Comparative Investigations of the Biodurability of Mineral Fibers in the Rat Lung

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The biodurability of various glass fibers, rockwool, and ceramic fibers was examined in rat lungs and compared with natural mineral fibers. Experiments were based on studies that have shown that the biodurability of fibers is one of the essential factors of the carcinogenic potency of these materials. Sized fractions of fibers were instilled intratracheally into Wistar rats. The evenness of distribution of fibers in the lung was checked by scanning electron microscopy (SEM) or careful examination of the fiber suspension before treatment. After serial sacrifices up to 24 months after treatment, the fibers were analyzed by SEM following low temperature ashing of the lungs. Parameters measured included number of fibers, diameter, and length distribution at the various sacrifice dates, so that analyses could be made of the elimination kinetics of fibers from the lung in relation to fiber length (F_L). Size selective plots of the fiber elimination correlated with fiber diameters enables the mechanism of the fiber elimination (dissolution, fiber breakage, physical clearance) to be interpreted. The half-time of fiber elimination from the lung ranges from about 10 days for wollastonite to more than 300 days for crocidolite. The biodurability of man-made vitreous fibers (MMVF) is between these values and is dependent on the chemical composition of the fibers and the diameter and length distribution. Results indicate that the *in vivo* durability of glass fibers is considerably longer than expected from extrapolation of published data on their *in vitro* dissolution rates. — Environ Health Perspect 102(Suppl 5):163–168 (1994)

Key words: biopersistence, mineral fibers, lung, rat

Introduction

The persistence of mineral fibers in the lung or the serosal tissue is thought to be related to the potency of tumor induction (*1,2*), but it is important to distinguish between persistence and biodurability of a fiber (*3*). Persistence can be defined as the long-term residence of fibers in the same location. High biodurability is a precondition for the persistence. In most investigations the entire lung was ashed and fibers were analyzed from this sample; therefore for practical reasons persistence and biodurability are used congruently, although for mechanistic reasons of tumor induction there is a difference.

The methodological approaches to evaluate the durability of fibers include *in vitro* and *in vivo* testing. *In vitro* investigations on the dissolution rate of fibers attempt to use a solution that simulates the environment of fibers in the lung. This is not possible with just one solution, because fibers may be located in areas where different conditions exist such as the alveolar space, in the interstitium, or in alveolar macrophages, where fibers would be subjected to the acid medium of lysosomes. *In vivo* investigations start with inhalation or intratracheal instillation of fibers into experimental animals after which the clearance of fibers from the lung is measured by serial sacrifices. The mechanisms responsible for clearance are dissolution, breakage of fibers, and physical clearance of whole fibers.

Element analysis may also provide important information on durability (*4*).

Clearly *in vivo* elimination of fibers may differ considerably from results obtained by *in vitro* dissolution, not only because of the complexity of the different dissolution conditions in the lung, but also because other important mechanisms are involved. The slower processes of breakage and alveolar clearance can be ignored for very soluble fibers, but often mineral fibers have quite a low *in vitro* solubility (*5*) and therefore investigation of *in vitro* dissolution alone may result in misleading conclusions.

Principles and Limitations of the Investigations of Biodurability

Application Method: Inhalation versus Intratracheal Instillation

The inhalation route is usually the appropriate method to deliver fibers to the lungs of experimental animals. Under certain circumstances, like the availability of a limited amount of sized fibers, intratracheal instillation may also be used (*6,7*). An advantage of the intratracheal instillation is that a precise starting point for kinetic analysis can be defined.

The evenness of the distribution of the fibers should be checked by SEM or by careful examination of the fiber suspension before treatment, to avoid the formation of granulomas, which were reported at an intratracheal injection dose of 20 mg (*7*). Comparison of results of inhalation studies with those where intratracheal instillation has been used should be done for validation.

Physical Mechanisms of Fiber Elimination

The key mechanisms for the physical elimination of fibers from the lung are mucociliary clearance; alveolar clearance, which is mostly mediated by alveolar macrophages; transport via lymphatic channels; and mechanical migration of fibers in lung tissue due to the respiratory tidal movement (*8*). These forces also may be responsible for the breakage especially of partly disintegrated fibers in the lung.

