Dose–Response Relationship of Fibrous Dusts in Intraperitoneal Studies

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The relationship between the number of fibers injected intraperitoneally and the occurrence of peritoneal mesotheliomas in rats was investigated using data from a series of carcinogenicity studies with several fibrous dusts. Based on observed tumor incidences ranging between 10 and 90%, the hypothesis of a common slope of dose–response relationships (parallel probit lines in probit analysis) cannot be rejected. In general, parallelism of probit lines is considered an indication of a common mode of action. Analysis of the shape of the dose–response relationship, with one apparent exception, shows virtually linear or superlinear behavior, i.e., from these data, there is no indication of a decrease in carcinogenic potency of an elementary carcinogenic unit at lower doses. — *Environ Health Perspect* 105(Suppl 5):1253–1256 (1997)

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Introduction

In addition to the question of possible carcinogenic potential, carcinogenicity testing can provide important information regarding dose–response relationships. Generally, based on consideration of probability calculation, three different curves can be distinguished: linear, sublinear, and superlinear. Linear type is approximately linear only in the low approximating 100% response range and is slightly bent to the right. The sublinear curve is sigmoidal over the whole range; the superlinear curve is more bent to the right than the linear type. A mathematical function that can be used to characterize the shape of the dose–response relationship is the Weibull cumulative distribution function (*1*): a value of unity for its shape parameter corresponds to a linear relation, which means that the potency of a single dose unit, i.e., the ratio of the carcinogenic response to the dose, is equal at all doses. A value for this parameter of greater than unity corresponds to a sublinear relation or a decreasing carcinogenic potency from higher to lower doses. Another model often considered is the probit (lognormal) model. Though the biological basis for its application to carcinogenicity data is doubtful, this type of analysis, commonly used in pharmacology and conventional toxicology, is often applied to the presentation and formal description of dose–tumor frequency data. This analysis can be particularly useful for estimation of relative potencies in the experimental range, especially when regression lines are parallel. In this paper, a probit analysis of the tumor frequencies of a recently completed series of intraperitoneal injection studies with several mineral fiber dusts is presented; additionally, dose–tumor frequency data were analyzed with regard to shape characteristics, using the Weibull model (*1*).

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Abbreviations used: df, degrees of freedom; MMVF, man-made vitreous fiber(s).

Materials and Methods

The data used for the analysis were taken from a series of three experiments in which a total of about 2000 male and female Wistar rats were injected ip either with suspensions of fibrous or nonfibrous dust or with saline only. After lifetime observation up to 30 months, they were examined for tumors in the abdominal cavity. One granular dust (silicon carbide), two asbestos dusts (crocidolite and tremolite) and eleven dust samples of man-made vitreous fiber(s) (MMVF) were administered. Five of the vitreous fiber types were fine fiber fractions from four commercial insulation wools and one experimental wool; the others were prepared by cutting and milling of wool from glass microfibers. Fiber types included some samples with similar size distribution and different chemical composition and some samples with different size distribution and the same chemical composition (Table 1). The total dust masses applied per animal ranged from 0.5 mg (crocidolite) to 1000 mg (glass fibers B-01 and granular silicon carbide). When multiple injections were administered per animal, the interval between injections was 1 or 2 weeks. Table 1 gives an overview of the dusts and doses used. According to the study protocol, tissue samples of all animals were examined histologically and animals were counted as positive if at least one tumor was found in the abdominal cavity and evaluated as mesothelioma. Further details of the experimental design and preliminary results have been previously described (*2*). A more detailed description of materials and methods applied in the carcinogenicity study and the final results with histological diagnoses is in preparation.

