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

Friedrich Pott,[1] Markus Roller,[2] Kenji Kamino,[2] and Bernd Bellmann[3]

[1]Medical Institute of Environmental Hygiene at the Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany; [2]Institute of Experimental Pathology, Hannover Medical School, Hannover, Germany; [3]Fraunhofer-Institute of Toxicology and Aerosol Research, Hannover, Germany

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 10^9 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 lung 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)

Key words: carcinogenicity, intraperitoneal, intratracheal, mineral fibers, biodurability, omentum majus

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.

This paper was presented at the Workshop on Biopersistence of Respirable Synthetic Fibers and Minerals held 7–9 September 1992 in Lyon, France.

Address all correspondence to Dr. Friedrich Pott, Medical Institute of Environmental Hygiene at the Heinrich-Heine-Universität Düsseldorf, Auf'm Hennekamp 50, D-4000 Düsseldorf, Germany.

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 carcinogens. E-glass fibers, for example, had produced a high tumor incidence when injected in ther 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 (5.8×10^9 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

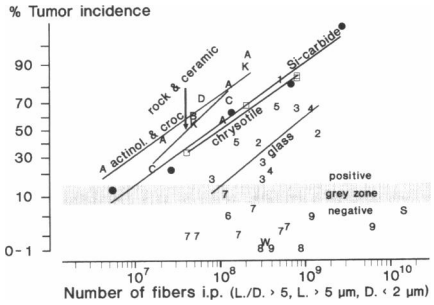

Figure 1. Dose-response relationship of several man-made and inorganic fiber types after intraperitoneal injection in rats. Symbols: A = actinolite asbestos, K = crocidolite asbestos, B = basalt (median L = 12 µm), D = 0.9 µm), D = diabase (median L = 16 µm, D = 1.0 µm), C = ceramic (2 types), (□) = chrysotile asbestos, (●) = silicon carbide, S = sepiolite Uicaluraro, W = wollastonite, 1–9 = glass microfibers (different sizes and chemical compositions, the regression line refers only to the measured points 1 to 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*).

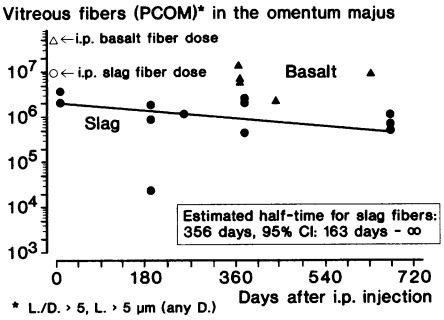

Figure 2. Fiber numbers in the omentum majus of female Wistar rats at various times after intraperitoneal injection of 50 mg slagwool (●) or 150 mg basalt wool (▲). For each point the number of fibers was calculated from a sample of the ashed omentum of one animal counted by phase contrast optical microscopy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

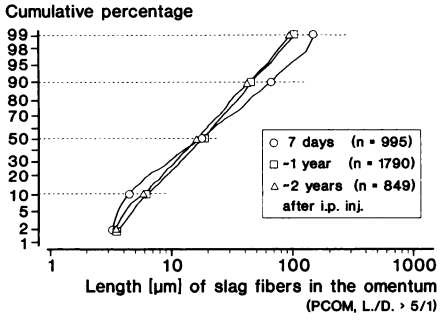

Figure 3. Length distributions of slag fibers recovered from the ashed omentum of Wistar rats injected intraperitoneally (pooled data; n = number of measured fibers).

(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 ceramic (aluminium 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? Replies 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 Majus 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 majus 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, Kißlegg), 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 majus 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 Methylene 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 filter 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 majus 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 each 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 omenta 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

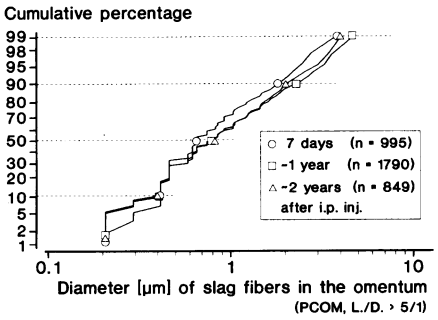

Figure 4. Diameter distributions of slag fibers recovered from the ashed omentum of Wistar rats injected intraperitoneally (pooled data; n = number of measured fibers).

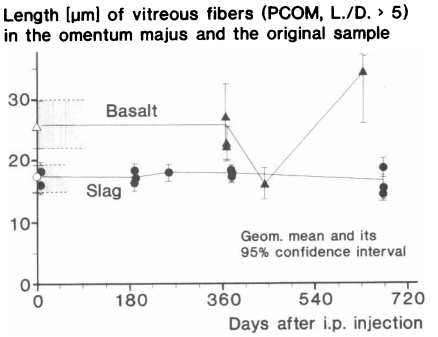

Figure 5. Mean lengths of slag and basalt fibers recovered from the ashed omenta of Wistar rats injected intraperitoneally and from the original samples. Each closed data point represents the results of measurements by phase contrast optical microscopy (PCOM) from one animal. The shaded areas indicate the 95% confidence intervals for the original samples.