The elimination of durable fibers from the lung is dependent on physical mechanisms, which are influenced by fiber length, fiber diameter, and fiber mass

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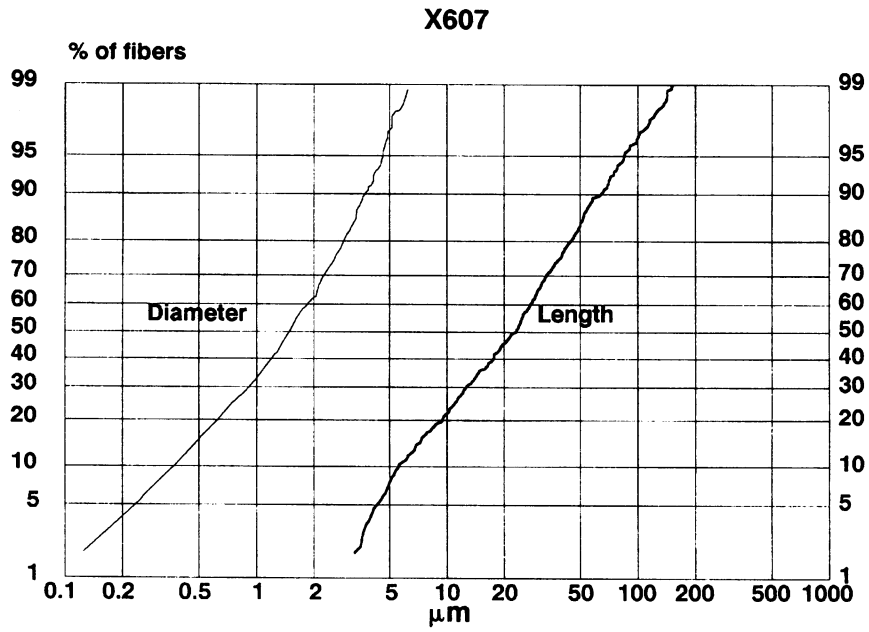

Figure 1. Diameter and length distribution of glass fiber×607.

retained in lungs. Alveolar macrophages can only phagocytize fibers up to 10 µm in length, prior to their removal by the ciliated airways (*9,10*).

Retained Mass

The phenomenon of "dust overloading" of the lung is observed with isometric particles at a lung burden of about 1 mg/g lung in rats (*11*). A similar effect was observed with fibers (*10*). Fibers may possess an "intrinsic" toxicity, which can affect the integrity of alveolar macrophages and thus their ability to eliminate fibers at a lower retained mass compared to isometric "nuisance" particles. As a result, the kinetics of fiber elimination from the lung are influenced not only by diameter and length distribution, but also by the retained fiber mass. The dependence of the physical clearance mechanisms on all these parameters makes it difficult to compare biodurability results obtained for different fiber types.

Methods of Characterizing the Fiber Elimination Process

To obtain an exact characterization of the fiber elimination process requires the total count of fibers retained in the lung and their length and diameter distribution for each date of serial sacrifices. Fiber length and diameter are usually log-normal distributions, and can be described on a log-normal plot (Figure 1), which can be plotted for each sacrifice date, for comparison with the original stock material.

It is possible to calculate half-times as a characteristic number, if, in a semilog plot, an approximation to a straight line is obtained. In this method, a regression line is calculated, based on the logarithm of the fiber mass or fiber number at each sacrifice date. The clearance rate k, and the 95% confidence limit are given by

$$t_{1/2} \pm t_{1/2}(CL) = \frac{ln2}{k \pm k_{SE}{}^{*}\hat{t}}$$

where k_{SE} represents the standard error of the regression coefficient and $\hat{t}$ is derived from the t distribution. Although this method leads to a considerable loss of information, it does make it possible to compare the elimination kinetics of different fiber types.

There are certain problems with this method, however. First, the dissolution kinetics is dependent on the fiber diameter, making it difficult to compare monodisperse fibers with log-normal distributed fibers, which in addition may have different breakage patterns. Second, with an increasing lung burden of particles, the fraction deposited in lung compartments from which there is low physical clearance will increase ("sequestered areas" [*12*]). This may be due to encapsulation of fibers by fibrotic tissue, leading to a considerable retardation of the elimination kinetics (*13*). Despite these shortcomings in the use of half-time, the method does enable different studies to be compared. Nevertheless, the complete set of information already mentioned is necessary for a more rigorous comparison of the biodurability of different fibers.

Materials and Methods

The sized glass fiber X607 (Manville Technical Center) consists of CaO: 38.3%, and SiO_2: 59.6%. The methods and results for this fiber are presented here as an example for other fibers. The source of the other materials and the methods used are described in detail elsewhere (*4,14–16*).

