Review of Animal/In Vitro Data on Biological Effects of Man-made Fibers

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This paper reviews the investigations with man-made fibers (MMF). Insulation wools: glasswool (GW), rockwool (RW), slagwool (SW), glass microfibers (GFM), glass filaments (GFI), and refractory ceramic fibers (RCF) have been used in experimental animals and in in vitro cell systems. A large heterogeneous number of fibers, methods of fiber preparation, size selection, aerosolization, fiber size, and fiber burden measurement were noted, rendering difficult a comparison between results. By inhalation, RCF and asbestos used as positive controls produced a significant tumor increase. In some studies, a low tumor yield was found after inhalation of insulation wools; when all inhalation data were gathered, a significant tumor increase was found with GW. However, it is difficult to draw definitive conclusions on the potential of other fiber types because, in addition to the different compositions of the fibers, differences in fiber number and sizes existed, especially in comparison with asbestos. Moreover, experiments using inoculation, especially by the intrapertoneal route revealed a carcinogenic potential of all fibers types but GFI and SW. In these two groups a small number of animals has been investigated and the fiber characteristics were sometimes irrelevant. So far, a relationship between the carcinogenic potency and fiber dimensions has been established. Other fiber parameters may be of importance (surface chemistry, biopersistence, fiber structure, for example) but further investigations are necessary to determine the correlations between these parameters and tumor incidence. In vitro experiments have emphasized the fiber characteristics identified in vivo as playing a role in the carcinogenic potency and should be developed as a better approach of the mechanismic effects of MMF. — Environ Health Perspect 102(Suppl 2):47–63 (1994).

Key words: alternative tests, animal experiments, asbestos, ceramic fibers, glass fibers, glasswool, glass filaments, lung cancer, man-made fibers, mesothelioma, rockwool, slagwool

Introduction

Man-made fibers (MMF) are a large group of fibers that include several subgroups. A number of classifications have been proposed based on the nature of the raw material used to make the fibers, preparation method, crystallinity and chemistry (1–3). According to Head and Wagg (1), MMF can be divided into four groups: insulation wools, continuous filaments, refractory fibers, and special purpose fibers. Insulation wools include glasswool (GW), slagwool (SW), and rockwool (RW). Within each group, crystallinity and chemistry may change and the range of fiber diameter decreases in the four groups according to the order listed above, special purpose fibers being the thinnest.

For about 15 years MMF have been subjected to in vivo and in vitro tests to determine whether the fibers are carcinogenic or fibrogenic and whether they have some carcinogenic or fibrogenic potential. In vitro experiments have been mainly carried out with rats but also, to a lesser extent, with other rodents (hamsters, mice, guinea pigs). The effects have also been observed in monkeys. In vitro experiments mainly dealt with cytotoxicity assays performed on different cell types but some transformation experiments have been also carried out. The in vivo toxicity of MMF has been studied using several routes of fiber administration: inhalation, intratracheal instillation, and inoculation, either intraperitoneal or intrapertoneal. Since a report by the International Agency for Research on Cancer (4), the in vivo toxicity of MMF has been recently summarized (5–8) in terms of human and animal exposure. So far, the risk for lung fibrosis seems low or negligible in humans. However, some animal experiments have indicated a fibrogenic and carcinogenic potential while other have not. The present paper compares the experimental conditions in the various studies to determine whether the differences can be explained on the basis of the fiber parameters suspected or demonstrated as causing adverse effects or whether other reasons are implicated. Moreover, we will evaluate the relevance of the in vivo data to studying MMF toxicity.

Animal Experiments with Insulation Wools

Experiments with Glasswool

This section reviews the experiments with GW or with other insulation glass fibers (GF) if no additional information on the glasswool was mentioned in the paper reviewed.

Inhalation and Intratracheal Instillation Studies. The effects of GW have been tested by inhalation on three rodent species: rat, hamster, and guinea pig (9–18). The results reported by Drew et al. (14) are not included here because the animals in that study were exposed for 10 weeks and sacrificed within 2 months after exposure, which did not permit the study of fibrosis and cancer. Similarly, the pulmonary reactions described in the preliminary report of Gross et al. (10) seem limited to a 12-month exposure with rats and only data with hamsters can been taken into consideration. Moreover, the study by Moorman et al. (17) should be interpreted with caution because it lacks information on survival times.

The summary of available characteristics of fibers used in inhalation experiments with rodents is reported in Table 1. In hamsters none of the three studies (10,11,16) showed tumor production after inhalation; however, tumors also were not found in positive control hamsters exposed to either 300 mg/m³ amosite (7 hamsters) or 7 mg/m³ crocidolite (70 hamsters) (Table 2). Only one study has been performed with guinea pig (11); few animals were exposed, two adenomas were detected in eight GF-exposed guinea pigs while no
Table 1. Summary of available characteristics of fibers used in inhalation experiments with rodents.

| Fiber designation | Methods | Dose | Fiber dimension |
|-------------------|---------|------|----------------|
|                   | F/ml    | mg/m³ | Length, µm | Diameter, µm | Reference |
| MMVF              | Ball milled/-/chamber | ND | 135 | 4.2 mg/m³ | average 10 | (10) |
| GW Saint Gobain   | Milling/Timrell Mark II/chamber | 94(70)³ | 21(15)³ | — | 58% >10³ 88% >5³ | (12,15) |
|                    | Ball milled/-/chamber | 6,540 | — | — | 11.2% >5³ 1.2³ | (11) |
| FG insulation QC  | Ring mill | — | 13.9 | 0.4 mg/m³ | >20 | 3.5 | (17) |
| FG insulation OC  | Grinder/-/chamber | — | 13.9 | 0.4 mg/m³ | >20 | <3.5 | |
| Series air filter media | — | — | 14.9 | 0.4 mg/m³ | >10 | <3.5 | |
| Insulsafe II, blowing | No milling/ | 100 | 10 | 600 p/ml | CML 37³ 99% >5³ 3.1³ 46% <1³ | (16) |
| Bldg insul with phenol | Timrell/nose only | 100 | 12 | 3,800 p/ml | CML 31 ± 3³ 94% >5³ 5.4³ 48% <1³ | (16) |
| Owens Corning high temperature | 25 | 9 | 775 p/ml | CML 114 ± 9³ 100% >5³ 6.1³ 20% <1³ | |
| English GW + resin | Milling/-/chamber | 267 | 29 | — | 52% [5/1]³ | 0.87³ [10/0.2]³ | (13) |
| Manville 901      | Sided | — | — | — | 47% [5/1]³ | 0.38³ [10/0.2]³ | |
| MMVF 10           | Nose only | 273 | 29 | — | GML 13.1 | GMD 1.26 | (18) |
| Certain Teed B    | Sided | — | — | — | GML 13.7 | GMD 0.69 | (18) |
| Certain Teed B    | Nose only | 41 | 28 | — | 60% >10³ 87% >5³ 23% <1³ | (12,15) |
| RW Saint Gobain   | Milling/Timrell Mark II/chamber | 14,023 | 10 | — | 52% >10³ 43% 0.1³ | (12,15) |
| RW Sweden         | Milling/-/chamber | 240 | — | — | 63% >10 | (13) |
| SW JV Spinner     | No milling/Timrell/ Nose only | 200 | 10 | 5,600 p/ml | CML 40 ± 6³ 95% >5³ 2.7³ 0.1³ | (16) |
| JM 100            | Milling/Timrell Mark II/chamber | 3,000 | 3 | 12,000 p/ml | 19% >10 | 0.45 | (16) |
| 104/475, tempstran | Ring mill grinder/-/ chamber | 576 | — | — | 50% [4.8/0.42]³ | — | (29) |
| 104/475, tempstran | Knife mill/Spurny Vibrating bed/nose only | 576 | — | — | 50% [4.8/0.42]³ | — | (29) |
| 104/475, Denver   | — | — | — | — | — | — | |
| JM 100            | No milling/Timrell/ nose only | 1,436 | — | — | 29% >10 | — | (13) |
| Al silicate glass | Steel rollers/ Timrell/chamber | 95 | 10³ | 380 p/ml | 30% >10 | 45% [5/1]³ | (38) |
| Kaolin            | —/nose only | 191 | 29 | — | 26 ± 20³ 1.1 ± 0.69³ | (39,40) |
| High purity       | — | 224 | 29 | — | 18 ± 15 | 1.0 ± 0.72³ | (39,40) |
| Zirconia          | — | 172 | 29 | — | 28 ± 20 | 0.86 ± 0.68³ | (39,40) |
| After service     | — | 166 | 30 | — | 11 ± 9 | 1.39 ± 0.75³ | (39,40) |
| PKT               | Ball milled/-/chamber | 6,530 | 73 | — | 5.5% >10³ 4.2³ 0.2³ | (11) |
| Titanate, Fybex   | Ball milled/-/chamber | 6,530–101,500 | 73–371 | — | 6.7³ 0.2³ | (11) |
| Saffil            | Ball milled/ Timrell/chamber | — | 3 | — | 15.5³ 2.7³ | (41) |
| Fibrefax carbonbundum | No milling/Timrell/ nose only | 200 | 12 | 6,600 p/ml | CML<30 ± 3³ 83% >10 | 1.8³ | (16) |

Abbreviations: FP/AG/ES, fiber preparation/aerosol generator/exposure system; NF, nonfibrous; CML, count mean length; GML, geometric mean length; CMD, count mean diameter; geometric mean diameter; MMVF, man-made fiber glass; GW, glasswool; GF, glass fibers; FG, fibrous glass; OC, Owens Corning; MMVF, man-made vitreous fibers; PKT, pigmented potassium titanate. *Mean. Median. ²Scanning electron microscopy measurement. ³Optical microscopy measurement. ⁴Respirable fibers. ¹[L/D] fiber dimensions see footnote b.
tumors were found in unexposed controls or in amosite-exposed animals. The number of animals studied in this experiment is too small to draw conclusions.

