Significance of Durability of Mineral Fibers for their Toxicity and Carcinogenic Potency in the Abdominal Cavity of Rats in Comparison with the Low Sensitivity of Inhalation Studies

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At the same time that carcinogenicity of very thin glass fibers after intrapleural and intraperitoneal (ip) administration was demonstrated (1,2), researchers found that gypsum fibers and HCl-leached chrysotile fibers were easily soluble in the peritoneal cavity. This led to the conclusion that the chemical composition of fibers was not responsible for the carcinogenesis but that the degree of carcinogenic potency of a fiber depended on the extent to which it retained its fibrous structure. A thin glass fiber with a low biodurability did not induce tumors after ip injection of a high dose, although the ip test had been criticized for being “overly sensitive.” The ip model has been the most successful for determining carcinogenicity of inorganic fibers and establishing dose-response relationships; but to determine the possibilities and limitations of this test model, very high doses of nonfibrous silicon carbide and of a slightly durable glass fiber type were injected ip in Wistar rats. No obviously acute or chronic toxic effect was observed in 90 weeks, but there was a 40% incidence of serosal tumors in the group treated with glass fibers. A pilot study on the persistence of slag fibers in the omentum of rats after ip injection showed a half-time of about 1 year. It was calculated that an ip injection of 10^6 fibers would lead to a concentration of fiber numbers in the ash of the omentum in the same range as the concentration in the lung after 2 years of inhalation exposure. The long-term inhalation study with fibers in rats has been called the “gold standard” for risk characterization. However, the tumor risk from inhalation of asbestos fibers in man has been estimated to be about 200 times higher per fiber than the risk in rats. The concentration of crocidolite fibers in the lungs of rats in an inhalation study that was negative was more than 1000-fold higher than the median concentration of amphibole fibers in the lungs of asbestos workers with mesotheliomas. This great difference clearly indicates that the sensitivity of the inhalation model is too low for the identification of the carcinogenic potential of mineral fibers. — Environ Health Perspect 102(Suppl. 5):145-150 (1994)

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Introduction — Early Results

When the carcinogenicity of very thin glass fibers after intrapleural and intraperitoneal (ip) administration was detected (1,2), three possible conclusions were discussed: that the fibrous shape of asbestos particles is the true cause of their carcinogenic effect in humans; that the chemical composition and surface properties are not decisive factors for the mechanism of fiber carcinogenesis; and that elongated nonasbestos particles in general may have a carcinogenic potential, as well as asbestos fibers, provided that the fibers are sufficiently long and thin and that their chemical composition enables the fibrous shape to be maintained for a sufficiently long period.

Early studies showed that gypsum fibers (2) and later, HCl-leached chrysotile fibers (3) dissolved in the intraperitoneal cavity a few days after injection and that the leached chrysotile was as acutely toxic as amorphous silicic acid. Those studies led to the conclusion that the chemical composition of a fiber, although not responsible for fiber carcinogenesis, did modify the carcinogenic potency from fiber type to fiber type, with variations that ranged between noncarcinogenicity and the carcinogenicity of crocidolite asbestos. When the fiber structure disappears by dissolution or disintegration, the carcinogenic properties also disappear, provided, of course, that the chemical components are themselves not carcinogenic. E-glass fibers, for example, had produced a high tumor incidence when injected in the peritoneal cavity. After treatment with 1.4 N HCl for 24 hr, however, a sample of the same fibers was not carcinogenic to the peritoneum. Leaching another sample of the same fibers with HCl for 2 hr reduced their carcinogenic potency, while NaOH treatment for 24 hr did not appear to alter their carcinogenicity (3,4). The reduction of carcinogenicity of acid-leached chrysotile in intrapleural experiments had been observed previously (5,6). However, the authors did not explain the effect by a reduced durability but rather by altered surface properties of the fibers and the loss of carcinogenic components by leaching. Neither hypothesis has yet been confirmed.

When a thin glass fiber with a low biodurability was produced and tested, no tumors were induced after intraperitoneal injection of a high dose (5.8 × 10^5 fibers in 100 mg) (7) even though this test model is reputed to be “overly sensitive.” In spite of the high dose, the adhesions of the abdominal organs were rather slight and indicated a short persistence of the fibers. The same fibers, after intratracheal instillation in rats, were found to have a half-time in the lung of 39 days (8). These results confirm the earlier hypothesis and should stimulate the man-made vitreous fiber
Vitreous fibers (P values in the omentum majorus

Figure 1. Dose-response relationship of several man-made and inorganic fiber types after intraperitoneal injection in rats. Symbols: A = actinolite asbestos, K = crocidolite asbestos, B = basalt (median L = 12 μm, D = 0.9 μm), D = diabase (median L = 16 μm, D = 1.0 μm), C = ceramic (2 types), (7) = chrysotile asbestos, (9) = silicon carbide, S = serpilite Uicalvario, W = wollastonite, 1-9 = glass microfibers (different sizes and chemical compositions). The regression line refers only to the measured points 1-6: 1 = M-104/E, 2 = M-100/475, 3 = 104/475, 4 = B-3K, 5 = B-3L, 6 = M-106, 7 = B-1 (median L = 7-18 μm, median D = 1-1.7 μm), 8 = B-2K, 9 = B-2L (7, 12, 13).

