A MATHEMATICAL MODEL OF FIBER CARCINOGENICITY AND FIBROSIS IN INHALATION AND INTRAPERITONEAL EXPERIMENTS IN RATS

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ABSTRACT

A hypothesis is presented that predicts the incidence of tumors and fibrosis in rats exposed to various types of rapidly dissolving fibers in an inhalation study or in an intraperitoneal (IP) injection experiment, for which the response to durable fibers has been determined. The model takes into account the fiber diameter and the dissolution rate of fibers longer than 20 μm in the lung, and it predicts the measured tumor and fibrosis incidence to within approximately the precision of the measurements.

The basic concept of the model is that a rapidly dissolving long fiber has the same response in an animal bioassay as a much smaller dose of a durable fiber. Long, durable fibers are considered to have special significance since no effective mechanism is known by which these fibers may be removed. In particular, the hypothesis is that the effective dose of a dissolving long fiber scales as the residence time of that fiber in the extracellular fluid. For example, a certain dose of a fiber that dissolves in one year acts like half that dose of a fiber that requires two years to dissolve. The residence time of a fiber is estimated directly from the average fiber diameter, its density, and the fiber dissolution rate as measured in simulated lung fluid at neutral pH.

The incidence of fibrosis in a recent series of chronic inhalation tests at the Research and Consulting Company (RCC) in Geneva, Switzerlend, is predicted well by the mathematical model. The observed lung tumor rates in these studies are consistent with this model. The model also predicts the incidence of mesothelioma in the IP model of Pott and colleagues.

The model allows one to predict, for an inhalation or IP experiment, what residence time and dissolution rate is required for an acceptably small tumorogenic or fibrotic response to a given fiber dose. For an inhalation test in rats at the maximum tolerated dose (MTD), such as the ones completed at RCC, the model suggests that less than 10% incidence of fibrosis would be obtained at the maximum tolerated dose of 1 μm diameter fibers if the dissolution rate were greater than 80 ng/cm^2/hr. The dissolution rate that would give no detectable lung tumors in such an inhalation test in rats is much smaller. Thus a fiber with a dissolution rate of 100 ng/cm^2/hr has an insignificant chance of producing either fibrosis or tumors by inhalation in rats even at the maximum tolerated dose used in the RCC study.

INTRODUCTION

There has been intense interest recently in determining what properties of certain fibers are responsible for producing respiratory disease in humans and in laboratory animals. The disease potential has long been linked to the dose, dimension, and durability of the fibers (Pott and Friedrichs, 1972; Stanton and Wrench, 1972). The various types of asbestos fibers, which are associated with mesothelioma, lung cancer, and fibrosis when inhaled, differ in each of these properties from insulation wool glass fibers, for example, which are not associated with these diseases when inhaled. Certainly the airborne fiber concentrations of asbestos to which workers were exposed many years ago (Liddell, 1991), were hundreds or thousands of times higher than that experienced by workers manufacturing or installing insulation glass fibers (Hesterberg and Hart, 1994; Jacob et al., 1992; 1993). The diameters of asbestos and insulation glass fibers are also markedly different, with asbestos fibers typically 0.1 to 0.2 μm or thinner, whereas airborne glass fibers typically average around 1 μm (Hesterberg and Hart, 1994), which is considerably less than the average in the product itself (Christensen et al., 1993).

Another property in which asbestos fibers and insulation wool glass fibers differ greatly, and the subject of this paper, is the dissolution rate of the fibers in the extracellular lung fluid. The dissolution rate of fibers can be measured in vitro in simulated lung fluid and the physical chemistry of the process is reasonably well understood (Potter and Mattson, 1991; Leineweber, 1984; Scholze, 1988). The dissolution rate constants measured in vitro depend significantly on the measurement conditions, and it is important to measure them in a way that is relevant to dissolution in the lung (Mattson, 1994a; 1994b). Therefore the in-vitro measurement methods used to determine the dissolution parameters given in this paper were tested by comparing the in-vitro results to glass fibers recovered from rat lungs at various times following intratracheal instillation. It was found that long fibers, for example, those 20 μm or longer, dissolve at the same rate in the lung as in vitro (Eastes et al., 1995). It was further found that the rate of disappearance of long (> 20 μm) glass, rock, and slag wool fibers was predicted by the dissolution rate measured in vitro by these methods (Eastes and Hadley, 1995). Short glass and asbestos fibers, on the other hand, do not appear to dissolve in the lung, but are cleared efficiently by macrophage-mediated physical action after inhalation (Eastes and Hadley, 1995).

It has long been believed that long fibers are the most biologically active, probably because their aerodynamic behavior allows fibers with much greater lengths than a macrophage can totally ingest, to enter the lower lung (Timbrell, 1976). Efficient macrophage-mediated clearance of these long fibers therefore cannot occur. If the long fibers are durable, they will accumulate if the exposure continues (Davis, 1994). Persistent long fibers can then result in incomplete or "frustrated" phagocytosis with leakage of macrophage contents, leading to chronic inflammation (Holt, 1987). The ability of a fiber to dissolve rapidly in the extracellular fluid would appear therefore to be an important means of reducing the dose of the most biologically active fibers to the lung parenchyma or to the pleura (Boffetta, 1994).

The model described here to predict the development of fibrosis or tumors is based on the hypothesis that a rapidly dissolving fiber acts like a much smaller dose of a durable fiber. A durable fiber is here considered to be one that does not dissolve during the lifetime of the species of interest, or about two years for the rat. For example, a given dose of a fiber that dissolves in one year is assumed to act like half that dose of a fiber that requires two years or more to dissolve.

The next section describes more precisely the nature of this mathematical model and the following one presents tests of its accuracy for an inhalation study and for intraperitoneal experiments in rats. The last section discusses a number of implications of the model.

THEORY

When animals are exposed to various doses of durable fibers, it is found that the tumor incidence is a function of the dose of the appropriate size of fiber (Stanton and Wrench, 1972). Accordingly, the starting point for this analysis is the assumption that the incidence of disease following administration of durable fibers is given by a function $f(X)$, where X is the dose according to some convention. For example, a logit form for $f(X)$ has been used to describe the mesothelioma incidence after intraperitoneal (IP) injection in rats (Pott et al., 1990a), as well as both lung tumors and fibrosis following inhalation in rats (Eastes and Hadley, 1994). However, in contrast to

these previous treatments, the present work establishes the dose-response function *f(X)* directly from the observed durable fiber results without the assumption of an analytical functional form.

The hypothesis proposed here is that the dose-response relation for durable fibers, *f(X)*, holds also for rapidly dissolving fibers, if the dose is adjusted by the fraction of the species lifetime that these long fibers remain in the lung. That is, the disease incidence f for dissolving fibers becomes

$$f = f(\alpha X), \tag{1}$$

where the adjustment factor α is defined as

$$\alpha = \frac{t_D}{t_L}, \tag{2}$$

the ratio of t_D, the time a fiber of diameter D remains in the lung, to the lifetime of the animal t_L. For the rat studies used here to test this hypothesis, the lifetime is taken to be two years.

The lifetime of a fiber t_D can be estimated from the rate law for fiber dissolution found in vitro and confirmed for fibers 20 μm or longer in vivo as well (Eastes et al., 1995). It has been found (Leineweber, 1984; Mattson, 1994a; Potter and Mattson, 1991; Scholze, 1988) that a wide variety of vitreous fibers dissolving in simulated lung fluid at nearly neutral pH, at a flow rate high enough to simulate the rapid removal of dissolution products in the lung, decrease in diameter at a constant rate given by

$$\frac{2k_{dis}}{\rho}, \tag{3}$$

where k_{dis} is the dissolution rate constant and ρ is the fiber density. It follows then that a fiber with initial diameter D decreases to zero diameter in a time

$$t_D = \frac{\rho D}{2k_{dis}}. \tag{4}$$

If the administered fibers do not all have the same diameter, then it is the average lifetime t_D that is required in Eq. (4). Since the fiber lifetime is proportional to the fiber diameter, the average fiber diameter is used in Eq. (4). Thus the diameter D should be understood to be the number weighted, arithmetic mean fiber diameter for a collection of fibers with a distribution of diameters.

Equation (1) along with Eqs. (2) and (4) provide a mathematical model to predict the incidence of disease at a given dose of long fibers X that dissolve at the rate given by the dissolution rate constant k_{dis}. The particular function f in Eq. (1) depends on the disease (fibrosis, lung cancer, or mesothelioma) and on the route of administration (inhalation or injection) in the particular bioassay being used, as well as on the units in which the dose is expressed.

