Durability of Ceramic and Novel Man-Made Mineral Fibers

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In vitro solubility testing is an important means of assessing the likely behavior of fibers that are respired and accumulate in the lung. The problem has been that such tests often do not mirror the dissolution and removal mechanisms seen in vivo. Comparison of iron and silica solubility values of various types of mineral fiber showed no obvious correlation. Treating a mineral fiber containing high levels of calcium with normal balanced salt solutions produces a precipitate of calcium phosphate on the fiber surface. This deposit was not seen in fibers isolated from the lung of exposed animals. New solutions have been developed and with variations in the methods of exposing fibers, results similar to those seen in vivo have been obtained. Suitable fluid phases have been examined in static and flow-through systems. The relationship of solubility to biological activity is discussed. — Environ Health Perspect 102(Suppl 5):67–71 (1994)

Key words: ceramic fiber, durability, Gamble's, superwool, synthetic body fluid

Introduction

The best measure of the potential effect of a novel fiber on an exposed human population is a properly conducted inhalation experiment in rats. The experiments sponsored by the Thermal Insulation Manufacturers Association (TIMA) at Research and Consulting Company (RCC) (1) and elsewhere (reported in this issue) are the best examples of such studies. These studies used specially size-selected fibers and demonstrated that, despite some differences that might still be attributed to fiber size, there are clear differences in biological effect due to the composition of the fiber and hence its persistence in vivo. Thus the experimental glass fibers have produced much less effect than the ceramic fibers. It therefore seems likely that a fiber that does not persist in its original state long enough to cause disease in rats should not be hazardous to humans.

Several problems arise with this type of experiment, not the least being that the activity of the size-selected fibers may overestimate the hazard of the commercial materials from which they have been isolated. However, the main problem is cost,

in terms of both finance and time. This precludes the easy use of inhalation experiments to assist in product development where the optimum composition of a fiber needs to be broadly defined to ensure both its usefulness in intended application and its lack of biopersistence.

We need systems that would enable the rapid prediction of a fiber's behavior in the body so that only a selection of fibers needs to be tested in long term in vivo experiments. We must also determine by how much the composition of a fiber can deviate from those already tested before we should consider them to be "biologically novel" materials. A number of in vitro systems have been used for several decades to study both the dissolution of fibers and the leaching of "trace" elements from them (2,3). The present study examines the effects of a variety of test systems on fiber dissolution and leaching, with particular reference to a mineral wool family designed to be more soluble than most other man-made mineral fibers. These fibers were originally known as X607 and are now sold as Superwool X607. X607 was examined in the same series of inhalation experiments as the TIMA fibers and found to produce no significant pathology (4).

Materials and Methods

The TIMA series of fibers were donated by the association. The Superwool fiber was either the X607 sample used in the RCC inhalation experiments (obtained from the Schuller Mountain Technical Center,

Denver, CO), or was produced with a similar composition at Thermal Ceramics de France, St. Marcellin-en-Forez, France, as Superwool B3 composition.

Table 1 gives the chemical compositions of fibers used in the studies described in this article. The fiber distribution for all the TIMA series was based on a nominal arithmetic mean fiber diameter of $1(\pm 0.3)$ µm, except for the asbestos fibers, which had means of approximately 0.3 µm. The Superwool B3 fibers, unscreened, had a fiber distribution typical of spun fiber production with an arithmetic mean of between 3.2 and 4.2 µm, depending on the measurement method.

A number of artificial physiological solutions (Gamble's, Kanapilly, Condradt & Scholtz, Synthetic Body Fluid [SBF], and variants of them) have been used to simulate the lung environment (Table 2).

Fibers were exposed either in a static system where a sample of fiber was suspended in the test solution and shaken at 37°C, or in a flow-through system. The flow-through system at Morgan Materials Technology is an eight-channel system used to evaluate the performance of up to four saline solutions in duplicate of 1.000 g of Superwool B3 fibers. Each channel was supplied with a 2-l plastic reservoir jar of saline solution, buffered where appropriate, by bubbling 5% CO_2/95% N_2 gas. The solutions were pumped by an eight-channel peristaltic pump at 10 ml/hr through eight 40 ml volume polythene tubes, within an incubation oven maintained at

Table 1. Chemical analyses[a].