Figure 2. Fiber numbers in the omentum majorus of female Wistar rats at various times after intraperitoneal injection of 50 mg slag wool (7) or 150 mg basalt wool (4). For each point the number of fibers was calculated from a sample of the ashed omentum of one animal counted by phase contrast optical microscopy (PCOM). The fiber numbers of the original samples in 50 mg of slag wool (7) and in 150 mg of basalt fibers (4) were similarly determined. The half-time and its 95% confidence interval (C.I.) were estimated from an exponential regression curve fitted to the data of slag fibers.

Cumulative percentage

Figure 3. Length distributions of slag fibers recovered from the ashed omentum of Wistar rats injected intraperitoneally (pooled data, n = number of measured fibers).

(MMVF) industry to produce fibers that are not more biodurable than necessary for their specific applications.

Although it is necessary to be very cautious in applying the results from intraperitoneal carcinogenicity studies to risk assessment, this test model was the first to show clearly differences in carcinogenic potency between long and short, and between durable and nondurable fibers. Although some researchers underestimate the importance of these results, we think that the possibilities of the relatively inexpensive and easily practicable intraperitoneal model are not fully utilized for research, and that many results obtained with this model can be extrapolated qualitatively to the lung.

The ip model has been used successfully for determining the dose-response relationships of several fiber types (Figure 1): the results show clear differences between some fiber types that depend simply on the numbers of fibers longer than 5 μm and thinner than 2 μm. How much these differences are due to deviation in fiber measurement, differences of fiber size, durability, or dust masses is still to be determined. For example, why were the relatively thick vitreous cellular (aluminum silicate) fibers and rock fibers significantly more carcinogenic than the thinner glass microfibers; conversely, why are they not more carcinogenic than the shorter actinolite and crocidolite fibers? Replots to these questions would increase the significance of the results as relevant factors for risk assessment (9, 10).

Pilot Study on Fiber Biodurability in the Omentum Majorus of Rats

The half-times of fibers in the lung after intratracheal instillation (8, 11) have been compared with serosal tumor incidence after intraperitoneal injection (7, 12, 13). To obtain information on fiber content in the omentum majorus over nearly two years after ip injection, a pilot study was performed using slag fibers, which were expected to have a relatively short survival compared with rock fibers.

Materials and Methods

Sedimented dust was collected from the beams in a packing room of a slagwool factory in 1977. The fibers were shortened by slight milling in a ball mill. Twelve female Wistar rats (Ivanovas, Kiblegg), aged 17 weeks, were injected intraperitoneally with 50 mg slag fibers suspended in 2 ml of saline using a 2.0-mm hypodermic needle. The animals were killed at intervals up to 673 days after ip injection. The omentum majorus of each rat was removed, frozen, and subsequently subjected to freeze-drying and low-temperature ashing. An aliquot of each ash specimen was suspended in 2 to 6 ml demineralized water, the suspension was colored with a few μl of Methylen Blue and 10 or 20 μl were filtered through a Millipore filter, Type GS 0.22 μm. The colored area on the filter was cut out and made transparent by acetone vapor, before being embedded in Kaiser's glycerine gelatin. The total fiber area was scanned manually by phase contrast optical microscopy at a magnification of ×400 and particle lengths and diameters were measured using a microcomputer-based video image analyzing system. All particles with an aspect ratio of >3:1 were counted. From the particle sizes and counts of about 300 fibers per filter, the fiber numbers per total organ ash, and per μg dry weight were calculated. Similar measurements were made on the omentum majorus from five randomly selected rats from a positive carcinogenicity test with basalt wool, and compared with the original dust samples. The animals had received five weekly ip injections of 30 mg dust, which resulted in a tumor incidence of 71% (7).

A half-time for slag fibers in the omentum was estimated from an exponential regression curve fitted to the data for slag fibers.