One feature of this model is that it has no adjustable parameters. Once the response *f(X)* to asbestos or to other durable fibers is known for a particular bioassay, then the response to any other fiber type is predicted from the dissolution rate constant, a property of the fiber composition measured in vitro. The implication of this model is that a lifetime exposure to a rapidly dissolving fiber acts like less than a lifetime exposure to a durable fiber. Since such less than lifetime exposures to durable fibers were also included in the inhalation studies, this feature provides a convenient way to test the model.

It remains to establish the meaning of the incidence f and the dose X. For lung cancer or mesothelioma, f is the fraction of animals diagnosed with this disease during the study. For fibrosis, a number of different indicators of the condition could be used. Here f is taken to be the fraction of animals that are found to have at least minimal fibrosis, defined as Wagner Grade 4 or above (McConnell et al., 1984).

The dose X should be the total number of "relevant" fibers to which the animal is exposed throughout the study. For a chronic inhalation study, it is considered here to be the total number of long fibers that are inhaled. This dose is not the same as the fiber lung burden at any time because the lung burden is the equilibrium value between the number of fibers continually inhaled and those continually removed by macrophage-mediated

physical clearance, or by dissolution. Therefore, the total number of respirable, long fibers in the aerosol, multiplied by the average air volume inhaled by the animal while exposed to the fibers, will be used here to characterize the total dose X. Respirable fibers are those that can be inhaled into the deep lung of the rat and are generally those less than 1 μm in diameter (Bernstein et al., 1995). The length of fibers that is relevant to lung disease is less clear, but it likely that fibers short enough to be engulfed and transported by alveolar macrophages are not associated with lung disease in the absense of significant overload (Davis, 1994). For the sake of definiteness, fibers longer than 20 μm were chosen in what follows as a surrogate for long fibers (Kuschner, 1987). Also, since the length distributions of the synthetic vitreous fibers in these studies were similar, the results were also similar whether 5 μm, 10 μm, or 20 μm was chosen as the minimum length of a long fiber. Thus the definition of relevant fibers used here is the number of aerosol fibers less than 1 μm in diameter and simultaneously greater than 20 μm in length.

For the intraperitoneal injection studies, the concept of dose is uncertain. It is clear that the injection of foreign materials into the sterile serosal cavity represents a very nonphysiological exposure. Not only are large quantities of materials injected as a bolus, but there is no anatomical filtration of fiber sizes, such as occurs after inhalation. Also, while there appears to be a biological basis for the unique aspects of long fibers in the lung, it is not clear if this is also relevant to the peritoneal or pleural cavities. For example, recent data have indicated that primarily the short fibers translocate to the pleural cavity following inhalation (Gelzleichter et al., 1995). Given this uncertainty, the model was tested simply by using the dose reported by the study authors, which was essentially fibers less than 2 μm in diameter and greater than 5 μm long (Pott et al., 1990a).

RESULTS

The hypothesis described in the previous section was tested by applying it to three different endpoints in rats: fibrosis and lung cancer after inhalation and mesothelioma following intraperitoneal injection. The inhalation studies were sponsored by the Thermal Insulation Manufacturers Association (TIMA) and were performed by the Research and Consulting Company (RCC) in Geneva (Hesterberg et al., 1993; Mast et al., 1993; McConnell et al., 1994). The intraperitoneal injection studies are those of Pott and coworkers (Pott et al., 1990a; 1990b; Pott, 1991).

In the RCC inhalation studies, separate groups of male Fischer 344 rats were exposed to different types of synthetic vitreous insulation wool fibers at different concentrations, to chrysotile, and to crocidolite asbestos for 6 hours per day, 5 days per week. Typically 3 to 6 rats were sacrificed at a time, at intervals from 3 months to over 2 years. The flow-through, nose only inhalation apparatus has been previously described (Bernstein et al., 1994).

The fiber types studied at RCC, against which this model was tested, along with their properties, are listed in Table 1. The dissolution constants k_{dis} given in Table 1 were measured in vitro (Mattson, 1992; 1994b; Potter and Mattson, 1991), except for the asbestos and RCF 1, which are measurements of fibers with compositions similar to those used at RCC. The average diameters of the fibers in Table 1 were taken from published reports (Hesterberg et al., 1993; Mast et al., 1993; McConnell et al., 1994). The densities of the fibers were measured (Christensen et al., 1993; Mattson, 1992).

Table 1. Fiber types in the RCC inhalation studies.

Code	Fiber Type	k_{dis}	D	ρ	t_D
		ng/cm^2/hr	[μm]	g/cm^3	[years]
CROC	Crocidolite	0.1*	0.28	3.25*	52
CHRYS	Chrysotile	0.2*	0.10	2.56*	7
RCF 1	Kaolin RCF	3*	0.80	2.58*	4

MMVF 10	JM 901 glass wool	300	1.40	2.52	0.07
MMVF 11	Glass wool	100	0.98	2.55	0.14
MMVF 21	Rock wool	20	1.11	2.79	0.9
MMVF 22	Slag wool	400	0.98	2.92	0.04

* Estimated from measurements of fibers with similar composition.

The lifetime of each fiber type was computed from Eq. (4) and is listed in Table 1. It is clear that the asbestos and RCF 1 dissolve slowly enough that they persist for the lifetime of the rats. Therefore, fibrosis and tumor incidence data for these three fiber types at different doses were used to establish *f(X)* for durable fibers.

The incidence of fibrosis was computed separately for each group of rats exposed to the same fiber at the same concentration for the same time. The dose X was expressed in units of 10^9 relevant fibers and was calculated by multiplying the airborne concentration of fibers less than 1 μm in diameter and greater than 20 μm long by the rat lung minute volume (here taken to be 100 cm^2/minute) and by the total exposure time for each group of rats. The dose-response of fibrosis to durable fibers in the RCC studies is shown in Fig. 1(a) in units of 10^9 fibers.

In order to determine whether the observed incidence of fibrosis or lung tumors in non-durable fibers agrees with that predicted by Eqs. (1) and (2), the durable fiber incidence *f(X)* is needed at all doses X, not just at the ones measured. Such a continuous function was obtained by grouping the doses for durable fibers into bins chosen to cover the range of the measured doses. The average fibrosis incidence for all doses in a bin becomes the estimate of the incidence at the midpoint dose for this bin. The standard error s of this estimate was estimated in two different ways, depending on the number of doses in the bin. If there were more than two experimental dose points in the bin, then the standard error was taken to be the standard error of the average in the bin (the standard deviation divided by the square root of the number of doses in the bin). If there were only one or two observed doses in the bin, which does not allow a reliable estimate of the standard error, then the standard error of the binomial distribution was used,

$$s = [f(1-f)/n]^{1/2}, \quad \textbf{(5)}$$

where f is the average incidence in the bin and n is the total number of animals at all doses within this bin. These averages and standard errors for each bin are shown connected by straight lines in Fig. 1(a).

The fibrosis incidence for non-durable fibers observed in these studies is plotted as a function of the adjusted dose α in Fig. 1(b). The **X** symbols are MMVF 10, 11, 21, and 22, in which the dose was adjusted by the factor α. The solid line in Fig. 1(b) is the same line as in Fig. 1(a). There is reasonable agreement between the observed (**X** symbols) and the predicted (solid red line) fibrosis incidence in Fig. 1(b).

A more quantitative representation of the agreement between the observed and predicted values is obtained by computing χ^2, the chi-squared statistic (Press et al., 1992),

