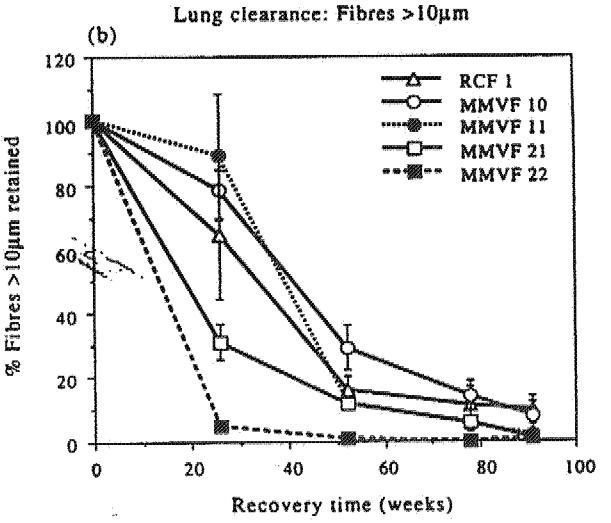
Lung clearance: Fibres >10μm
(b)
% Fibres >10μm retained
Recovery time (weeks)
RCF 1
MMVF 10
MMVF 11
MMVF 21
MMVF 22
0
20
40
60
80
100
120

Fig. 7 (b).

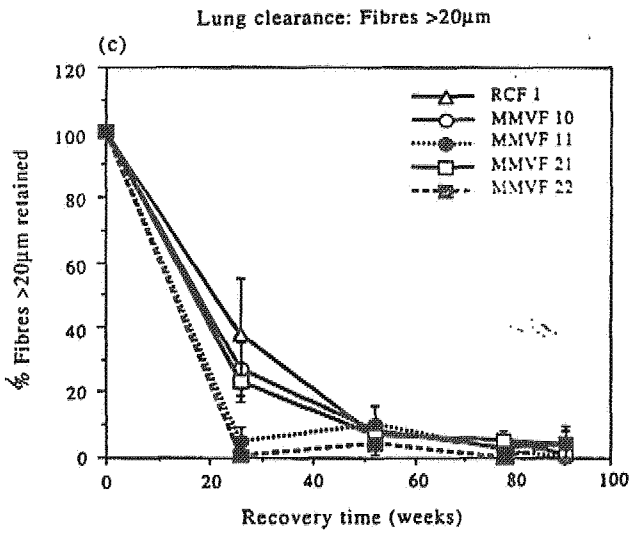


Fig. 7. Disappearance of (a) WHO fibres, (b) fibres > 10 μm in length and (c) fibres > 20 μm in length from the lungs of animals held without exposure for different recovery times. Both length categories of the two fibre glasses (MMVFs 10 and 11) appeared to clear from the lung more slowly than RCF and the two mineral wools. The percentage of fibres retained in the lungs was calculated by taking the ratio of lung fibres in recovery animals divided by the number of fibres in continuously exposed animal lungs (see Fig. 6). For fibres > 20 μm in length, RCF cleared the most slowly, followed by MMVFs 10 and 21.

MMVF types appeared to clear more rapidly than the shorter fibres; and (ii) the long fibres of RCF 1 cleared more slowly than MMVFs 10 and 21. The most rapidly cleared long fibres were MMVFs 11 and 22.

## DISCUSSION

Previous inhalation studies of fibre glass using rodents agree with the findings of the Research and Consulting Company (RCC) studies. Fibre glass has been tested by inhalation in guinea pigs (Gross *et al.*, 1970), hamsters (Lee *et al.*, 1981; Smith *et al.*, 1987) and rats (Gross *et al.*, 1970; Lee *et al.*, 1981; Wagner *et al.*, 1984; McConnell *et al.*, 1984; Mitchell *et al.*, 1986; Muhle *et al.*, 1987; Le Bouffant *et al.*, 1987; Smith *et al.*, 1987). In spite of FG lung burdens in excess of several hundred thousand fibres $mg^{-1}$ dry lung tissue none of these studies identified a significant increase either in fibrosis or in neoplasms following glass fibre inhalation. In three of the above studies, the chronic inhalation toxicity of rock and slag wool were also examined (Wagner *et al.*, 1984; Le Bouffant *et al.*, 1987; Smith *et al.*, 1987). As was seen with fibrous glass, all three studies demonstrated no tumorigenic response by this route of exposure.

