Investigation on the Durability of Man-made Vitreous Fibers in Rat Lungs

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Two types of sized stonewool with median lengths of 6.7 and 10.1 µm and median diameters of 0.63 and 0.86 µm, and crocidolite with fibers of median length of 4.8 µm and median diameter of 0.18 µm were instilled intratracheally into female Wistar rats. A single dose of 2 mg in 0.3 ml saline was used for the stonewool samples and 0.1 mg in 0.3 ml saline for crocidolite. The evenness of distribution of fibers in the lung was checked by scanning electron microscopy (SEM). Five animals per group were sacrificed after 2 days, 1, 3, 6, and 12 months. After low-temperature ashing of the lungs about 200 fibers per animal were analyzed by SEM for length and diameter. The number and mass of fibers in the total lung were calculated. For the stonewool samples the decrease in the number of fibers in the lung ash followed approximately first order kinetics resulting in half-times of 90 and 120 days. The analysis of fiber number and diameter of different length fractions was used to estimate the contribution of three processes of fiber elimination: transport by macrophages for short fibers, breakage of fibers, and dissolution of fibers. (The process of transport by macrophages was found fastest for fibers with length <2.5 µm). For the elimination of critical fibers with length >5 µm, the breakage and dissolution were the most important processes. The breakage of fibers was predominant for one of the stonewool samples. The preferential type of the mechanism of fiber elimination is dependent on chemical composition and size distribution. — Environ Health Perspect 102(Suppl 5):185–189 (1994)

Key words: biopersistence, man-made vitreous fibers, stonewool, crocidolite, rat, intratracheal instillation

Introduction

The durability of fibers in the lung is one important criterion of carcinogenic potential. A parallel study of in vivo durability (1) and carcinogenicity investigated by the intraperitoneal test (2) did not show a significant tumor rate by this method for fibers with retention half-times of approximately 40 days.

Biodurability studies with stonewool fiber have been published only for samples with relatively thick fibers. For example, SG stonewool with a median diameter of about 2.0 µm was used in a 12-month inhalation study with rats (3). From the fiber retention data up to 16 months after termination of exposure, half times of approximately 200 days can be calculated.

A half-time of about 280 days was reported for fiber retention data up to 24 months after intratracheal instillation of stonewool with a median diameter of 1.8 µm (4). A similar half-time was found for a glasswool in the same study.

In a study of solubility of stonewool fibers with a median diameter of 1.1 µm and a median length of 28 µm, fibers with length >20 µm, analyzed by light microscopy up to 18 months after intratracheal instillation had an unchanged median diameter; but the fibers had become thinner at their ends, indicating a low solubility (5,6).

In this study the biodurabilities of sized samples of a commercial stonewool composition (MMVF21) and of a modified stonewool with increased alumina content (stonewool HT) were analyzed and compared with a crocidolite sample.

Materials and Methods

The test substances were a basalt-based stonewool (MMVF21) and stonewool HT fiber, both of known chemical composition (7). A special preparation of UICC crocidolite with an increased fraction of long fibers was used as positive control with an expected high durability.

A small sample of each test material was suspended in doubly-distilled water, sonicated, and filtered onto a Nucleopore filter (pore size 0.2 or 0.4 µm). Part of the filter was mounted on an aluminum stub and sputtered with approximately 30 nm of gold, then analyzed by a Cambridge Stereoscans 360 scanning electron microscope (SEM). Two or three magnifications were used to enable the measurement of both the longest and the thinnest fibers with sufficient precision; at each magnification, fiber length limits were set to avoid double counting. The length and diameter of about 400 fibers of stock samples were measured. The calculated number of critical fiber (L > 5 µm, D < 2 µm, L/D > 5/1), their percentiles of length, and diameter are given in Table 1.

Two milligrams of fibers per rat for stonewool samples and 0.1 mg of crocidolite, were each suspended in 0.3 ml of 0.9% NaCl solution and instilled intratracheally in a single dose into lungs of female Wistar rats, body weight approximately 200 g. Five animals per group were sacrificed after 2 days, 2 weeks, 1, 3, 6, and 12 months for the stonewool groups, and after

| Fiber | Fiber length, µm | Fiber diameter, µm |
|-------|------------------|--------------------|
| Critical fibers/µg | L>5 µm, D<2 µm | 10%< | 50%< | 90%< | σ ² | 10%< | 50%< | 90%< | σ ² |
| Crocidolite long | 212 | 1.5 | 4.8 | 12.3 | 2.5 | 0.10 | 0.18 | 0.31 | 1.55 |
| MMVF21 | 29 | 3.4 | 6.7 | 15.3 | 1.7 | 0.32 | 0.63 | 1.21 | 1.72 |
| Stonewool HT fiber | 17 | 4.5 | 10.1 | 27.9 | 1.9 | 0.43 | 0.66 | 1.62 | 1.72 |

*Fiber definition: L/D > 5. *Geometric standard deviation.