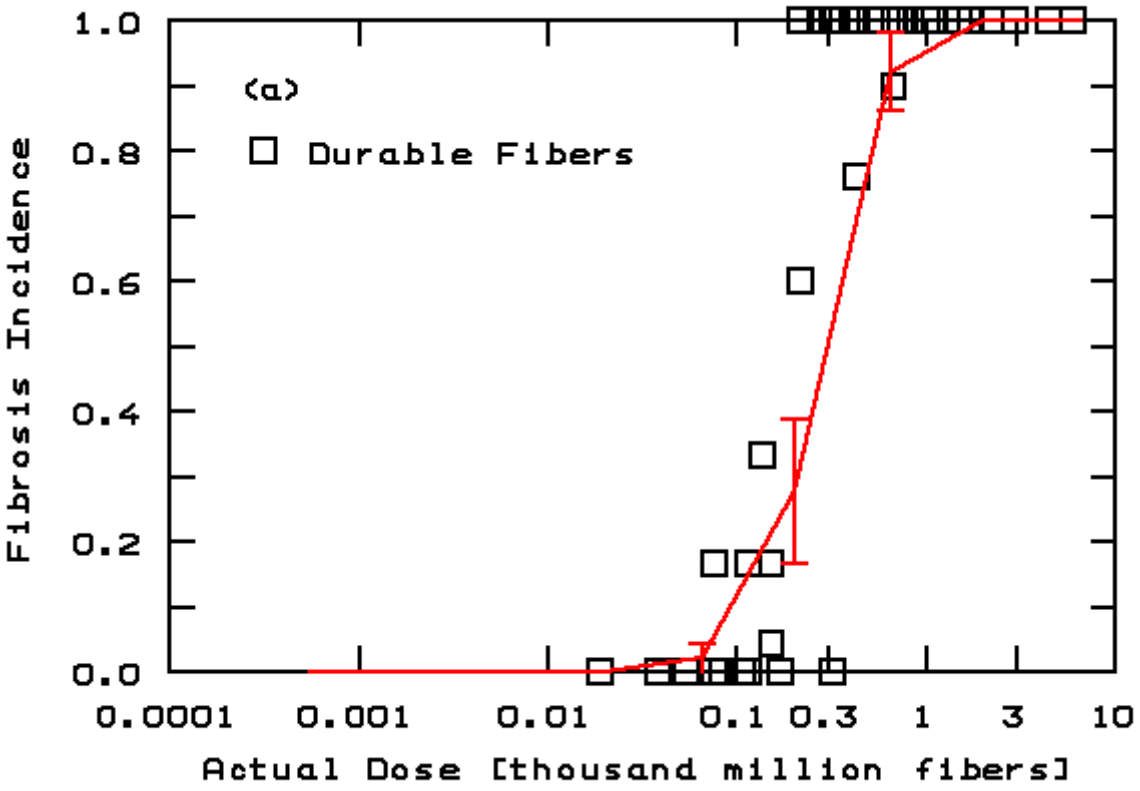
$$\chi^2 = \sum_i \frac{[F_i - f(\alpha X_i)]^{1/2}}{S_i^2 + s_i^2}. \quad \textbf{(6)}$$

Here F_i is the fibrosis incidence observed for a non-durable fiber at adjusted dose αX_i and S_i is its estimated standard error from Eq. (5). The predicted incidence is $f(\alpha X_i)$ obtained from the lines in Fig. 1(a) interpolated between the bin midpoints, and s_i is the standard error in this bin. The value of χ^2 over all of the 114 non-durable fiber doses in Fig. 1(b) is shown in Table 2, along with the associated probability of the null hypothesis. P is the probability that the differences between the observed (**X** symbols) and the predicted values (lines) in Fig. 1(b) would arise merely by chance when the hypothesis is correct. Probabilities larger than about 0.05 are good evidence that the model predicts the observed values to within the errors involved in the experimental data.

TABLE 2. Statistics for the model predictions.

Disease	Route of Administration	Number of Samples	Model with Dose Adjustment		No Dose Adjustment	
			χ^2	P	χ^2	P
Fibrosis	Inhalation, RCC	114	109	> 0.62	4300	$< 10^{-5}$
Lung Tumors	Inhalation, RCC	12	17	0.16	24	0.02
Mesothelioma	IP Injection,	23	35	0.051	3500	$< 10^{-5}$

Useful insight into the nature of this model is given by comparing Fig. 1(a) and (b) with the companion Fig. 1(c), in which the same fibrosis incidence data are plotted as a function of the actual, unadjusted dose. The actual dose on the horizontal axis of Fig. 1(c) does not take into account the short lifetime of the rapidly dissolving fibers (**X** symbols). These rapidly dissolving fibers shift to the right in Fig. 1(c) compared to Fig. 1(b), whereas the durable fibers (squares) are in the same place in both figures. Clearly there is no consistent dose-response relation in Fig. 1(c), except for the durable fibers (squares). The chi-squared statistic and its probability for the non-durable fibers in Fig. 1(c) are shown in the last two columns of Table 2. The large value of χ^2 and the vanishingly small probability confirm what is evident in Fig. 1(c), that the long fiber dose alone does not explain the fibrotic response. However, when the dose for each rapidly dissolving fiber is reduced according to the lifetime of that fiber, the values agree with the dose response relation in Fig. 1(b) within the variation inherent in the experiments.


(a)
Durable Fibers
Fibrosis Incidence
1.0
0.8
0.6
0.4
0.2
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0.0001
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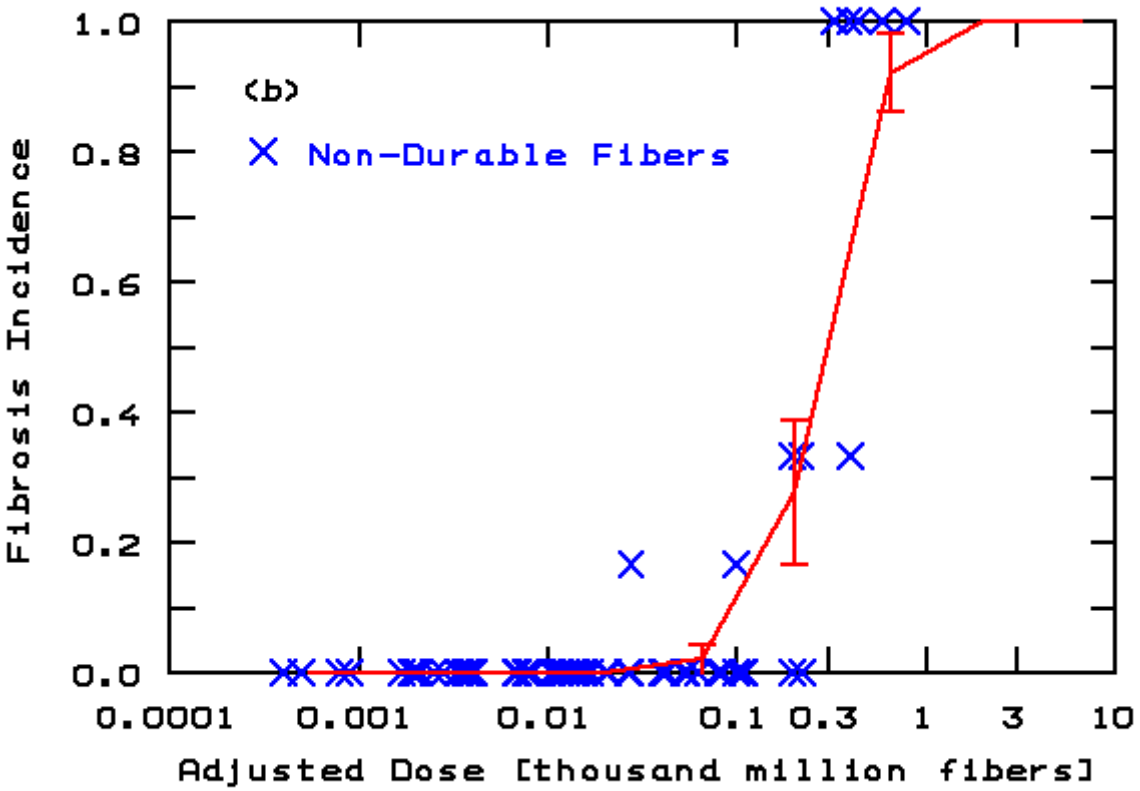
0.01
0.1
0.3
1
3
10
Actual Dose [thousand million fibers]


(b)
Non-Durable Fibers
Fibrosis Incidence
1.0
0.8
0.6
0.4
0.2
0.0
0.0001
0.001
0.01
0.1
0.3
1
3
10
Adjusted Dose [thousand million fibers]