To characterize the samples, the fibers were suspended in water, briefly sonicated (<1 min), and filtered onto a Nuclepore filter. Fiber number and size distribution were determined from transmission electron microscopy (TEM) or SEM photos. For the majority of the samples, two or three magnifications were chosen so that both the longest and the thinnest fibers could be measured with sufficient precision. To avoid double counting, different fiber length limits were set for the counts at each magnification. The size distribution was approximated to log-normal for all fiber types and was classified by the limits for length and diameter of 10, 50, and 90% of the fiber number.

Two milligrams of fibers, suspended in 0.3 ml of 0.9% NaCl solution were instilled intratracheally into 200 to 220 g female Wistar rats (Central Institute for Laboratory Animal Breeding, Hannover). Three to six rats of each group were sacrificed. After drying, the lungs were subjected to low-temperature ashing; the ash was briefly suspended in water and filtered on a Nuclepore filter (pore size, 0.2 µm, 0.4 µm for glass fibers). The lungs of each animal were analyzed as separate samples. For fibers with short residence times, only samples of the earlier sacrifice dates were analyzed, whereas for persistent fibers samples up to two years after treatment were investigated. Usually 200 fibers per animal were analyzed for size distribution. Further details are presented elsewhere (*17*).

Results

We analyzed fibers in the ashed lung of rats that had received the glass fiber X607 by intratracheal instillation 2 and 14 days, 1, 2, 3, and 6 months before sacrifice; the elimination kinetics were plotted logarithmically (Figure 2). Calculated half-times, which are short compared with other glass fibers, are given in Table 1.

