

BIO PERSISTENCE OF SYNTHETIC MINERAL FIBERS AS A PREDICTOR OF CHRONIC INTRAPERITONEAL INJECTION TUMOR RESPONSE IN RATS

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In December 1997 the European Commission (EC) adopted Directive 97/69/EC (O.J. L 343/19 of 13 December 1997), in which criteria were established for the classification and labeling of synthetic mineral fibers. This directive was derived based upon an extensive program evaluating current scientific knowledge on fiber pathogenicity and its relationship to the biopersistence of long fibers. Within this context, the biopersistence of fibers longer than 20 μm was found to be a good predictor of the lung burden and early pathological changes in chronic inhalation studies with fibers as well as of the tumor response in chronic intraperitoneal studies with fibers. The analysis that provided the scientific basis for the relationship of biopersistence to the chronic intraperitoneal (ip) results is presented in detail. Analysis of the relationship of biopersistence clearance half-times to ip tumor response shows a statistically significant relationship of ip tumor response to not only the number of fibers injected, but also the median length of the fibers injected and their solubility (clearance half-time). The results show that the biopersistence half-times as determined by intratracheal instillation ($T_{1/2}$ of WHO fibers or weighted $T_{1/2}$ of fibers with $L > 20 \mu\text{m}$) and as determined by inhalation (weighted $T_{1/2}$ of fibers with $L > 20 \mu\text{m}$) are equivalent predictors of the ip results. From these ip studies, fibers that can be exonerated from classification as carcinogens in Europe have a relative tumorigenic potency in the ip cavity of between 66 and 2500 times less than fibers that have been shown to produce a significant increase in tumors following chronic inhalation exposure. In addition, based upon the ip results, there is no statistical difference between the EC and the other fiber exoneration criteria, such as the German Gefahrstoffverordnung of 1999.

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In December 1997 the European Commission (EC) adopted Directive 97/69/EC (O.J. L 343/19 of 13 December 1997, European Commission, 1997) in which criteria were established for the classification and labeling of synthetic mineral fibers. This directive was derived based upon an extensive program evaluating current scientific knowledge on fiber pathogenicity and its relationship to the biopersistence of long fibers. Within this context, the biopersistence of fibers longer than 20 μm was found to be a good predictor of the lung burden and early pathological changes in chronic inhalation studies with fibers as well as of the tumor response in chronic intraperitoneal studies with fibers. This article presents the background and scientific basis for the relationship of biopersistence to the chronic intraperitoneal injection tumor response. A parallel publication presents the basis for the relationship of biopersistence to the chronic inhalation results (Bernstein et al., 2001).

The mesothelioma-producing potential of asbestos fibers was first demonstrated in animals by the implantation/injection of fibers into the pleural cavity of rats (Wagner, 1963; Wagner & Berry, 1969). Subsequently, Stanton and Wrench (1972) showed by implantation in the pleural cavity and Pott and Friedrichs (1972) by injection in the peritoneal cavity that fiber shape was important and that fibers can produce tumors if they are sufficiently long, thin, and durable. In the years since, the intraperitoneal (ip) injection route was used more extensively than pleural implantation due to its relative simplicity. For the same reason that the ip test became of interest in that it could show the relationship of fibers to mesothelioma, it later became controversial in that many fibers that were not seen to be carcinogenic by chronic inhalation exposure were shown to be capable of producing tumors when injected into the peritoneal cavity (Bernstein, 1993; Muhle & Pott, 2000). There were two primary reasons for this controversy. First, in many early chronic inhalation studies, the importance of the fiber diameter and length was not fully considered. As a result, the diameters of many of the synthetic mineral fibers used were such that many of the fibers were not rat respirable, with the result that the animals were not well exposed. In addition, the stock fiber (of both asbestos and the synthetic mineral fibers) was often ground to reduce the size distribution from the commercial bulk, and in the process the length was reduced considerably to the point where there were few fibers longer than 20 μm in the aerosol. The second reason was that with the ip test the natural route of exposure is bypassed, as fibers are injected directly into the ip cavity and with this all the mechanisms by which the lung can remove, dissolve, or break fibers, thereby reducing or eliminating potential pleural cavity exposure are also bypassed. With the ip test, there is no physiologically imposed maximum concentration to which the animals can be exposed and therefore the ip test itself does not have an inherent limit that would provide an indication as to when a fiber is safe or should be

classified as a carcinogen (Eastes & Hadley, 1994). Only comparison with known carcinogens either from epidemiological studies or from well-performed chronic inhalation studies can provide a basis for assessing a safety margin.

This article develops the relationship between the biopersistence of synthetic mineral fibers as determined following either inhalation or intratracheal instillation exposure and the tumor response in the ip tests. In doing so, a basis is provided for the assessment of the ip test in distinguishing fibers of different solubilities.

More than 150 ip studies have been reported in the scientific literature (Fogel et al., 1998), providing a good basis for evaluating the relationship of biopersistence to ip tumorigenic response. Not only have these studies used a wide variety of different fiber types and solubilities, they also used a wide variety of different fiber doses and fiber dimensions. While associations have been reported between fiber dose injected and response, the fiber dimensions and in particular fiber length have not been systematically incorporated into a model relating biopersistence to ip response.

The associations that have been reported previously have used linear regression over ranges of fiber length (TRGS 905, 1994/1998). In our analysis we have compared linear regression to logistic regression and have found that in all cases logistic regression explained a notably greater percentage of the variance than linear regression. This should not be unexpected, as an analysis involving a continuous dependent variable, such as body weight, would generally require the use of a linear regression model. An analysis (as presented below) using individual-level data and having a dichotomous (two possible values: either a one or zero) dependent variable such as the presence or not of tumors generally requires the use of a logistic regression model.

METHODS

As presented in Bernstein et al. (2001), the concept of the biopersistence of long fibers being important to pulmonary response of fibers developed in parallel to the use of the ip test. While many authors put forth a relationship to solubility and sometimes to fiber dimension, as mentioned earlier, this was not performed systematically over a wide range of fiber types and lengths. The methods developed for assessing the biopersistence and the analysis of the clearance half-times are also presented in (Bernstein et al. (2001).

When examining the relationship of biopersistence to chronic ip effects, only those fibers that were included in the ECB biopersistence analysis and for which the complete data set were made available were considered (European Chemical Bureau, 1997a: ECB/TM/11(97)). Nine

fiber types were included with a total of 24 exposure groups. The range of the solubility of the fiber compositions extended from a relatively insoluble synthetic vitreous fiber (RCF) to one of the most soluble fibers (Bayer 01). Thus the ip data set provided a suitable range in fiber solubility for assessing relationships of chronic ip toxicity response to biopersistence. This range was similar to that used in the assessment of biopersistence to chronic inhalation toxicity (Bernstein et al., 2001).

The subset of fibers included in the analyses presented in this report were as follows: RCF 1 (Ceramic), MMVF 21 (Stone wool), MMVF 11, (Glass wool), MMVF 10 (Glass wool), MMVF 22 (Slag wool), Wollastonite, B01/09 (Bayer fiber), C (Soluble glass), and G (Soluble stone).

The full database of chronic ip studies in the rat was graciously provided by Charles Morscheidt of the St. Gobain Corporation and has been included in the report to the ECB (European Chemicals Bureau, 1997b: ECB/TM/15(97)). The subset used in this analysis is included in the Appendix. This database was found to be the only unified compilation of the numerous ip studies that have been performed. In these studies, the fibers injected were described; however, no systematic analysis of the number or size of fibers remaining in the ip cavity was performed.*

The ip chronic study database consists of studies on 70 different fiber names with many fibers investigated using multiple doses and includes both natural and synthetic mineral fibers. A total of 143 studies were included in the Morscheidt database, which ranged in solubility from asbestos to soluble synthetic mineral fibers.

Chronic Intraperitoneal Injection Studies

The data were analyzed using logistic regression (SAS Institute, Inc., 1989, 1989–1996). As previously indicated, ordinary (least squares) linear regression is not well suited to predict a binary outcome that is characteristic of the ip studies where there is either a tumor or no tumor. There are three problems with using the (least squares) linear regression model:

1. The error terms (e) are heteroskedastic (heteroskedasticity occurs when the variance of the dependent variable is different with different values of the independent variables): $\text{var}(e) = p(1 - p)$, where p is the probability that $\text{EVENT} = 1$. Since p depends on x ; the “classical regression assumption” that the error term does not depend on the x s is violated.
2. e is not normally distributed because x takes on only two values, violating another “classical regression assumption.”

*The majority of these studies were reported by Prof. Friedrich Pott (retired, Leverkusen, Germany) and his colleagues, and reprints of these articles and a compilation of the chemistry of the fibers tested were provided by Dr. Peter Wardenbach (Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, Dortmund, Germany).

3. The predicted probabilities can be greater than 1 or less than 0; which can be a problem if the predicted values are used in a subsequent analysis. Some people try to solve this problem by setting probabilities that are greater than (less than) 1 (0) to be equal to 1 (0). This amounts to an interpretation that a high probability of the event (nonevent) occurring is considered a sure thing.