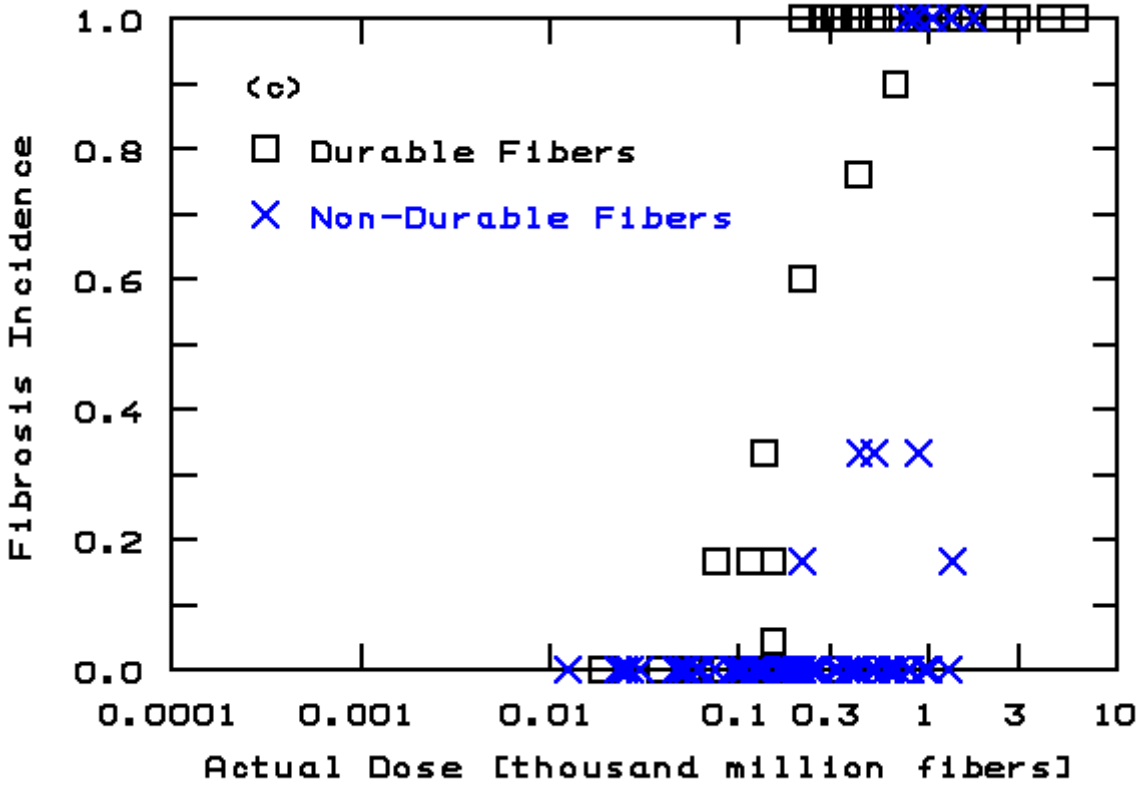
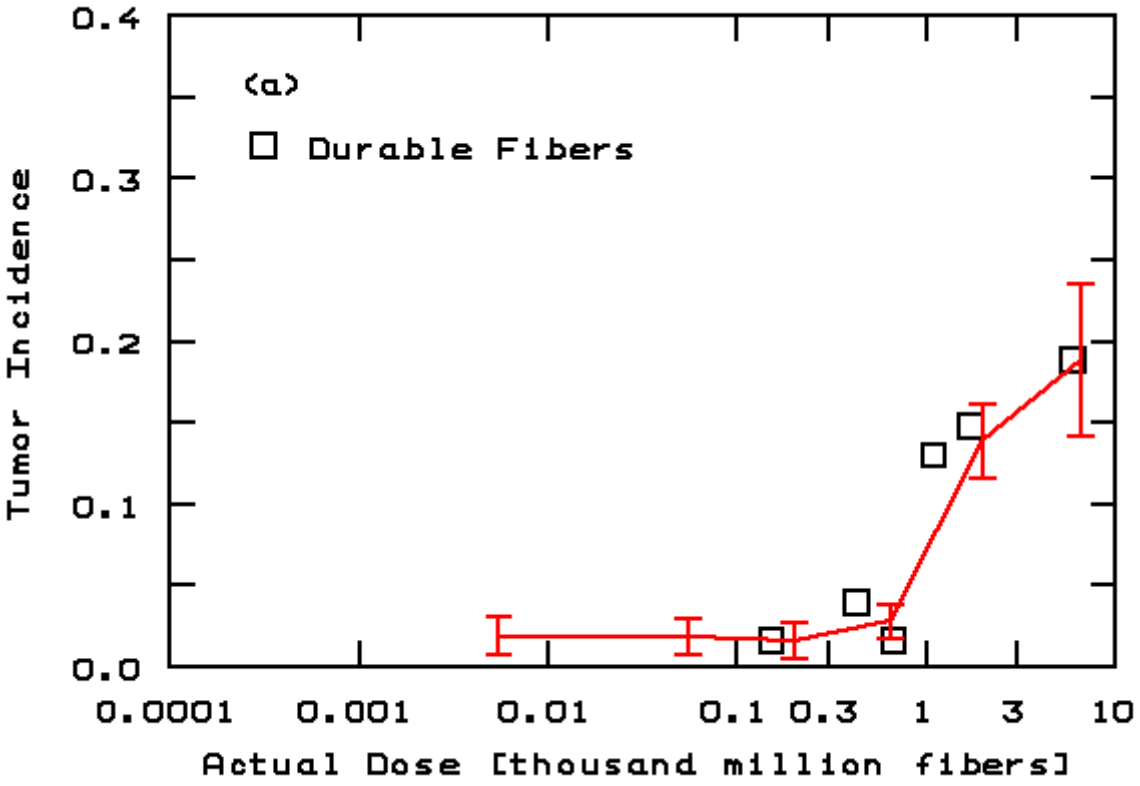
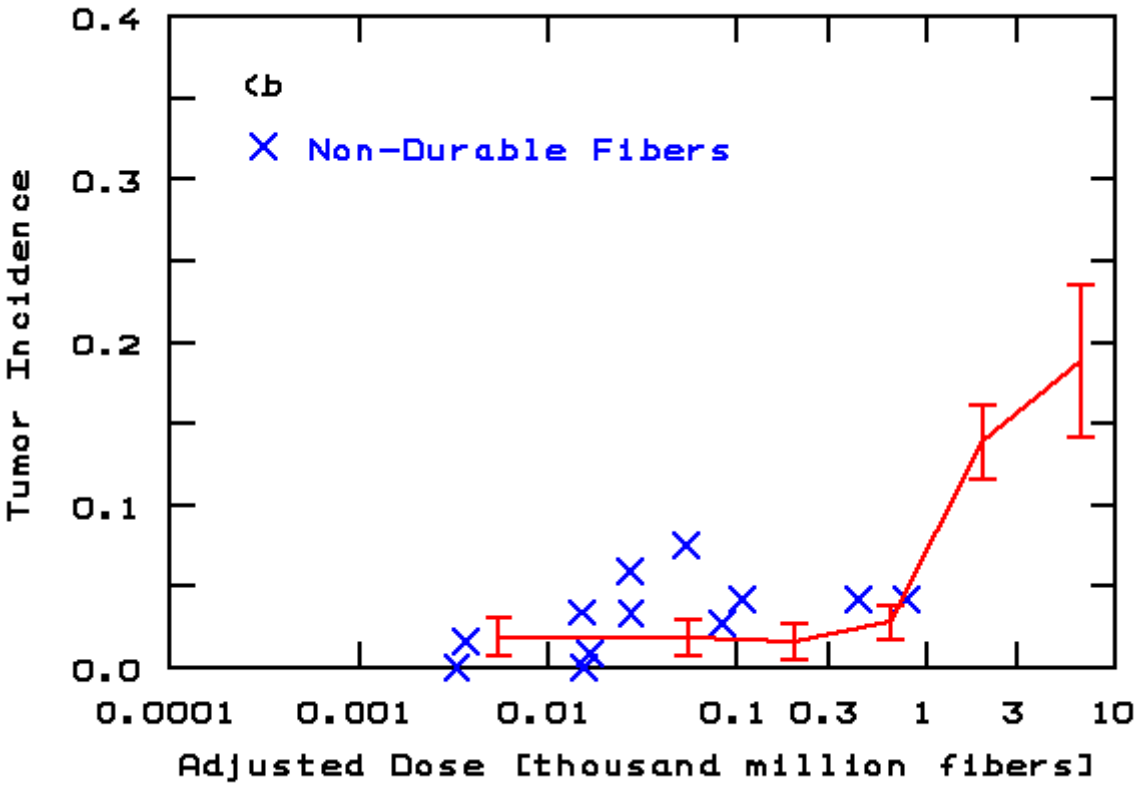


FIGURE 1. Observed incidence of Wagner Grade 4 or higher fibrosis in the RCC chronic inhalation study in rats, **(a)** as a function of the actual dose of durable fibers, and **(b)** as a function of the adjusted dose of non-durable fibers. The solid red line, which is determined by the durable fiber data in **(a)**, is the predicted incidence for non-durable fibers in **(b)**. In **(c)**, the observed fibrosis incidence is plotted as a function of the actual dose, without adjusting for the shortened lifetime of the non-durable fibers (**X** symbols). The data displayed in these three graphs are available separately as a tab delimited text file by clicking here.