						Fiber type						
Chemical	Superwool B3	Superwool (X607)	TIMA MF10	TIMA MF11	TIMA MF21	TIMA MF22	TIMA RCF1[b]	TIMA RCF2[b]	TIMA RCF3[b]	TIMA RCF4[b]	TIMA UICC Crocidolite
SiO_2	60.0	59.6	57.5	63.4	46.2	38.35	53	54	50	53	50.87
Al_2O_3	1.0	0.18	5.1	3.88	13.0	10.55	45	46	35	45	0.05
CaO	34.0	38.3	7.5	7.45	16.9	37.5	—	—	—	—	0.71
MgO	4.4	0.56	4.13	2.82	9.25	9.9	—	—	—	—	3.41
Na_2O	—	0.03	14.95	15.45	2.64	0.38	<1	—	—	<1	5.62
K_2O	—	—	1.06	1.32	1.25	0.45	<1	—	—	<1	0.07
B_2O_3	—	—	8.75	4.45	—	—	—	—	—	—	—
MnO	—	—	—	0.01	0.16	0.7	—	—	—	—	0.05
Fe_2O_3	—	0.06	0.07	0.25	7.0	0.3	—	—	—	—	37.7
ZrO_2	—	0.36	0.03	0.03	0.03	0.06	—	—	15	—	—
TiO_2	—	0.02	0.01	0.06	2.95	0.45	—	—	—	—	0.01
SrO	—	—	—	0.01	0.07	0.05	—	—	—	—	—
SO_3	—	—	0.12	0.33	0.23	1.81	—	—	—	—	0.12

[a] Numbers indicate the percentage of each fiber's weight attributable to each chemical. [b] Nominal compositions.

37°C ± 2°C. 30 ppm of Oil Aid 82 (Water Management & Gamlen Ltd., Droitwich) was added to prevent fungal and bacterial growth in the saline solutions.

The dissolution of silica from the fibers was estimated by atomic absorption using a Thermo Jarrell Ash Smith–Hiefje II machine. For Gamble's and SBF, only SiO_2 was determined. For Saline 3 and modified SBF, CaO, MgO and SiO_2 were determined. SiO_2 was determinable up to concentrations of 250 ppm with no dilution. However, both Ca and Mg required dilutions of ×10 or ×20. The Ca and Mg values were then converted to CaO and MgO values. In all cases standards were prepared prior to analysis and 0.1% KCl was added to prevent ionic interference.

For the dissolution of iron the fibers were suspended in 0.03 M sodium acetate buffer at a concentration of 20 mg/ml and incubated in a shaking water bath for 4 days at 37°C. The fibers were sedimented by centrifugation for 10 min at 14,000 rpm and the supernatants filtered through 0.45 μm membrane filters. The clarified supernatants were then mixed with an equal volume of 10 mM Ferrozine (Aldrich Chemical Co) and incubated at room temperature for 30 min. Absorbance at 562 nm was measured against a reagent blank and concentrations of iron were calculated from a standard curve obtained with solutions of $FeSO_4$.

Scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS) were performed on an ISI ABT-60 with a PGT IMIX EDS analysis system incorporating an omega ultra-thin window efficient for light element detection. All the analyses and micrographs were taken at 8 keV, on carbon coated samples prepared on a Polaron coater/evaporation unit. The fibers were mounted in methyl methacrylate resin and polished to 1 μm diamond on an Abrumin polishing machine, to enable analysis of the fiber cross sections.

Results

The dissolution of a range of fibers was studied in the static system. The materials differed significantly in the solubility of silica from the fiber (Figure 1), with the Superwool (X607) being the most soluble. The solubility of the crocidolite asbestos in this study is exaggerated since it consists of much finer fibers. All the other materials have similar size distributions.

The problem of having a relatively low ratio of test solution to fiber surface area for the soluble Superwool fiber can be seen in Figure 1. The dissolution of SiO_2 within experimental error does not alter after 48 hr presumably because the solution is saturated in terms of the equilibrium kinetics for the removal of silica from the fiber.