Table 1. Size distribution of test material.
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the chealinstillation with the sample, Stonewool prepared for initial geometry.

Table 2. Size distribution of test materials.

| Test material | Sacrifice date | Fibers counted | 10%< | 50%< | 90% | \( \sigma_a \) | 10%< | 50%< | 90% | \( \sigma_a \) |
|---------------|---------------|---------------|------|------|----|---------|------|------|----|---------|
| Crocidolite long | Initial material | 498 | 1.5 | 4.8 | 12.3 | 2.5 | 0.10 | 0.18 | 0.31 | 1.55 |
| | 2 days | 1031 | 2.0 | 5.3 | 13.7 | 2.1 | 0.09 | 0.17 | 0.30 | 1.67 |
| | 6 months | 1006 | 2.3 | 6.1 | 14.3 | 2.2 | 0.09 | 0.19 | 0.31 | 1.73 |
| | 12 months | 1060 | 2.3 | 5.2 | 13.0 | 1.9 | 0.07 | 0.16 | 0.29 | 1.93 |
| MMVF 21 | Initial material | 324 | 3.4 | 6.7 | 15.3 | 1.7 | 0.32 | 0.63 | 1.21 | 1.72 |
| | 2 days | 704 | 2.9 | 6.8 | 15.9 | 1.9 | 0.27 | 0.62 | 1.19 | 1.95 |
| | 1 month | 726 | 3.0 | 6.9 | 17.1 | 1.9 | 0.24 | 0.62 | 1.24 | 2.10 |
| | 3 months | 733 | 3.1 | 7.1 | 17.1 | 2.0 | 0.28 | 0.66 | 1.22 | 1.97 |
| | 6 months | 799 | 3.5 | 7.4 | 17.3 | 1.8 | 0.29 | 0.69 | 1.24 | 1.95 |
| | 12 months | 777 | 3.0 | 6.5 | 14.0 | 1.8 | 0.24 | 0.60 | 1.07 | 2.07 |
| Stonewool HT fiber | Initial material | 325 | 4.5 | 10.1 | 27.9 | 1.9 | 0.43 | 0.85 | 1.62 | 1.72 |
| | 2 days | 837 | 5.0 | 11.2 | 29.4 | 1.9 | 0.43 | 0.87 | 1.65 | 1.78 |
| | 1 month | 799 | 4.3 | 9.6 | 24.8 | 1.9 | 0.49 | 0.95 | 1.77 | 1.69 |
| | 3 months | 805 | 5.1 | 9.7 | 23.9 | 1.6 | 0.60 | 1.16 | 1.67 | 1.69 |
| | 6 months | 729 | 5.6 | 12.3 | 30.2 | 1.8 | 0.75 | 1.50 | 2.30 | 1.72 |
| | 12 months | 440 | 3.3 | 9.5 | 26.4 | 2.2 | 0.43 | 1.19 | 2.62 | 2.21 |

*Weighting by number of fibers, fiber definition: L/D > 5. * Geometric standard deviation.

2 days, 6 and 12 months for crocidolite. After sacrifice, the lungs were isolated, oven-dried at 105°C and ashed at low temperature. That this procedure did not alter the size distribution of test materials was shown by comparing lung ash samples from rats sacrificed 2 days after intratracheal instillation with the corresponding initial test materials (Table 2). A fraction of the ashed lung was suspended in filtered water, filtered on a Nuclepore filter (pore size 0.2 or 0.4 µm) within 15 min, and prepared for analysis by SEM. For each sample, 200 fibers were measured on SEM video prints or photos, the size distribution of the fibers was analyzed, and the total number of fibers per lung was calculated for each animal. The volume of the particles was estimated assuming cylindrical geometry. Clearance kinetics were calculated using a regression analysis of logarithm of number or mass of fibers versus time after instillation for individual animals. The resulting clearance rate constants \( k \) with their 95% confidence limit were transformed to the corresponding half-times \( t_{1/2} \) by: \( t_{1/2} = \ln 2/k \).

Results

SEM examination of the distribution of fibers in the lung two days after intratracheal instillation of MMVF21, showed fibers in the main bronchi, on the epithelium of the distal segments of bronchioles and in alveoli. No agglomerations of fibers were found.

