Investigation of the Biodurability of Wollastonite and Xonotlite

Bernd Bellmann and Hartwig Muhle
Fraunhofer Institute of Toxicology and Aerosol Research, Hannover, Germany

The in vivo durability of wollastonite materials, coated and uncoated, and of xonotlite was tested. Wollastonite is an anhydrous natural silicate and xonotlite is a hydrated synthetic calcium silicate. UICC crocidolite was used as a positive control with high durability. Using a dry-sizing technique, fractions from the stock materials were prepared according to the definition of "thoracic particulate mass" and "respirable particulate mass" of the American Conference of Governmental Industrial Hygienists. Fibers were instilled intratracheally into female Wistar rats, and the evenness of their distribution in the lung was checked by scanning electron microscopy (SEM). After serial sacrifices at 2 and 14 days, 1, 3, and 6 months, and low temperature ashing of the lung, the fibers were analyzed by SEM. The number and size distribution of fibers were investigated. The total number of crocidolite fibers decreased with a half-time of 240 days, but the number of fibers >5 µm in length was unchanged after 6 months. The elimination kinetics of wollastonite fibers from the lung were relatively fast, with half-times of 15 to 21 days. The coating of wollastonite in Wollastocote had no effect on this elimination process. For the thoracic fraction of wollastonite, the elimination from the lung was as fast as for the respirable particulate fraction. The elimination kinetics of xonotlite from the lung was very fast. This material consisted of single crystals of acicular morphology with a median length of 1.3 µm and of agglomerates of these crystals. More than 99% of single crystals and about 85 to 89% of the agglomerates were already eliminated 2 days after instillation. — Environ Health Perspect 102(Suppl 5):191-196 (1994)

Key words: biopersistence, wollastonite, xonotlite, crocidolite, rat, intratracheal instillation

Introduction

The durability of fibers in the lung is one important criterion for assessing their possible carcinogenicity. In a previous biodurability study of three samples of wollastonite with sacrifice dates at 2 and 30 days, half-times of about 15 days were observed (1). These half-times are very short compared to approximately 40 days found for special glass fibers (2) that showed no carcinogenicity in the intraperitoneal test (3). In the present study specific fractions of commercial fiber samples of wollastonite with and without surface modification and of xonotlite, a synthetic mineral of similar composition, were investigated.

Biological durability studies should use the fraction of fibers that could be deposited in lungs of humans. In designing the fiber sizing, the definition of "thoracic particulate mass" and "respirable particulate mass" was taken from published threshold limit values (4).

It has been estimated that about 95% of lung cancer in man originates in the tracheobronchial region (5). Fibers retained in this area are most likely to be relevant for health effects (6), so that the two fractions of fine airborne fibers that would be able to penetrate the lung had to be separated from the coarse material.

Materials and Methods

Wollastonite is a naturally occurring calcium metasilicate (CaSiO₃) which, on milling in a rotating plate attrition mill, gives acicular fragments. The wollastonite samples in this study were NYAD G wollastonite, a high aspect ratio product, and NYAD 1250, which has a fine particle size grade with lower aspect ratio. NYAD G Wollastocote 1001, and NYAD G Wollastocote 2075 (NYCO Inc., Willsboro, NY) were manufactured from NYAD G by surface modification. The test material for xonotlite (Ca₂SiO₅(OH)₃) was a commercial product (Promax, Netherlands, trademark Promaxon). The samples for analysis consisted of single crystals of acicular morphology with a median length of approximately 1.3 µm, and of agglomerates of these crystals. UICC crocidolite was used as positive control with high durability.