Different elements dissolve from the fiber; and the solubility of iron and silica were not correlated (Figure 2), although

Table 2. Composition of four artificial physiological solutions.

Component	Concentration in g/l			
	Gamble's	Saline 3	Synthetic body fluid	Modified SBF
$NaHCO_3$	2.571	2.268	0.353	0.353
Na_2SO_4	0.079	—	0.071	0.071
$(NH_4)HPO_4$	—	—	0.132	0.132
$CaCl_2 2H_2O$	0.255	—	0.368	—
$MgCl_2 \cdot 6H_2O$	0.212	—	0.305	—
$HCl(35.4\%)$	—	—	4.640	4.640
$(CH_2OH)_3CNH_2$	—	—	6.050	6.050
KCl	0.311	—	0.224	0.224
$NaCl$	6.171	6.780	8.161	8.161
Na_2HPO_4	0.148	0.171	—	—
$CH_3COONa \cdot 3H_2O$	1.065	—	—	—
NH_4Cl	—	0.535	—	—
$Na_3citrate \cdot 2H_2O$	—	0.059	—	—
Glycine	—	0.450	—	—
H_2SO_4	—	0.049	—	—
pH	7.8	7.6	7.5	7.5

Figure 1. The dissolution of silica from some man-made mineral fibers. The TIMA series of fibers was used MF10 and MF11, glass fibers; MF21, rockwool; MF22, slagwool; and UICC crocidolite. The curve for ceramic fibers was broadly similar to the one for MF21 and 22, and has been omitted for clarity.
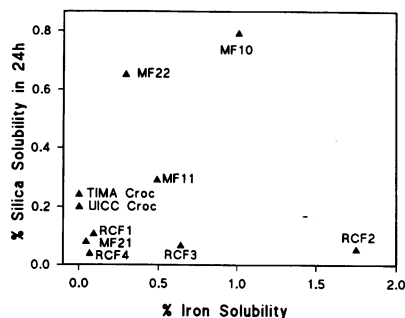

Figure 2. The dissolution of iron and silica expressed as a proportion of the total in the fiber. There is no correlation between the two measures of solubility. The fibers are the TIMA series: RCF—refractory ceramic fibers; MF—glass-, rock-, and slagwools; Croc—crocidolite asbestos.

the conditions used were different in the two assays since at pH 7.4 too little iron dissolves for quantification.

Examination of the Superwool fibers in several systems showed that calcium phosphate would deposit on their surface; this result agrees with results at the Schuller Mountain Technical Center. This phenomenon was not seen during *in vivo* residence of the fibers (TW Hesterberg, personal communication). It was therefore decided to alter the incubation conditions to attempt to mimic the *in vivo* observations.