Table 3 presents the analysis of fibers in the ashed lungs for sacrifice dates 2 days, 1, 3, 6, and 12 months after intratracheal instillation. A logarithmic plot of the number of fibers versus time (Figure 1) indicated that the elimination of fibers can be described approximately by first order kinetics, defined by only one parameter, the half-time (Table 4).

No significant change was observed in the size distribution of fibers in the lung ash up to 12 months, with the exception of the diameter distribution for the Stonewool HT fiber, which shifted to thicker fibers with time (Table 2).

Discussion

Decrease in the number of fibers is influenced by three processes: mechanical clearance of short fibers, breakage of longer fibers, and dissolution of fibers. Only fibers with length up to about 10 µm can be engulfed completely by macrophages. For anthophyllite and crocidolite the fastest clearance in rats was found for fibers below 5 µm in length (4,8). Anthophyllite fibers >17 µm were not cleared from the lung in humans (9). These results suggest that fibers >20 µm in length will disappear only by breakage or dissolution. To estimate the contribution of each of these processes, an analysis of the number of fibers of different fiber length fractions (Figure 2; Tables 5, 6) and of different diameter fractions (Figure 3) was performed. Fiber breakage causes a shift to a shorter length fraction within the same diameter fraction without changing the cumulative length (Figure 3). Dissolution of fibers will result in a shift to thinner diameter fractions without changing the length distribution. Mechanical clearance of fibers should remove fractions of the same length regardless of the diameter.
Table 4. Half-time and 95% confidence limit (CL) of the elimination of fibers.

| Test material | Half-time calculated from | Number of particles | Number of fibers (L>5 μm, D<3 μm) | Mass of particles |
|---------------|---------------------------|---------------------|-----------------------------------|-------------------|
|               |                           | Mean (95% CL)       | Mean (95% CL)                     | Mean (95% CL)     |
| Crocidolite long |                           | 665 (320–∞)         | 695 (354–∞)                      | 683 (364–∞)       |
| MMVF21       |                           | 257 (195–370)       | 291 (221–406)                    | 249 (187–372)     |
| Stonewool HT |                           | 111 (98–127)        | 98 (86–112)                      | 150 (126–185)     |

[Figure 2. Decrease in number of fibers of different length fractions in the lung ash after intratracheal instillation.]

ter fractions. This clearance would be greatest for length fractions <5 to 10 μm and minimal for fractions >20 μm in length.

For crocidolite, data from the sacrifice dates 2 days, 6 and 12 months after instillation are available. From 2 days to 6 months, a relatively fast clearance was found for the length fraction > 40 μm, indicating that long crocidolite fibers were broken in the lung (Figure 2). The relatively fast clearance of the fibers < 5 μm in length is due to the mechanical clearance of these fibers. From 6 to 12 months, the number of fibers decreased only for length fractions >20 μm, whereas for length fractions <20 μm the number of fibers increased only for diameter fraction <0.2 μm. SEM photos from sacrifice dates of 6 and 12 months showed that long and thick fibers often split at their ends into several thin fibrils <0.2 μm in diameter which would explain the increase in the number of thinner fibers. For fibers >0.2 μm in diameter the number was constant from 6 to 12 months, indicating the high durability of these crocidolite fibers.

MMVF21 fibers in the length fractions >20 μm were eliminated the fastest as a result of the breakage of these long fibers. From 3 to 12 months the elimination of thicker fibers, >1.25 μm in diameter, was significantly faster than that of thinner fibers, resulting in a shift to fractions of thinner diameter, due probably to reduction by dissolution. The elimination of fibers <5 μm in length is the result predominantly of mechanical clearance.

For the stonewool HT fiber, the elimination of all length fractions >10 μm was relatively fast; but the highest elimination rate was found for the thinner diameter fractions.

SEM photos showed some fibers whose diameter was not constant all along the fiber due to corrosion. In in vitro tests with this stonewool sample (7) the dissolution rate at pH 4.8 was much faster than at pH 7.7, and pH 4.8 corresponds to the pH in the phagolysosomes of the macrophages (10). The parts of fibers with thinner diameter were approximately 5 to 10 μm in length, so that part could have been within a macrophage, leaving the thicker parts outside. The thinner sections would be the likely sites for fiber breakage, so that first the thin fibers and later the thicker ones might break to smaller fragments. Those smaller fragments could either be eliminated by mechanical clearance or lose their characteristic fiber shape and disappear. This may also explain the shift to thicker
Table 5. Mean diameter of fibers of different length fraction of test materials in the lung ash.