Few or no tumors were found in rats (Table 2); however, in all studies combined, a total of 1290 rats were exposed; 29 developed tumors. When all groups from all of the experiments were combined the difference was significant (p<0.05) in comparison to air or unmanipulated control animals ($\chi^2$ test). A highly significant increase in the number of tumors was obtained with the positive asbestos controls (p<0.0001) (Table 3). The use of combined data from the different studies allows one to emphasize statistical differences because of the greater number of animals in the analysis. However, because the weight of each study depends on the number of animals included in it, general conclusions on the response to a given fiber type should be made with caution. However, the conclusions seem sound because low or medium numbers of tumors (i.e., 3 to 12) were found in all of the studies.

A summary of the inhalation experiments with rats where data on the cumulative dose of fibers were available is reported on Table 2. The number of fibers present in the GW aerosol was lower than that of asbestos fibers. Experiments using GW and GF have been performed with doses of 9 to 30 mg/m$^3$, except in the experiment by Gross et al. (10) in which doses up to 135 mg/m$^3$ were reported (Table 1). In this latter paper, however, the lack of information on the number of fibers per unit weight and the absence of positive controls make it difficult to conclude whether the absence of effect is related to an absence of carcinogenic potential of the fibers or to some other parameters (fibers of nonrespirable size for instance).

The diameter of any fiber type used in inhalation experiments is very important because fibers thicker than 1.0 $\mu$m have a poor chance of being deposited in the rodent respiratory airways. In a number of inhalation experiments, the diameter of some or all of the fibers in the sample was too large to permit them to be deposited in the rat lung. In several experiments the diameter was greater than 1.0 $\mu$m; Smith et al. (16) indicated that the count mean diameter (CMD) was 1.4, 1.4, and 3 $\mu$m for Insulafine II, building insulation and Owens Corning fibers, respectively, with a mean of 3.1, 5.4, and 6.1, respectively, and a percentage of less than 50%<1 $\mu$m (Table 1). It is likely that under such circumstances, the probability that a part of the fiber sample would be deposited in the rodent lung was low. However, Lee et al. (11) who used fibers of lower mean diameter, 1.2 $\mu$m, observed two adenomas. Comparison of studies reported by Lee et al. (11), Hesterberg et al. (18), and Smith et al. (16) show that not only fiber dimensions but also the exposure doses were different (Table 2). The concentration in Lee et al. (11) was more than 60 times that in Smith et al. (16); moreover, the cumulative dose at the end of the exposure time was higher in the experiment by Lee et al.: 35.3 x 10$^5$ fibers against 3.12 to 0.78 x 10$^5$ fibers in the experiment by Smith et al. Aerosol fiber concentrations were only twice in the experiment by Hesterberg et al. (18) but the fiber diameter was considerably thinner. A larger number of respirable fibers might account for the occurrence of some tumors in the Lee and Hesterberg experiments (11,18); however, the tumor yield was so low that only suggestions can be made. Comparison of studies with MMF is also very difficult because of the various methods used for fiber preparation and differences in aerosol generation and exposure systems. A summary of these procedures is given in Table 1. The results obtained with monkeys (17) are difficult to interpret in terms of fibrogenic and carcinogenic potential because of the short duration of exposure; the investigation lasted only 18 months.

Some tissue disorders other than fibrosis or respiratory cancer have been reported. Moorman et al. (17) did not observe lung tumors but did find a significant rate of mononuclear cell leukemia in rats exposed to GF. This disease was not found in unexposed rats. However, contradictory results were obtained by Drew et al. (14), who observed an unspecific monocytic leukemia in rats after chronic exposure to GF. In the preliminary report by Gross et al. (10), heavily collagenized stroma was observed after a 12-month exposure.

Intratracheal instillations with GW or GF have been carried out in two experiments using a total of 83 hamsters (10,19). A third report was not taken into consideration because of the high rate of deaths at 12 months (9). One lung carcinoma out of 20 hamsters inoculated with 10 mg (5 inoculations of 2 mg each) GF with a mean diameter of 0.65 $\mu$m (90% <0.2 $\mu$m) was detected in one experiment (19). No positive controls were tested; no tumors were observed in 20 untreated controls. In rats, seven tumors were observed among 322 rats inoculated with 5 to 35 mg GF (10,14). The instillation method consisted of 1 to 10 inoculations. The tumor yield in GW-treated rats (2.1%) was not significantly different from that of saline controls (6/171=3.5%). Positive controls were tested only in one experiment (14), in which one adenocarcinoma was found out of 46 rats treated with crocidolite. The differences were not significant (Table 3).

Intraperitoneal Inoculation Studies. By intraperitoneal implantation Stanton et al. (20,21) have defined fiber characteristics related to the carcinogenic potency. According to these authors the higher probability of tumor formation was associated with fibers >8 $\mu$m long and >0.25 $\mu$m in diameter and longer than 4 $\mu$m and thinner than 1.5 $\mu$m. Two other studies have been carried out using a total of 83 rats (Table 2) (13,22). Only one mesothelioma was found in one study (13) against six mesotheliomas obtained with chrysotile in this study (12.5%). The dimensions of the fibers were as stated; the total number of fibers inoculated was 196 x 10$^8$ with asbestos and 5.1 x 10$^8$ and 9.8 x 10$^8$ with GW, with and without binder; the number of fibers longer than 5 $\mu$m was 196 x 10$^8$ for asbestos and 2.2 and 4.2 x 10$^8$ for GW. Therefore, both the total number and number of "long" fibers inoculated were higher with asbestos than with GW. When the two studies reported by Wagner et al. (13,22) are gathered, a total of 116 rats were inoculated with chrysotile; 43% showed mesothelioma, p<0.001 (Tables 2 and 3).

In contrast with intraperitoneal inoculation, intraperitoneal inoculation resulted in a high rate of tumors (23). All three GF types tested produced carcinoma, mesothelioma, or sarcoma (Table 2). A total of 366 rats were treated with different doses and 159 rats exhibited tumors (43.4%) in a dose-dependent manner. Similarly, 45.7% of the rats treated with asbestos (chrysotile or crocidolite) formed tumors. In these experiments, 72 controls, either saline-treated or untreated, have been used; no tumors were found (Table 3). Considering only data obtained with rats, based on inoculation studies performed with several samples, one can conclude to a tumorigenic response with GW depending on the fiber dimensions.

Slagwool Experiments

Inhalation Studies in Rodents. The effects of SW have been studied by inhalation with two rodent species, hamsters, and rats. No tumor was observed in 69 hamsters and crocidolite used as positive control was not tumorigenic (0/70) (16) (Tables 3 and 4).
Table 2. Experiments with glasswool and other glass fibers in hamsters and rats