A number of simulated body fluid systems have been evaluated, the main ones represented by the solutions given in Table 2. The pH of the solution exiting from the tubes containing the fibers under test was monitored; for the high solubility, high alkaline earth content Superwool fibers, the buffering from the 5% CO_2/95% N_2 gas was ineffective for solutions that relied
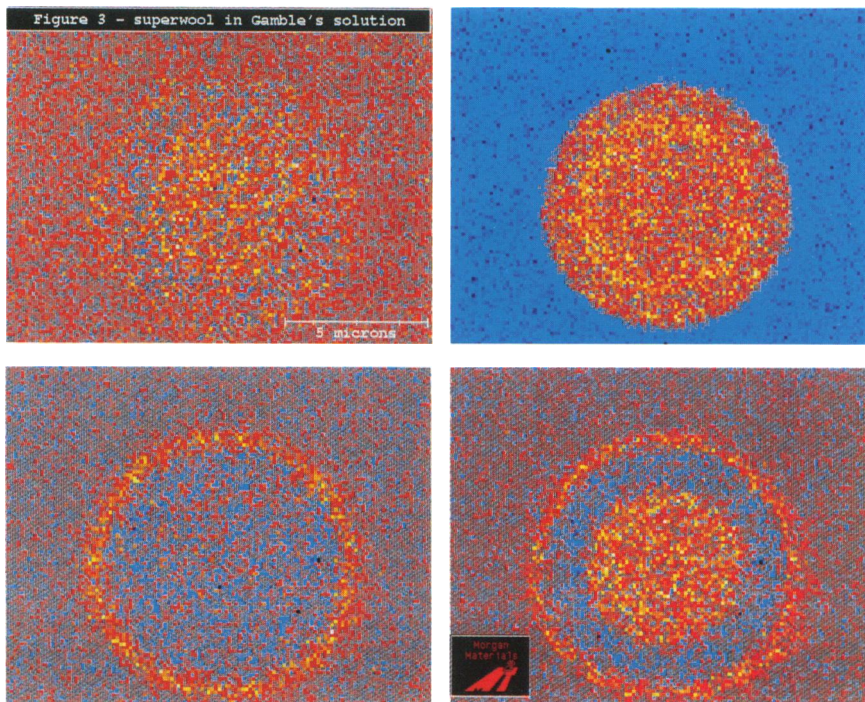