Dose–response relationships were investigated for the analysis presented here. Dose was defined as the number of fibers with length > 5 µm, diameter < 2 µm, and length/diameter > 5/1. This definition of dose applies to both fiber types (long and thick and short and thin) in Table 1, but comprises different proportions of particles contained in the dust samples. Mass dose refers to the total amount of dust injected. Response was defined as proportion of rats with peritoneal mesothelioma(s) among the number of rats examined. Because no systematic differences between sexes were found, the numbers of male and female rats were combined. The probit model was fitted to the data under the constraint of a common slope. Goodness of fit was tested using the χ^2 statistic. Generally, lack of fit of such a constrained model can be due to lack of homogeneity or lack of parallelism.

Table 1. Fibrous dusts and mass doses applied in a series of carcinogenicity studies from 1990 to 1992 (number of injections × dust mass/injection, mg).

Fibrous dusts containing relatively long and thick fibers[a]	Fibrous dusts containing relatively short and thin fibers[b]
Glass fibers[c] B-01-0.9 (5×25, 10×25, 20×25, 40×25)	
B-09-2.0 (3×50, 9×50)	B-09-0.6 (2×50, 6×50)
B-20-2.0 (1×6, 1×18, 2×30)	B-09-0.6 (1×3.5, 1×8.5, 1×25, 3×25)
Glass[d] MMVF11 (2×35, 6×30)	Glass fibers[d] M-753-104 (1×17, 1×50)
Stone[d] MMVF21 (2×30, 5×30)	
Slag[e] MMVF22 (1×20, 1×50, 3×50)	Asbestos
M-Stone (1×8.5, 1×25.5, 2×42.5)	Crocidolite (5×0.1)
R-Stone-Experimental 3 (4×28.5, 9×28.5)	Tremolite (1×3.3, 1×15)

[a]Particles with aspect ratio > 5/1, median length 8–17 μm, median diameter 0.7–1.2 μm. [b]Particles with aspect ratio > 5/1, median length 2–4 μm, median diameter 0.2–0.5 μm. [c]All B-prefix fibers are glass fibers. The first number (B-01, B-09, B-20) represents a code for the chemical composition. The composition of B-20 is similar to stone fibers MMVF21. The second number indicates the nominal median diameter of the original sample. [d]The designations MMVF11 and -21 originate from the Thermal Insulation Manufacturers Association (TIMA, Stamford, CT). TIMA made the samples available for scientific purposes. The dusts were prepared from the thinnest fraction of typical commercial insulation wools and were also used in inhalation experiments in the laboratories of Research and Consulting Company (RCC), Geneva, Switzerland. Glass fibers M-753-104 are produced as microfibers, i.e., with a relatively small diameter and a chemical composition similar to MMVF11. [e]The chemical composition of this dust sample, bought in 1990 from Manville Technical Center (Denver, CO), is practically identical to the slag-wool designated MMVF22 in inhalation experiments carried out in the RCC laboratories, Switzerland.

Because χ^2 was significant for the constrained model when all data sets were included in our analysis, an unconstrained fit was done and an F-test performed for differentiation between departure from homogeneity and departure from parallelism (*3,4*). Additionally, a similar analysis was performed using the Weibull model (*1*) instead of the lognormal model. The functional form of the Weibull model is $P = 1 - \exp(-a_i x^b)$, where x is dose and P is response. Using the maximum likelihood method, separate values for the parameter a_i, which depends on the carcinogenic potency of a dust sample, were estimated for the individual dusts ($i = 1, 2, \ldots$, number of dusts analyzed), but b, which characterizes the shape of the curve, was constrained to be equal for all dusts. This method is equivalent to estimation of parallel lines in a complementary log–log plot where b represents the slope of the lines.

To illustrate the behavior of the dose–response relationship with regard to the assumption of a common shape, the doses of each dust i were normalized by multiplication with $a_i^{1/b}$ so that the dusts are treated as equally potent, but variation of the data points around the curve shape remains. The normalization factor can serve as a measure of relative carcinogenic potency. The procedure is equivalent to movement of the regression lines of transformed data along the x axis to a common position.