In order to test this model for lung cancer in the RCC studies, a dose-response relation for the durable fibers must be established. Since there were so few lung tumors (adenomas plus carcinomas) in these inhalation studies, the data were grouped differently than for the fibrosis results. All of the animals exposed to a given fiber type for one year or more at a single exposure rate were grouped together in the same way that these results have been reported previously (Hesterberg et al., 1993; Mast et al., 1993; McConnell et al., 1994). Since most of these animals were exposed for two years, the dose for each group was calculated for a two year exposure, except for the group exposed to crocidolite, most of which were exposed for 44 weeks. The tumor incidence for these durable fibers is plotted as a function of the dose in Fig. 2(a). The square symbols are, from left to right, the four dose rates of RCF 1, crocidolite, and chrysotile fibers. The average tumor incidence in the bins and its standard error is shown by the lines connecting points with error bars in Fig. 1(a). To obtain incidence values in bins at doses below what was measured, linear interpolation was done between the measured values and the tumor incidence of the air controls at zero fiber dose. The average tumor incidence of all of these air controls was 0.019, similar to the average tumor incidence at the lowest doses of durable fibers in Fig. 1(a).

The results from the non-durable fiber exposed animals are shown in Fig. 2(b). The model correctly predicts that the rapidly-dissolving MMVF 10, 11, 21, and 22 (**X** symbols) have a tumor rate indistinguishable from the air-exposed controls. The chi-squared statistic for the 12 non-durable groups is shown in Table 2 with an associated probability that would be expected from random variation alone.


(a)
Durable Fibers
Tumor Incidence
0.4
0.3
0.2
0.1
0.0
0.0001
0.001
0.01
0.1
0.3
1
3
10
Actual Dose [thousand million fibers]


(b
Non-Durable Fibers
Tumor Incidence
0.4
0.3
0.2
0.1
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Adjusted Dose [thousand million fibers]

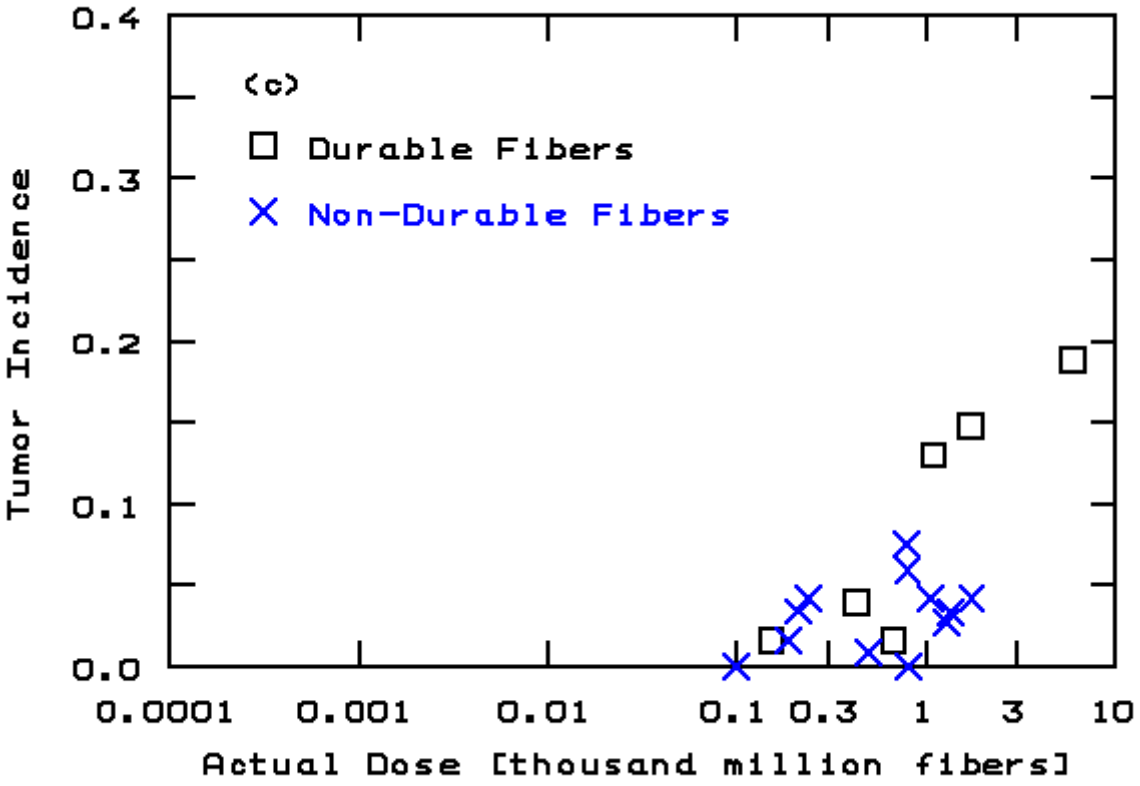


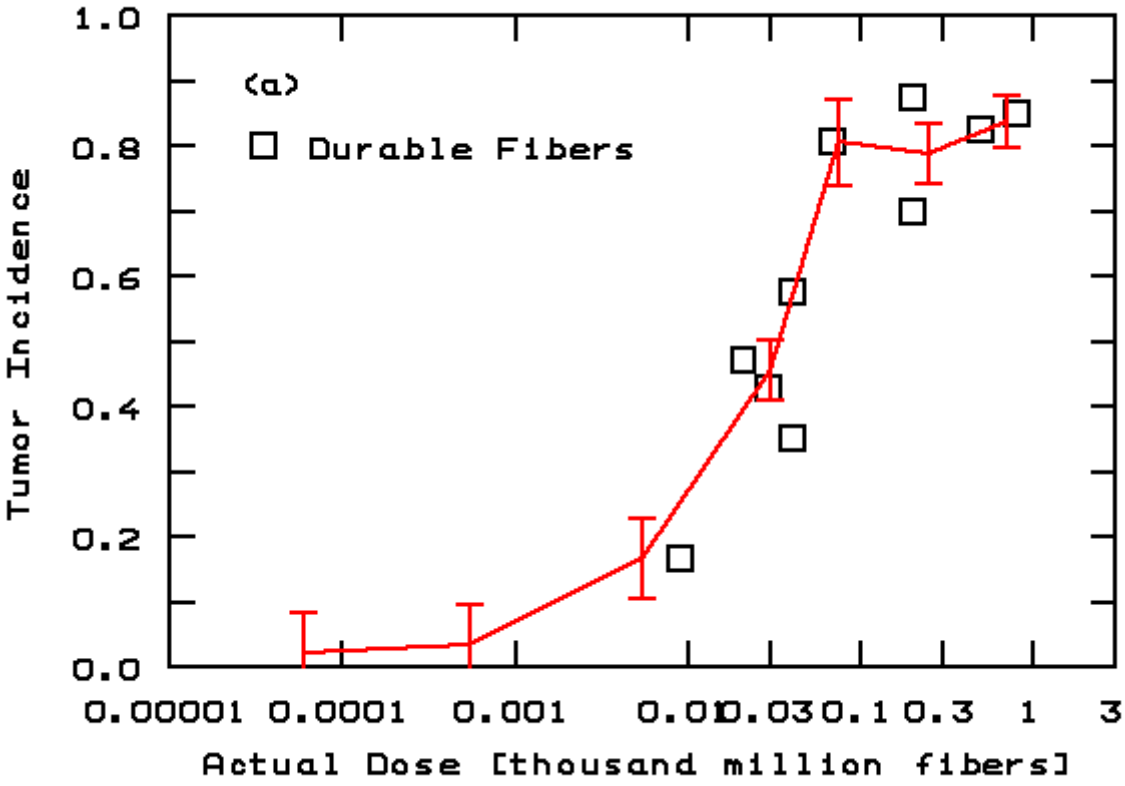
FIGURE 2. Observed incidence of adenomas and carcinomas in the RCC chronic inhalation study in rats, **(a)** as a function of the actual dose of durable fibers, and **(b)** as a function of the adjusted dose of non-durable fibers. The solid red line, which is determined by the durable fiber data in **(a)**, is the predicted incidence for non-durable fibers in **(b)**. In **(c)**, the observed tumor incidence is plotted as a function of the actual dose, without adjusting for the shortened lifetime of the non-durable fibers (**X** symbols). The data displayed in these three graphs are available separately as a tab delimited text file by clicking here.

It is of interest to know the predictive power of this model if it is applied to the doses of fibers longer than 10 μm or even 5 μm, rather than those over 20 μm in length presented here. Graphs corresponding to fibers longer than 10 μm and longer than 5 μm appear virtually the same as Figs. 1 and 2. This result comes about because the synthetic vitreous fibers tested in the RCC studies were prepared with very similar length and diameter distributions, at least on the scale of Figs. 1 and 2. Thus redrawing with length cutoff values of 5 or 10 μm merely changes the scale but not the relative locations of the synthetic fiber doses. The asbestos doses move to larger values relative to the synthetic vitreous fibers as the length cut-off decreases, because these preparations contained many more short fibers. However, the asbestos fibers simply define the large dose end of the durable fiber dose-response. They do not affect the prediction for the non-durable fibers tested. The predictive quality of this model is roughly the same whether 20, 10, or 5 μm fibers are chosen to test it. This fact does not suggest that all of these lengths are equally potent, merely that the differences cannot be detected with these fiber preparations. Information about the length dependence must be obtained from experiments with the same fiber in preparations with significantly different lengths.