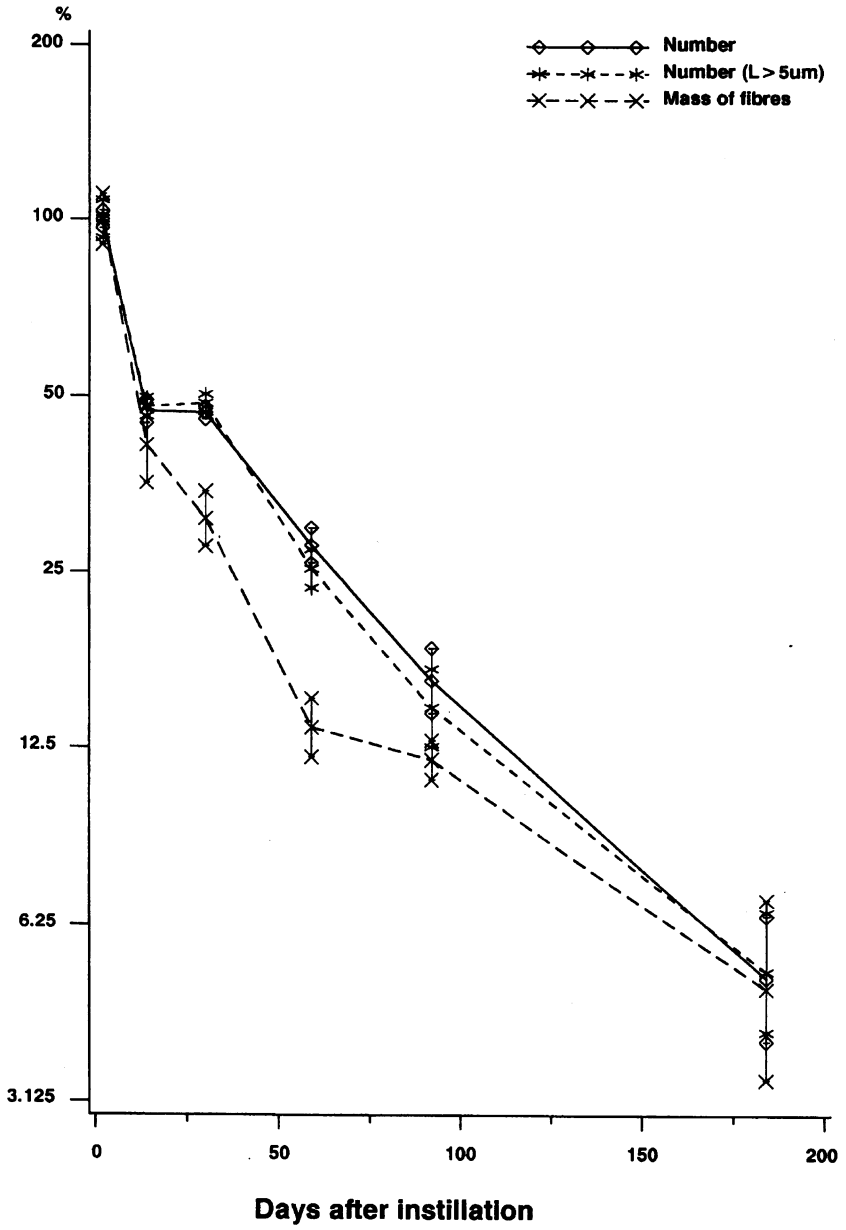

Figure 2. Decrease of the number and mass of the glass fiber (type × 607) in the lungs with time after intratracheal instillation.

Clearance kinetics for five fractions of increasing fiber length were also plotted (Figure 3) and the corresponding half-times were calculated (Table 2). The half-time for fibers >20 μm in length was short, indicating that breakage and dissolution were responsible for the elimination of this fraction. For particles < 2.5 μm in length the half-time calculated from the mass of particles was 68 days, corresponding to the value of the alveolar clearance of insoluble isometric particles, which was approximately 60 days (*11*).

The data from a series of fibers that had been studied earlier (*4,14*) were recalculated in similar fashion (Tables 3, 4).

The results from recalculation were markedly different for glass fiber 104/E, since in the earlier calculations only the values after 1 and 180 days were considered. As the fiber count had increased after 365 days, these values were incorrectly omitted. The original data set is given in Table 5. Another methodological limitation in the earlier experiments was due to the low fiber number remaining after 1 and 2 years, necessitating very long and tedious examination of the SEM and TEM photographs of the filtered fibers. Parts of the earlier study (*4*) are being repeated, this time adopting current methodology; five animals are sacrificed at each time interval, and in each lung 200 fibers will be analyzed, making a total of 1000 fibers, which should be sufficient for rigorous statistical analysis.

Discussion

Intratracheal instillation must be performed very carefully to avoid agglomerations of particles and to ensure an even distribution of the particles in the lung. It has been shown that instillation of 2 mg of fiber repeated 10 times resulted in a distribution in the lung very similar to that resulting from inhalation (*7*). In the present studies a single intratracheal instillation of 2 mg was used, and SEM examination indicated a quite even distribution. Recent results with long fibers show, however, that even 2 mg of fibers may result in agglomerations. The use of multiple instillations was suggested to achieve optimum dust distribution with a limitation of the doses administered to a maximum of 0.6 mg/g fresh weight of lung (*18*).

In another study of the glass fiber × 607, a half-time of 77 days was calculated (*19*), compared with 46 days reported here.

A reason for this difference may be the low statistical power, due to the limited number of animals used in the inhalation study, and because intervals examined were only one and two years after exposure (*19*). It is recommended that in chronic inhalation studies larger numbers of animals are necessary in retention and clearance experiments. A further difference between the two studies is that the fibers were administered to the animals during one year in the inhalation experiment (*19*), whereas in the present study, fibers were given in a single treatment. The long period of administra-

Table 1. Half-time with 95% confidence limit (CL), of the elimination of fibers (type × 607) from rat lung.

Half-time (days) calculated from					
Number of fibers (All dimensions)		Number of fibers, L >5 μm, D <3 μm		Mass of particles	
Mean	(95% CL)	Mean	(95% CL)	Mean	(95% CL)
47	(41–54)	46	(41–54)	48	(39–63)