Table 1. Size distribution of classified fibers. *

| Test material          | Fibers counted | Fiber length, µm | Fiber diameter, µm | Aerodynamic diameter, µm |
|------------------------|----------------|------------------|--------------------|-------------------------|
|                        |                | 50% < νd          | 50% < νd           | 50% < νd               |
| NYAD G (thor)          | 276            | 4.3 ± 1.8        | 0.85 ± 2.05        | 7.95 ± 1.78            |
| NYAD G (alv)           | 381            | 3.2 ± 1.6        | 0.63 ± 1.70        | 5.74 ± 1.91            |
| NYAD 1250 (thor)       | 111            | 1.8 ± 1.5        | 0.44 ± 1.37        | 6.77 ± 2.48            |
| NYAD 1250 (alv)        | 134            | 1.7 ± 1.4        | 0.40 ± 1.42        | 2.43 ± 1.61            |
| NYAD G Wollastocote 1001 (thor) | 338          | 3.7 ± 1.6        | 0.77 ± 1.98        | 7.98 ± 2.03            |
| NYAD G Wollastocote 1001 (alv) | 266          | 2.8 ± 1.6        | 0.56 ± 1.61        | 3.61 ± 1.55            |
| NYAD G Wollastocote 2075 (thor) | 318          | 4.3 ± 2.0        | 0.83 ± 1.83        | 9.17 ± 1.80            |
| NYAD G Wollastocote 2075 (alv) | 373          | 3.5 ± 1.6        | 0.70 ± 1.61        | 5.26 ± 1.67            |
| Promaxon (thor) fibers | 395            | 1.1 ± 1.6        | 0.19 ± 1.61        | 1.09 ± 1.57            |
| Promaxon (alv) fibers  | 476            | 1.3 ± 1.7        | 0.22 ± 1.83        | 1.10 ± 1.44            |
| Promaxon (thor) agglomeratesa | 250          | 4.0 ± 1.5        | 2.24 ± 1.46        | 6.21 ± 1.79            |
| Promaxon (alv) agglomeratesa | 47            | 3.6 ± 1.3        | 2.41 ± 1.41        | 3.52 ± 1.41            |

Abbreviations: alv, alveolar fraction; thor, thoracic fraction. a Fiber definition: aspect ratio > 3/1. b Weighting by number of fibers. c Weighting by mass of fibers. d Geometric standard deviation. e Using particles with aspect ratio > 1/1.

This paper was presented at the Workshop on Biopersistence of Respirable Synthetic Fibers and Minerals held 7-9 September 1992 in Lyon, France.

Address correspondence to Dr. Bernd Bellmann, Fraunhofer Institute of Toxicology and Aerosol Research, Nikolai-Fuchs-Str. 1, D-30625 Hannover, Germany. Telephone 49 511 5350452. Fax 49 511 5350155.
To get a thoracic particulate mass and respirable particulate mass of the test materials, sizing was done in two steps. In the first step the material was aerosolized by a combination of a dust-feeding system and a high pressure, high-velocity dispersion nozzle into a buffer chamber where big clumps of powder were removed. The airborne fibrous material then was routed into a two-stage, heavy grain load impactor, described elsewhere (7). Depending on the operating conditions of this device, the total aerosol was split into two fractions, coarse and fine, at a preset value of the aerodynamic diameter of the fibers. The noninhalable coarse particles were collected on impaction plates impregnated with vacuum oil, leaving the fine particle fraction to be sampled downstream of the separator by a membrane filter. The fine material was removed mechanically from the membrane filter. 

For each sample a small fraction was suspended in double-distilled water, sonicated and filtered on to a Nuclepore filter (pore size 0.2 or 0.4 μm). A part of the filter was mounted on an aluminum stub and sputtered with approximately 30 nm of gold. These samples were analyzed by a Cambridge Stereoscan 360 scanning electron microscope (SEM), with a magnification to enable the measurement of both the longest and the thinnest fibers with sufficient precision. The length and the diameter were measured of some 400 particles of each sample of sized materials. The length, diameter, and calculated aerodynamic diameter (8) are presented in Table 1.

Two milligrams of fibers, suspended in 0.3 ml of 0.9% NaCl solution, were instilled intratracheally in a single dose into the lung of a female Wistar rat with a body weight of about 200 g. Five animals per group that had received either wollastonite or crocidolite were sacrificed after 2 days, 2 weeks, 1, 3, and 6 months; those that had received crocidolite were sacrificed after 2 days and 6 months.

After sacrifice, the lungs were isolated, oven-dried at 105°C and subjected to low-temperature ashing, which did not influence the size distribution of test materials. This was confirmed by comparing lung ash samples from the lungs of rats two days after intratracheal instillation with the original test materials. A fraction of the lung ash was suspended in filtered water and filtered on a Nuclepore filter (pore size 0.2 or 0.4 μm) within 15 min. These samples were prepared and analyzed by SEM as described for the characterization of the test materials. For each lung ash sample 200 fibers were measured on SEM videoprints and the total number of fibers per lung was calculated. For samples with low fiber content, 50 SEM screens were analyzed. The size distribution of the fibers was also analyzed. From the shape of the fibers, the volume of the particles was estimated assuming cylindrical geometry. Clearance kinetics were calculated from a regression analysis of the logarithm of number or mass of fibers versus time after instillation for individual animals.

**Results**

Examination of the distribution of fibers in the lung by SEM 2 days after intratracheal instillation showed fibers in the main bronchi, on the epithelium of the distal segments of bronchioli, and in alveoli; no large agglomerations of fibers were found. The results indicated the evenness of fiber distribution in the lung.