| Route of exposure (species) | Control | GW or GF | Asbestos | Reference |
|-----------------------------|---------|----------|----------|-----------|
| Inhalation (hamsters)        |         |          |          |           |
| Tumors (%)                  | 1/17 (0.6) | 0/257 (0) | Amosite or crocidolite<sup>a</sup> | (10,14,17) |
| Dose, F/ml                  |         | 654.0    |          |           |
| Cumulative dose x 10<sup>5</sup> |         | 35.3     |          |           |
| Tumors (%)                  | 0/48 (0) | 6 Ad (2) | GS<sup>d</sup> |           |
| Dose, F/ml                  |         | 255      |          |           |
| Cumulative dose x 10<sup>5</sup> |         | 4.6      |          |           |
| Inhalation (rats)            |         |          |          |           |
| Tumors (%)                  | 0/19 (0) | 1 AdCa (2) | GS<sup>d</sup> |           |
| Dose, F/ml                  |         | 654.0    |          |           |
| Cumulative dose x 10<sup>5</sup> |         | 35.3     |          |           |
| Tumors (%)                  | 0/48 (0) | 1 Ad (2) | GS<sup>d</sup> |           |
| Dose, F/ml                  |         | 255      |          |           |
| Cumulative dose x 10<sup>5</sup> |         | 4.6      |          |           |
| Inhalation (rats)            |         |          |          |           |
| Tumors (%)                  | 0/47 (0) | 1 Ca45 (2) | GS<sup>d</sup> |           |
| Dose, F/ml                  |         | 94       |          |           |
| Cumulative dose x 10<sup>5</sup> |         | 2.4      |          |           |
| Intratracheal (hamsters)     |         |          |          |           |
| Tumors (%)                  | 0/40 (0) | 1/83 (1.5) | GS<sup>d</sup> |           |
| Intratracheal (rats)         |         |          |          |           |
| Tumors (%)                  | 6/171 (3.5) | 7/339 (2.1) | GS<sup>d</sup> |           |
| Intrapleural (rats)          |         |          |          |           |
| Tumors (%)                  | 0/24 (0) | 1/83 (1.2) | GS<sup>d</sup> |           |
| Intraperitoneal (rats, 1)    |         |          |          |           |
| Tumors (%)                  | 0/0     | 1/83 (1.2) | GS<sup>d</sup> |           |
| Dose, F/ml                  |         | 2 mg     |          |           |
| Tumors (%)                  | 0/0     | 2M, 3S/36 (11.1) | GS<sup>d</sup> |           |
| Dose, F/ml                  |         | 10 mg    |          |           |
| Tumors (%)                  | 0/0     | 20M, 3S/32 (7/19) | GS<sup>d</sup> |           |
| Dose, F/ml                  |         | 4 x 25 = 75 mg | GS<sup>d</sup> |           |
| Tumors (%)                  | 0/0     | 17M, 3S/3 (7/4) | GS<sup>d</sup> |           |
| Dose, F/ml                  |         | 2 mg     |          |           |
| Tumors (%)                  | 0/0     | 36M, 4Ca, 15/77 (53.2) | GS<sup>d</sup> |           |
| Dose, F/ml                  |         | 10 mg    |          |           |
| Tumors (%)                  | 0/0     | 47M, 8S/7 (7/1) | GS<sup>d</sup> |           |
| Dose, F/ml                  |         | 2 x 25 = 50 mg | GS<sup>d</sup> |           |
| Tumors (%)                  | 0/0     | 12M, 1Ca, 15/37 (37.8) | GS<sup>d</sup> |           |
| Dose, F/ml                  |         | 20 mg    |          |           |

Abbreviations: Ad, adenoma; AdCa, adenocarcinoma; Ca, carcinoma; M, mesothelioma; S, sarcoma. <sup>a</sup>Number of animals with tumors/total number of animals. <sup>b</sup>Size determined by electron microscopy. <sup>c</sup>Size determined by optical microscopy. <sup>d</sup>+, binder; --binder. <sup>e</sup>Amosite: 300 mg/m<sup>3</sup>, 7 hamsters and crocidolite: 7 mg/m<sup>3</sup>, 70 hamsters. <sup>f</sup>Only results obtained with the highest dose are indicated in the table.
Only one study has been reported with rats in which no tumorigenic effect was found (Table 4) (16). The SW sample was composed of fibers with a mean diameter of 2.7 using a scanning electron microscope (SEM) and contained a great number of nonfibrous (NF) particles (NF/F = 28) (Table 1). Three tumors, including one mesothelioma, were observed with crocidolite but no tumors were detected in 184 control rats (59 air control and 125 unmanipulated controls) (Table 3). The cumulative dose, as indicated by the number of airborne fibers, was different 6.2 $\times 10^{5}$ with SW, against 94 $\times 10^{5}$ with crocidolite (200 F/ml and 3000 F/ml, respectively). Thus, the number of asbestos fibers to which animals were exposed was greater than the number of MMF, and conversely, the fiber diameter was generally smaller than that of MMF.

**Intranasal Inoculation Studies** No tumors were found following intranasal inoculation of 20 mg of SW, while mesothelioma occurred for an equivalent weight of chrysotile (13) (Table 4). The number of fibers inoculated was greater with chrysotile than with SW, as was the number of fibers longer than 5 $\mu$m. The dose of SW inoculated was 0.02 $\times 10^{8}$ (10/0.2)* and 0.36 $\times 10^{8}$ [5/1] for fibers with binder and 0 [10/0.2] and 0.52 $\times 10^{8}$ [5/1] for fibers without binder. The number of chrysotile fibers inoculated was greater than that of SW, as was the number of fibers $>5$ $\mu$m long (196 $\times 10^{5}$ against 1.2 $\times 10^{5}$); there is no mention of the diameter but it is very likely that all chrysotile fibers have a diameter smaller than 1.0 $\mu$m. However, neither SW nor chrysotile produced a significant increase in tumor formation (Table 3).

By intraperitoneal inoculation, in spite of studies showing a response of 10 tumors in 136 animals inoculated (7.3%), the increase is not significant compared to control animals, in which no tumors were detected (24,25) (Tables 3 and 4). Since these papers present no data on fiber size, comparison with positive controls is not possible.

In summary, SW fibers did not produce significant tumor increase. Some mesotheliomas and sarcomas were observed, only after intraperitoneal inoculation. From the experiments reported here, it is, however, difficult to safely conclude an absence of carcinogenic potential because asbestos did not produce tumors except following intraperitoneal inoculation.

**Rockwool Experiments**

*Inhalation and Intratracheal Instillation Studies with Rodents.* Inhalation of RW results in a low tumor yield (Table 5) since only two tumors were found in a total 95 rats (2.1%); this enhancement was not significant in comparison with untreated controls, in which no tumors were found in 95 rats. Eleven tumors (11.5%) were observed in positive controls ($p<0.001$; Tables 3, 5) (12,13,15). As noted above for the other fiber types, fiber number was lower with RW than with chrysotile. In the study by Le Bouffant et al. (12,15), size was determined by optical microscopy. The cumulative dose of chrysotile fibers was about 300 times more than that of RW, suggesting a larger number of "respirable" chrysotile fibers. It is likely that greater differences in fiber number existed and would have been found using transmission electronic measurements. Therefore, the number of respirable fibers may account for the higher tumor yield with asbestos than with the RW samples. Differences in cumulative number of respirable fibers in the experiment reported by Wagner et al. (13) was less, ratio of about 1:20; since size was measured by SEM, it is likely that it is a better representation of the difference in the number of "respirable" fibers than when optical measurement would be.

Hamsters were inoculated with RW fibers (19) using a repetitive procedure of five inoculations of 2 mg each. No significant rate of tumors was produced (Table 3) but the number of animals was low (20 hamsters).

**Intraserosal Inoculation Studies** Following intraperitoneal inoculation, closer rates of mesothelioma were found in rats treated with RW (5.2%) and in rats treated with asbestos (12.5%) (13) (Tables 3 and 5). As with SW treatment, the number of RW fibers inoculated was much lower than that of asbestos (either total number of fibers or fibers longer than 5 $\mu$m). However, the increase of tumors was not significant for either RW or asbestos (Table 3).

Intraperitoneal inoculation resulted in significant production of tumors both following injection of RW or asbestos (Table 5). Of the 349 rats inoculated with different RW fibers, a total of 125 formed tumors (35.8%, $p<0.001$ treated/un-treated) (24–27). With asbestos the percentage of rats with tumors was 51.8% (Table 3) ($p<0.001$).

In summary, the effects of RW fibers have been tested mainly in rats. Significant tumor enhancement was found after intraperitoneal inoculation. Although some tumors were reported after inhalation or intrapleural inoculation, the rate was not statistically significant. As discussed above for the other MMF, it is difficult to give a clear-cut interpretation of the results because of the different doses of exposure when compared to asbestos and because of the small number of animals tested by intratracheal instillation and intrapleural inoculation.

**Animal Experiments with Glass Microfibers**

Many studies have investigated the effect of glass microfibers (GMF); all routes of exposure have been used: inhalation, intratracheal instillation, and intrapleural or intraperitoneal inoculation.

*Inhalation and Intratracheal Instillation Studies* Seven papers have reported the results of six inhalation experiments carried out in rodents (12,13,15–17,28,29). No significant increase of tumor rate has been found in rats (Table 3); the six studies have investigated a total of 515 rats exposed to GMF doses ranging from 14,023 F/ml (12,15) to 576 F/ml (29) (Table 1). The corresponding asbestos controls were 167,938 F/ml and 241 F/ml with chrysotile or 2011 F/ml with crocidolite.