The results from two previous RCF inhalation studies (Davis *et al.*, 1984; Smith *et al.*, 1987) differ from the more recent RCC studies presented here. Davis *et al.* (1984) reported RCF exposure of rats resulted in an average of 5% pulmonary fibrosis, pulmonary tumours in eight of 48 rats, and one peritoneal mesothelioma. The lower fibrosis and tumour response in the Davis study may have resulted from the lower exposure concentration used, 8.4 mg $m^{-3}$ compared to 30 mg $m^{-3}$ in the present study. In addition, the use of fibres that were not presized, the use of whole-body

exposure, or the fibre generation technique, which may have crushed some of the fibres, could account for the lack of consistency with the present study. Smith *et al.* (1987) exposed hamsters and rats to RCF at 200 fibres $cm^{-3}$, 6 h per day, 5 days per week, for 24 months. The rat study showed no significant increase in neoplasms and minimal pulmonary fibrosis in 22% of the exposed animals. In the hamster study, RCF produced only one mesothelioma in 50 animals and no fibrosis was observed. It is difficult to explain why there was little response to RCF in the Smith studies, but it may be related to the different aerosol and exposure technology used or to the low exposure level, 12 mg $m^{-3}$ compared to 30 mg $m^{-3}$ in the present study.

Previous intracavitary injection studies do not agree with the findings of the inhalation studies: intracavitary injection of nearly all compositions of MMVF has resulted in serosal cancer in animals (IARC, 1988; Vu, 1988). However, injection of fibres bypasses the normal defence mechanisms of the lung and can produce abnormal fibre distribution, fibre clumping and overload doses (Hesterberg *et al.*, 1991). Furthermore, when fibres are injected into the pleura or peritoneum of an animal, leaching, degradation, fragmentation or any other transformations are unlikely to be the same as they are after inhalation. The weaknesses of intracavitary injection studies of fibrous materials limit their relevance for human risk assessment (IPCS, 1988; Vu, 1988; NIEHS, 1989).

The primary purpose of the RCC inhalation studies was to provide information which would be useful in predicting possible human health risks of exposure to different compositions of MMVFs. These studies also present a unique opportunity to gain a greater understanding of the fibre characteristics that are the critical toxicological determinants, because the experimental design resulted in pulmonary region deposition of large numbers of the different fibre types having comparable dimensions. The major focus of the present paper is to characterize the fibres in the exposure aerosols and in the lungs of animals chronically exposed to the different compositions of MMVF, and to attempt to determine whether fibre dose, dimension or durability could explain the observed differences in lung and pleural pathology.

As shown in Table 3, the aerosol exposure concentrations of the different MMVFs could not explain the differences in their toxicity to the lung. Although the exposure to RCF (WHO fibres $cm^{-3}$) was the lowest of the five fibre types, it was the only MMVF that produced an elevation in lung tumours and mesothelioma. Neither could the dimensions of the RCF fibres in the aerosol explain its greater toxicity to the lung, since the length and diameter distributions of RCF were comparable to those of the other MMVF types (Fig. 3). It should, however, be kept in mind that the RCF fibre preparation contained more non-fibrous particulates than the other MMVF types. What effect this might have had on the pathological effects of RCF is not as yet known. It was previously shown that exposure of rats to non fibrous $TiO_2$ and asbestos significantly enhanced the induction of lung tumours and mesotheliomas compared to rats exposed to asbestos alone (Davis *et al.*, 1991).