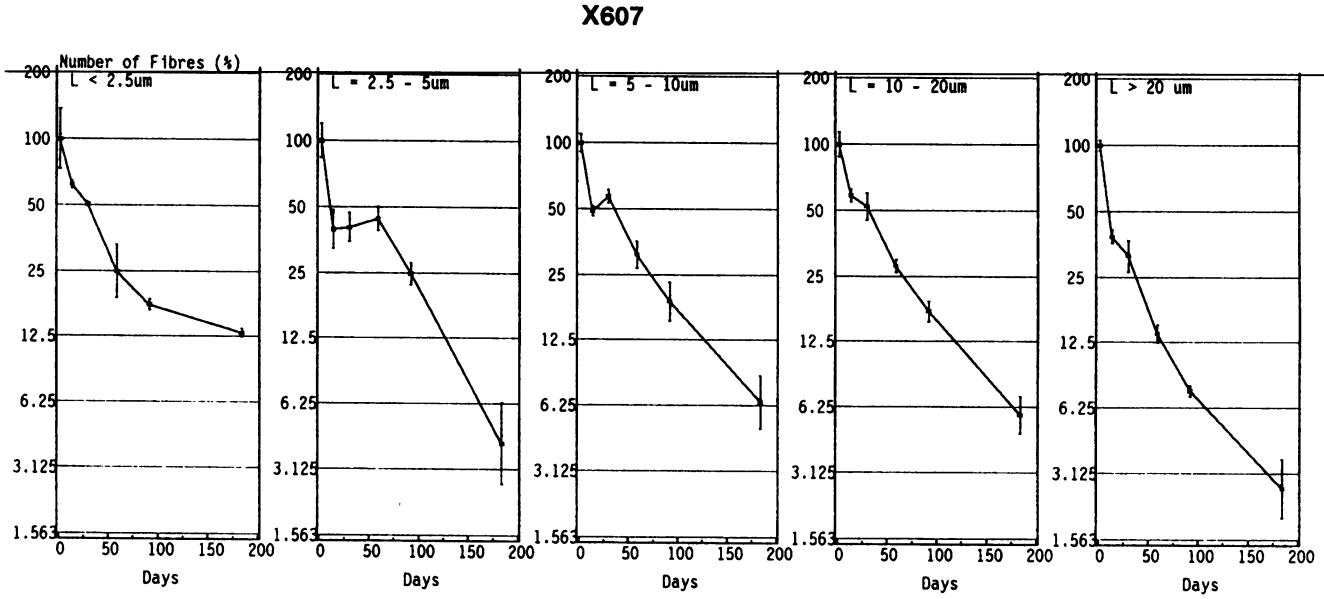

Figure 3. Decrease with time of the number of fibers of fiber type × 607, according to category of fiber length.

Table 2. Half-time, with 95% confidence limit (C.L.), for elimination of fibers of different length fractions of glass fiber X607 in rat lung ash.

	Half-time (days) of different fiber length fraction											
	<2.5 µm		2.5–5 µm		5–10 µm		10–20 µm		>20 µm		All	
	Mean	(95% C.L.)	Mean	(95% C.L.)	Mean	(95% C.L.)	Mean	(95% C.L.)	Mean	(95% C.L.)	Mean	(95% C.L.)
Calculation from:												
Number of fibers	64	(45–115)	46	(37–60)	50	(43–61)	47	(42–53)	39	(33–47)	47	(41–54)
Mass of particles	68	(37–445)	49	(36–75)	62	(48–87)	50	(45–58)	47	(36–66)	48	(39–63)

tion could permit the migration of particles into slow clearance compartments in the lungs.

Calculation of clearance half-times from results of another inhalation experiment (*20*) using the same methods as those described here are given in Table 3, and these are in relatively good agreement with the half-times derived from intratracheal instillation experiments (Table 4). One glass microfiber (104/475), however, appears to have a very long half-time of 1794 days for fibers >5 µm in length, but the 95% confidence limit of 301 days–∞ would suggest that the number of experimental animals and the number of fibers analyzed may have been too low for adequate statistical analyses.

When the dissolution rates of 30 glass fibers of different composition were studied at pH 7.4, a 1000-fold difference was observed between the most durable fiber, with a rate of 0.9 ng/cm²/hr and the least durable glass fiber type, with a rate of 869.9 ng/cm²/hr (*5*). Where the dissolution rate was measured *in vitro* and the biodurability was determined *in vivo* for the

Table 3. Elimination of fibers from the lung after inhalation.

						Half-time (days), with 95% CL calculated from			
Fiber type	Duration of exposure, days	Aerosol concentration, mg/m³	Length (% fibers with L>5 µm)	Retained fiber mass, mg/lung	Sacrifice dates, days	Number of fibers (all), fibers/lung	Number of fibers, f_L>5 µm fibers/lung	Mass of fibers, g/lung	Reference
Glass fiber, M 104/475	365	3	32	0.8	0, 365	585 (309–∞)		184 (146–252)	(*14*[a])
Crocidolite		2.2	10	0.7	0, 365	301 (116–∞)	∞ (179–∞)	827 (110–∞)	
Chrysotile, Calidria		6	10	0.3	0, 365	*b*	337 (85–∞)	96 (46–∞)	
Glasswool	365	5	17	0.51	0, 30, 210, 485	182 (56–∞)		329 (201–903)	(*20*[a])
Rockwool			20	0.25	0, 30, 210, 485	203 (82–∞)		185 (99–1444)	
Glass fiber, M 100/475			5.6	2.48	0, 30, 210, 485	250 (174–444)		268 (129–∞)	
Chrysotile Canada			14	0.29	0, 30, 210, 485	236 (91–∞)		198 (82–∞)	

[a] Recalculation (1992), this article . [b] Increase of fiber number by splitting.