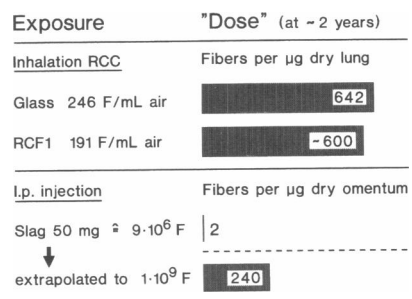

Figure 6. "Exposure–dose relationships." Comparison of vitreous fiber concentrations in the dry mass of the rat lung after inhalation and in the dry mass of the rat omentum after ip injection. The data for 50 mg slagwool correspond to the fiber numbers shown in Figure 1, the concentration for a theoretical number of 1–10^9 injected fibers has been linearly extrapolated from these data.

fiber sizes was detected, but the phase contrast of the fibers decreased in the first months of the experiment. The results do not support the earlier hypothesis that slag fibers could not be durable enough for the induction of tumors.

The concentration of 2 slag fibers/µg dry omentum found in this study was compared 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 10^9 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 0.004×10^9 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: SiO_2 = 60.7%, B_2O_3 = 3.2%, Na_2O = 15.4%, CaO = 16.5%, MgO = 3.2%, K_2O = 0.7%, FeO + Fe_2O_3 = 0.2%, Al_2O_3, 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.

Wistar rats (WU/Kißlegg-Iva: WIWU, 8–10 weeks) were repeatedly injected intraperitoneally under CO_2 anesthesia with dust suspensions in 2 ml buffered 0.9% sodium chloride solution. Silicon carbide was injected in two doses (5 × 50 mg and 20 × 50 mg) at intervals of two weeks into 48 female and 72 male rats. Glass fibers were administered in two doses (20 × 25 mg at intervals of two weeks and 40 × 25 mg weekly) to 54 male rats per group. Animals were killed when in bad health. Some died spontaneously, some were lost after anesthesia. After macroscopical post mortem examination of the abdominal cavity, parts of tumors or organs with macroscopically suspected tumor tissue were fixed in formalin and prepared for histological examination on paraffin-embedded H & E stained sections. The trunk was fixed in formalin and stored for possible further examination.

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 13×10^9 fibers influence the body weight or mortality, but the three mesotheliomas 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

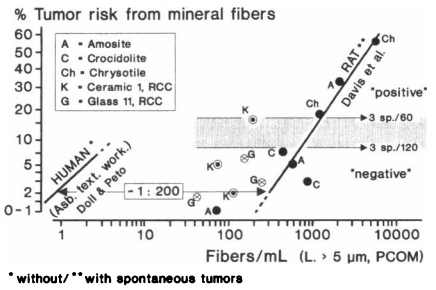

Figure 7. Tumor risk from mineral fibers of men and rats. The risk to humans from asbestos has been derived by linear extrapolation from the predicted numbers of asbestos-induced deaths due to lung cancer and mesothelioma occurring before the age of 80 among men in chrysotile textile manufacture exposed to a level of 0.25 f/ml 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.

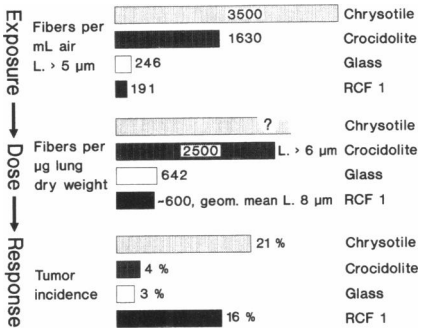

Figure 8. Exposure, dose (organ concentration) and tumor incidence from long-term inhalation studies in rats with chrysotile (Hesterberg, unpublished data) crocidolite (*23,25*) and ceramic fibers RCF 1 (*14*).

difference between the tumor response of the two dose groups will continue to increase or will decrease in the last months of the experiment.

No findings to date plausibly explain the hypothesis that the carcinogenicity of fibers after intraperitoneal injection of doses up to about 10^9 fibers in 250 mg dust is an artifact and not relevant for humans because of the unphysiologic exposure, bolus effect or so-called overload carcinogenesis. It is more likely that the ip model identifies the carcinogenic hazard of fibers for humans much more reliably than long-term inhalation studies in rats. Such studies are very insensitive compared with the effects seen in humans. In considering all the data of the ip model there are no signs of "overcultivated sensitivity." On the contrary, an enlarged sensitivity is desirable, e.g. for testing of fibers about 2 µm in diameter. Carcinogenicity of such fibers can only be detected if the carcinogenic potency per fiber is not lower than that of thinner fibers and if the dust sample does not contain a substantial proportion of other particles.