For all wollastonite test materials, the initial mass found in the lung 2 days after instillation was approximately 0.6 to 2 mg calculated from the shape and density of particles. Six months later the relative mass of test material was 0.02 to 0.5% of the initial lung burden. The logarithmic plot of the number or mass of wollastonite fibers versus sacrifice date (Figures 1–3), shows that the elimination of fibers follows first order kinetics, which can be defined by only one parameter, the half-time. For all wollastonite test materials in this study, calculated half-times were between 15 and 24 days (Table 2). No difference in half-times was found between NYAD G Wollastocoat and the coated NYAD G 1001, but the half-time for NYAD G Wollastocoat 2075
was significantly higher, 21 days for the thoracic fraction. The median diameter of this material was some 24% greater than that of the thoracic fraction of NYAD G, a percentage similar to the 26% difference in their half-time. This suggests that the longer half-time could be related to the greater diameter of NYAD G Wollastocoat 2075 fibers.

For crocidolite the number of fibers >5 μm in length was unchanged during 6 months after intratracheal instillation.

Two days after instillation of xonotlite, very few fibers were observed in the lungs. The estimated mass of single fibers was <10 μg per lung. Only 0.4% of the mass fraction of single fibers present in the injected test materials were detectable in the thoracic fraction, and 0.08% in the alveolar fraction. Since the number of fibers or agglomerates was very low, or even zero, in animals sacrificed 14 days and 1 month after instillation, means of five animals were used for regression analysis. No fibers or agglomerates were detected at the 3-month sacrifice date. Estimated half-times for the elimination of the fibers were 4 to 9 days. It was not possible to calculate a half-time from the very rapid 2-day clearance.

Discussion

The half-times for the elimination of wollastonite and xonotlite samples were very short compared both to crocidolite fibers and man-made mineral fibers (2,9). These results confirm the assumption that lack of carcinogenicity after intraperitoneal injection of 100 mg wollastonite in rats could be explained by low durability in vivo (10).

The half-times for the elimination of wollastonite fibers and xonotlite crystals are much shorter than the unimpaired retention half-time of insoluble spherical particles such as toner or PVC particles, for which half-times of approximately 80 days were reported (11). This suggests that mechanical clearance mediated by macrophages could be only of minor importance for wollastonite, for which dissolution of fibers must be the important clearance process. Since wollastonite is acid labile, dissolution would be fastest in the phagolysosomes of the macrophages, where the pH is approximately 4.8 (12). Two days after instillation, the number of wollastonite fibers in the lung was about 100 to 200 × 10⁶. The total number of macrophages in a rat lung is about 15 × 10⁶ (13), giving a mean of approximately 10 wollastonite fibers per macrophage. Because the fraction of fibers >10 μm in length is 10% or less, most of the fibers could be phagocytized completely by macrophages.

For all the wollastonite samples in this study, the half-time for elimination of the mass of fibers was very similar to that for total number of fibers, but the decrease of mass from day 2 to day 14 was higher for the thoracic fractions of all the wollastonite samples than for the corresponding alveolar fractions (Figure 3). This effect could be explained by mucociliary clearance of large particles with an aerodynamic diameter >10 μm, which were found only in the thoracic fractions. A fast decrease of the aerodynamic diameter from day 2 to day 14, detected only for the thoracic fractions, tends to confirm this hypothesis.

The decrease of number of fiber >5 μm in length is faster than the decrease of total number of fibers for all wollastonite samples up to 1 month (Figures 1, 2). That could be due to breakage of fibers, which would tend to increase the number of fibers <5 μm in length.

The elimination kinetics of the xonotlite fibers was at least one order of magnitude faster than that of wollastonite fibers. That may have been due to the fast dissolution of the xonotlite crystals, which have small diameter and large surface. Only about 0.5% of the xonotlite crystals was >5 μm in length. The very fast dissolution of xonotlite fibers in the lung can explain...
Table 2. Half-time and 95% confidence limit (CL) of the elimination of fibers. *

| Test material | Number of fibers | Number of fibers, L > 5 μm | Mass of particles |
|---------------|-----------------|-----------------------------|-------------------|
|               | Mean (95% CL)   | Mean (95% CL)               | Mean (95% CL)     |
| UICC Crocidolitea | 226 (146-613) | ∞ (325-∞) | ∞ (389-∞) |
| NYAD G (thor) | 17 (16-18)     | 18 (15-21) | 19 (17-22) |
| NYAD G (alv)  | 17 (15-18)     | 18 (16-22) | 18 (16-20) |
| NYAD 1250     | 16 (15-17)     | 19 (16-24) | 16 (14-18) |
| NYAD 2075     | 15 (13-16)     | 18 (14-26) | 17 (16-18) |
| NYAD Wollastocoat 1001 (thor) | 16 (15-17) | 16 (13-21) | 16 (14-17) |
| NYAD Wollastocoat 1001 (alv) | 18 (16-18) | 19 (16-23) | 17 (16-18) |
| NYAD Wollastocoat 2075 (thor) | 21 (18-26) | 22 (18-27) | 24 (19-33) |
| NYAD Wollastocoat 2075 (alv) | 19 (18-21) | 19 (17-21) | 20 (18-22) |