The cumulative dose, all fiber size considered, was 5.7 $\times 10^{5}$ with GMF (one tumor) and 20 $\times 10^{5}$ with asbestos (one tumor with crocidolite) (29); 360 $\times 10^{5}$ GMF (no tumor) and 4400 $\times 10^{5}$ in the corresponding chrysotile control (nine tumors) (12,15). It was impossible, due to the lack of standardization of methods used to measure fiber dimensions, to determine if the percentage of tumor was dependent on the cumulative number of fibers of a size relevant to penetrate in the respiratory airways. It is likely that the different tumor rates observed with positive control fibers are related to a higher cumulative dose or to the fiber size, and alternatively, to the methods of preparation, aerosolization, and exposure. These differences might account for the lack of activity of crocidolite in the inhalation experiments involving hamsters (Table 2) reported by Smith (16) in which only 3% of the fibers had a length $>10 \mu$m; those were 19% in the JM 100 sample (Table 4).

In experiments using monkeys, Goldstein et al. (30,31) exposed baboons up to 30 months, and Moorman (17) exposed Cyanomologus for 18 months; these durations...
of exposure are probably too short; longer exposures would have been required to detect neoplasms in such animals. Intrapulmonary instillations have been made in hamsters (32,33) and in rats (16,25). Both species produced significant numbers of respiratory tumors while none of the untreated animals exhibited tumors (Tables 3 and 6). However, tumor enhancement was observed in a study with rats in which the animals were treated with 20 x 0.5 mg (25) while no tumors resulted from the same total dose administered under a different schedule, 5 x 2 mg (16). Similarly, hamsters treated once with 26 mg of JM104 (32) did not develop tumors, while 27 and 35% tumors were obtained after instillation of 8 x 1 mg of JM104 (33). The clumping of fibers when high doses are instilled might account for the different results. Alternatively, the differences may stand in several parameters including the total dose and the dose per instillation. For this method if too many fibers are instilled they form clump and not penetrate in the lung or clump once deposited in the lung. This often results in a granuloma formation which effectively insulated the fibers from further toxic responses. In addition, it is unknown whether repetitive traumatism may act as an enhancer of tumor formation.

**Intraperitoneal Inoculation Studies.** Glass microfibers have also been inoculated in rats via intrapleural or intraperitoneal routes (Table 3). Intrapleural inoculation resulted in a significant increase of mesotheliomas compared to saline controls (p<0.02); the same result was also obtained after inoculation with asbestos (p<0.001) fibers. A significant enhancement of tumor production was obtained (p<0.001) following intraperitoneal inoculation with both GMF and asbestos, compared to controls (24,25). In the published papers, there is no mention of the amount of fibers per unit weight; thus, the cumulative dose cannot be calculated and it is not possible from these data to link the results to one or the other of the fiber parameters. However, Pot et al. (34) have proposed that the probability of tumor formation was dependent on fiber dimensions and fiber durability.

To summarize, in vivo studies carried out with GMF have demonstrated a carcinogenic potential using several routes of exposure, except inhalation. By the intrapulmonary route the data are in agreement with a carcinogenicity dependent on fiber dimensions (20,21,35) and on other parameters (35,36). To explain data obtained by intraperitoneal inoculation, Pot et al. suggested that durability is another important parameter (34,37). JM 100 and JM 104/475 have been used in inhalation studies; no tumors resulted in spite of the thin diameter of the fiber which allowed deposition into the lung. The lung deposition of JM fibers has been confirmed by studies of lung burden (12,16); in each study, a higher number of JM 100 than asbestos, used as positive control, has been detected in spite of an equivalent or lower cumulative dose exposure (Table 7). The results may indicate that the retention of GMF is greater than that of asbestos (either crocidolite or chrysotile) or that GMF might generate more fibers after deposition because of fiber degradation. In contrast, Mühle et al. (29) reported a lower retention of JM 104/475 versus crocidolite resulting from a lower exposure in terms of

### Table 3. Summary of experimental data and statistical analyses.

| Fiber | Inhalation | Intratracheal instillation | Intraperitoneal inoculation | Intrapleural inoculation |
|-------|------------|----------------------------|----------------------------|-------------------------|
| Rat   |            |                            |                            |                         |
| GW and GFM | 2.2%       | 0.03                       | 2.1%                       | NS                      |
| Asbestos | 17.2%      | 0.0001                     | 2.2%                       | NS                      |
| Controls | 0/164      | 2/171                      | 0/24                       | 0/72                    |
| SW | 0           | NS                         | No data                    |                          |
| Asbestos | 5.0%       | NS                         | 12.5%                      | NS                      |
| Controls | 0/5966     | 0/162                      | 0/24                       | 0/46                    |
| RW | 2.1%       | NS                         | No data                    |                          |
| Asbestos | 11.5%      | NS                         | 12.5%                      | NS                      |
| Controls | 0/64      | 0/24                       | 0/24                       | 5/256                   |
| GMF | 0.4        | NS                         | 8.9%                       | 0.001                   |
| Asbestos | 11.7%      | 0.001                      | 27.4%                      | 0.001                   |
| Controls | 3/579      | 0/190                      | 0/56                       | 9/442                   |
| RCF | 3.5%       | 0.01                       | 0 NS                       | 3.8%                    |
| Asbestos | 14.7%      | 0.001                      | 8%                         | 0.04                    |
| Controls | 0/299      | 0/150                      | 0/48                       | 2/291                   |
| RCF | 9.6%       | 0.001                      | cf RCFa                    | cf RCFa                 |
| Asbestos | 13.1%      | 0.001                      | cf RCFa                    |
| Controls | 2/424      |                            |
| GFil | No data    | No data                    | No data                    |                          |
| Asbestos | No data    | No data                    |
| Controls | 3/498      | No data                    |
| Hamster |            |                            |                            |                         |
| GF and GW | 0          | NS                         | 1.5%                       | NS                      |
| Asbestos | 0/400      | No data                    |
| Controls | 0/400      |                            |
| SW | 0           | NS                         | No data                    |                          |
| Asbestos | 0          | NS                         | No data                    |
| Controls | 1/170      |                            |
| RW | No data    | 0%                         | NS                         | No data                 |
| Controls | 0/20       |                            |
| GMF | 0%          | NS                         | 25.4%                      | 0.001                   |
| Asbestos | 0%         | NS                         |
| Controls | 2/170      | 0/59                       |
| RCF | 3.5%       | 0%                         | NS                         | 19.4%                   |
| Asbestos | 0%         | 74.0%                      |
| Controls | 1/191      | 0/169                      |
| RCF | 16.7%      | 0.001                      | cf RCF                      |
| Asbestos | 0%         | NS                         |
| Controls | 1/293      |                            |

Abbreviations: GW, glasswool; GF, glass fibers; SW, slagwool; RW, rockwool; GMF, glass microfibers; RCF, refractory ceramic fibers; GFil, glass filaments; NS, not significant. *Except Stanton et al. (20). †Including the RCC study. aNumber of animals with tumors/number of sham or untreated animals tested.
Table 4. Experiments with slag wool in hamsters and rats.

| Route of exposure (Animal species) | Control | SW | Asbestos | Reference |
|-----------------------------------|---------|----|----------|-----------|
| Inhalation (hamsters)             | 1/170 (0.6) | 0/69 (0) | Crocidolite | 94 (16) |
| Tumors %                         | 0/70 (0) | 6.2 |          |           |
| Cumulative dose x 10⁻⁵             |          |    |          |           |
| Inhilation (rats)                 | 0/184 (0) | 0/55 (0) | Crocidolite | 94 (16) |
| Tumors %                         | 0/3 + 1/60 (5) | 6.2 |          |           |
| Cumulative dose x 10⁻⁵             |          |    |          |           |
| Intraperitoneal (rats)            | 0/24 (0) | 0/48 (0) | SW₁⁵ | Chrysotile (9.5/64/48) |
| Tumors %                         | 0/69 (0) | 6.5 |          |           |
| Dose (0.5 ml)                     | 20 mg | 20 mg | 20 mg |          |
| Cumulative dose x 10⁻⁸             |          |    |          |           |
| Total fibers                      |          | 3.6 | 6.5     | 196      |
| Sized fibers (5/1)¹                |          | 0.36 | 0.52 |          |
| Intrapleural (rats)               | 0/48 (0) | 2/41 | 6/59 | 2/96 | 9/44 | 26/44 | 35/44 |
| Tumors %                         | (5) | (8) | (2) | (21) | (59) | (80) | (24,25) |
| Dose (2 x 2 ml)                    | 5 mg | 40 mg | 40 mg | 0.4 mg | 2 mg | 10 mg |          |

N.D. not determined. ¹ Number of animals with tumors/total number of animals. ² Number of fibers [L > 5 μm/ B < 5 μm]. ³ binder; ⁴ = binder.

total fibers and a greater retention of long fibers as a result of a higher exposure to longer fibers; the standard deviation study, however, makes it difficult to be sure that this assumption is accurate.