The model was also tested against the mesothelioma incidence at various doses of fibers injected into the peritoneal cavity by Pott and coworkers (Pott et al., 1990a; 1990b; Pott, 1991). The dose X was taken to be the number of "critical fibers" (in units of 10^9), where "critical fibers" are defined (Pott et al., 1990a) as those with diameter 2 μm or less, length 5 μm or more, and aspect (length/diameter) ratio 5 or greater. Data were used from several sources (Pott et al., 1990a; 1990b; Pott, 1991), but only those experiments were used for which the fiber could be identified precisely enough to estimate its dissolution constant and its average diameter (Bellmann et

al., 1990; Muhle et al., 1990), values needed to compute t_D in Eq. (4). The fiber types used were those designated B1, B2, B3, Chrysotile, Crocidolite, 104E, 475, and Ceramic fiber in the various length and diameter preparations in the references just cited. As before, the observed tumor incidence-dose relationship for the very durable fibers, 3(a), was used to establish *f(X)* which is shown as solid lines with standard error bars at the bin midpoints. As before, the values for bins at doses lower than measured for durable fibers were obtained by interpolation. The zero-dose tumor incidence was taken to be 0.02 (Pott et al., 1990a). The tumor incidence for animals injected with rapidly dissolving fibers is shown in Fig. 3(b). They agree generally with the value predicted by this model (lines). The chi-squared statistic for all of the non-durable fiber doses in Fig. 3(b) has a probability of 0.051, a lower value than obtained with the inhalation experiments but still not unreasonable. If these tumor incidence data are all plotted as a function of the actual dose, instead of the adjusted dose for durable fibers in Fig. 3(b), then Fig. 3(c) is obtained. Again, there is no consistent dose-response relation in Fig. 3(c), as indicated by the large χ^2 and vanishingly small probability (Table 2). Such a relation appears in Fig. 3(b) when the dose of each rapidly dissolving fiber is reduced to account for its shorter residence time.

The proposed model appears to function both for mesothelioma incidence in Fig. 3(b) and for fibrosis in Fig. 1(b), in spite of the fact that Figure 3(b) is for a different disease in a different organ by a different route of administration of the fibers. The fact that these three diseases and two routes of administration are predicted by the same model suggests that fiber dissolution may affect them all in the same way, but to a different degree. The most striking difference between mesothelioma following intraperitoneal injection and fibrosis following inhalation is that the incidence of mesothelioma rises more slowly with intraperitoneal dose than the inhalation results. There appears to be a sharp drop in the incidence below a certain dose for fibrosis and possibly for lung tumors following inhalation compared to IP injection. Whether this behavior is caused by the nature of the disease or the route of administration is not known.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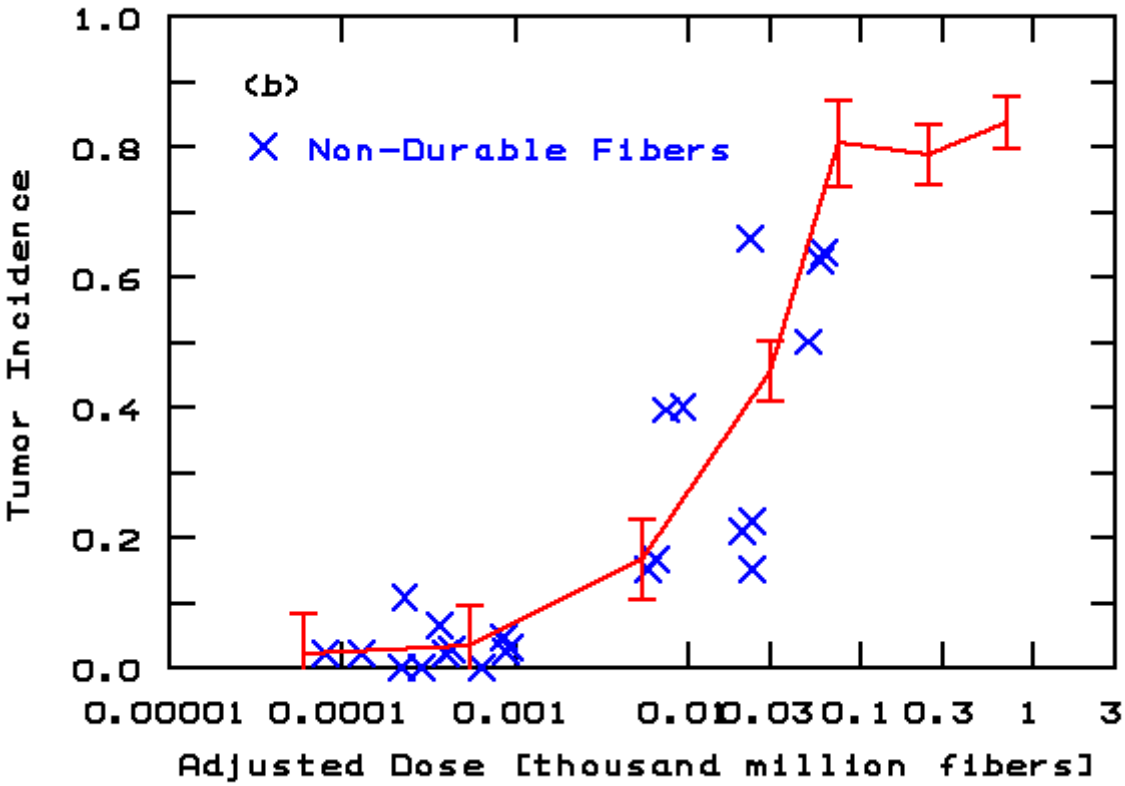
Actual Dose [thousand million fibers]


(b)
Non-Durable Fibers
Tumor Incidence
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Adjusted Dose [thousand million fibers]

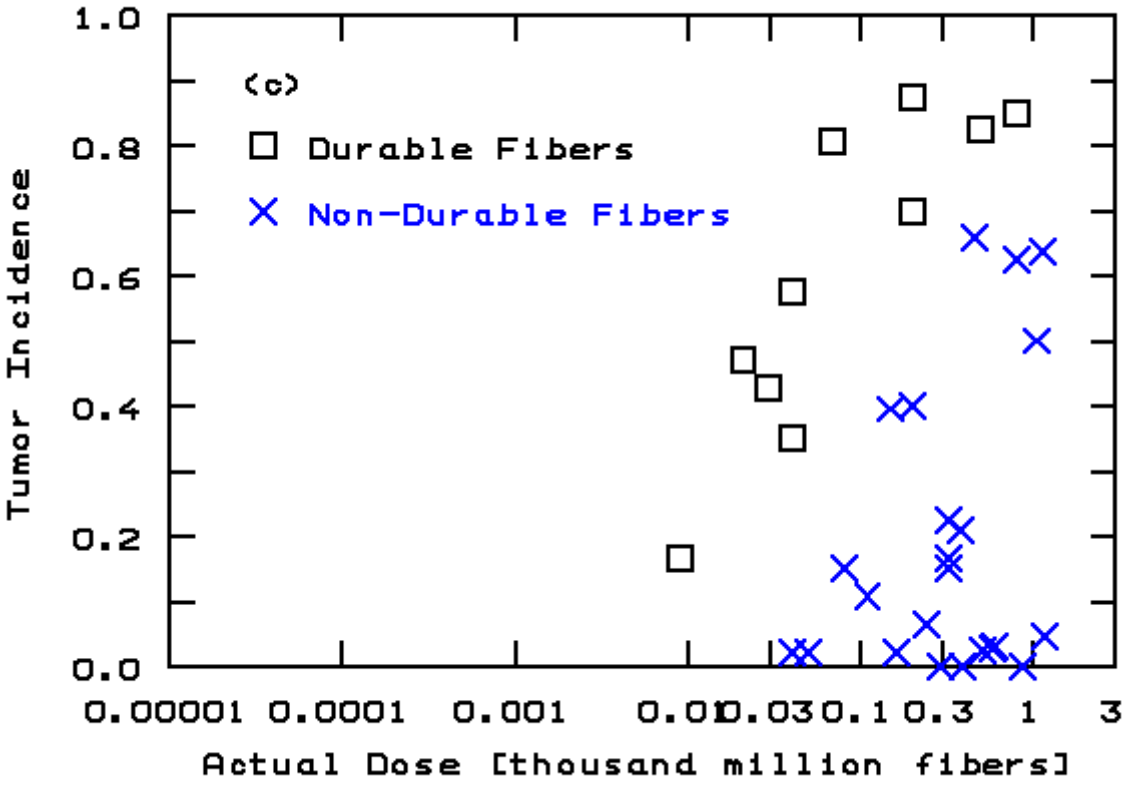

FIGURE 3. Observed incidence of tumors in a series of intraperitoneal injection experiments by Pott and coworkers, **(a)** as a function of the actual dose of durable fibers, and **(b)** as a function of the adjusted dose of non-durable fibers. The solid red line, which is determined by the durable fiber data in **(a)**, is the predicted incidence for non-durable fibers in **(b)**. In **(c)**, the observed tumor incidence is plotted as a function of the actual dose, without adjusting for the shortened lifetime of the non-durable fibers (**X** symbols). The data displayed in these three graphs are available separately as a tab delimited text file by clicking here.