The logistic regression model solves these problems:

$$p = \exp(b_0 + b_1 X) / [1 + \exp(b_0 + b_1 X)]$$

where p is the proportion at each value of the explanatory variable X , b_0 and b_1 are numerical constants to be estimated, and \exp is the exponential function.

The logistic regression model is simply a nonlinear transformation of the linear regression. The "logistic" distribution is an S-shaped distribution function that is similar to the standard normal distribution (which results in a probit regression model) but easier to work with in most applications (the probabilities are easier to calculate). The logit distribution constrains the estimated probabilities to lie between 0 and 1.

Logistic regression was therefore used to determine if the tumor response in the ip test could be predicted from:

- The number of fibers injected into the ip cavity.
- The median length of the fibers injected.
- The biopersistence clearance half-times (inhalation and intratracheal instillation).

Table 1 lists the analyses that were performed.

The goodness of fit of each set of predictors was measured in two ways. First, a chi-square variable for covariates was calculated. This variable is the difference between twice the negative logarithm of the likelihood ($-2 \log L$) in the model with and without predictors. Asymptotically, it follows a χ^2 distribution with three degrees of freedom, and it measures the importance of the set of predictors for the prediction of the response variable.

As a second method, two analogues to the R^2 of linear regression were calculated, one for grouped or mean values and the other for individual values of tumor response. These analogues are defined as follows:

R^2 Grouped Assume we have observations i , $1 \leq i \leq n$, where observation i is from a group of rats receiving the same fibers in the same dose. Also assume that in observation i we have observed a percentage of tumors y_i , while the model predicted a probability of tumors π_i . Then R^2 is defined as

TABLE 1. Analyses performed

Dependent variable		Set of predictors	
ip Tumor response	ln(Fiber number injected)	Median fiber length	Biopersistence half-time (days), IT WHO fibers
ip Tumor response	ln(Fiber number injected)	Median fiber length	Biopersistence half-time (days), IT W- $T_{1/2}$ of fibers with $L > 20 \mu\text{m}$
ip Tumor response	ln(Fiber number injected)	Median fiber length	Biopersistence half-time (days), INH W- $T_{1/2}$ of fibers with $L > 20 \mu\text{m}$
ip Tumor response	ln(Fiber number injected)	Median fiber length	Biopersistence half-time (days), INH W- $T_{1/2}$ of slow-phase fibers with $L > 20 \mu\text{m}$

$$R^2 = 1 - \frac{\sum(y_i - \pi_i)^2}{\sum(y_i - \bar{y})^2}$$

With this definition of R^2 , we usually get larger values of R^2 than with the R^2 individual, which is defined next.

R^2 Individual Here each individual rat constitutes an observation. This means that y_i is either 0 or 1, depending on whether this individual rat as a tumor or not. Assume the model predicted a probability of tumors π_i for observation i . Then R^2 is defined as

$$R^2 = 1 - \frac{\sum(y_i - \pi_i)^2}{\sum(y_i - \bar{y})^2}$$

Note that R^2 can never be 1, because the difference $y_i - \pi_i$ will either be $-\pi_i$ or $1 - \pi_i$, both of which are generally not 0. With this definition of R^2 , we usually get smaller values of R^2 , especially if there are observations with intermediate π_i . This would hold even if the model could predict the probabilities perfectly well.

RESULTS

The median length of the fibers ranged from 1.4 to 20 μm . While the actual length distributions were not available, summary information on some of the fibers indicated that fibers considerably longer were often present (BIA, 1998). The fiber number injected ranged from 30,000 to 20,000,000,000. The distribution of the fiber number injected over the range of studies approximated a lognormal distribution, and as such a log transformation was used in the analysis. The data used in these analyses are included in the table in the Appendix.

The logistic function used in this analysis was:

$$\text{ip tumor response} = \frac{\exp\{b_0 + b_1 \times \text{Length} + b_2[\ln(\text{NoFib})] + b_3 T_{1/2}\}}{1 + (\exp\{b_0 + b_1 \times \text{Length} + b_2[\ln(\text{NoFib})] + b_3 T_{1/2}\})}$$

where Length is the median length of the fibers injected (μm), $\ln(\text{NoFib})$ is the natural logarithm of the number of fibers injected, and $T_{1/2}$ is the clearance half-time in days.

Association of Intratracheal Instillation Biopersistence and Intraperitoneal Tumorigenic Response

The results from the logistic regression using as predictors of the ip tumor response the median fiber length, the natural logarithm (\ln) of the number of fibers injected, and the intratracheal instillation biopersistence clearance half-times for the WHO fibers and the fibers longer than 20 μm are shown in Tables 2 and 3, respectively. Unfortunately, the intratracheal

TABLE 2. Prediction of percent tumors with fiber number injected, median fiber length, and IT-WT_{1/2}/WHO

A. Model fitting information and testing global null hypothesis beta = 0								
Criterion	Intercept only	Intercept and covariates	Chi-square for covariates					
AIC	1066.125	634.725						
SC	1070.968	654.096						
-2 LOG L Score	1064.125	626.725	437.400 with df ($p = .0001$)					
			428.273 with df ($p = .0001$)					
B. Analysis of maximum likelihood estimates								
Variable	df	Parameter estimate	Standard error	Wald chi-square	Pr > chi-square	Standardized estimate	Odds ratio	t Statistic
INTERCPT	1	-9.2759	1.0204	82.64	.0001			
Length	1	0.1443	0.0425	11.5119	.0007	0.303427	1.155	3.40
ln(FIBNO)	1	0.4043	0.058	48.5198	.0001	0.674705	1.498	6.97
IT-WT _{1/2} /WHO	1	0.0121	0.00162	55.5164	.0001	0.623797	1.012	7.47
Chi-square				R^2_{Grouped}		$R^2_{\text{Individual}}$		
437.4				.901		.493		
C. Association of predicted probabilities and observed responses								
Concordant = 88.90%					Somers D = 0.8			
Discordant = 9.00%					Gamma = 0.817			
Tied = 2.10%					Tau-a = 0.304			
(166,822 pairs)					c = 0.9			

TABLE 3. Prediction of percent tumors with fiber number injected, median fiber length, and IT-WT_{1/2} of fiber with $L > 20 \mu\text{m}$

A. Model fitting information and testing global null hypothesis $\beta = 0$								
Criterion	Intercept only	Intercept and covariates	Chi-square for covariates					
AIC	1066.125	645.587						
SC	1070.968	664.958						
-2 LOG L	1064.125	637.587	426.538 with 3 df ($p = .0001$)					
Score			412.634 with 3 df ($p = .0001$)					
B. Analysis of maximum likelihood estimates								
Variable	Parameter df	Parameter estimate	Standard error	Wald Chi-square	Pr > Chi-square	Standardized estimate	Odds ratio	t Statistic
INTERCPT	1	-9.4705	1.0118	87.6149	.0001			
Length	1	0.1874	0.0425	19.486	.0001	0.394035	1.206	4.41
ln(FIBNO)	1	0.4034	0.0558	52.1791	.0001	0.673301	1.49	7.23
IT-WT _{1/2} L20	1	0.0151	0.00235	41.2019	.0001	0.554401	1.015	6.43
Chi-square				R^2_{Grouped}		$R^2_{\text{Individual}}$		
426.5				.875		.483		
C. Association of predicted probabilities and observed responses								
Concordant = 89.1%				Somers D = 0.802				
Discordant = 8.8%				Gamma = 0.82				
Tied = 2.1%				Tau-a = 0.305				
(166,822 pairs)				c = 0.901				

half times were available only for part of the data. Therefore, the analysis in Tables 2 and 3 is based on only nine fiber types.

The slow phase of the intratracheal (IT) clearance half-times for both the WHO fibers and the fibers longer than $20 \mu\text{m}$ were also examined and provided approximately the same fit to the data and hence were not included in detail.

The t statistic, which is the ratio of the parameter estimate divided by the standard error (see Tables 2 to 3), provides an indication of the relative importance of each of the predictors. The t statistic is the square root of the Wald chi-square, and the p values from the Wald chi-square hold for the t statistic as well. For the prediction that includes the IT-WT_{1/2}WHO, the clearance half-time and the number of fibers are nearly of the same importance with the length being slightly less.

The $R^2_{\text{individual}}$ as defined earlier for these variables to predict the ip tumor response was 0.493 and the R^2_{Grouped} was .901 using the IT-WT_{1/2}WHO

clearance half times. When using the IT-WT_{1/2}L20 clearance half-times, the $R^2_{\text{Individual}}$ was .483 and the R^2_{Grouped} was .875. This indicates that both sets of predictors are rather similar. As said before, the fact that the $R^2_{\text{Individual}}$ values are relatively small does not indicate that the model does not fit well. The chi-square for covariates are 437.4 and 426.5, respectively. At three degrees of freedom this indicates a highly significant influence of the sets of covariables (p value .0001).