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 that 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/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/ml 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

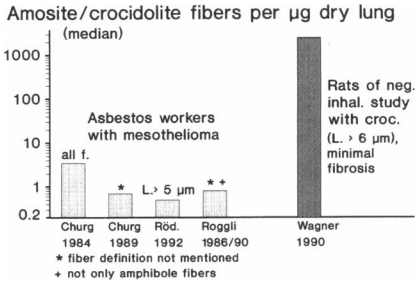

Figure 9. Concentration of amphibole fibers in the lung of asbestos workers with mesothelioma and in the lung of rats of a "negative" inhalation study that resulted in 1 mesothelioma of rats (*25,28–32*).

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 carcinogenic 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.

REFERENCES

1. Stanton MF, Wrench C. Mechanisms of mesothelioma induction with asbestos and fibrous glass. J Natl Cancer Inst 48:797–821 (1972).
2. Pott F, Friedrichs KH. Tumoren der Ratte nach ip Injektion faserförmiger Stäube. Naturwissenschaften 59:318 (1972).
3. Pott F, Matscheck A, Ziem U, Muhle H, Huth F. Animal experiments with chemically treated fibres. Inhaled particles VI. Ann Occup Hyg 3(Suppl 1):353–359 (1988).
4. Pott F, Schlipköter HW, Ziem U, Spurny K, Huth F. New results from implantation experiments with animal fibres. In: Biological Effects of Man-made Mineral Fibres. Proceedings of a WHO/IARC Conference, Vol 2. Copenhagen:World Health Organization Regional Office for Europe, 1984;286–384.
5. Morgan A, Davies P, Wagner JC, Berry G, Holmes A. The biological effects of magnesium-leached chrysotile asbestos. Br J Exp Pathol 58:465–473 (1977).
6. Monchaux G, Bignon J, Jaurand MC, Lafuma J, Sebastien P, Masse R, Hirsch A, Goni J. Mesotheliomas in rats following inoculation with acid-leached chrysotile asbestos and other mineral fibres. Carcinogenesis 2:229–236 (1981).
7. Pott F, Roller M, Rippe RM, German P-G, Bellmann B. Tumours by the intraperitoneal and intrapleural routes and their significance for the classification of mineral fibres. In: Mechanisms in Fibers Carcinogenesis. Proceedings of a NATO advanced research workshop on mechanisms in fibre carcinogenesis held October 22–25, 1990, in Albuquerque, New Mexico. (Brown RC, Hoskins JA, Johnson NF, eds), NATO ASI Series. Series A, Life Sciences, Vol 223, New York:Plenum Press, 1991;547–505.
8. Muhle H, Bellmann B, Pott F. Durability of various mineral fibres in rat lungs. In: Mechanisms in Fibers Carcinogenesis. Proceedings of a NATO advanced research workshop on mechanisms in fibre carcinogenesis held 22–25 October 1990, in Albuquerque, New Mexico. New York:Plenum Press, 1991;181–187.
9. Pott F. The fibrous particle–a carcinogenic agent. Ramazzini Newsletter 1:28–52 (1992).
10. Pott F. Testing the carcinogenicity of fibers in laboratory animals: results and conclusions. In: Contemporary Issues in Fiber Toxicology (Warheit D, ed). New York: Academic Press, 1993;395–424.
11. Bellmann B, Muhle H, Pott F, König H, Klöppel H, Spurny K. Persistence of man-made mineral fibres (MMMF) and asbestos in rat lungs. Ann Occup Hyg 31:693–709 (1987).
12. Pott F, Ziem U, Reiffer F-J, Huth F, Ernst E, Mohr U. Carcinogenicity studies in fibres, metal compounds and some other dusts in rats. Exp Pathol (Jena) 32:129–152 (1987).
13. Pott F, Roller M, Ziem U, Reiffer F-J, Bellmann B, Rosenbruch R, Huth F. Carcinogenicity studies on natural and man-made fibres with the intraperitoneal test in rats. In: Non-Occupational Exposure to Mineral Fibres. (Bignon J, Peto J, Saracci R, eds). IARC Scientific Publications No. 90, Lyon:International Agency for Research on Cancer, Lyon, 1989;173–179.
14. TIMA Letter of May 4, 1992 to Document Control Officer, Office of Toxic Substances, Environmental Protection Agency. Re: Respirable Fibrous Glass Chronic Multidose Inhalation Study—Preliminary Final Results. Thermal Insulation Manufacturers Association, Stamford, CT, 1992.
15. Beck EG, Bruch J, Friedrichs K-H, Hilscher W, Pott F. Fibrous silicates in animal experiments and cell-culture—morphological cell and tissue reactions according to different physical chemical influences. In: Inhaled Particles III, Vol 1 (Walton WH, ed). Old Woking, Surrey/England:Unwin, 1971;477–487.
16. Goodglick LA, Kane AB. Cytotoxicity of long and short crocidolite asbestos fibers *in vitro* and *in vivo*. Cancer Res 50: 5153–5163 (1990).
17. WHO Consultation on Man-made Fibres: Validity of methods for the Assessment of Carcinogenicity of Fibres 0242g, limited distribution. Executive Summary of a WHO consultation held 19–20 May 1992 in Copenhagen. Copenhagen:World Health Organization, August 1992.
18. McClellan RO, Miller FJ, Hesterberg TW, Warheit DB, Bunn WB, Dement JM, Kane AB, Lippmann M, Mast RW, McConnell EE, Reinhardt CF. Approaches to evaluating the toxicity and carcinogenicity of man-made fibers. Reg Toxicol Pharmacol 16:321–364 (1992).
19. Doll R, Peto J. Effects on Health of Exposure to Asbestos. Health and Safety Commission. Her Majesty's Stationery Office, London, 1985.
20. Davis JMG, Beckett ST, Bolton RE, Collings P, Middleton AP. Mass and number of fibres in the pathogenesis of asbestos-related lung disease in rats. Br J Cancer 37:673–687 (1978).
21. Davis JMG, Addison J, Bolton RE, Donaldson K, Jones AD. Inhalation and injection studies in rats using dust samples from chrysotile asbestos prepared by awet dispersion process. Br J Exp Pathol 67:113–129 (1986).
22. Davis JMG, Jones AD. Comparisons in the pathogenicity of long and short fibres of chrysotile asbestos in rats. Br J Exp Pathol 69:717–737 (1988).
23. Wagner JC, Skidmore JW, Hill RJ, Griffiths DM. Erionite exposure and mesotheliomas in rats. Br J Cancer 51:727–730 (1985).
24. Wagner JC, Griffiths DM, Munday DE. Experimental studies with palygorskite dusts. Br J Ind Med 44:749–753 (1987).
25. Wagner JC. Biological effects on short fibers. DHHS NIOSH Publ. No. 90–108, Part 2. In: Proceedings of the VIIth International Pneumoconiosis Conference, 23–26 August, 1988 Pittsburgh, PA. Washington, DC:U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, 1990;835–839.
26. McConnell EE, Wagner JC, Skidmore JW, Moore JA. A comparative study of the fibrogenic and carcinogenic effects of UICC Canadian Chrysotile B asbestos and glass microfibre (M 100). In: Biological Effects on Man-made Mineral Fibres, Vol 2.