DISCUSSION

Inhalation of durable fibers has been associated with disease in both humans and experimental animals, while numerous inhalation studies with soluble fibers have not found an association with disease in rodents. Interest in the role of fiber durability in possible biological effects of airborne fibers has been growing rapidly. The model presented here is an attempt to couple knowledge of fiber dissolution with the basic anatomical discrepancies between the length of inhaled fibers and the phagocytic capability of the alveolar macrophage. Absent efficient macrophage clearance, long fibers remain in the lung where their physical presence may lead to chronic effects. Since rapid dissolution serves to remove long fibers, it is reasonable to assume that the resultant reduction in effective dose of long fibers may indeed play a major role in potential effects.

When interpreting the chi-squared statistics for the model predictions in Table 2, it should be noted that χ^2 and its associated probability depend not only on the differences between the observed and predicted values, but are also a sensitive function of the estimated errors in these values from Eq. (6). If one "in a 'fit' of conservatism" (Press et al., 1992) estimates the errors to be too large, than any model will appear to predict the observations, at least within the assumed errors. On the other hand, if one sufficiently underestimates the standard errors S_i compared to the true values, then no model will appear to give acceptable results. The method used here tends to underestimate S_i and s_i in Eq. (6) for two reasons: First, the use of the standard error of the mean to estimate s_D

is appropriate only if the incidence $F(X_i)$ does not change much over the range of X_i in the bin. If it does, then the standard error estimates the standard deviation of the mean incidence of the bin, not the larger variation within the bin. For a rapidly increasing incidence with dose, such as for fibrosis in Fig. 2, the standard error so calculated appears to seriously underestimate the variation in several bins in the knee of the curve. The second underestimate is the assumption of the binomial distribution to obtain S_i Eq. (5), which most likely yields a lower bound for the standard error of the observed incidence F_i. In view of these likely underestimates of the experimental errors, it is somewhat surprizing that this simple scaling of the dose without adjustable parameters leads to such quantitatively good agreement. It speaks to the unavoidably large variations inherent in animal experiments. On the other hand, the experimental errors are small enough to show that dose and dimension alone do not explain the responses to the different fibers, as seen in the last two columns of Table 2.

So far this work has focused on the calculation of the fibrosis or tumor incidence in rats exposed to a given fiber type at a specified dose to compare with that actually observed in an animal study. Given the agreement, it is apparent that the model can also be used in the opposite way -- to predict how large a dissolution constant a fiber must possess to avoid producing disease in a given animal experiment. Such an estimate was obtained by solving Eq (1) for α at a given actual dose X and sufficiently small incidence f. This value of α was used then to determine k_{dis} from Eqs. (2) and (4). This value of k_{dis} will be called the "no-disease limiting" dissolution constant.

The estimate of the no-disease limiting k_{dis} is sensitive to the incidence value that is considered to be insignificant and the corresponding dose. The average fiber diameter and the fiber density also play a role, although a relatively minor one. Some reasonable assumptions used in the estimates given here are listed in Table 3. For the RCC inhalation studies, the dose was the number of long fibers at the highest dose for the longest exposure time, averaged over all synthetic vitreous fibers studied. Since these RCC studies were performed at the maximum tolerated dose (MTD), it sets an approximate upper bound on fiber intake. A minimum significant fibrosis incidence of 10% was chosen because anything less would likely be undetected in these studies, since generally only six animals were evaluated at interim sacrifices. The density 2.5 g/cm^3 and fiber diameter 1 μm of a typical airborne glass insulation wool fiber were used in this estimate. Since there were more animals in the groups for which lung tumors were reported, a lower tumor incidence could be detected. For the purposes of this illustration, a minimum incidence of 4%, or about twice the rate of the air controls, was chosen. For IP experiments, a minimum incidence of 10% at 10^9 injected fibers has been suggested (Pott et al., 1990a).

TABLE 3. Assumptions for estimating the no-disease limiting k_{dis}.

Disease	Route of Administration	Dose [10^9 fibers]	Minimum Incidence
Fibrosis	Inhalation, RCC	1.25*	10 %
Lung Tumors	Inhalation, RCC	1.25*	2 %
Mesothelioma	IP Injection	1.0	10 %

* For 20 μm or longer fibers in a rat lifetime inhalation study at MTD

The calculated no-disease limiting k_{dis} for the diseases tested are shown in Table 4. These data suggest that fibrosis occurs at a lower adjusted dose, and therefore at a higher k_{dis} than is required for tumor formation. The same conclusion is reached from a comparison of Fig. 1 for fibrosis, in which the incidence rises above zero at a dose just below 0.1 thousand million, with Fig. 2 for lung tumors, where the incidence rises above background at

just under 1 thousand million fibers. Fibrosis is therefore a more sensitive indicator of irreversible lung damage than the development of tumors.

TABLE 4. Calculated no-disease limiting k_{dis}.

Disease	Route of Administration	k_{dis} [ng/cm^2/hr]
Fibrosis	Inhalation, RCC	84
Lung Tumors	Inhalation, RCC	11
Mesothelioma	IP Injection	2400

The data in Table 4 also suggest that the no-disease limiting k_{dis} is much higher for the intraperitoneal injection studies than for the inhalation studies. This prediction is consistent with results (Smith et al., 1987; Wagner et al., 1984) of studies in which a fiber produced disease following IP injection yet did not induce significant effects following inhalation. The reason for these differences is unknown. One possible explanation may be that the large bolus injections into a sterile body cavity compared to a relatively homogeneous distribution of fibers throughout the lung over a period of time may lead to alterations in dissolution processes or in the fiber environment. For example, Collier et al. (1994) have reported that, after a few weeks in the peritoneal cavity, the dissolution of long fibers appears to stop or to proceed at a much reduced rate. Slag wool fibers, which generally dissolve rapidly in the lung, were also reported to have an unexpectedly long residence time following IP injection (Pott et al., 1994). This increased persistence of long fibers in the peritoneal cavity in contrast to the lung may be significant. Collier et al. additionally reported that, at doses injected into the peritoneal cavity above 1.5 mg, binding to the peritoneal organs is no longer proportional to the dose, and the fibers are found free in the peritoneal cavity in clumps or nodules loosely attached to the peritoneal organs. The possible role and relevance of these fiber nodules is unknown.

It is also important to note that IP studies relate only to the development of mesothelioma at the site of injection. An expert committee of the World Health Organization noted (WHO, 1992): "If mesotheliomas are produced, it becomes essential to determine whether inhaled fibers can penetrate to the pleura or peritoneum in a sufficiently unmodified state to allow this carcinogenic potential to be expressed." Considering the rapid pulmonary dissolution and clearance of long fibers with a dissolution rate constant of 100 ng/cm^2/hr or more (Eastes and Hadley, 1994; Eastes et al., 1995), coupled with the absence of mesothelioma in any glass wool fiber inhalation study, or in any human epidemiology studies of glass wool production workers, it seems logical that rapid pulmonary dissolution of fibers does indeed modify their biological potential.

The no-disease limiting k_{dis} values presented in Table 4 provide an estimate of what dissolution rate is required of 1 μm, long fibers to avoid disease in these specific animal studies. Another way to present this information that gives an indication of the error of the estimates is to compute the disease incidence by this model for the maximum doses of 1 μm diameter, >20 μm long fibers listed in Table 3 as a function of the dissolution rate constant, just as was done to obtain the lines with standard error bars in Figs. 1-3. The results are shown in Fig. 4 for the three diseases and routes of administration. The interpolated standard errors are shown in Fig. 4 as vertical lines spaced arbitrarily closely so as to indicate the variations involved. Although the standard errors of the fibrosis estimates are fairly large, the steepness of its slope in Fig. 4 makes it clear that it would be very unlikely to observe any disease after this exposure to a fiber with a k_{dis} of 100 ng/cm^2/hr or higher. The dividing line in k_{dis} for lung tumors after inhalation is less clear because the background rate is large compared to what it is desired to detect. Even so, it is clear that the no-disease limiting k_{dis} for lung tumors is much less than 100 ng/cm^2/hr.