Animal Experiments with Ceramic Fibers

Inhalation and Intratracheal Instillation Studies with Refractory Ceramic Fibers

Inhalation studies with refractory ceramic fibers (RCF) have been performed on rats and hamsters. Differences existed between the percentage of tumors observed in the different studies (11, 16, 38-40). Overall, a significant production of tumors was obtained with rats (p < 0.001) as well as with hamsters (p < 0.001) (Tables 3 and 7). However, the percentages of tumor formation in the different studies ranged between 0% and 16%. Asbestos did not produce respiratory tumors in hamsters.

Refractory ceramic fibers produced lung tumors (11, 16, 38-40) and mesotheliomas (40) in rats. The highest rates of respiratory tumors were obtained in a series reported by Hesterberg et al. (40). In hamsters, of 41 tumors, 40 were mesotheliomas (11, 40); only one adenoma has been reported, with the highest dose of Fybec (371 mg/m³) (11). A much lower incidence of mesotheliomas was obtained by Lee (11) (7%) than by Hesterberg et al. (40), who reported that the rate reached 42%. Since the cumulative dose in the former study (101,500 F/ml) was even higher than the latter (215 F/ml), the different results may be related to the methods of aerosolization and exposure. The significant overall response to RCF in hamster is due to the study reported by Hesterberg et al. (39) (Table 8).

In rats, RCF as well as asbestos induced a significant increase of tumors, but the tumor yield was higher in the study reported by Hesterberg et al. (64/500) than in the other experiments (9/256) (Table 8); the difference between the two studies was significant (p < 0.001). However, when the asbestos results were compared, no significant difference was found in the two sets of experiments (p > 0.20). The lack of effect observed in several studies may be due to the irrelevant fiber diameter: median diameters of 2.75 and 3.7 μm (41), mean diameter of 1.8 μm (16) by comparison with 0.86 to 1.39 μm in the studies reported by Hesterberg et al. (39,40) and finally to a lower dose of exposure in terms of fibers available for lung deposition. The study by Lee et al. (11) with thin fiber (0.2 μm diameter) showed a dose-response except with the highest dose where overload effect may have modified the survival time since rats showed a dose-related mortality. No data are given on the mean diameter of the fibers in the experiment by Davis et al. (38) but 45% were less than 1 μm in diameter and more than 5 μm in length; that may account for the small tumor yield (8.3%).

Intratracheal instillation of RCF was made in two studies (16, 19). No tumors were detected in 42 hamsters or in 22 rats while 74% of hamsters and 8% of rats formed tumors after instillation of asbestos.

The chemical composition of one sample was K₄Ti₂O₁₃ (19).

Intraperitoneal inoculation studies

Inoculation experiments resulted in tumors in several experiments (Table 8) (16, 21, 22, 38, 42, 43). In the studies of intraperitoneal inoculation, two experiments entailed either a low yield of tumor (9.7%) (22) or no tumors (44) (Table 8). Only data on fiber diameter are available, they indicate a thinner diameter in the former experiment (0.5–1 μm) than in the latter (>2 μm). Stanton et al. (21), however, concluded that the probability of tumor formation was dependent on the number of fibers of characteristic size (above), an assumption that is not invalidated by the other results.

Smith reported high rates of tumors (83%) (16) after intraperitoneal inoculation of 25 mg, but Davis (38) found only 9% tumors after inoculation of the same dose. It is possible in these experiments to compare the number of fibers inoculated that were longer than 10 μm regardless of the diameter; this was 3.6 × 10⁶ in Smith (16) and 0.72 × 10⁶ in Davis (38) using SEM determination. Therefore, different tumor yields might result from different doses of relevant fibers. Maltoni et al. (42) found five tumors in a group of 40 rats treated with 10 mg of fibrefrax but no tumors after inoculation of 1 or 5 mg. In the absence of sufficient data on the number of fibers per unit weight, it is difficult to establish whether the differences are due to the number of "long" and "thin" fibers injected or to other parameters.

Animal Experiments with Glass Filaments

After intraperitoneal implantation glass filaments (GFil) did not produce mesotheliomas (21). According to the authors, this was related to the lack of fibers of relevant size, as discussed above with GW and RCF. One paper by Pott (45) has not been taken into consideration because it is a review of results previously published by Pott and others. Another paper reports data obtained by Pott et al. (25) in which GFil were injected intraperitoneally in rats as was granular glass. Diameter of fibers was high, in the order of 4, 5, or 7 μm. No difference in the percentage of tumors was found between treated and untreated animals (Table 3). Therefore, the results indicate that the glass filament samples were
not carcinogenic. Doses injected were sufficient to expect a carcinogenic effect but the fiber size could explain the results. Indeed, according to the data 50% of fibers had a diameter >3.7 μm (ES.3), >5.5 μm (ES.5), and >7.4 μm (ES.7). If one recalls the correlation coefficients reported by Stanton et al. (21), a positive correlation between cancer incidence and fiber size was found only with fibers <1.5 μm in diameter.

**In Vitro Experiments with Man-Made Fibers**

**Studies with Insulation Wools**

The *in vitro* cytotoxicity of GW and RW have been assessed in an immortalized human mesothelial cell line. Both fiber types reduced the cell viability in a descending order: thin RW, coarse GW, milled RW, milled GW and coarse RW. In general, the MMF samples were less toxic than asbestos (46).

**Studies with Glass Microfibers**

Several *in vitro* studies have been performed with JM fibers, mainly codes 100, 104, and 110. Different types of tests have been used that investigated mainly cytotoxicity, genotoxicity, and transformation. Code 100 fibers, in comparison with glass beads, were tested on macrophages to study cytotoxicity and production of superoxide by the cells (47). Glass beads were found to be less active than glass fibers in triggering cells to produce -O₂ at comparable weight concentrations.

**Studies with Long or Short and Thick or Thin Fibers**

Two papers report investigations of the genotoxicity of several types of fiber glass, including code 100 and/or code 110 on different cell types (48,49). Chromosomal abnormalities were detected with JM 100 fibers but not with JM 110 on rodent cells incubated with 10 μg/ml of fibers; no increase in chromosome aberrations and polyploidy were found in primary human fibroblasts or lymphoblastoid lines. According to the authors, the different responses observed between human and rodent cells might be related to the less efficient DNA repair of rodent cells compared to human cells. An additional explanation might be connected with the number of fibers ingested by the different cell types. This parameter is not generally considered in the interpretation of data because it is very difficult to obtain.

It is generally reported that long fibers are more toxic than short fibers. Tilkes and Beck (50) studied the effect of JM 104 and JM 100, fine and ultrafine fractions produced by a colloidal chemical method, on growth and viability of ascite tumor cells. It was observed that the toxicity of both fractions was of the same range on a per weight basis, but on a per number of fiber basis, the longer fibers had greater toxicity. In addition, the ultrafine fraction of JM 100 exhibited a lower toxicity than its fine counterpart on a per number of fibers basis. A length- and dose-dependent cytotoxicity of the fibers was also found in a lung rodent macrophages system (51). Using the same samples of fibers, another test, detection of sister chromatid exchanges (SCEs) in hamster peritoneal cells, Fisher (52) also concluded that a given number of short fiber fractions was less effective than the longer fiber fractions.

Two papers have also reported studies performed with JM 100 and JM 110 fibers. Brown et al. (53) tested the two samples, as well as respirable fractions of these samples using cytotoxicity, and genotoxicity assays. Results were compared on the basis of both weight and number of fibers. On a per weight basis, the cytotoxicity of JM 110 was nil or weak but selecting the respirable fractions resulted in increased biological activity. JM 100 fibers exhibited a greater cytotoxicity than code 110. However, the number of fibers contained in a given weight was not the same in the different

| Inhalation | Control | RW | Asbestos | Reference |
|------------|---------|----|----------|-----------|
| Tumors (%) | 0/47 (0) | 0/47 (0) | 0/47 (19) | (12,19) |
| Total fibers | — | 1.1 | 4.000 | |
| Sized fibers [10/any] | — | 0.66 | 218 | |

| Inhalation | Control | RW | Asbestos | Reference |
|------------|---------|----|----------|-----------|
| Tumors (%) | 0/48 (0) | 2/48 (4) | 12/48 (25) | (13) |
| Total fibers | — | 4.4 | 69.7 | |
| Sized fibers [10/0.2] | — | 0.09 | 1.7 | |

| Intraperitoneal (rats) | Tumors (%) | 5/256 (1.9) | 125/349 (35.8) | 86/166 (51.8) | (24-27) |