Results and Discussion

Absolute fiber numbers per omentum were plotted against the time after injection (Figure 2). The relatively large variations of the fiber concentrations in the omentum may be due to inhomogeneous suspension of the injected fibers, to the difficulty of injecting very long and thick fibers, and to the different deposition patterns on the serosa of the organs and the peritoneum parietalis. Nevertheless, a trend of decreasing numbers of slag fibers during the observation time can be observed, and an estimated half-time for slag fibers was calculated to be 356 days, with a 95% confidence interval of 163 days—∞. There were insufficient data to estimate a half-time for basalt fibers, but their behavior seemed similar to that of the slag fibers. Figures 3 and 4 show the size distributions of the slag fibers in the omentum of the animals killed at 7 days, 1 year, and 2 years after injection. The geometric mean lengths of the slag fibers from each of 12 rats and of the basalt fibers from each of five rats are given in Figure 5. No important change in the
pared to the fiber concentrations in the lungs of rats from the Research and Consulting Company (RCC) inhalation study (14) on man-made vitreous fibers (Figure 6). It can be calculated that an ip injection of 10³ fibers would produce concentrations of 240 fibers/µg dry omentum, which is in the same range as the concentrations of vitreous fibers in the lung after 2 years of inhalation exposure. Provided that the dissolution rate of mineral fibers were similar in the lung and in the peritoneal cavity, the latter has a great advantage for studying biodurability or solubility in vivo, since bronchial clearance of intact fibers makes the determination of the dissolution rate complex. However, intraperitoneal studies with mineral fibers have shown that the migration of short fibers to the lymph nodes is substantial (15).

The present pilot study shows that it is, in principle, possible to explore the fiber durability in the abdominal cavity by analyzing the fibers retained in omentum majus.

**Carcinogenicity Study with High Doses of Nonfibrous Silicon Carbide and of Slightly Durable Glass Fibers**

The lowest ip injected fiber dose that resulted in a significant increase in serosal tumors in the abdominal cavity of rats was 0.004 × 10⁶ fibers >5 µm in length in 10 µg actinolite dust (11). When several groups of rats were treated with 250 mg of nonfibrous dusts or fibers >3 µm in diameter or <3 µm in length, no carcinogenic effect could be detected. To learn more about the possibilities and limitations of the intraperitoneal carcinogenicity test model, very high doses of nonfibrous silicon carbide and of low-durable glass fibers were injected.

**Materials and Methods**

Nonfibrous silicon carbide (type NF 2) was used (provided by Elektroschmelzwerk Kempten GmbH, Munich). Glass fiber dust was prepared by picking into pieces the bulk material of Bayer ATF-3101 and dry milling in a ball mill. Its chemical composition had been given as: SiO₂ = 60.7%, B₂O₃ = 3.2%, Na₂O = 15.4%, CaO = 16.5%, MgO = 3.2%, K₂O = 0.7%, FeO + Fe₂O₃ = 0.2%, Al₂O₃, BaO, ZnO = 0%. The characteristics of the particles given by scanning electron microscopy were: number of "critical fibers" (L/D > 5/1, L > 5 µm, D < 2 µm) 26 per ng; lengths: 10% < 3.6 µm, 50% < 7.4 µm, 90% < 16 µm; diameters: 10% < 0.41 µm, 50% < 0.72 µm, 90% < 1.23 µm.

**Results**

One year after the first ip injection of silicon carbide, the average body weight of the rats injected with 20 × 50 mg was about 5% lower in both sexes than in the control group injected 20 times with 2 ml saline. Six months later this difference was between 7 and 8% in both sexes. The mortality was less than 20% in 90 weeks in all silicon carbide groups. No serosal tumors were found in the abdominal cavity of 35 histopathologically examined rats. The body weight of the two groups injected with glass fibers was not lower than in the other groups. Within 87 weeks after the first injection, three rats were diagnosed with serosal tumors in the group treated with 20 × 25 mg glass fibers, and 20 serosal tumors were found in the group treated with 40 × 25 mg.

**Discussion**

Observations 90 weeks after the start of the experiment do not indicate any obviously acute or chronic toxic effect in male and female rats due to 1000 mg nonfibrous silicon carbide dust administered intraperitoneally. Neither did 500 mg glass fibers containing 13 × 10⁶ fibers influence the body weight or mortality, but the three mesothelias indicate a carcinogenicity that may become statistically significant. The 1000 mg dose is already associated with a tumor incidence of about 40%, and it will be very important for the final interpretation to see whether the relatively large
The risk to humans from asbestos has been derived by linear extrapolation from the predicted numbers of asbestos-induced deaths due to lung cancer and mesothelioma occurring before the age of 80 among men in chrysotile textile manufacture exposed to a level of 0.25 t/m³ at the age of 20 to 45 years (19). Data of the inhalation studies with asbestos in rats (20–22). Data of the inhalation studies of glass and ceramic fibers in the RCC Laboratories (Hesterberg unpublished data, 14). Above the gray zone, tumor incidences are significantly increased (“positive”) with groups of 60 rats and 3 spontaneous lung tumors (sp.) in the control group. Above the lower limit of the gray zone, tumor incidences are “positive” with groups of 120 rats and 3 spontaneous lung tumors (sp.) in the control group.