The prediction in the logistic model uses a linear combination of the predictor variables, and transforms this linear combination into a prediction of proportions by the use of the logistic curve. Therefore, a graph for the prediction based upon these three predictors can be produced as shown in Figures 1 and 2, where the observed proportion of rats with tumors is

**The Predicted Versus the Observed Proportion of Tumours
(Fiber Number Injected, Median Fiber Length and IT-WT_{1/2}WHO)**

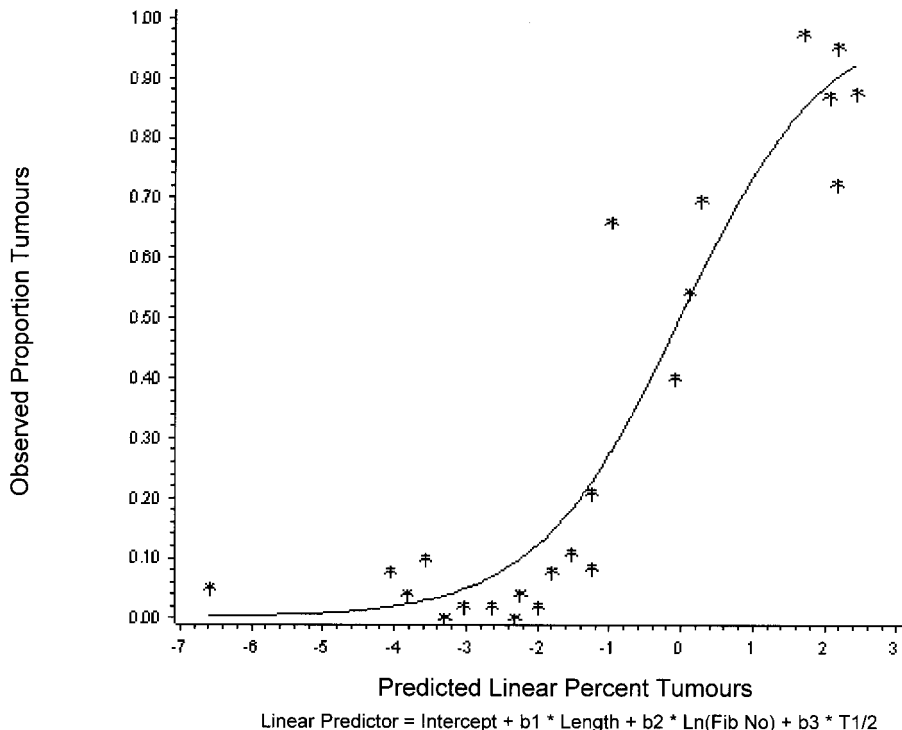


FIGURE 1. Graph of the prediction using logistic regression of the percent tumors in the chronic ip studies with fiber number injected into the ip cavity, the median fiber length of the fibers injected, and the weighted $T_{1/2}$ of the WHO fibers as determined by intratracheal instillation biopersistence studies. Plotted are the observed proportion of rats with tumors against the linear combination of the predictor variable that is used to calculate the predictions (Linear predictor = intercept + $b_1 \times$ Length + $b_2 \ln(\text{Fib No}) + b_3 T_{1/2}$). The chi-squared value of 437.4 indicates a highly significant influence of the sets of covariables (p value .0001, 3 degrees of freedom).

**The Predicted Versus the Observed Proportion of Tumours
(Fiber Number Injected, Median Fiber Length and IT-WT_{1/2} L>20 μm)**

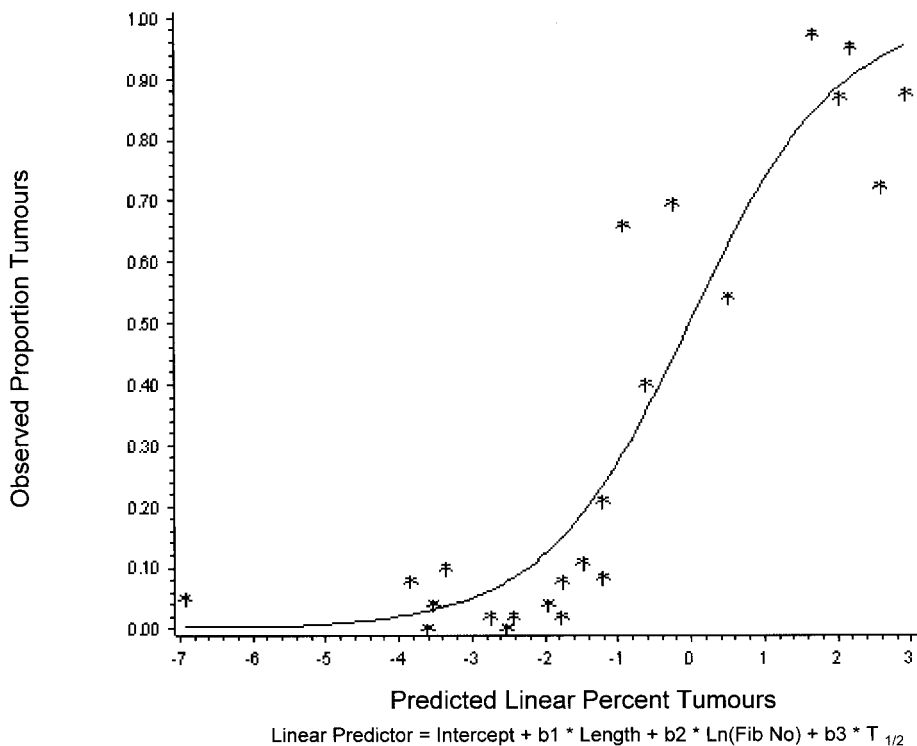


FIGURE 2. Graph of the prediction using logistic regression of the proportion of tumors in the chronic ip studies with fiber number injected into the ip cavity, the median fiber length of the fibers injected, and the weighted $T_{1/2}$ of the fibers with $L > 20 \mu\text{m}$ as determined by intratracheal instillation biopersistence studies. Plotted are the observed proportion of rats with tumors against the linear combination of the predictor variable that is used to calculate the predictions (Linear predictor = intercept + $b_1 \times \text{Length} + b_2 [\ln(\text{Fib No})] + b_3 T_{1/2}$). The chi-squared value of 426.5 indicates a highly significant influence of the sets of covariables (p value .0001, 3 degrees of freedom).

plotted against the linear combination of the predictor variable that is used to calculate the predictions (Linear predictor = intercept + $b_1 \times \text{Length} + b_2 [\ln(\text{Fib No})] + b_3 T_{1/2}$).

Association Between Inhalation Biopersistence and Intraperitoneal Tumorigenic Response

Similarly, the results from the logistic regression using as predictors of the ip tumor response the median fiber length, the natural logarithm of the number of fibers injected, and the inhalation biopersistence clearance half-times for the weighted $T_{1/2}$ of fibers with $L > 20 \mu\text{m}$ and the slow-phase $T_{1/2}$ of fibers longer than $20 \mu\text{m}$ are shown in Tables 4 and 5. Here

the half-times were available for all fiber types in the study. Therefore, the analysis in Tables 4 and 5 is based on a larger set of observations.

The *t* statistic (see Tables 4 and 5), which provides an indication of the relative importance of each of the predictors, indicates that the inhalation biopersistence half-times are the most important predictor, followed by the fiber number injected and length.

The *R*² as defined earlier for these variables to predict the ip tumor response was .311 (.750 grouped *R*²) using the inhalation biopersistence

TABLE 4. Prediction of percent tumors with fiber number injected, median fiber length, and INH-WT_{1/2} of fibers with *L* > 20 μm (using the complete data set)

A. Model fitting information and testing global null hypothesis beta = 0								
Criterion	Intercept only	Intercept and covariates	Chi-square for covariates					
AIC	3137.72	2309.494						
SC	3143.602	2333.024						
-2 LOG L Score	3135.72	2301.494	834.226 with 3 df (<i>p</i> = .0001)					
			711.630 with 3 df (<i>p</i> = .0001)					
B. Analysis of maximum likelihood estimates								
Variable	Parameter df	Parameter estimate	Standard error	Wald chi-square	Pr > chi-square	Standardized estimate	Odds ratio	<i>t</i> Statistic
INTERCPT	1	-9.5276	0.4983	365.5925	.0001			
Length	1	0.1386	0.0134	107.6989	.0001	0.341191	1.149	10.34
Ln(FIBNO)	1	0.4369	0.0322	183.6162	.0001	0.536425	1.548	13.57
INH-WT _{1/2} L20	1	0.0526	0.00251	438.5108	.0001	0.80697	1.054	20.96
		Chi-square		<i>R</i> ² _{Grouped}		<i>R</i> ² _{Individual}		
		834.2		.750		.311		
C. Association of predicted probabilities and observed responses								
Concordant = 83.30%				Somers D = 0.679				
Discordant = 15.40%				Gamma = 0.688				
Tied = 1.30%				Tau-a = 0.273				
(1,411,794 pairs)				c = 0.84				
D. Chi-squared and <i>R</i> ² values for percent tumors with fiber number injected, median fiber length, and INH-WT _{1/2} of fibers with <i>L</i> > 20 μm (using only the same fiber data that were available for the intratracheal instillation analyses)								
		Chi-square		<i>R</i> ² _{Grouped}		<i>R</i> ² _{Individual}		
		428.0		.879		.483		