Proceedings of a WHO/IARC Conference held 20–22 April 1982 in association with Joint European Medical Research Board and Thermal Insultation Manufacturers Association, Copenhagen: World Health Organization, 1984;234–252.
27. Vu V. Refractory ceramic fibers: EPA's risk assessment and regulatory status. In: Proceedings of the Toxicology Forum 1992. Annual Winter Meeting. Washington, DC, 1992;400–416.
28. Churg A, Wiggs B. Fiber size and number in amphibole asbestos-induced mesothelioma. Am J Pathol 115:437–442 (1984).
29. Churg A, Wright JL. Fibre content of lung in amphibole- and chrysotile-induced mesothelioma: implications for environmental exposure. In: Non-Occupational Exposure to Mineral Fibres. (Bignon J, Peto J, Saracci R, eds). IARC Scientific Publications No. 90, Lyon:International Agency for Research on Cancer, 1989;314–318.
30. Rödelsperger K, Gerhard J, Brückel B, Arhelger R, Woitowitz H-J. Fall-Kontroll-Studie zum Gehalt anorganischer Fasern im Lungenstaub von Mesotheliom-Patienten. In: Arbeitsmedizin für eine gesunde Umwelt. Arbeitsmedizin in der Land-und Fortswirtschaft. Bericht über die 31. Jahrestagung der Deutschen Gesellschaft für Arbeitsmedizin. (Shäcke G, Ruppe K, Vogel-Sührig C, eds). Verhandlungen der Deutschen Gesellschaft für Arbeitsmedizin. Stuttgart: Gentner, 1992;233–237.
31. Roggli VL, Pratt PC, Brody AR. Asbestos content of lung tissue in asbestos-associated diseases: a study of 110 cases. Br Ind Med 43:18–28 (1986)
32. Roggli VL. Human disease consequences of fiber exposure: a review of human lung pathology and fiber burden data. Environ Health Perspect 88:303 (1990).