Figure 3. Elemental X-ray maps of Superwool B3 fiber cross-sections after a 6-week flow-through test with Gamble's solution. Top left, Mg; top right, Si; bottom left, P; bottom right, Ca. Color key: white (highest concentration), yellow, orange, red, light blue, dark blue, black (lowest concentration)
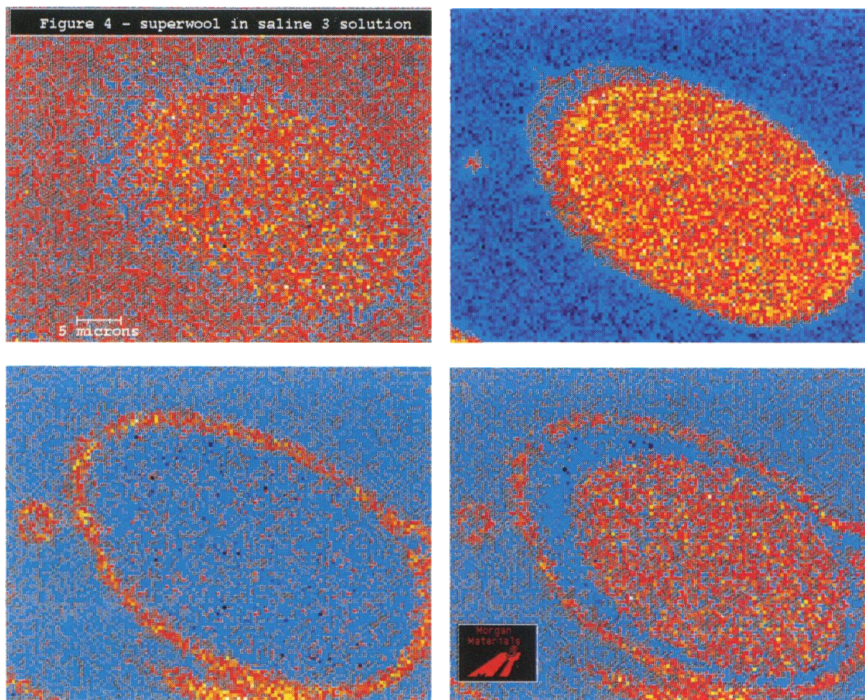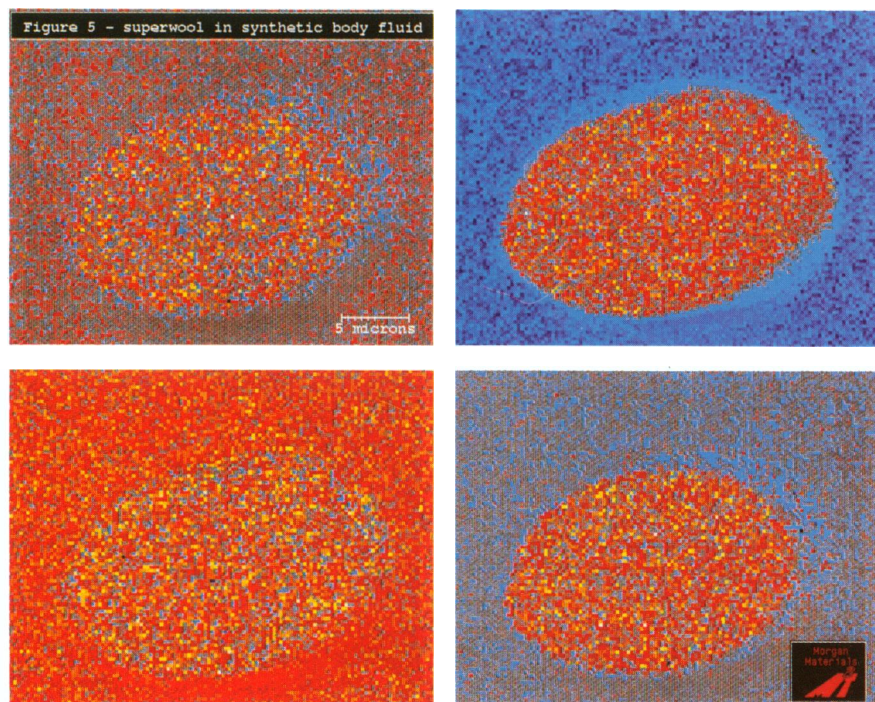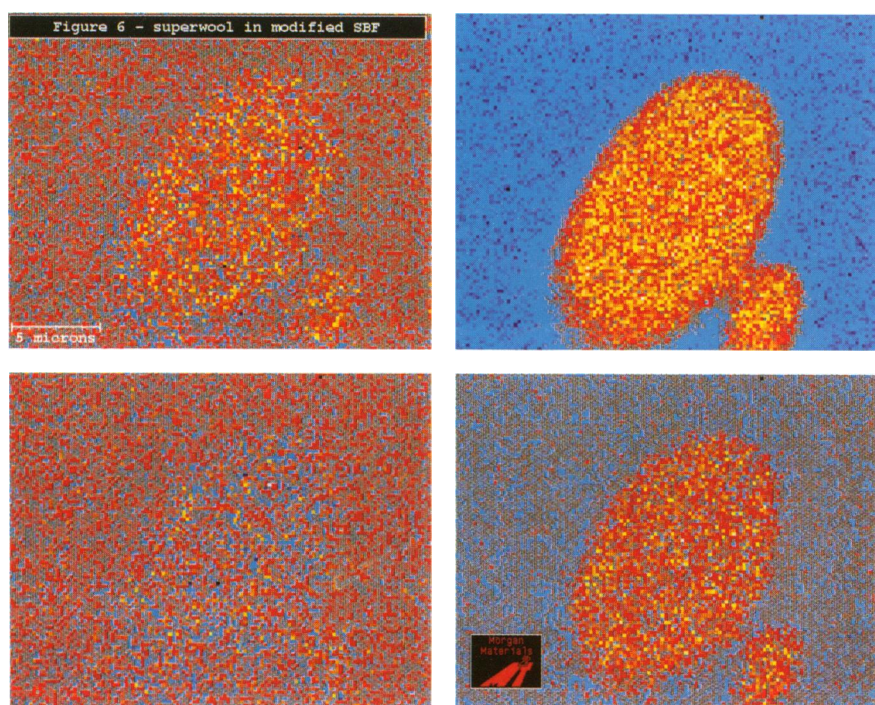