TABLE 5. Prediction of percent tumors with fiber number injected, median fiber length, and INH "slow-phase" $T_{1/2}$ of fibers with $L > 20 \mu\text{m}$

A. Model fitting information and testing global null hypothesis $\beta = 0$								
Criterion	Intercept only	Intercept and covariates	Chi-square for covariates					
AIC	3137.72	2376.402						
SC	3143.602	2399.933						
-2 LOG L Score	3135.72	2368.402	767.317 with 3 df ($p = .0001$)					
			607.680 with 3 df ($p = .0001$)					
B. Analysis of maximum likelihood estimates								
Variable	Parameter df	Parameter estimate	Standard error	Wald chi-square	Pr > chi-square	Standardized estimate	Odds ratio	t Statistic
INTERCPT	1	-11.1182	0.5525	404.9137	.0001			
Length	1	0.2025	0.0132	235.9392	.0001	0.498452	1.224	15.34
Ln(FIBNO)	1	0.4993	0.0344	210.7201	.0001	0.612994	1.648	14.51
INH-SF $T_{1/2}$ L20	1	0.0225	0.00107	438.208	.0001	0.855646	1.023	21.03
Chi-square				R^2_{Grouped}		$R^2_{\text{Individual}}$		
767.3				.720		.296		
C. Association of predicted probabilities and observed responses								
Concordant = 82.40%				Somers D = 0.662				
Discordant = 16.20%				Gamma = 0.672				
Tied = 1.40%				Tau-a = 0.266				
(1,411,794 pairs)				c = 0.831				
D. Chi-squared and R^2 values for percent tumors with fiber number injected, median fiber length, and INH "slow phase" $T_{1/2}$ of fibers with $L > 20 \mu\text{m}$ (using only the same fiber data that were available for the intratracheal instillation analyses)								
Chi-square				R^2_{Grouped}		$R^2_{\text{Individual}}$		
400.2				.860		.471		

weighted clearance half-time (IH-WT $_{1/2}$ L20) and .296 (.720 grouped R^2) using the inhalation biopersistence slow-clearance half-time (IH-LGT $_{1/2}$ L20). As shown in the Discussion, the lower R^2 for the IH half-times does not show that the prediction with IH half-times is worse than with ip half-times. This is illustrated in Tables 4D and 5D, where only the subset of data available for the IT biopersistence comparison to the ip results was used and similar chi-squared and R^2 values were obtained. It also should be noted

that the chi-square value of 834.2 for IH-WT_{1/2}L20 and that of 767.3 for IH-LGT_{1/2}L20, at 3 degrees of freedom, show a very highly significant influence of the predictors.

The prediction in the logistic model uses a linear combination of the predictor variables, and transforms this linear combination into a prediction of proportions by the use of the logistic curve. Therefore, a graph for the prediction based upon these three predictors can be produced as shown in Figures 3 and 4, where the observed proportion of rats with tumors is plotted against the linear combination of the predictor variable that is used to calculate the predictions (Linear predictor = intercept + $b_1 \times \text{Length} + b_2 [\ln(\text{Fib No})] + b_3 T_{1/2}$).

**The Predicted Versus the Observed Proportion of Tumours
(Fiber Number Injected, Median Fiber Length and INH-WT_{1/2} L>20 μm)**

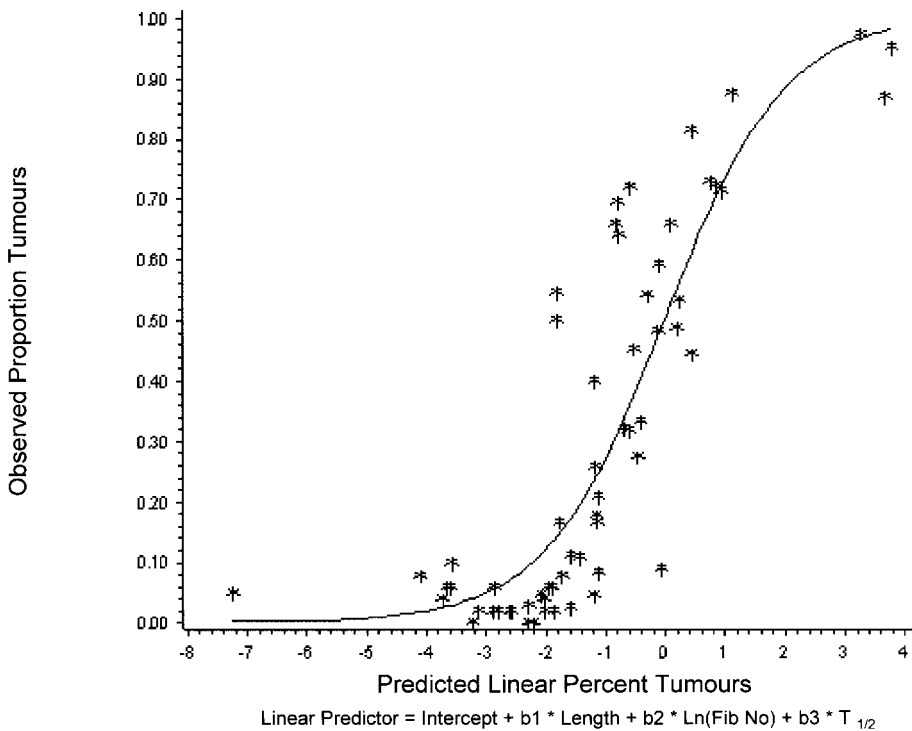


FIGURE 3. Graph of the prediction using logistic regression of the proportion of tumors in the chronic ip studies with fiber number injected into the ip cavity, the median fiber length of the fibers injected, and the weighted $T_{1/2}$ of the fibers with $L > 20 \mu\text{m}$ as determined by inhalation biopersistence studies. Plotted are the observed proportion of rats with tumors against the linear combination of the predictor variable that is used to calculate the predictions (Linear predictor = intercept + $b_1 \times \text{Length} + b_2 [\ln(\text{Fib No})] + b_3 T_{1/2}$). The chi-squared value of 834.2 indicates a highly significant influence of the sets of covariables (p value .0001, 3 degrees of freedom).

**The Predicted Versus the Observed Proportion of Tumours
(Fiber Number Injected, Median Fiber Length and INH-SFT_{1/2} L20)**

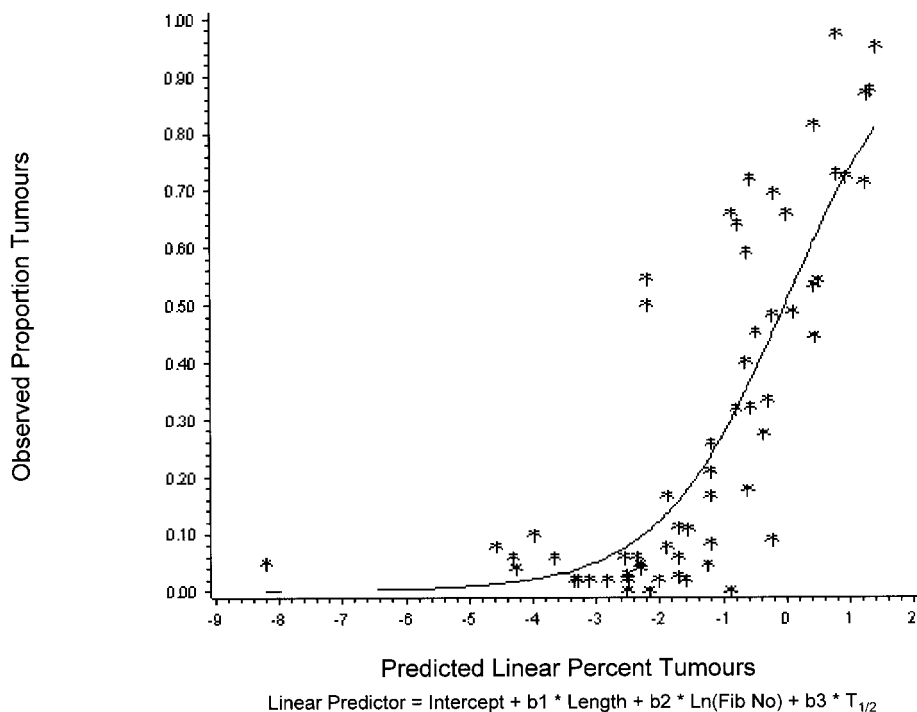


FIGURE 4. Graph of the prediction using logistic regression of the proportion of tumors in the chronic ip studies with fiber number injected into the ip cavity, the median fiber length of the fibers injected, and the “slow-phase” $T_{1/2}$ of the fibers with $L > 20 \mu\text{m}$ as determined by inhalation biopersistence studies. Plotted are the observed proportion of rats with tumors against the linear combination of the predictor variable that is used to calculate the predictions (Linear predictor = intercept + $b_1 \times$ Length + $b_2 \ln(\text{Fib No}) + b_3 T_{1/2}$). The chi-squared value of 767.3 indicates a highly significant influence of the sets of covariables (p value .0001, 3 degrees of freedom).