*Number of animals with tumors/total number of animals. Number of fibers [>μm/ <0.5μm]. Size determined by optical microscopy. Size determined by electron microscopy. Binder, binder.*

| Route of exposure | Control | RW | Asbestos | Reference |
|------------------|---------|----|----------|-----------|
| Inhalation | None | RW | Chrysotile | (12,19) |
| Tumors (%) | 0/47 (0) | 0/47 (0) | 0/47 (19) | (12,19) |
| Dose F/ml (mg/m³) | — | 41 (28) | 167,338 (15) | |
| Cumulative dose x 10⁵ | — | 1.1 | 4.000 | |
| Total fibers | — | 0.66 | 218 | |
| Sized fibers [10/any] | — | 0.09 | 1.7 | |

| Intraperitoneal (rats) | Tumors (%) | 5/256 (1.9) | 125/349 (35.8) | 86/166 (51.8) | (24-27) |

*Number of animals with tumors/total number of animals. Number of fibers [>μm/ <0.5μm]. Size determined by optical microscopy. Size determined by electron microscopy. Binder, binder.*

Table 5. Experiments with rock wool in rats.

| Route of exposure | Control | RW | Asbestos | Reference |
|------------------|---------|----|----------|-----------|
| Inhalation | None | RW | Chrysotile | (12,19) |
| Tumors (%) | 0/47 (0) | 0/47 (0) | 0/47 (19) | (12,19) |
| Dose F/ml (mg/m³) | — | 41 (28) | 167,338 (15) | |
| Cumulative dose x 10⁵ | — | 1.1 | 4.000 | |
| Total fibers | — | 0.66 | 218 | |
| Sized fibers [10/any] | — | 0.09 | 1.7 | |

Table 6. Summary of intratracheal studies with glass microfibers.

| Animal species | Fiber type | Fiber length | Dose, n times x mg | Tumor rate | % | Reference |
|----------------|------------|--------------|--------------------|------------|---|----------|
| Hamster | JM 104 | — | 1 x 26 | 0/64 | 0 | (32) |
| Hamster | Crocidolite | — | 1 x 26 | 0/64 | 0 | |
| Hamster | JM 104 | 50% > 7 μm | 8 x 1 | 1/136 | 35 | (33) |
| Hamster | JM 104 | 50% > 4.2 μm | 8 x 1 | 30/138 | 27 | |
| Hamster | Crocidolite | 50% > 2.1 μm | 8 x 1 | 18/22 | 13 | |
| Hamster | T02 | — | 8 x 1 | 2/125 | 1.5 | |
| Rat | 104/475 | — | 20 x 0.5 | 5/34 | 15 | (29) |
| Rat | Crocidolite | — | 20 x 0.5 | 15/25 | 43 | |
| Rat | Saline | — | 20 x 0.3 ml | 0/40 | 0 | |
| Rat | JM 100 | 19% >10μm | 5 x 2 | 0/22 | 0 | (19) |
| Rat | Crocidolite | 3% >10 μm | 5 x 2 | 2/25 | 8 | |
| Rat | Controls | — | — | 0/150 | 0 | |
samples, and the conclusions changed when the results were compared on the basis of the number of fibers. Thus, it appears that a given number of JM 100 fibers is less toxic than the same number of JM 110 fibers. When fibers >10 μm of length were considered the same trend was found. Hesterberg and Barrett (54) also tested these samples in cytotoxicity and transformation assays on Syrian hamster embryo cells (SHE). The authors reported that thin fibers (JM 100, diameter 0.13 μm) were more cytotoxic and transformant than thick fibers (JM 110, diameter 0.8 μm) on a per-weight basis. However, when cytotoxicity was determined according to the number of fibers, JM 110 fibers were 40-fold more cytotoxic than JM 100 fibers. Thus, to compare results with several samples of MMF on in vitro cell systems, it is important to consider both fiber weight and fiber number.

The different toxicities and transforming potencies of JM00 versus JM 110 fibers has also been shown by Mikalsen et al. (55) using the SHE cell assay. On the basis of weight JM 100 was about 15 to 20 times more toxic than JM 110, but the first sample contained about 60 times more total fibers than the second.

### Studies with Milled and Unmilled Fibers

Several papers have indicated that fiber milling results in a decrease of the fiber activity on the in vitro cell systems. Ririe et al. (56) reported that milling reduced the cytotoxicity of JM 100 fibers tested on rat tracheal epithelial cells. Hesterberg and Barrett (54), and Hesterberg et al. (57) reported that milling of JM 100 fiber glass resulted in a reduction of the cytotoxicity, genotoxicity and transformation of SHE cells. In the former report, milling resulted in a length decrease: from 9.5 to 1.7 μm or 16.0 to 0.95 μm (two separate experiments) without changing the diameter (0.13 vs 0.11 and 0.18 vs 0.19 μm). Since the same amount of fiber was incubated with the cells, it is evident that, on a per-number basis, milled fibers were much less toxic than unmilled fibers. The fibers were dispersed with a polytron tissue grinder to perform a good dispersion of the sample before use. The reduced transforming potency of milled versus unmilled JM 100 fibers is not due to a reduction of the number of fibers ingested by the cells, as demonstrated by Hesterberg et al. (57).

### Studies with Ceramic Fibers

Ceramic fibers have been used in some in vitro experiments (64-67). In vitro experiments were designed to detect only cytotoxicity of the fibers. However, a good correlation exists between in vivo and in vitro findings concerning the effect of size.

Brown et al. (60) have included a ceramic sample in a study of correlations between in vivo effects and in vitro asbestos

| Fiber | Exposure | Cumulative dose of fibers | Lung burden/g dry tissue weight | Reference |
|-------|----------|---------------------------|-------------------------------|-----------|
| JM 100 | 1.436F | 2.6 x 10^6 | 2.1 mg | (13)F |
| Chr | 3.832F | 7.0 x 10^6 | 0.4 mg | (13)F |
| JM 100 | 14.023F | 3.6 x 10^7 | 15.2 mg | (12)F |
| Chr | 167.538F | 4.3 x 10^7 | 1.7 mg | (12)F |
| JM 100 | 3.000F | 9.4 x 10^6 | 1.9 x 10^6 F | (16)F |
| Cr | 3.000F | 9.4 x 10^6 | 3.9 x 10^6 F | (16)F |
| JM 104 | 576 ± 473F | 5.7 x 10^5 | (3.1 ± 1.6) x 10^6 F | (29)F |
| 252 (5 μm L) | 2.5 x 10^3 | 7.0 x 10^6 F (>5) | (29)F |
| 2,011 ± 835F | 2.0 x 10^6 | (5.6 ± 2.7) x 10^6 F | (29)F |
| 162 (5 μm L) | 1.6 x 10^5 | 5.6 x 10^6 F (>5) | (29)F |

*Total fiber/mL. *F x m l⁻¹ x hr. A multiplication factor of 1.4 x 10⁴ is necessary to convert into total number of fibers inhaled by a rat. *Electron microscopy F/ml. *Optical microscopy F/ml. *Exposure: 24 months. *Lung burden expressed per lung; exposure: 12 months.

### Table 7. Glass microfiber studies: lung burden after end of exposure.

| Route of exposure | Control | RCF | Asbestos | Reference |
|------------------|---------|-----|----------|-----------|
| Inhalation (rats) | Tumors (%) | 0/299 (0) | 9/256 (3.5) | 31/211 (14.7) | (11,16,32,39,42-44) |
| | Tumors (%) | 2/125 (1.6) | 64/500 (12.8) | 13/125 (10.4) | (40) |
| Inhalation (hamsters) | Tumors (%) | 1/191 (0.52) | 5/142 (3.5) | 0/77 (0) | (11,16,38,39,42-44) |
| | Tumors (%) | 0/102 (0) | 36/130 (27.7) | 0/102 (0) | (40) |
| Intrapleural (rats) | Tumors (%) | 0/48 (0) | 3/79 (3.8) | 51/116 (44) | (21,22,44) |
| | Tumors (%) | 2/291 (0.7) | 72/276 (26.1) | 204/371 (55) | (16,38) |

*Number of animals with tumors/total number of animals. *The experiment was not achieved when the review was completed. *p < 0.01 treated/untreated; **p < 0.001 treated/untreated.
cytotoxicity; even though they found an association between fiber length and cytotoxicity, certain fibers such as the ceramic sample showed disparate results, thus confirming that parameters other than fiber length likely account for the biological response. With the ceramic fiber type a higher activity was obtained in vitro than would have been expected from the in vivo response.

Discussion

In general, studies with rodents show a very low tumor yield after inhalation of MMF, sometimes but not always contrasting with asbestos results obtained in the control series. It is difficult to determine the exact origin(s) of the differences between results obtained with MMF and asbestos because MMF and asbestos differ in fiber composition, fiber number and size distribution. Moreover, it is even difficult to compare MMF of the same category because the chemistry of the MMF investigated was seldom reported (14,19,30). In addition, differences in the dusting procedures and methods used to characterize the fibers also made comparison between studies difficult. However, some conclusions can be drawn and some results can be discussed.