Results and Discussion

In 433 rats treated with saline or granular silicon carbide (250 or 1000 mg), two rats with mesotheliomas were found—one among 69 male rats receiving 20×2 ml saline and one among 47 female rats receiving 250 mg silicon carbide. No mesotheliomas were found among 45 female and 70 male rats treated with 1000 mg silicon carbide. With most of the fibrous dusts, including two types of asbestos and different types of MMVF, statistically significantly increased and dose-dependent frequencies of peritoneal mesotheliomas were produced.

For the probit analysis, those experimental groups that did not provide information for a test of parallelism, e.g., crocidolite, were not considered. Crocidolite was tested in two of the experimental series, albeit as a positive control with the same dose, thus providing no relevant information about the curve shape. The same is true for MMVF21 data and R-Stone-Experimental 3. The latter fiber type produced no tumor with the lower dose and only one mesothelioma among 35 rats with the higher dose, which is not significantly different from the background. Therefore, these data allow virtually any curve shape, from no dose–response relationship at all to a parallel probit line. A tumor frequency of 97% was obtained even with the lower dose of MMVF21. Tumor frequencies near 100% are rarely observed in animal experiments because at any relatively early time point an animal may die from causes other than cancer, which makes a 100% response impossible. With the higher dose of MMVF21, a few rats died relatively soon without tumor development, perhaps due to fibrosis from the relatively high fiber dose. Therefore, the lower tumor frequency (87%) is likely due to nontumor related mortality. Similarly, the 97% tumor frequency with the lower dose of MMVF21 must be considered a maximum response; these data also allow any curve shape or slope of probit line. Furthermore, these data provide only limited information about the carcinogenic potency because the same maximum response may, theoretically, have also occurred at much lower doses. Because of these limitations, data from crocidolite, R-Stone-Experimental 3, and MMVF21 were not included in the analysis, although parallel probit lines may be appropriately drawn through these data. Inclusion of these data would not change the result of a parallelism test.

We fitted the probit model to all other groups, including glass fiber B-01-0.9, under the constraint of a common slope, which gave a χ^2 of 39.4, thus indicating a significant departure of the data from the model (degrees of freedom [df] = 17, $p = 0.002$). However, this does not mean that the hypothesis of parallelism must be rejected—the F-test differentiating variances due to departure from parallelism and homogeneity (after independent fit of the unconstrained model to each data set) gave an insignificant value of 2.29 (df1 = 9, df2 = 8, $p = 0.13$). It was obvious, and can be seen in Figure 1, that the lack of fit is explained to a great extent by an apparently different behavior of the glass fiber B-01-0.9. Such different behavior may be considered plausible since this dust, which has a relatively low durability, was tested with the highest dose of 1000 mg. Therefore, the analysis was repeated excluding the B-01-0.9 data. No significant deviation of the data from the model with a common slope was found ($\chi^2 = 14.4$, df = 13, $p = 0.35$).

This was also true ($\chi^2 = 17.2$, $p = 0.19$) when the Weibull model was fitted to the same data under the constraint of a common shape parameter, i.e., slope of the complementary log–log lines. An estimate of 0.73 was obtained for shape parameter b. For a better visual comparison of the single dust groups with the common shape,

data sets of the dusts (except B-01-0.9) were normalized using $b = 0.73$ and the method described above. Figure 2 shows the resulting slightly superlinear curve and also a curve fitted separately to the B-01-0.9 data, which resulted in a sublinear shape ($b = 2.4$).

These dose–response relationships are in good agreement with the results of earlier ip studies, both in terms of parallelism and curve shape. Although results from experiments conducted at various times were used, we found that the probit lines fitted independently to data sets of several dusts did not show markedly different slopes (*5*). As mentioned above, the slope of a dose–response line in a transformed plot (probit, logit, complementary log–log plot, etc.) is related to the shape of the corresponding dose–response function graph plotted on linear axes. Therefore, it is not surprising that in earlier studies with similar probit lines, similar shapes of the Weibull function were found (*6*). In principle, these shapes tended to be more strongly superlinear than those found in the recent study (e.g., chrysotile and silicon carbide whiskers $b = 0.5$, actinolite and crocidolite $b = 0.6$).