TABLE 6. Performance of some predictors when used on the part of the data where all variables were available

Set of predictors	Chi-square ^a	R^2_{Grouped}	$R^2_{\text{Individual}}$
ln(Fiber no.), length, IT-WT _{1/2} WHO	437.4	.901	.494
ln(Fiber no.), length, IT-WT _{1/2} L20	426.5	.875	.483
ln(Fiber no.), length, INH-WT _{1/2} L20	428.0	.879	.483
ln(Fiber no.), length, INH-SFT _{1/2} L2	400.2	.860	.471

^aThe chi-square values are all highly significant; however, they are not statistically different from one another.

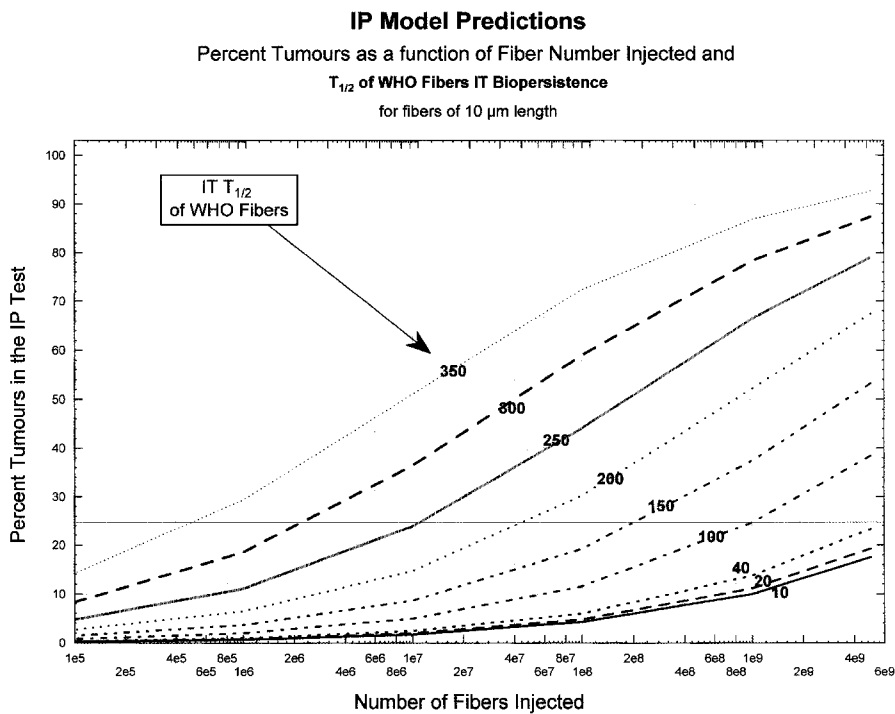


FIGURE 5. Using the derived logistic regression functions, each graph shows the relationship of the $T_{1/2}$ of WHO fibers as determined by IT biopersistence, to IP tumor response if the fibers injected in each experiment were all of the same length (10 μm). A line has been drawn through the 25% IP tumor response level (TD25), which has been used previously for comparative purposes (TRGS 906). The 10- μm length was chosen based on the mean length for the fibers in the IP studies in these analyses, which was 8.2 μm for those studies included in the inhalation biopersistence and 12 μm for those studies included in the IT biopersistence analyses.

DISCUSSION

The results from the logistic regression analysis show that the combination of the median fiber length, the natural log of the number of fibers injected, and the biopersistence clearance half-time provide a good prediction of the number of tumors that will occur in the ip test.

To make a comparison possible, we have repeated the analysis with the inhalation half-times reported in Tables 4 and 5 for the subset of fiber types for which all half times were available (summary of predictors presented in Table 4D and 5D). Table 6 summarizes the three indicators of goodness of fit for the models for this subset of the observations. It shows that there is not much difference between using the different biopersistence half-times as predictors in conjunction with the length and number of fibers injected. The first set [$\ln(\text{Fiber no.})$, Length, and $\text{IT-WT}_{1/2}$ WHO] appears to be slightly better, but this is well within the range of random fluctuations. The fact that the R^2 values are better for the reduced data

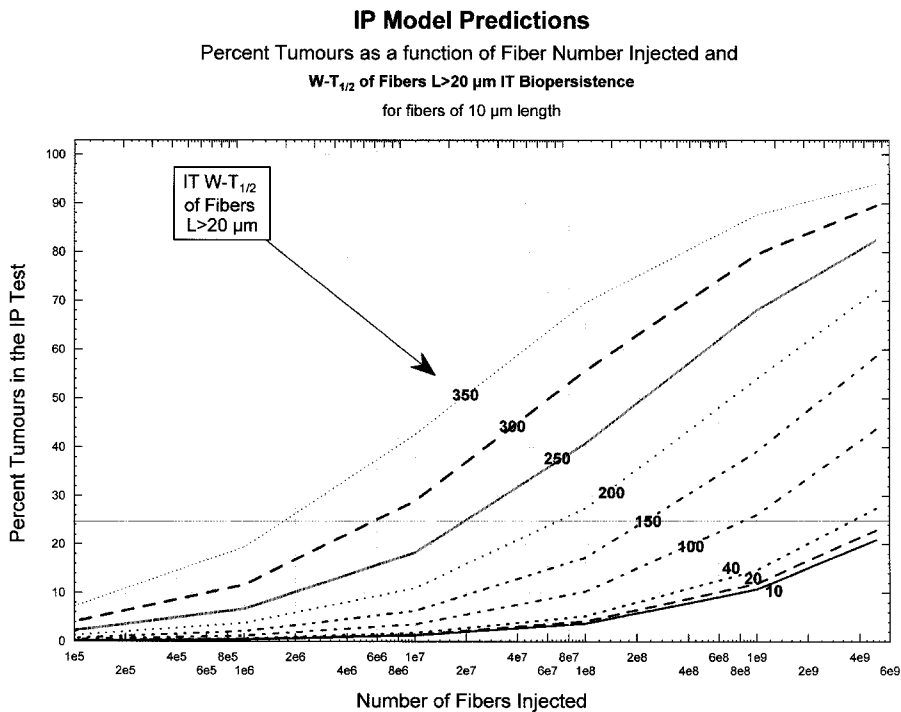


FIGURE 6. The graph shows the relationship as in Figure 5, but for weighted $T_{1/2}$ of fibers with $L > 20 \mu\text{m}$ as determined by IT biopersistence.

set for the inhalation half-times (Tables 4D and 5D) than for the larger data set (Tables 4 and 5, A–C) does not imply that the additional fiber types are harder to predict. The lower R^2 can be explained by the fact that the additional fiber types give more intermediate predictions between 0 and 1.

As has been shown in the preceding logistic regression analysis, not only does fiber number injected influence the ip tumor response, but also the length of the fibers injected and the biopersistence of the fibers also have a significant effect on tumor response. Accordingly, comparison of the relative difference in ip potency of different compositions (solubilities) of fibers requires that the length of the fibers injected be standardized.

In the following comparisons of the relative potency of different fiber types, the relationship of biopersistence to ip tumor response was examined for $10\text{-}\mu\text{m}$ fibers using the logistic regression functions derived above. The $10\text{-}\mu\text{m}$ length was chosen based on the mean length for the fibers in the ip studies in these analyses, which was $8.2 \mu\text{m}$ for those studies included in the inhalation biopersistence and $12 \mu\text{m}$ for those studies included in the IT biopersistence analyses. Figures 5 to 8 illustrate what the relationship of each biopersistence half-time type (inhalation, intratracheal instillation, etc.) to ip tumor response would be if the fibers injected in each experiment were all of the same length ($10 \mu\text{m}$). A line has been drawn through

the 25% ip tumor response level (TD25), which has been used previously for comparative purposes (TRGS 906).

As mentioned earlier, there is no inherent threshold in the ip test that can be used to exonerate fibers from their pathological response, as any fiber can be shown to be carcinogenic by increasing the fiber dose injected. In TRGS 906, it was reported that a technical limit was encountered of not being able to inject more than 1.5 g of fiber into the ip cavity. This limit was not, however, related to inherent pathological response to this quantity of fiber. With this limit, one of the most soluble fibers known, the Bayer 01/09, was shown to produce tumors when 1×10^{10} fibers were injected. Thus, the only way to evaluate the relative potency of different fibers in the ip test is either based upon those fibers that have been shown using epidemiological studies to be human carcinogens or based upon fibers that have been shown to produce tumors in inhalation carcinogenicity studies in animals.

When considering human epidemiological studies, the only fibers that have been shown to clearly produce mesothelioma have been the amphibole asbestos fibers and erionite (Hodgson & Darnton, 2000; Barris et al., 1987). The clearance half-time of the amphibole asbestos, amosite, from an inhalation biopersistence study has been reported as $W-T_{1/2} = 460$ days (Hesterberg et al., 1998). From Figure 7, the difference in TD25 between amosite and fibers which are exonerated by the EC ($W-T_{1/2} = 10$ days) would be greater than 1,000,000.