Long-term inhalation is a realistic method of experimental dusting since it is similar to the situation encountered by humans. When all data obtained with MMF in rodents were gathered and considered independently in terms of technical conditions, significant respiratory lung tumor enhancement was found with GW in rats and with RCF, in both rats and hamsters, suggesting a carcinogenic potential at least for fibers submitted to the analysis. Inhalation data show both tissue specificity and species specificity.

RCF exposure resulted in respiratory tumors; rats had a lower rate of mesothelioma (10.4%) than lung cancer (89.6%). Hamsters, however, had a higher rate of mesotheliomas (97.6%) than lung tumors (2.4%). Differences might be due to species and tissue specificities. It can be suggested that the differences in the type of tumor are related to differences in fiber migration in rats compared to hamsters allowing a better transloduction of RCF toward pleura in hamsters. Alternatively, the fact that hamsters produce more asbestos bodies than rats ([16]; TW Hesterberg, personal communication) may account for the low lung carcinogenicity of inhaled RCF in this rodent species. However, data obtained with asbestos show that hamsters only develop lung tumors after intratracheal instillation (16); therefore the predominance of mesotheliomas following inhalation of RCF does not imply that hamster cannot develop lung tumor. It remains to be demonstrated whether the differences in tumor location are dependent on the fiber type and/or the route of exposure.

In rats, RCF produced fewer tumors than asbestos (Table 2). In contrast, RCF produced more tumors than asbestos in hamsters, since no tumors were found in a total of 179 hamsters treated with chrysotile, amosite, or crocidolite in independent studies (11,16,40). This might be due to a species specificity as well as to a fiber specificity.

Positive asbestos controls produced significant enhancement of tumor yield in rats in all group of studies except those involving SW (Table 3). The positive controls were included in a total of 10 experiments (11–13,15,16,18,28,29,38–41) and a total of 480 rats were exposed to chrysotile (12,13,15,18,28,29,38–40), 108 to crocidolite (16,38), and 16 to amosite (11). Significant increase in tumor yield was observed in seven of eight studies in which chrysotile was used and one of two studies in which crocidolite was used. The type of tumor was not always given but only one mesothelioma has been reported following treatment with chrysotile (18) and one with crocidolite (16). An overall 14.9% of tumors was found in rats exposed to asbestos, in contrast with 1.9% (15/777) in unexposed or sham-exposed rats. Therefore, the hypothesis of a carcinogenic potential of asbestos, both crocidolite and chrysotile, was consistent with data obtained in inhalation studies.

The fact that SW, RW, and GMF do not produce significant tumor enhancement by inhalation may be related to several issues such as fiber size and number, as well as fiber type.

One of the differences between asbestos and MMF is the diameter. Fibers too thick to be deposited in the rodent respiratory airways were sometimes used, as summarized on Table 1. To be relevant, inhalation requires the use of respirable particles. The size, especially diameter, of the particles convenient for rodents is smaller than that of significance for human exposure. It is generally admitted that the maximal size to use with rodents is 2 μm of aerodynamic equivalent diameter.

If the total number of fibers to which animals are exposed is taken into consideration, it can be argued that this number is lower with MMF than with asbestos. This does not imply that the number of fibers available to be deposited in the lung was similarly lower but it does suggest that more asbestos fibers may have been deposited in the lung in comparison with MMF. However, it does not seem possible to increase the MMF concentration to which animals were exposed since exposure was probably at the maximum tolerated dose. Short- and long-term studies and determination of lung burden could give information on the proportion of deposited versus inhaled fibers. The differences of experimental design between experiments reflect the evolution of knowledge in studies of the toxic effects and mechanistic action of fibers.

So far, lung burden determined after 24 months of exposure in three experiments (12,16,18) indicated a greater number of
asbestos fibers in the lung than of GW (Table 9). However this result is not observed with all MMF; in GMF experiments, in spite of the lower GMF fiber concentration in the aerosol and in spite of a higher cumulative dose, the lung burden may be lower than that of asbestos (Table 7). The lung burden has been studied in several experiments; Smith et al. (16) found that fiber recovery in rats was to about 400 times lower with MMF than with crocidolite, except for JM 100. In the same way, the number of MMF except JM 100 recovered in the lungs of hamsters was about 10 to 10^4 less than in crocidolite. In one study with GW (12) the cumulative dose of respirable chrysotile fibers was 4.3 \times 10^3 against 2.4 \times 10^3 with GW (Table 9). It resulted a higher lung burden in chrysotile-exposed animals, and a tumor yield of 9 versus 1 of 45 rats in each group. Because of the low number of tumors, it is not possible to conclude to a greater potential of one fiber type in comparison with the other; a larger number of rats would have been necessary to produce statistical differences. Several data have been reported concerning lung burden after exposure to GMF (Table 7). Le Bouffant et al. (12) reported that the cumulative dose of JM 100 was lower than that of chrysotile but the count was made using optical rather than electronic analysis; exposure resulted in a greater amount of JM 100 in the lung than of chrysotile. It is possible that the different method of fiber determination may, at least partially, explain the discrepancies. The cumulative dose of sized [10/any] fibers was approximately the same: 190 \times 10^3 and 210 \times 10^3. Since the fiber diameter of JM 100 was small (43% <0.1 \mu m), it is likely that the JM 100 fibers could be deposited in the respiratory airways; therefore the results would suggest a better retention of JM 100 or a fragmentation. However, we do not always know the confidence level of the lung burden measurement so the differences reported in this experiment between JM 100 and asbestos may be not significant. Moreover, the number of fibers retained after 24 months may have poor significance in terms of number of fibers related to the biological effects, because the important damage caused by the fibers may occur at the time of deposition and interaction with respiratory cells. Recent data on stimulation of mesothelial cells shortly after exposure to asbestos may argue for a short-term effect (TW Hesterberg, personal communication; (68)). Finally, JM 100 did not produce tumors, in contrast with chrysotile (nine tumors), possibly due to a lower potential of JM 100 in comparison with chrysotile. Similarly Smith et al. (16) observed that higher fiber recovery in JM 100-treated rats in comparison with crocidolite-treated rats was not associated with higher tumor yield since no tumors were detected following exposure to JM 100; three tumors including one mesothelioma, were found in rats treated with crocidolite. Even if the number of tumors observed with JM 100 fibers is not significant in comparison with untreated controls, these results should be given attention.

Mühle et al. (29) have exposed rats to JM104 and crocidolite (Table 7); the total number of crocidolite fibers to which animals were exposed was higher than that of JM104 but the cumulative dose of sized fibers was not highly different if we consider that crocidolite fibers were less than 0.42 \mu m in diameter. The calculation gives 3 \times 10^3 fibers of JM104 [4.8/0.42]; (50% of total fibers) and 2 \times 10^3 [4.5/any] for crocidolite (10% of total fibers); it resulted approximately the same number of fibers recovered. Low yield of tumors (1 tumor) was found in both cases, giving rates of 0.9% and 2%, respectively, suggesting comparable carcinogenic potency in these experimental conditions.

Intratracheal instillations resulted mostly in significant tumor enhancement with asbestos as well as with GMF in rats and hamsters. When tumors occurred they generally followed multiple instillations (Table 6). It has been found that instillation of a great number of fibers is not convenient because of the clumping of fibers; small doses have to be inoculated to permit a better distribution of the fibers in the respiratory airways. The absence of effect reported with GF (10.14) (Table 3) does not seem due to this parameter since several inoculations have been made but might be related to the dose, to the short survival time, or to the nature of the fibers. Intratracheal instillations of crocidolite resulted in 8% of tumors in rats and 74% in hamsters (16).

Recently, it has been suggested that the intrapleural route is a good approach to the human risk of mesothelioma (69). By intrapleural inoculation, it has been concluded that the probability of tumor formation was dependent on fiber size, based on results obtained with MMF and asbestos (21); this assumption was confirmed recently (35,36,71). Asbestos used as positive control entailed a globally significant tumor enhancement, except in SW and RW studies (13). However, although mesotheliomas were found in 12.5% of rats inoculated with asbestos, the lack of statistical significance is due to the small number of animals. The results obtained with SW and RW do not negate the results by Stanton (20,21), since the number of size relevant fibers, either SW or RW, was at least 30 [5/any] to 50 [5/1] times lower than that of asbestos (Tables 4 and 5).