However, careful comparison of the historic ip data with recent data is necessary because slightly different modes of evaluation of positive animals were used. In the earlier studies, under macroscopic examination, only tissues thought to contain tumorous lesions were examined histologically. Microscopic examinations were performed by different pathologists; tumors that were diagnosed as sarcomas (not organ specific, no lymphosarcomas or hemangiosarcomas) and mesotheliomas were considered exposure related. In the early studies a few carcinomas were also counted. In the recent study, tissue samples from macroscopically inconspicious rats were examined microscopically. Specimens were taken by the pathologist (K. Kamino) and mesotheliomas were counted. A sarcoma diagnosis was rare in this series. Furthermore, a pathology working group [Davis et al. (*7*)] reviewed a sample of 840 slides from 223 rats from this study in 1996. Approximately 20 rats were selected at random from each experimental group. The charge of the committee was to review critically the morphologic diagnoses of peritoneal tumors. The committee reached a final agreement in 99% of the cases. The panel concluded that the morphological pattern of the tumors was consistent with what has appeared in the literature as malignant mesothelioma; the final diagnoses

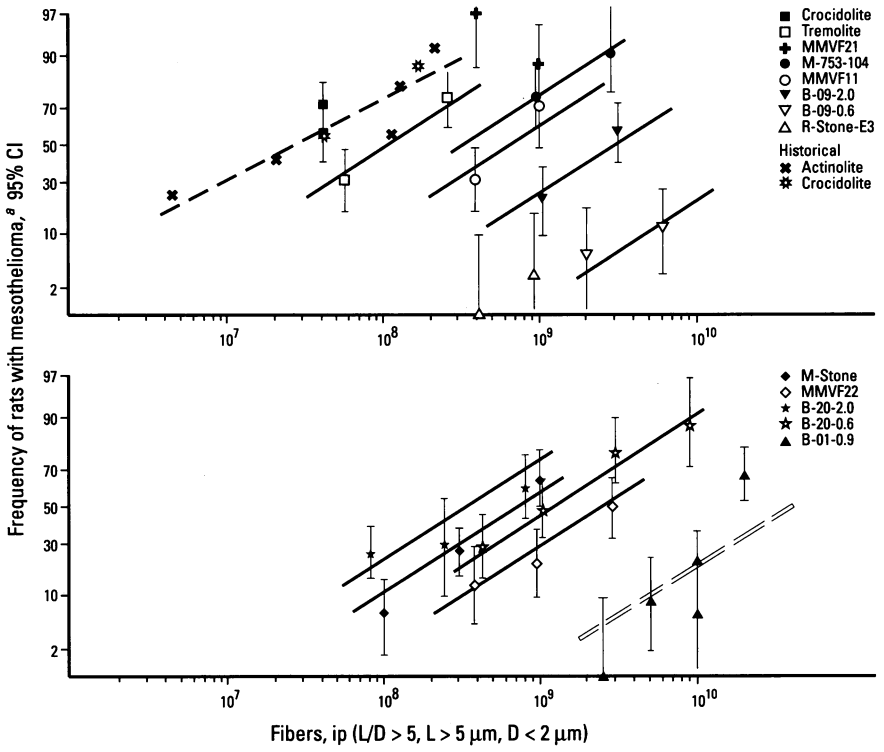

Figure 1. Probit analysis of the relationships between injected number of fibers and frequency of rats with peritoneal mesothelioma in ip injection studies. Data from three series of experiments (1990–1992) were combined for analysis and presented in two separate panels for clarity. For comparison, results from asbestos dusts depicted previously (*6*) have been added. The corresponding broken line has been independently fitted to the combined historical data of actinolite and crocidolite. [a]Historical data: mesothelioma/sarcoma. Abbreviations: L, length; D, diameter.