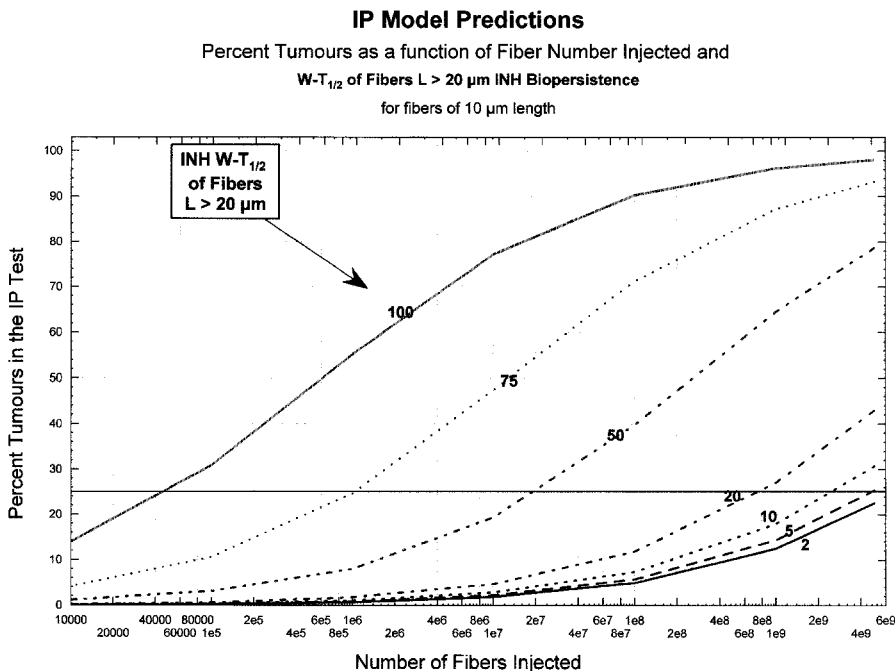


FIGURE 7. The graph shows the relationship as in Figure 5, but for the weighted $T_{1/2}$ of fibers with $L > 20 \mu\text{m}$ as determined by inhalation biopersistence.

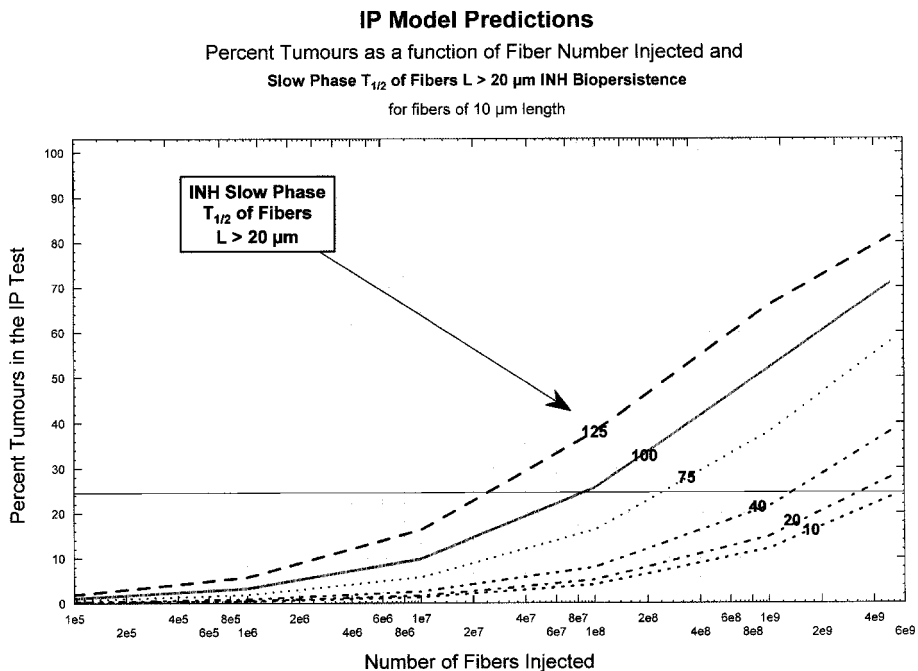


FIGURE 8. The graph shows the relationship as in Figure 5, but for the slow-phase $T_{1/2}$ of fibers with $L > 20 \mu\text{m}$ as determined by inhalation biopersistence.

Comparison can also be made based upon fibers shown to produce a significant level of tumors compared to air treated controls in animal inhalation toxicology studies. In such inhalation studies, RCF 1 and E-glass have been shown to produce a statistically significant elevated tumor level in the high-dose exposure groups (Mast et al., 1995; Cullen et al., 2000).

Table 7 summarizes the TD25 values for the three criteria currently used in Europe for the exoneration of fibers as carcinogens. Two of these criteria are specified by EC Directive 97/69, and the third is specified by the current German ordinance on fibers (Gefahrstoffverordnung, 1999). As Table 7 is derived from Figures 5–8, the fiber length was fixed to $10 \mu\text{m}$ as described. For comparison, the clearance half-times for RCF 1 and E-glass are also presented for each type of biopersistence half-time (intratracheal instillation $T_{1/2}$ of WHO fibers; intratracheal instillation $W-T_{1/2}$ of fibers with $L > 20 \mu\text{m}$; and inhalation $W-T_{1/2}$ of fibers with $L > 20 \mu\text{m}$).

From Table 7, it is clear that each of these biopersistence half-times predicts a similar number of fibers for producing a TD25 ranging from 3.4 to 5×10^9 fibers (these values are well within the statistical variation of the data). Thus, based upon the ip results, the three criteria are not statistically different. Accordingly, the German regulation does not improve worker protection over that of the EC regulation (as has been sometimes stated).

The ratio of the TD25 for each exoneration criteria to the TD25 for RCF 1 and E-glass ranges from 2500 to 250 using the German ordinance cri-

teria for IT $T_{1/2}$ of WHO fibers; 100 to 66 using the EC IT W- $T_{1/2}$ of fibers with $L > 20 \mu\text{m}$; and 85 to 1100 using the EC INH W- $T_{1/2}$ of fibers with $L > 20 \mu\text{m}$. These differences are not considered to be statistically different (considering the standard errors of the parameter estimates, Tables 2–5) and most likely reflect the variation in the respective studies.

From these ip studies, fibers that can be exonerated from classification as carcinogens in Europe have a relative tumorigenic potency in the ip cavity of between 66 and 2500 times less than fibers that have been shown to produce a significant increase in tumors following chronic inhalation exposure.

CONCLUSIONS

Analysis of the relationship of biopersistence clearance half-times to ip tumor response shows a statistically significant relationship of ip tumor

TABLE 7. Comparison of the TD 25

Criteria	Corresponding half-times (days)	ip Model using length = 10 μm , number of fibers injected and the $T_{1/2}$ as specified		
		IT $T_{1/2}$ of WHO fibers	IT W- $T_{1/2}$ of fibers $L > 20 \mu\text{m}$	INH W- $T_{1/2}$ of fibers $L > 20 \mu\text{m}$
EC exoneration: inhalation biopersistence, fibers $L > 20$	10 (INH W- $T_{1/2}$ $L > 20$)	—	—	3,400,000,000
EC exoneration: intratracheal biopersistence, fibers $L > 20$	40 (IT W- $T_{1/2}$ $L > 20$)	—	4,000,000,000	—
German exoneration: intratracheal biopersistence WHO fibers	40 (IT $T_{1/2}$ WHO)	5,000,000,000	—	—
RCF 1	40 (INH W- $T_{1/2}$ $L > 20$)	—	—	40,000,000
RCF 1	230 (IT W- $T_{1/2}$ $L > 20$)	—	40,000,000	—
RCF 1	300 (IT $T_{1/2}$ WHO)	2,000,000	—	—
E-glass	68 (INH W- $T_{1/2}$ $L > 20$)	—	—	3,000,000
E-glass ^a	218 (IT W- $T_{1/2}$ $L > 20$)	—	60,000,000	—
E-glass ^a	226 (IT $T_{1/2}$ WHO)	20,000,000	—	—

^aE-glass IT half-times from Bellmann and Muhle (1995), pp. 28–29.

response to not only the number of fibers injected, but also the median length of the fibers injected and their solubility (clearance half-time). The results show that the biopersistence half-times as determined by intratracheal instillation ($T_{1/2}$ of WHO fibers or weighted $T_{1/2}$ of fibers with $L > 20 \mu\text{m}$) and as determined by inhalation (weighted $T_{1/2}$ of fibers with $L > 20 \mu\text{m}$) are equivalent predictors of the ip results. From these ip studies, fibers that can be exonerated from classification as carcinogens in Europe have a relative tumorigenic potency in the ip cavity of between 66 and 2500 times less than fibers that have been shown to produce a significant increase in tumors following chronic inhalation exposure. In addition, based upon the ip results, there is no statistical difference between the EC and other fiber exoneration criteria (e.g., the German Gefahrstoffverordnung, 1999).