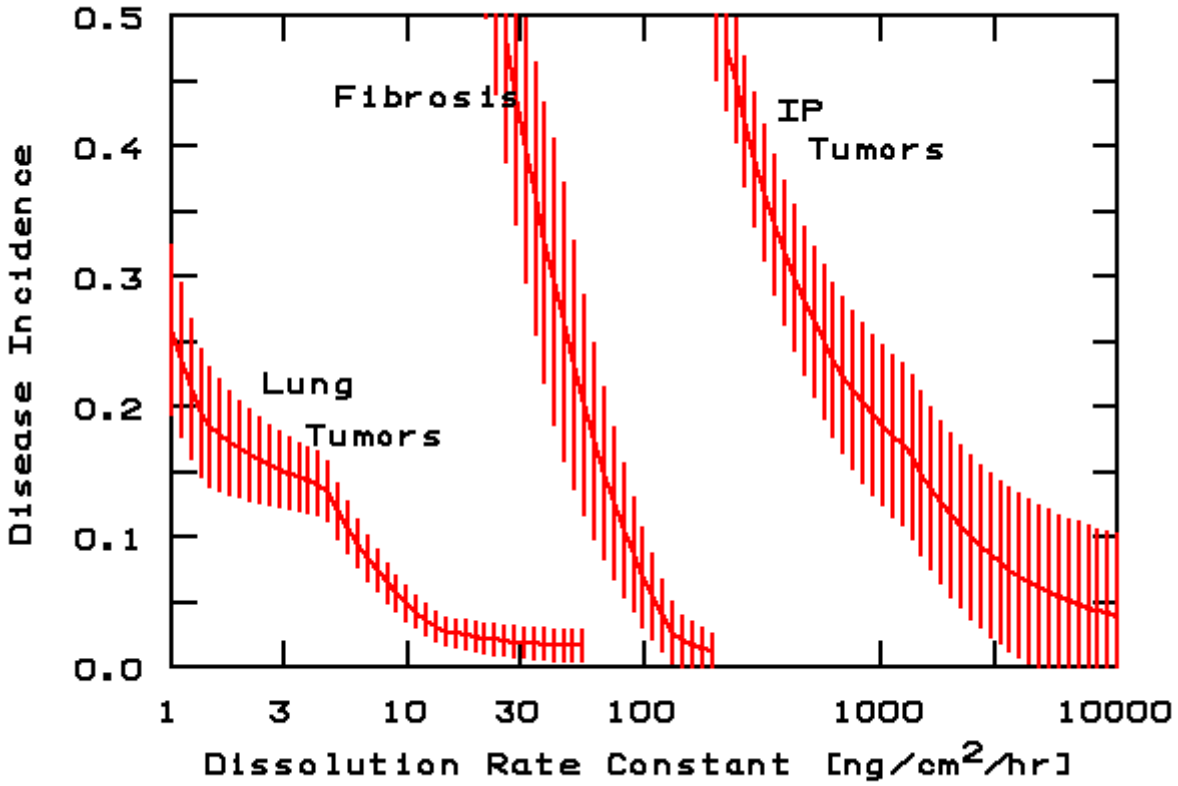

FIGURE 4. Predicted incidence of the indicated disease at the maximum doses from Table 3 of 1 μ diameter fibers as a function of the fiber dissolution rate constant. The estimated standard errors associated with the predicted incidence (solid red lines) are shown as arbitrarily spaced vertical red lines.

It is of interest that one additional fiber, known as X607, has been tested by inhalation at RCC. This fiber had similar dimensions and doses to the other synthetic vitreous fibers studied. The dissolution rate constant of X607 is as high or higher than any of the fibers considered here. The study was negative for both fibrosis and lung tumors and thus is consistent with the model predictions (Hesterberg, 1992).

It would appear from these considerations that, to be free of respiratory disease in a state-of-the-art, RCC-type rat inhalation study at MTD, an insulation wool fiber should have a dissolution rate constant of 100 ng/cm^2/hr or higher. This conclusion is based on these considerations:

- This dissolution constant is predicted to be sufficient to make fibrosis unlikely at doses even higher than what has been tested at RCC.
- The recommended dissolution constant is the same as that of an insulation wool fiber (MMVF 11) which was tested at RCC with no fibrosis or significant lung tumors observed.
- A dissolution rate that is high enough to avoid fibrosis in these rat inhalation studies is larger than needed to avoid lung tumors in the same studies.
- Inhalation is the route of exposure of fibers in humans, and therefore a chronic inhalation bioassay is the relevant model for assessing potential human respiratory effects (WHO, 1992).

An additional aspect of the model is that, for the range of fibers examined, primarily the synthetic vitreous insulation wool fibers, the predicted response does not depend on the fiber family (that is, slag wool, rock wool, or glass wool), but rather on the dissolution rate of the fibers. Since dissolution provides an effective means of reducing the number or dose of the most biologically active fibers, it may not be too surprising that the model appears to provide good estimates of the effects of fibers regardless of their composition, other than as it relates

to the dissolution rate. For example, the model would suggest that respirable E glass fibers, which have a dissolution rate constant similar to RCF 1, would produce results similar to those observed for RCF 1 in an RCC-type inhalation study, if their doses and length and diameter distributions were similar. E glass is a glass fiber composition with electrical resistance properties that is used primarily in large, non-respirable diameter continuous filaments.

There are a number of precautions to be understood when interpreting the predictions of this model. First, it must be remembered that the model has been shown to have predictive value only for the specific species studied at these high doses in these specific kinds of experiments, for which a dose-response relation is available for durable fibers in the same bioassay. For different animals in a different apparatus, the durable fiber response would be different and it is difficult to predict how it would change.

Another caution about this model is that, while it predicts the results for fairly rapidly dissolving insulation wool fibers, it contributes little to the understanding of the carcinogenic properties of very durable fibers such as various forms of asbestos. This model predicts that all very durable fibers would yield the same tumor incidence at the same dose of the same size fibers. This is apparently not true for chrysotile and crocidolite in humans for example. It is possible that, in short-lived animal species like the rat, both chrysotile and crocidolite behave as though they are infinitely durable. However, in the much longer life span of humans, the increased dissolution rate of chrysotile compared to crocidolite may be significant. For fibers that are very durable compared to the lifespan of the study, there are likely other factors that contribute to respiratory disease besides the dissolution of long fibers. Such effects could be included in this model if they were sufficiently understood. The fact that the present model predicts lack of disease potential for rapidly dissolving fibers suggests that, when dissolution is appreciable over the life of the study, it dominates the fibrogenicity and carcinogenicity of the fibers.

It is interesting to note that this model works even though Eq. (4) assumed by it is an idealization of the actual dissolution process. There is evidence that some fibers may not dissolve completely uniformly or may dissolve in an incongruent fashion (Potter and Mattson, 1991), which could lead to fiber breakage. The model does not preclude these events, but rather suggests that the dissolution rate constant captures these possibilities and thereby serves as a predictor for biological activity.

It is significant that, since dissolution is a physical chemical process, it is likely that dissolution proceeds in rodents and humans at the same rate. Given the longer latency periods seen in disease induction in humans coupled with the longer lifespan, and lower metabolic rate, it is suggested that fibers which dissolve rapidly enough in the rodent lung so as not to induce disease in an inhalation study at MTD, pose no significant risk to humans from inhalation of the low levels of airborne fibers associated with the typical uses of insulation wools.

The mathematical model presented and tested here has been found to predict the diseases that occur in rats exposed to large doses of fibers both in inhalation and in intraperitoneal experiments. The model suggests that synthetic vitreous fibers with a dissolution rate constant of 100 ng/cm^2/hr or more would not result in fibrosis in a well-conducted, RCC-type animal inhalation study at MTD. To induce tumors, a fiber would have to be much more durable. This model provides a tool for the assessment of the potential health effects of untested fibers.

The idea that dissolution is an important aspect of the potential biological activity of fibers is certainly not new, being in the literature for at least twenty years (Stanton and Wrench, 1972). Until now, however, there has not been a means to incorporate dissolution into the estimation or prediction of biological activity of rapidly-dissolving fibers in a bioassay. It is probable that, as more data become available from additional inhalation studies, particularly studies involving different length preparations of the same fiber type, improvements to the model may be appropriate. The ability of the model to predict results of both inhalation and intraperitoneal studies suggests that the concept of dose adjustment based on dissolution rate is appropriate to the complex biological events leading to chronic effects in these studies.

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