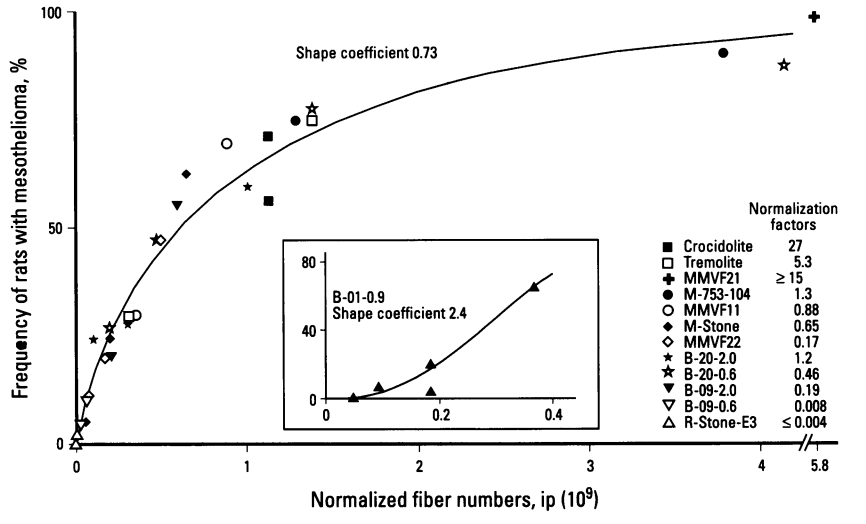

Figure 2. Dose–response relationships after ip injection of various fibrous dusts, plotted on linear scales. The curve of the main plot represents a Weibull model with a common shape parameter for most of the tested dusts. The insert shows a sublinear curve for B-01-0.9 fibers. The dose values of B-01-0.9 have been normalized using the same *b*-value as for the other dusts so that the scale of the *x* axis is comparable to the main plot. The curve shown has been fitted to the normalized data. Differences in the carcinogenic potencies of the dusts that are lost during the procedure of normalization are extracted within the normalization factors, which can serve as a measure of the relative carcinogenic potencies of the dusts.

of the panel agreed with the published results (*2*) in more than 98% of the cases.

It is unclear if the differences in evaluation may be related to some slightly different curve shapes. It seems more important that with all study designs, sublinear dose–response relationships were found only in exceptional cases. In the historical studies, 19 analyzed datasets revealed that only 3 were sublinear and only 1 had a *b*-value > 2; this was a dataset with only two dose levels and low tumor frequency, and therefore large statistical uncertainty. Among the six datasets with three or more dose levels, no sublinear curve was found. Thus, the data do not provide evidence of decreasing carcinogenic potency of a dust unit at lower doses or evidence of a threshold dose. In the present study, the injected dust mass was relatively high in some groups, e.g., 150 to 450 mg for B-09-2.0. However, with an even higher dose of 1000 mg nonfibrous silicon carbide, no mesothelioma was observed among 115 rats. In other groups, the doses were substantially lower, e.g., 3.3 to 15 mg for tremolite. The specific fiber content (number of fibers per mass unit of dust) was also different for the individual dusts. If the induction of the mesotheliomas was mainly linked to some mass load of the peritoneum, we would have expected different slopes dependent on the mass required for induction of comparable tumor frequencies. However, similar curve shapes were obtained for dose–response relationships when dose was defined as the number of fibers with certain dimensions. In the earlier studies, asbestos dusts that were used included a mass as low as 0.01 mg actinolite. Figure 1 shows that the dose–response relationship obtained is not substantially different from more recent ones. Together with the finding that no mesothelioma was observed with a high mass dose of 1000 mg nonfibrous dust, this analysis is in agreement with the assumption that the mechanism responsible for the mesotheliomas in this experimental system is specific for the fibrous shape of the particles administered.

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