REFERENCES

- Baris, I., Simonato, L., Artvinli, M., Pooley, F., Saracci, R., Skidmore, J., and Wagner, C. 1987. Epidemiological and environmental evidence of the health effects of exposure to erionite fibres: a four-year study in the Cappadocian region of Turkey. *Int. J. Cancer* 39(1):10-17.
- Bellmann, B., and Muhle, H. 1995. Biobeständigkeit verschiedener Mineralfasertypen in der Rattenlunge nach intratrachealer Applikation, Bundesanstalt für Arbeitsschutz, 44061 Dortmund, Druck und Verlag: Wirtschaftsverlag NW, Bremerhaven, Germany. ISBN 3-89429-567-8.
- Bernstein, D. M. 1993. An evaluation of the use of an inhalation model versus intraperitoneal injection model for the assessment in rats of the carcinogenicity of natural and man-made vitreous fibers. *Zentralbl. Arbeitsmed.* 43(4):120-128.
- Bernstein, D. M., Riego-Sintes, J. M., Ersboell, B. K., and Kunert, J. 2001. Biopersistence of synthetic mineral fibers as a predictor of chronic inhalation toxicity in rats. *Inhal. Toxicol.* 13(10):823-849.
- BIA. 1998. Report 2/1998. Fasern—Tests zur Abschätzung der Biobeständigkeit und zum Verstaubungsverhalten, Muhle, H., Sebastian, K., Nies, E. HVBR, Sankt Augustin, Germany, www.hvbr.de, ISBN 3-88383-462-9.
- Cullen, R. T., Searl, A., Buchanan, D., Davis, J. M. G., Miller, B. G., and Jones, A. D. 2000. Pathogenicity of a special-purpose glass microfiber (E glass) relative to another glass microfiber and amosite asbestos. *Inhal. Toxicol.* 12(10):977.
- Eastes, W., and Hadley, J. G. 1994. Role of fiber dissolution in biological activity in rats. *Regul. Toxicol. Pharmacol.* 20:104-112.
- European Chemicals Bureau. 1997a. ECB/TM/11(97), 4 March 1997. Report by David M. Bernstein, Data Analysis of IT and INH Biopersistence Data, submitted to the Joint Research Centre, Environment Institute, European Chemicals Bureau, Ispra, Italy.
- European Chemicals Bureau. 1997b. ECB/TM/15(97), 11 June 1997. Report by David M. Bernstein, Correlation Between Short Term Biopersistence and Chronic Toxicity Studies, submitted to the Joint Research Centre, Environment Institute, European Chemicals Bureau, Ispra, Italy.
- European Commission. 1997. O.J. L 343/19 of 13 December 1997. Commission Directive 97/69/EC of 5 December 1997 adapting to technical progress for the 23rd time Council Directive 67/548/EEC on the approximation of the laws regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.
- Fogel, P., Morscheidt, C., Hanton, D., and de Meringo, A. 1998. A formula for predicting the tumor incidence in intraperitoneal experiments with mineral fibers. *Inhal. Toxicol.* 10(9):875-893.
- Gefahrstoffverordnung. 1999. GefStoffV, Künstliche Mineralfasern, Verordnung zum Schutz vor gefährlichen Stoffen, vom 15. November 1999, Neufassung 1999: BGBl. I S. 2233, Anhang V Nr. 7, ab 1.10. 2000: statt 65 Tage gilt 40 Tage. Hesterberg, T. W., Chase, G., Axten, C., Miller, W. C., Musselman, R. P., Kamstrup, O., Hadley, J., Morscheidt, C., Bernstein, D., and Thevenaz, P. 1998. Biopersistence of synthetic vitreous fibers and amosite asbestos in the rat lung following inhalation. *Toxicol. Appl. Pharmacol.* 151(2):262-275.

- Hodgson, J. T., and Darnton, A. 2000. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. *Ann. Occup. Hyg.* 44(8):565–601.
- Jones, A. D., Miller, B. G., Cullen, R. T., Searl, A., Davis, J. M. G., Buchanan, D., Donaldson, K., Soutar, C. A., and Bolton, R. E. 1997. The Colt fibre research programme: aspects of toxicological risk assessment. *Ann. Occup. Hyg.* 41(suppl 1):244–250.
- Lambré, C., Schorsch, F., Blanchard, O., Richard, J., Boivin, J. C., Hanton, D., Grimm, H.-G., and Morscheidt, C. 1998. An evaluation of the carcinogenic potential of five man-made vitreous fibers using the IP test. *Inhal. Toxicol.* 10:995–1021.
- Mast, R. W., McConnell, E. E., Anderson, R., Chevalier, J., Kotin, P., Bernstein, D. M., Thevenaz, P., Glass, L. R., Müller, W. C., and Hesterberg, T. W. 1995. Studies on the chronic toxicity (inhalation) of four types of refractory ceramic fiber in male Fischer 344 rats. *Inhal. Toxicol.* 7(4):425–467.
- Muhle, H., and Pott, F. 1991. Faserige Stäube—tierexperimentelle Ergebnisse (Fibrous dusts—Results of studies in experimental animals). *Krebserzeugende Stoffe in der Umwelt* Düsseldorf: VDI-Verlag, 1991, (VDI-Berichte 888). ISBN 3-18-090888-2, S.273–292.
- Muhle, H., and Pott, F. 2000. Asbestos as reference material for fibre-induced cancer. *Int. Arch. Occup. Environ. Health* 73(suppl.):S53–S59.
- Pott, F., and Friedrichs, K. H. 1972. [Tumors in the rat following intraperitoneal injections of fibrous dust]. *Naturwissenschaften* 59(7):318.
- Pott, F., Friedrichs, K. H., and Huth, F. 1976. Ergebnisse aus Tierversuchen zur kanzerogenen Wirkung faserförmiger Staube und ihre Deutung im Hinblick auf die Tumorentstehung beim Menschen. *Zentralbl. Bakt. Hyg. I. Abt. Orig.* B162:467–505.
- Pott, F., Schlipköter, H. W., Ziem, U., Spurny, K., and Huth, F. 1984. New results from implantation experiments with mineral fibers. In *Biological effects of mineral fibers*, vol. 2, pp. 286–302. Copenhagen, WHO.
- Pott, F., Ziem, V., Reiffer, F.-J., Huth, F., Ernst, H., and Mohr, U. 1987. Carcinogenicity studies on fibers, metal compounds, and some other dusts in rats. *Exp. Pathol.* 32:129–152.
- Pott, F., Roller, M., Ziem, U., Reiffer, F. J., Bellmann, B., Rosenbruch, M., and Huth, F. 1989. Carcinogenicity studies on natural and man-made fibers with the intraperitoneal test in rats. In *Non-occupational exposure to mineral fibers*, eds. J. Bignon, J. Peto, and R. Saracci, vol. 90, pp. 173–179. Lyon: IARC.
- Pott, F., Roller, M., Rippe, R. M., Germann, P.-G., and Bellmann, B. 1991. Tumors by the intraperitoneal and intrapleural routes and their significance for the classification of fibers. In *Mechanisms of fiber carcinogenesis*, eds. R. C. Brown, J. A. Hoskins, and N. F. Johnson, pp. 547–565. New York: Plenum Press.
- Roller, M., Pott, F., Kamino, K., Althoff, G. H., and Bellmann, B. 1996. Results of current intraperitoneal carcinogenicity studies with mineral and vitreous fibres. *Exp. Toxicol. Pathol.* 48(1):3–12.
- Roller, M., Pott, F., Kamino, K., Althoff, G. H., and Bellmann, B. 1997. Dose-response relationship of fibrous dusts in intraperitoneal studies. *Environ. Health Perspect.* 105(Suppl. 5):1253–1256.
- SAS Institute, Inc. 1989. *SAS/STAT User's Guide*, version 6, 4th ed., vol. 2. Cary, NC: SAS Institute, Inc.
- SAS Institute, Inc. 1989–1996. *SAS Proprietary Software Release 6.12*. TS045. Cary, NC: SAS Institute, Inc.
- Smith, D. M., Ortiz, L. W., Archuleta, R. F., and Johnson, N. F. 1987. Long-term health effects in hamsters and rats exposed chronically to man-made vitreous fibers. *Ann. Occup. Hyg.* 31:731–750.
- Stanton, M. F., and Wrench, C. 1972. Mechanisms of mesothelioma induction with asbestos and fibrous glass. *J. Natl. Cancer Inst.* 48(3):797–821.
- TRGS 905. 1994/1998. Technische Regeln für Gefahrstoffe 905. Classification of Dusts from Natural and Man-Made Mineral Fibers. Bundesarbeitsblatt Nr. 6, s.57. 1994 and Bundesarbeitsblatt 5, 72, 1998, Bundesministerium für Arbeit und Sozialordnung. Stuttgart: Verlag W.-Kohlhäuser.
- TRGS 906. 1995/1997. Technische Regeln für Gefahrstoffe 906—Begründungen zur Bewertung von Stoffen der TRGS 905, BArbBl. 10/95, S. 46; 4/97, S. 64, Bundesministerium für Arbeit und Ausgabe, September 1995.
- Wagner, J. C. 1963. Asbestosis in experimental animals. *Eur. J. Ind. Med.* 20:1.
- Wagner, J. C., and Berry, G. 1969. Mesotheliomas in rats following inoculation with asbestos. *Br. J. Cancer* 23(3):567–581.