Figure 4. Elemental X-ray maps of Superwool B3 fiber cross-sections after a 6-week flow-through test with Saline 3 solution. Top left, Mg; top right, Si; bottom left, P; bottom right, Ca. Color key: white (highest concentration), yellow, orange, red, light blue, dark blue, black (lowest concentration)

only on the gas for a buffering effect. For these solutions the pH rose toward 8.0, which occasionally caused a blocking of the tubes with a calcium phosphate deposit.

This effect was most marked for Gamble's solution; Saline 3 showed the pH change, but to a lesser extent. In experiments where the quantity of buffer (Dimethanolamine)

Figure 5. Elemental X-ray maps of Superwool B3 fiber cross-sections after a 6-week flow-through test with Synthetic Body Fluid (SBF). Top left, Mg; top right, Si; bottom left, P; bottom right, Ca. Color key: white (highest concentration), yellow, orange, red, light blue, dark blue, black (lowest concentration)

Figure 6. Elemental X-ray maps of Superwool B3 fiber cross-sections after a 6-week flow-through test with Modified SBF. Top left, Mg; top right, Si; bottom left, P; bottom right, Ca. Color key: white (highest concentration), yellow, orange, red, light blue, dark blue, black (lowest concentration)

pared to those tests where true buffering of the solution was maintained.

Figures 3 to 6 show elemental X-ray maps of Superwool B3 fiber cross sections after a 6-week flow-through test in Gamble's, Saline 3, SBF, and Modified SBF. Both Gamble's and Saline 3 show similar behavior. Pronounced CaO leaching is apparent, and leaves a SiO_2-rich outer surface; also the deposition of a calcium phosphate layer is highly visible. However, neither SBF version resulted in calcium phosphate deposition, but both appear to produce some slight preferential leaching of CaO. When the level of buffer in the SBF solutions was lowered by a factor of 2 or more, a calcium phosphate layer appeared in the elemental maps, similar to that on fibers exposed to Saline 3.

Figure 7 shows the percent weight loss versus time as calculated from SiO_2 loss for Gamble's and SBF, and both SiO_2 and total loss ($CaO + MgO + SiO_2$) for Saline 3 and SBF modified. Removal of Ca and Mg salts (the difference between SBF and modified SBF) did not affect the fiber dissolution rate markedly. The relative ranking of the same fiber in different test solutions is not greatly affected whether the SiO_2 dissolution or the total oxide dissolution is compared.

Discussion

The dissolution of various elements from mineral fibers is readily studied *in vitro* and simple methods distinguish between the characteristics of different fibers. However, the qualitative results of such laboratory tests do not necessarily match the observations seen in animals. Several generalizations on how to obtain results similar to those seen *in vivo* could be drawn from our studies of Superwool, including the following:

- The pH of the test solution should be maintained at 7.4 ± 0.2 throughout the test.
- The stability of the test solution to precipitation, particularly in the presence of leached ions, is crucial.
- The volume of test solution must be in proportion to the surface area of the fiber and to duration of the test. The consequences of this are as follows:
  - Screening tests that use "static" small volume sample tubes (typically 50 ml of solution) should not normally be run for more than 24 hr.
  - Studying the mechanism of dissolution requires either large volume
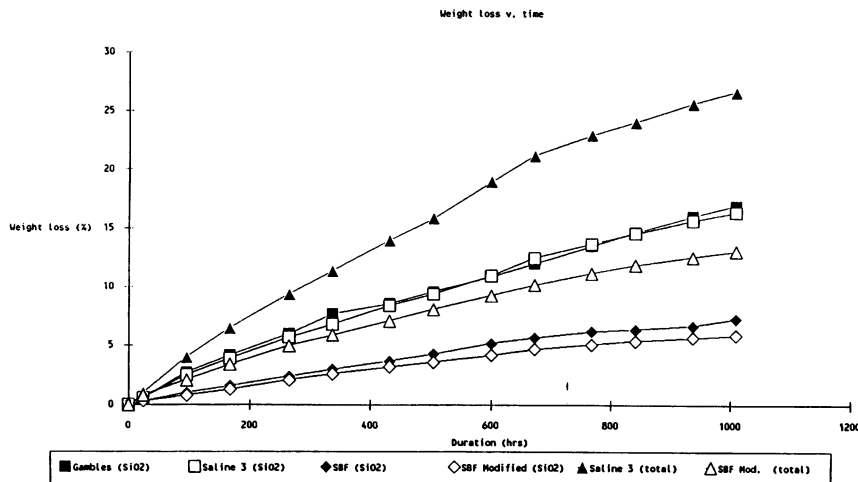
in the SBF solutions was lowered by a factor of 2 or more, the pH was seen to rise. When the pH of the solution was not con-trolled in the tube containing the fiber sample, the dissolution rates were approximately doubled for Superwool B3 com-

Weight loss v. time

Figure 7. Percentage weight loss vs time for Superwool fiber in different simulated body fluids.

obtained *in vivo* might reveal something of the conditions to which it had been exposed.