When individual experiments are considered, the results obtained with MMF as well as with control fibers indicate that intrapleural inoculation produces a higher tumor yield than the inhalation procedure, but the overall results are close to those obtained by inhalation in terms of significance (Table 3). The differences likely result from lower number of animals investigated by intrapleural inoculation. The rate of spontaneous pleural tumors should be 0.3% to 0.8% according to Ilgren (72). These data were obtained from a database gathering large series of several hundreds of animals where the percentage of pleural mesotheliomas did not exceed 0.4% (EB Ilgren, personal communication). In the experiments depicted here, the background of pleural mesotheliomas is very low, even after intrapleural inoculation. No mesotheliomas were found in untreated or sham-treated rats (104 animals) but 3 were found (0.5%) if the study of Stanton et al. (21) is taken into consideration (592 rats studied). However, in this experiment, tumors were defined as pleural sarcomas and the method used to apply fibers was different since fibers dispersed in hardened gelatin were implanted by thoracotomy. Therefore, the probability of observing a pleural tumor by intrapleural inoculation in a small group of rats is negligible and the occurrence of one mesothelioma in a group of treated animals suggests the need for complementary studies.

Intraperitoneal inoculation of fibers resulted in significant enhancement of tumors with GW, RW, GMF and RCF (but not with SW and GF). Intraperitoneal inoculation gave the highest incidence of tumors among the methods used to determine the carcinogenic potency of fibers. Fibrefrax, one of the RCFs tested by means of three routes of administration was much more carcinogenic in rats by the intraperitoneal route than by the other methods, since 70% of tumors were produced in rats after inoculation of 5 \times 9 mg of fibers (43) and 83% with 25 mg, or 4.2 \times 10^8 total fibers (16). These Fibrefrax fibers did not produce tumors by intratracheal instillation of 5 \times 2 mg (16), nor after inhalation of a cumulative dose of 6.2 \times 10^3 fibers.
Results obtained in rats with RW from Sweden as well as with JM 100 expressed the same trend. Two adenomas (4.2%) were detected after inhalation of RW (13) while 45 tumors (71.4%) arose after intraperitoneal inoculation of 75 mg, and 6 (13.3%) were observed with 10 mg of a finer sample (25). No tumors (12,15,16) and 1 adenocarcinoma occurred after inhalation of JM 100 (13) in comparison with 32% (16), and about 40% following intraperitoneal inoculation (25). However, the results from the various studies remain difficult to compare because of the different characteristics of fibers used in inhalation versus intraperitoneal experiments but similar results have been previously obtained with other fiber types, tremolite and brucite. These particles induced 93 and 96% of mesotheliomas after intraperitoneal injection and about 51 and 13% of tumors by inhalation (73).

The reasons for this higher response by the intraperitoneal route are not well defined. However, it seems likely that the high number of fibers deposited in a short period of time (lasting for one to several inoculations) at the surface of the peritoneum partly accounts for the higher yield of tumors arising by this route of administration. If the number of fibers injected can be more than $10^3$ higher than the cumulative number of fibers given by inhalation, the total number of fibers given to a rat by inhalation is about 1. $4 \times 10^4$ greater than the cumulative dose; thus, rats would have inhaled more fibers than they have received intraperitoneally. However, the final result is that more fibers have been given intraperitoneally because the deposition rate by inhalation does not exceed a few per cent of the particles to which the rat has been exposed.

According to the papers by Port and co-workers, which have been summarized recently (27,34,37), carcinogenic potential is dependent on the number of fibers $>5 \mu m$ in length and $<2 \mu m$ in diameter and on the durability of these fibers. As far as intrapleural method, the effect of size might be related to the translocation of the small particles toward other sites rendering them unavailable to exert an effect, as well as to the intrinsic potential of long and thin fibers. Goodglick and Kane (74) have reported that preventing the clearance of short fibers resulted in cytotoxicity in mice inoculated intraperitoneally. The results of several experiments have been reported where fibers were treated in such a way that their size was modified. Fractioning of rockwool (Sweden) resulted in a decrease in the tumor rate, a response attributed to a reduction of the fiber size; both length and diameter were reduced by the preparation method (25). However, these experiments, are difficult to compare because 75 mg of the long fibers were injected (71.4% of tumors) while 10 mg of the shorter fibers were injected and resulted in 13.3% of tumors. A dose-response for glass fibers has been suggested in other studies (34).

The importance of fiber durability in terms of chemical stability on the incidence of peritoneal tumors has not been well documented. The concept of durability is complex, including the notions of epuration, migration and chemical stability. According to Pott et al. (34) the carcinogenic potency is related to the fiber durability but it is not clear whether this term refers to half-life of the fibers, solubility, or combination of both parameters; nor are described the methods to assess these parameters.

Production of oxygen derivatives by the surface active iron is another possibility to account for carcinogenicity of some fiber types. In this context, the carcinogenic potency of iron at the MMF surface is lower than that of asbestos (73).

A major difference between intraperitoneal and intrapleural methods concerns the rate of tumors in untreated or in saline-treated animals. By intraperitoneal inoculation, this rate was zero in control series belonging to experiments that assessed MMF carcinogenic potency; some sarcomas were formed following the implantation procedure of Stanton et al. (21). A maximal rate of 6% was observed by Pott et al. (25) in a series of 15 experiments involving intraperitoneal inoculation of MMF. The mean tumor rate was 2.5% (204 rats observed) and the tumors included sarcomas (3/5), mesotheliomas (1/5), and carcinomas (1/5). This level is in agreement with a previous paper in which the incidence of spontaneous mesotheliomas in rats was 0.9–1.7% in a series of several hundred animals (70). According to Port's findings, a tumor rate of 15% is not significant if $10^7$ fibers $>5 \mu m$ in length have been injected (37). Tumors other than mesothelioma were observed and the diagnosis after intraperitoneal injection of particles indicated that sarcomas were found most often while few carcinomas were found least often.

To summarize, the results obtained with insulation wools are difficult to interpret in terms of carcinogenic and fibrogenic risk, because of the great differences between fiber characteristics, even within a given group of fibers and between experiments. However, several studies have shown a significant increase in tumor incidence following intraperitoneal administration of GW, RW and GF. The absence of effect of SW and GF may be related to fiber dimensions; the few studies carried out with these fiber types do not allow us to make conclusions. So far, it seems that the length and thickness of fibers play a role in tumor formation with all routes of administration, with a greater carcinogenic potential for long and thin fibers. This is observed with different fibers but it is not possible to determine the comparative potential of the different fibers. No clear-cut conclusion can be made on the relevance of the concept of durability; further experiments are necessary to determine the effects of the various parameters, including, among others, biopersistence, oxidants production, surface area, solid rod or fibrillar structure in the pathological processes.

In inhalation studies, significant effects have been found with GW, RCF, and asbestos, but only RCF produced significant tumor yield, as did asbestos in some individual studies. One might ask if it is relevant to compare asbestos to other fibers. To compare the hazard of man-made versus natural fibers, it would be necessary to know if the number of airborne fibers produced under occupational conditions generate different levels of airborne fibers.

In vitro experiments are very useful to study the mechanisms of action of the fibers. The results obtained with in vitro systems have emphasized the fiber characteristics identified in vivo as playing a role in carcinogenic potency, indicating that these tests are useful to study the effects of particles and could be used on a larger scale. A number of cell systems are presently available (including macrophages, tracheal cells, mesothelial cells, epithelial cells, for example), and additional studies should be encouraged to study the response of in vitro assays to other fiber parameters suggested as possibly relevant to the toxic potency.

Some experiments may be suggested to fill in gaps in our knowledge on the effects of MMF on biological systems. Fibers of
well controlled and similar sizes but of different solubility could be inoculated in different species to determine the role of the fiber solubility in the biological response.

For problems arising from inhalation, only thin fibers should be investigated, i.e., of a diameter allowing their deposition in the lungs. In inhalation experiments, the dimension will depend on the animal species tested. Thus, continuous filaments do not seem to be of major concern because of their diameter. However, some of them could penetrate the upper airway; thus some studies might investigate interactions between epithelial cells (e.g., nasal cells) and fibers.

Long-term experiments with insulation wools of a given size but of different solubilities should be tested by inhalation in several species. Indeed, only one species has been generally tested, except with some RCF samples. A correlation should be made between pathogenicity in one hand and solubility and lung burden in the other. However, these experiments would need a very large number of animals to show statistical differences between results obtained with different fibers since only a small percent of animals with pathological changes can be expected.

Concerning refractory fibers, only ceramic fibers have been tested on a large scale. Refractory ceramic fibers may be more useful than insulation wools to study the correlation between pathogenicity and lung burden, solubility and other fiber parameters since the tumor yield is generally greater than that obtained with insulation wools; this would allow for the use of smaller groups of animals.

Finally, the correspondence between hazard to humans and risk evaluated in animals has to be studied because the enhancement of exposure dose, the fiber selection etc. that are necessary to detect a pathogenic effect in animals create specific conditions that have to be compared to the human situation. Moreover, the samples should be tested in cell systems in vitro to provide data allowing comparison between in vitro and in vivo results and the greater use of alternative tests.

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