APPENDIX. Data table

Fiber	Reference	Median diameter (μm)	Median length (μm)	Number fibers injected × 10 ³	In (fiber number)	Mass injected (mg)	Tumor fraction
B0109	Roller et al., 1996	0.7	9	10,000,000	16.12	500	0.21
B0109	Roller et al., 1996	0.7	9	20,000,000	16.81	1000	0.66
B01-09	Roller et al., 1997	0.7	9	2,500,000	14.73	125	0.08
B01-09	Roller et al., 1997	0.7	9	5,000,000	15.42	250	0.11
C	Lambré et al., 1998	0.44	12	10,000	9.21	0.7	0.10
C	Lambré et al., 1998	0.44	12	3,000	8.01	2.1	0.08
C	Lambré et al., 1998	0.44	12	100,000	11.51	7	0.02
C	Lambré et al., 1998	0.44	12	500,000	13.12	35	0.02
Wollastonite	Muhle and Pott, 1991	0.71	5.6	93,000	11.44	30	0.00
Wollastonite	Pott et al., 1989	1.1	8.1	430,000	12.97	100	0.00
G	Lambré et al., 1998	0.52	10.5	10,000	9.21	1.1	0.04
G	Lambré et al., 1998	0.52	10.5	70,000	11.16	7.7	0.02
G	Lambré et al., 1998	0.52	10.5	500,000	13.12	55	0.04
Slagwool	Pott et al., 1984	0.18	2.7	30	3.40	5	0.05
MMVF22	Jones et al., 1997	1.1	20	1,000,000	13.82	129.6	0.54
MMVF11	Roller et al., 1996	0.77	14.6	400,000	12.90	70	0.40
MMVF11	Roller et al., 1996	0.77	14.6	1,000,000	13.82	180	0.70
MMVF21	Jones et al., 1997	1.1	17.7	1,000,000	13.82	183.1	0.95
MMVF-21	Roller et al., 1997	1.02	16.9	400,000	12.90	60	0.97
MMVF-21	Roller et al., 1997	1.02	16.9	1,000,000	13.82	150	0.87
RCF1	Jones et al., 1997	0.88	17.8	1,000,000	13.82	110.9	0.88
RCF1	Jones et al., 1997	0.86	15.9	1,000,000	13.82	188.8	0.72
F	Lambré et al., 1998	0.57	9.9	10,000	9.21	1.1	0.06
F	Lambré et al., 1998	0.57	9.9	70,000	11.16	7.7	0.02
F	Lambré et al., 1998	0.57	9.9	500,000	13.12	55	0.06
A	Lambré et al., 1998	0.5	8.8	10,000	9.21	0.7	0.06
A	Lambré et al., 1998	0.5	8.8	30,000	10.31	2.1	0.02
A	Lambré et al., 1998	0.5	8.8	100,000	11.51	7	0.02
A	Lambré et al., 1998	0.5	8.8	500,000	13.12	35	0.06
H	Lambré et al., 1998	0.51	13	10,000	9.21	1.1	0.06
H	Lambré et al., 1998	0.51	13	70,000	11.16	7.7	0.02
H	Lambré et al., 1998	0.51	13	500,000	13.12	55	0.18
MMVF10	Jones et al., 1997	1.1	15	1,000,000	13.82	144.4	0.59
JM104/475	Pott et al., 1991	0.14	2.3	320,000	12.68	2	0.17
JM104/475	Pott et al., 1989	0.15	2.6	680,000	13.43	5	0.64
JM104/475	Pott et al., 1987	0.18	3.2	57,000	10.95	0.5	0.17
JM104/475	Pott et al., 1987	0.18	3.2	228,000	12.34	2	0.26
JM100475	Pott et al., 1984	0.24	1.4	50,000	10.82	2	0.05
JM100475	Pott et al., 1984	0.33	2.4	280,000	12.54	2	0.05
JM100/475	Pott et al., 1987	0.33	2.4	1,300,000	14.08	10	0.45
JM106475	Pott et al., 1976,	0.47	2.2	120,000	11.70	10	0.03
JM106475	Pott et al., 1976,	0.47	2.2	24,000	10.09	2	0.00

Number animals with tumors	Total number animals	Clearance half-times (days)					
		Intratracheal instillation biopersistence				Inhalation biopersistence	
		W $T_{1/2}$ WHO	Slow $T_{1/2}$ WHO	W $T_{1/2}$ $L > 20 \mu\text{m}$	Slow $L > 20 \mu\text{m}$	W $T_{1/2}$ $L > 20 \mu\text{m}$	Slow $L > 20 \mu\text{m}$
10	48	18	18	5	5	2.2	2.2
33	50	18	18	5	5	2.2	2.2
3	39	18	18	5	5	2.2	2.2
4	37	18	18	5	5	2.2	2.2
5	51	21	21	10	10	5	5
4	51	21	21	10	10	5	5
1	51	21	21	10	10	5	5
1	51	21	21	10	10	5	5
0	50	45	45	13	36	10	94
0	54	45	45	13	36	10	94
2	51	18	64	17	79	6	6
1	51	18	64	17	79	6	6
2	51	18	64	17	79	6	6
2	41	77	77	44	44	8	30
13	24	77	77	44	44	8	30
16	40	155	155	61	137	13	48
16	23	155	155	61	137	13	48
19	20	276	276	184	184	92	92
37	38	276	276	184	184	92	92
33	38	276	276	184	184	92	92
21	24	296	296	233	233	41	87
13	18	296	296	233	233	41	87
3	51					9	9
1	51					9	9
3	51					9	9
3	51					13	48
1	51					13	48
1	51					13	48
3	51					13	48
3	51					16	59
1	51					16	59
9	51					16	59
13	22					25	25
8	48					48	139
34	53					48	139
5	30					48	139
8	31					48	139
2	44					48	139
2	44					48	139
24	53					48	139
1	40					48	139
0	40					48	139

(Table continues on next page)

APPENDIX. Data table (*continued*)

Fiber	Reference	Median diameter (μm)	Median length (μm)	Number fibers injected $\times 10^3$	ln (fiber number)	Mass injected (mg)	Tumor fraction
JM106475	Pott et al., 1976	0.47	2.2	120,000	11.70	10	0.11
JM106475	Pott et al., 1976	0.47	2.2	24,000	10.09	2	0.03
JM106475	Pott et al., 1976	0.47	2.2	1,200,000	14.00	100	0.72
JM100475	Smith et al., 1987	0.4	4.9	400,000	12.90	25	0.32
JM100475	Jones et al., 1997	0.32	4	1,000,000	13.82	8.3	0.33
JM104E	Pott et al., 1976	0.2	10	12,000	9.39	2	0.27
JM104E	Pott et al., 1976	0.2	10	60,000	11.00	10	0.53
JM104E	Pott et al., 1976	0.2	10	300,000	12.61	50	0.71
JM104E	Pott et al., 1984	0.26	3.5	430,000	12.97	10	0.49
JM104E	Pott et al., 1984	0.26	4	290,000	12.58	10	0.66
JM104E	Pott et al., 1984	0.26	4	58,000	10.97	2	0.32
JM104E	Pott et al., 1987	0.29	4.8	505,000	13.13	5	0.44
JM104E	Pott et al., 1987	0.29	4.8	505,000	13.13	5	0.81
JM104E	Pott et al., 1984	0.29	4.8	1,010,000	13.83	10	0.73
JM104E	Pott et al., 1984	0.39	2.7	300,000	12.61	10	0.09
JM104E	Pott et al., 1987	0.3	3.5	4,300	8.37	10	0.50
JM104E	Pott et al., 1987	0.3	3.5	4,300	8.37	10	0.55
JM100E	Pott et al., 1987	0.32	4.4	155,000	11.95	2	0.48

Number animals with tumors	Total number animals	Clearance half-times (days)					
		Intratracheal instillation biopersistence				Inhalation biopersistence	
		W $T_{1/2}$ WHO	Slow $T_{1/2}$ WHO	W $T_{1/2}$ $L > 20 \mu\text{m}$	Slow $L > 20 \mu\text{m}$	W $T_{1/2}$ $L > 20 \mu\text{m}$	Slow $L > 20 \mu\text{m}$
4	36					48	139
1	34					48	139
23	32					48	139
8	25					48	139
8	24					48	139
20	73					68	180
41	77					68	180
55	77					68	180
19	39					68	180
29	44					68	180
14	44					68	180
20	45					68	180
44	54					68	180
27	37					68	180
4	45					68	180
13	26					68	180
18	33					68	180
26	54					68	180