Abbreviations: alv, alveolar; thor, thoracic. * Calculation from sacrifice dates 2, 14 days, 1, 3 and 6 months after instillation. a Calculation from sacrifice dates 2 days and B months only.

Figure 3. Decrease in calculated mass of wollastonite fibers in the lung ash after intratracheal instillation.

why these fibers did not induce lesions in the lung, whereas attapulgite, chrysotile, and Fiberfax fibers did induce lesions 1 month after intratracheal instillation of 1, 5, or 10 mg/lung in rats (14).

Conclusion

The elimination of wollastonite fibers from the lung followed first order kinetics, and was predominantly due to the dissolution of fibers, with very short half-times of 15 to 21 days. The elimination of xonotlite crystals from the lung was even faster.

The relatively fast dissolution of the test materials, which are all composed of a "calcium silicate" base, should minimize the health effects related to respirated fibers.

REFERENCES

1. Muhle H, Bellmann B, Pott F. Durability of various mineral fibers in rat lungs. In: Mechanisms in Fiber Carcinogenesis (Brown RC, Hoskins JA, Johnson NF, eds). NATO ASI Series. New York: Plenum Press, 1991.

2. Bellmann B, Muhle H, Pott F. Study on the durability of chemically different glass fibers in lung of rats. Zbl Hyg 190:310–314 (1990).

3. Pott F, Schlipkötter H-W, Roller M, Rippe RM, Germann P-G, Mohr U, Bellmann B. Carcinogenicity of glass fibers with different durability. Zbl Hyg 189:563–566 (1990).

4. ACGIH. Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. American Conference of Governmental Industrial Hygienists. Cincinnati, OH, 1990–1991, 46.

5. Greenberg SD. Carcinomas of the peripheral airways. In: Lung Carcinomas (Dowell EM, ed). New York: Churchill Livingstone, 1987:286–309.

6. Pott F. A hypothesis for explaining the syncarcinogenic effect of cigarette smoke and asbestos. In: Biological Interaction of Inhaled Mineral Fibers and Cigarette Smoke (Wehner AP, ed). Columbus: Batelle Press, 1989:51–62.

7. Muhle H, Koch W, Bellmann B. Acute and subchronic effects of intratraehally instilled nickel containing particles in hamsters. In: Health Hazards and Biological Effects of Welding Fumes and Gases (Stern RM, Berlin A, Fletcher AC, Järvisalo J, eds). New York: Excerpta Medica, 1986:337–340.

8. Harris RL, Fraser DA. A model for deposition of fibers in the human respiratory system. Am Ind Hyg Assoc J 37:73–89 (1976).

9. Bellmann B, Muhle H, Pott F, König H, Klöppel H, Spurny K. Persistence of manmade mineral fibres (MMMF) and asbestos in rat lungs. Ann Occup Hyg 31:693–709 (1987).
10. Pott F, Ziem U, Reiffer F-J, Huth F, Ernst H, Mohr U. Carcinogenicity studies on fibres, metal compounds, and some other dusts in rats. Exp Pathol 32:129–152 (1987).

11. Muhle H, Bellmann B, Creutzenberg O, Heinrich U, Mermelstein R. Dust overloading of lungs: investigation of various materials, species differences, and irreversibility of effects. J Aerosol Med 3(Suppl 1):S-111–S-128 (1990).

12. Kreyling WG, Nyberg K, Collier CG, Camner P, Heilmann P, Lundborg M, Matejkova E. Interspecies comparison of phagolysosomal pH in alveolar macrophages. Inhal Toxicol 3:91–100 (1991).

13. Bellmann B, Muhle H, Creutzenberg O, Mermelstein R. Recovery behaviour after dust overloading of lungs in rats. J Aerosol Sci 21:377–380 (1990).

14. Lemaire I, Dionne PG, Nadeau D, Dunnigan J. Rat lung reactivity to natural and man-made fibrous silicates following short-term exposure. Environ Res 48:193–210 (1989).