In many studies the acid leaching of chrysotile asbestos has been shown to reduce its biological activity (5) even though many fibers remain in the inoculum used. Such leached fibers may be less able to interact with their biological target(s) or their strength may be reduced, which enables them to fragment into biologically inactive material. In either case, it seems that a fiber that is prone to such leaching *in vivo* is less likely to be harmful and that this susceptibility to dissolution can be predicted from simple *in vitro* test results.

There are numerous reports that elements such as iron dissolve more rapidly from some fibers than others, some authors have suggested that this leached iron could be deleterious as it would be free to take part in free radical-generating reactions (6). This could be responsible for the high radical generating activity of rockwool, reported by Fournier et al. (7). However, the relevance of this activity to pathogenesis is unclear since presumably the leached elements would be removed from the site of leaching and leave an iron-depleted and hence inactive fiber. This longer-term loss of activity is not likely to be readily seen *in vitro* using the current short-term test systems. We should therefore expect more short-term studies to be carried out on previously leached material.

"static" tanks (several liters) or dynamic flow-through systems. In the latter systems it is important that the flow rate is above a crucial minimum value so that the test is carried out in a regime where the dissolution rate is not a function of flow rate.

- The silica content of the test solution is a fair indication of the dissolution rate of the fiber, although, if possible, other leachable elements should also be analyzed, because leach rates are rarely in the same ratios as the elements occur in the fiber.
- The elemental profile through fibers should be obtained by microanalysis (EDS) after the completion of testing.

There is no common protocol for the *in vitro* testing of fiber dissolution, so differences between laboratories cannot adequately be ascribed to any particular part of the methods used. Despite this lack of standardization, the same general trends and differentiation of fibers can be obtained, although absolute values vary quite markedly. Most important, by the choice of test solution and test method the fiber end-product after testing can closely match those recovered from *in vivo* experiments. The physicochemical conditions to which fibers are actually exposed *in vivo* are largely unknown and, in any case, must vary with their intra- or extracellular location. In fact, study of the actual nature of the leached product

REFERENCES

1. Hesterberg TW, Mast R, McConnell EE, Vogel O, Chevalier J, Bernstein DM, Anderson R. Chronic inhalation toxicity and oncogenicity study of refractory ceramic fibers in Fischer 344 rats. Toxicologist 11:85 (1991).
2. Morgan A, Holmes A. Neutron activation techniques in investigations of the composition and biological effects of asbestos. In: Proceedings of the International Conference on Pneumoconiosis, Johannesburg (Shapiro HA, ed). New York:Oxford University Press,1970;52–56.
3. Oberdoerster G, Boose CH, Pott F, Pfeiffer U. *In vitro* dissolution rates of trace elements from mineral fibres. In: The *in vitro* Effects of Mineral Dusts (Brown RC, Gormley IP, Chamberlain M, Davies R, eds). London:Academic Press,1980;183–189.
4. Hesterberg TW, Miiller WC, Mast R, McConnell EE, Bernstein

DM, Anderson R. Relationship between lung biopersistence and biological effects of man-made vitreous fibers after chronic inhalation in rats. Environ Health Perspect 102(Suppl 5): 133–137 (1994).
5. Morgan A, Davies P, Wagner JC, Berry G, Holmes A. The biological effects of magnesium-leached chrysotile asbestos. Br J Exp Path 58:465–473 (1977).
6. Lund LG, Aust AE. Iron mobilization from asbestos by chelators and ascorbic acid. Arch Biochem Biophys 278:60–64 (1990).
7. Fournier J, Guignard J, Nejjari A, Zalma R, Pezerat H. The role of iron in the redox surface activity of fibers. Relation to carcinogenicity. In: Mechanisms in fibre carcinogenesis, NATO ASI Series, Series A: Life sciences, Vol 223 (Brown RC, Hoskins JA, Johnson NF, eds). New York:Plenum Press, 1991;407–414.