Table 4. Elimination of fibers from the lung after intratracheal instillation.

Fiber type	Dose, mg	Length (%fibers F_L>5 µm)	Length Median	Sacrifice, dates, days	Half-time (days), with 95% CL calculated from Number of fibers, (all), fibers/lung	Number of fibers, F_L>5 µm, fibers/lung	Mass of fibers, g/lung	Reference
Glass fiber 104/E	2	12	2.1	1, 180, 365	131 (66–∞)	131 (77–458)	140 (83–456)	(4[a])
Glass fiber 104/475	2	28	3.2	1, 365	589 (224–∞)	1794 (301–∞)	115 (75–241)	
Glass fiber 104/753	2	12	2.7	1, 365, 730	137 (107–191)	172 (118–313)	123 (80–264)	
Glasswool	2	55	16	1, 365, 730	237 (120–8553)	272 (114–∞)	173 (73–∞)	
Rockwool	2	85	25	1, 365, 730	258 (120–∞)	283 (128–∞)	∞ (215–∞)	
Ceramic fiber	2	75	13	1, 180	190 (33–∞)	304 (38–∞)	378 (20–∞)	
Crocidolite UICC	2	1.5	1.0	1, 365	185 (96–2459)	976 (214–∞)	345 (139–∞)	
Chrysotile (Zimbabwe)	2	4	1.3	1, 30, 180, 365, 548, 730	∞	∞	5000 (772–∞)	
Glass fiber B-1M	2	94	10.7	1, 30, 180, 365, 730	102 (96–109)	106 (98–115)	105 (88–132)	(15[a])
Glass fiber B-2L	2	57	6.0	1, 30, 180, 365	37 (35–39)	39 (36–42)	51 (42–64)	
Glass fiber B-3L	2	60	5.6	1, 30, 180, 365, 730	221 (179–291)	240 (184–346)	296 (214–480)	
Wollastonite 1	2	83	8.4	2, 30	11 (9–13)	11 (9–13)	10 (7–16)	(16)
Wollastonite 2	2	57	5.6	2, 30	13 (11–18)	10 (7–15)	8 (6–12)	
Wollastonite 3	2	52	5.4	2, 30	11 (8–14)	12 (9–18)	11 (6–69)	
Wollastonite samples								
NYAD G thoracic fraction	2	32	3.2	2, 14, 30, 90, 180	17 (16–18)	18 (15–21)	19 (17–22)	(17)
NYAD G alveolar fraction	2	19	4.3	2, 14, 30, 90, 180	17 (15–18)	18 (16–22)	18 (16–20)	

[a] Recalculation (1992), this paper.

Table 5. Elimination of the glass fiber 104/E from the rat lung (*4*).

Sacrifice date, days	Animal no.	Fibers counted	Fibers, 10^6/lung	Fibers (L>5 µm) 10^6/lung
1	3	137	613	193
	23	180	680	196
	24	220	1544	406
180	17	120	74	34
	18	109	54	26
	19	102	56	26
365	14	110	140	32
	15	49	68	29
	16	84	194	50

same fiber type, a considerably longer half-time was found in the *in vivo* investigations (*5,21*). In the *in vitro* study (*5*), a 1 µm fiber (type no. 27) had a dissolution-rate constant of 596.5 ng/cm²/hr corresponding to complete dissolution in less than 15 days. In contrast, in the *in vivo* study (*21*) about 10% of this fiber was recovered from the lungs after one year, corresponding to a half-time of about 100 days.

A glass fiber of a composition identical with technically used glasswool or thermal isolation (type no. 7) had a dissolution-rate constant of 49.2 ng/cm²/hr, slower than the fiber type no. 27 by a factor of 12 (*5*). Consistent with these data, an *in vivo* durability investigation with a very durable glass fiber with a dissolution-rate constant of 2 ng/cm²/hr, gave an unchanged fiber number in the lungs after 12 months (*21*).

These comparisons support the view that investigations *in vivo* of biodurability provide more relevant data than *in vitro* investigation of dissolution rates.

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