| Sacrifice date | <2.5 | 2.5-5 | 5-10 | 10-20 | 20-40 | >40 | All |
|----------------|------|-------|------|-------|-------|-----|-----|
|                | Mean | SD    | Mean | SD    | Mean | SD  | Mean | SD  |
| Crocidolite long |      |       |      |       |      |     |      |     |
| 2 days         | 0.16 | 0.01  | 0.16 | 0.01  | 0.19 | 0.01| 0.22 | 0.02|
| 6 months       | 0.17 | 0.03  | 0.18 | 0.01  | 0.20 | 0.01| 0.23 | 0.01|
| 12 months      | 0.13 | 0.01  | 0.14 | 0.01  | 0.19 | 0.02| 0.24 | 0.02|
| MMVF21         |      |       |      |       |      |     |      |     |
| 2 days         | 0.63 | 0.04  | 0.76 | 0.04  | 0.84 | 0.02| 0.89 | 0.09|
| 1 month        | 0.61 | 0.05  | 0.75 | 0.03  | 0.86 | 0.08| 0.86 | 0.13|
| 3 months       | 0.60 | 0.05  | 0.74 | 0.03  | 0.84 | 0.05| 0.88 | 0.11|
| 6 months       | 0.61 | 0.09  | 0.74 | 0.06  | 0.81 | 0.06| 0.93 | 0.09|
| 12 months      | 0.52 | 0.03  | 0.66 | 0.05  | 0.76 | 0.05| 0.79 | 0.10|
| Stonewool HT   |      |       |      |       |      |     |      |     |
| 2 days         | 0.69 | 0.08  | 0.93 | 0.03  | 1.01 | 0.05| 1.04 | 0.09|
| 1 month        | 0.71 | 0.08  | 0.90 | 0.06  | 1.07 | 0.04| 1.27 | 0.07|
| 3 months       | 0.77 | 0.05  | 1.01 | 0.07  | 1.15 | 0.03| 1.46 | 0.07|
| 6 months       | 0.91 | 0.21  | 1.14 | 0.04  | 1.45 | 0.04| 1.90 | 0.06|
| 12 months      | 0.59 | 0.06  | 0.91 | 0.09  | 1.54 | 0.10| 2.06 | 0.16|

Table 6. Half-time for elimination of fibers of different length fraction of test materials in the lung ash.

| Test material | <2.5 | 2.5-5 | 5-10 | 10-20 | 20-40 | >40  | All   |
|---------------|------|-------|------|-------|-------|------|-------|
| Crocidolite long | 379  | (305–∞) | 372  | (335–∞) | 812  | (402–∞) | 392  | (202–6536) | 217  | (155–382) | 665  | (320–∞) |
| MMVF21        | 207  | (136–379) | 251  | (185–392) | 318  | (229–520) | 279  | (217–381) | 191  | (148–271) | 158  | (121–227) | 257  | (195–376) |
| Stonewool HT  | 118  | (79–231) | 132  | (106–175) | 104  | (90–122) | 99   | (88–114) | 92   | (79–111) | 98   | (87–113) | 111  | (88–127) |

Figure 3. Decrease of cumulative length of fibers of different diameter and length fractions normalized to the cumulative length of all fibers per animal in the lung ash of sacrifice date day 2. The values are given in parts per thousand below the bars. (A) crocidolite long; (B) MMVF21; (C) Stonewool HT.
This may also explain the shift to thicker diameter fractions.

In *in vitro* studies for MMVF21 the dissolution rate at pH 4.8 was also higher than at pH 7.7, but only by a factor of about 2 (7), which accords with the observation that the shape of fibers in the lung ash was relatively regular for all MMVF21 fibers up to 12 months after sacrifice. It was observed, however, that some long fibers were thinner at the ends than in the middle (6). The higher dissolution rate of stonewool HT at acid pH compared with MMVF21 could be due to the substitution of magnesium and some silica by aluminum. For the glasswool fiber (MMVF11) the *in vitro* dissolution rate was lower at acid pH than at pH 7.7 (7,11).

In another study, glasswool fibers with high solubility at pH 7.7 showed a rapid decrease in fibers longer than 10 μm, while the shorter fibers were much more durable (12), due to the slower dissolution of the phagocytized short fibers in the acid pH of the macrophages.

**Conclusions**

The breakage of fibers with length >40 μm was a common phenomenon for MMVF21, stonewool HT, and crocidolite. For stonewool HT, breakage of fibers was detected for all fractions >10 μm in length. Both breakage and the dissolution of fibers are important reasons for the decrease of number of critical fibers with length >5 μm. The overall elimination rate of fibers increased in the order crocidolite < MMVF21 < stonewool HT, although the mean diameter of samples increased in the same order.

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