Lung deposition patterns of the five MMVF types could not explain differences in pathogenicity; lung burdens were similar for each of the test fibres. In each case, the fibres that were recovered from the rat lung had even narrower length and diameter ranges than the aerosol fibres (Fig. 4). It is valid to assume that most of the fibres recovered from the lungs were in the pulmonary region or interstitium, because the animals were killed at least 24 h after the exposure was stopped, allowing clearance of

the upper airways by mucociliary mechanims. The numbers of WHO fibres and fibres $>10$ $\mu$m in length in the lung were similar for each of the different MMVF types [Figs 5(a) and (b)]. However, greater numbers of long fibres ($>20$ $\mu$m long) were found in the lungs of rats exposed to RCF 1 and MMVF 21 (rock wool) than in those exposed to the other fibre types [Fig. 5(c)]. Even though lung concentrations of long MMVF 21 fibres were higher than those of long RCF 1 fibres, lung fibrosis occurred much later for MMVF 21 (18 months vs 6 months for RCF 1) and no mesotheliomas or significant increase in lung tumours were observed for MMVF 21. This indicates that the lung pathogenic potential of a fibre may be determined by more than its dose and dimensions.

Examination of the lung clearance patterns for the different MMVFs revealed some interesting findings. First, it is clear that the long fibres ($>20$ $\mu$m long) disappeared more rapidly than shorter fibres (Fig. 7). Perhaps the long fibres were breaking and contributing to the short fibre populations. Secondly, lung clearance patterns for the five MMVFs could not account for differences in the pathogenicities of the test fibres. Figures 7(a) and (b) show the per cent of WHO fibres and of fibres $>10$ $\mu$m in length retained in the lung after various recovery periods for the five MMVFs. The two fibre glasses (MMVFs 10 and 11) took longer to disappear or to clear from the lung than did RCF, perhaps because the lung macrophage clearance mechanisms were more inhibited in the fibreglass exposures. However, the long fibres ($>20$ $\mu$m long) disappeared more slowly from the RCF 1-, MMVF 21- and MMVF 10-exposed rats than from rats exposed to the other fibre types. In general, this agrees with the greater accumulation of long fibres in rats exposed to RCF 1 and MMVF 21 [Fig. 5(c)]. Perhaps MMVF 10-exposed rats did not accumulate high levels of long fibres because the aerosolized fibre was somewhat shorter and thicker than the other fibre types (Fig. 3). The fact that RCF 1 and MMVF 21 disappeared from the lung at similar rates provides further support for the contention that the pathogenicity of a fibre is dependent upon more than simply the dose, dimension, and the durability of the fibres in the lung.

In a previous paper on this series of studies it was reported that fibre glass and RCF reached similar levels in the lung during continuous exposure and appeared to clear from the lung at comparable rates (Hesterberg *et al.*, 1994). Fibres recovered from the lungs of animals exposed for only 13 weeks and held without further exposure for 91 weeks (recovery animals) were analysed for chemical change using energy-dispersive spectroscopy (EDS) with scanning electron microscopy. These analyses of recovery lungs showed that much of the alkalis and alkaline earth components had leached from the fibre glass over time. However, only a slight change in RCF chemistry was observed. These findings indicate that the leaching of fibres and the resultant change in chemical composition, especially the surface chemistry, may be an important determinant of the biological activity of MMVFs. The importance of chemical composition to the toxic potential of fibres has been recently reviewed (Guthrie and Mossman, 1993). In addition, fibres that are more rapidly leached of their alkalis and alkaline earths may be more readily broken in the lung, which might explain why the more leachable fibre compositions show the most rapid disappearance of long fibres. More studies are required to determine if a fibre's ability to be leached is a critical determinant of its ultimate toxicity to the lung.

It is interesting to note that, in the RCC study series, chrysotile asbestos produced

no lung cancer or mesotheliomas in hamsters, but in rats it produced the entire spectrum of asbestos-associated lung disease observed in humans, including pulmonary fibrosis, lung tumours and mesothelioma. For this reason, if a single species is chosen for assessing the human health risk of fibre inhalation, the rat would be the most appropriate choice. This is in agreement with the consensus opinion of a workshop of fibre toxicology experts (McClellan *et al.*, 1992). However, because of its greater pleural mesothelioma response after RCF inhalation (41%), the hamster has been recommended as a second species for evaluating the human health risk of fibres (Vu and Dearfield, 1993; Vu, 1994).

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