**The Inhalation Model is no “Gold Standard” for the Identification of the Carcinogenic Hazard of Fibers**

We argue here, as on previous occasions, why we consider the serosal test a better method for detecting the carcinogenic potential of inorganic fibers than the long-term inhalation study.

According to a recent World Health Organization (WHO) “consultation group” (17)—and at variance with other authors (18)—the human evidence, rather than animal inhalation experiments, had to be regarded as the ultimate “gold standard” against which animal studies have to be validated. In this case, we must compare the tumor risk in humans and rats after exposure to airborne asbestos. Relevant data are presented in Figure 7. The left hand curve shows the cancer risk of workers in chrysotile textile manufacture derived from the estimates of Doll and Peto (19). This dose-response curve might be called the pure “Gold Standard,” although there are some uncertainties there, too. The right-hand curve shows the tumor risk for rats derived from inhalation studies (20–22); the difference in the two dose-response relationships can be estimated to be 1 to 200. The difference increases if only malignant tumors are evaluated. Inhalation studies with chrysotile have confirmed the results of Davis (20–22), Wagner (23–25) and McConnell (26) which demonstrated that the inhalation model in rats is relatively insensitive for asbestos fibers compared with their carcinogenicity in man. Recent results of inhalation studies in the RCC laboratories with vitreous ceramic fibers and glass fibers (Hesterberg, unpublished data) show a relatively low concentration of about 200 ceramic fibers per mL induced a significant increase in lung tumor incidence (Figure 7). This concentration is low compared with the positive asbestos groups, and could be explained by the greater fiber length, if indeed the fibers reaching the lung are also very long. However, the long fibers are also usually thick and would therefore be deposited to a large degree in the nose of rats. The average fiber length in the exposure atmosphere was about 20 μm, but the geometric mean fiber length in the lung was reported to be 8 μm (Figure 8). The length distribution of crocidolite fibers in the lung in Wagner’s study (25) is probably similar to that of the ceramic fibers, because the figure of 2500 crocidolite fibers/μg lung dry weight referred only to fibers longer than 6 μm, and the concentration of fibers longer than 10 μm was about 350/μg/ml air. The higher deposition rate of crocidolite fibers compared to ceramic fibers can be explained by their smaller aerodynamic diameter.

From the available data, we can compare chrysotile, crocidolite, ceramic, and glass fibers in terms of the relationship between exposure, dose, and response (Figure 8). A relatively low exposure concentration of about 200 ceramic (RCF 1) fibers/μl giving a dose of about 600 fibers/μg lung dry weight, with a geometric mean fiber length of 8 μm, is associated with a tumor incidence of 16%. The crocidolite data come from studies of three inhalation experiments with UICC crocidolite. The results were almost all negative (23–25), in spite of the high concentration (2500/μg lung dry weight) of fibers longer than 6 μm that were identified in the lung ash after the experiment. This concentration is more than 1000-fold higher than the median concentration of amphibole fibers found in the lungs of asbestos workers with mesothelioma (Figure 9), which emphasizes the relatively low sensitivity of the inhalation model.

Based on the data reproduced in Figure 8, it would appear that the vitreous ceramic fibers (RCF 1) are not only more carcinogenic than the crocidolite fibers of similar lengths in the lung (25), but also are more carcinogenic per fiber than the asbestos fibers used by Davis [Figure 7, and (20–22,27)]. However, these conclusions
cannot be affirmed definitely because there are substantial uncertainties in the experimental data. Moreover, the data relating to test glass fibers (14) were insufficient to permit a firm conclusion that they were not carcinogenic. Compared with the exposure–dose–response relationship of asbestos, the dose of glass fibers was low, and a positive effect was thus unlikely. Nevertheless, the carcinogenicity potency per glass fiber could be some 30% or more of the carcinogenicity of the ceramic fibers tested. Note, though, that the inhalation test in rats is unsuitable for testing mineral fibers with a diameter >1 μm, which are quite capable of reaching the human bronchial tree.

In conclusion, preventive measures should be based on risk estimates derived from epidemiological studies of asbestos workers, introducing correction factors wherever necessary for each fiber type depending on deposition, translocation, biodurability, and other properties which together determine the cancer risk. This proposal has been outlined (9,10). Even if the difference between the dose-response relationships for exactly comparable asbestos fibers in humans and rats is not 1 to 200 as indicated in Figure 7 but, say, only 1 to 50, it is obviously unjustified to conclude that long-term inhalation studies with fibers provide the most appropriate data for risk characterization in humans. Ignoring very important data from epidemiologic studies on asbestos is neither good policy in research nor sound practice for the prevention of cancer from fibers.

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