

Significance of the Biodurability of Man-made Vitreous Fibers to Risk Assessment

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It is generally agreed that the biodurability of man-made vitreous fibers is a major factor for the characterization of potential health effects. As there is currently no standardization of experimental protocols to determine biodurability, the results of the clearance assays have not been used up to now for regulatory purposes. Methods used to analyze biodurability in animal models are short-term inhalational exposure and intratracheal instillation of rat respirable fibers. Both test methods have strengths and limitations for regulatory purposes. We outline recommended procedures for standardized biodurability assays that can be used to compare different fiber types. In animal experiments, biodurability is difficult to separate from biopersistence, as mucociliary and macrophage-mediated clearance occur simultaneously with dissolution and disintegration. For intratracheal instillation, a sized rat respirable sample must be used. Precautions should be taken to prevent aggregation of fibers in the lungs. Although from a scientific point of view questions remain about quantifying the influence of fiber length, diameter, dose, and exposure route, consistent data on the biodurability of vitreous glass fibers are available which may be used for regulatory purposes. — *Environ Health Perspect* 105(Suppl 5):1045–1047 (1997)

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Introduction

It is generally agreed that the biodurability of man-made vitreous fibers (MMVF) is a major factor for the characterization of potential health effects (1). According to this working hypothesis, faster removal of MMVF from lungs leads to a decreased risk of fiber-induced health effects. As there is currently a lack of standardized experimental protocols to determine biodurability, the results of clearance kinetics have not yet been used for regulatory purposes.

It should be noted that the definition of biodurability differs from that of biopersistence. Biopersistence is defined as the ability of a fiber to remain in the lung in spite of the lung's physiological clearance mechanisms. These defense mechanisms are *a*) transportation of entire particles by the mucociliary

escalator and by alveolar macrophages, *b*) dissolution of fibers, and *c*) disintegration.

Biodurability includes only the removal of fibers from lungs by dissolution and disintegration. Biopersistence and biodurability of fibers are often used as synonyms, which leads to misunderstandings. Fibers that persist in the lung for long periods of time have to be durable, but durable fibers can be removed from the lungs by physical transportation, e.g., by ciliary clearance and macrophage-mediated clearance. In animal experiments, biodurability of fibers is difficult to determine, as the physical removal of entire fibers is also involved. For risk assessment, however, focus should be on biodurability and not on biopersistence, as the fraction of fibers removed by physical

transportation may differ in experimental animals, compared to humans.

Toxicokinetics of Fibers

Fibers differ from nonfibrous particulates in their deposition probabilities at various surfaces of the respiratory tract. They may also have different retention times at deposition sites, as well as different clearance pathways (1,2).

Deposition

Deposition of long MMVF primarily occurs in rats in the ciliated airways (3). The authors concluded from their calculation that, in rats, a fiber having a diameter of 1 μm and a length of 20 μm has about a 5-fold higher deposition fraction in the tracheobronchial region than in the pulmonary region.

Clearance

The fate of fibers deposited on surfaces within the respiratory system depends on the site of deposition and the characteristics of the fibers.

Physical transportation refers to the movement of the intact fiber along the epithelial surface from alveoli and terminal bronchioles to the respiratory bronchioles. Alveolar macrophages play a dominant role in this process. The elimination of durable fibers from the lung is dependent on fiber length, fiber diameter, and fiber mass retained in the lungs. Alveolar macrophages can only completely phagocytize fibers up to about 10 μm in length, prior to their removal via the ciliated airways (4,5). In addition, fibers may be translocated *a*) to the ciliated epithelium at the terminal bronchioles, *b*) into and through the epithelium, or *c*) along lymphatic drainage pathways.

Dissolution is the process of removal by dissolving a fiber that is dependent on the chemical composition of the fiber (2). For vitreous fibers this is a complex process that includes network dissolution and leaching the various components such as alkali ions (6).

Disintegration includes processes such as the subdivision of fibers into shorter segments. This may result from local dissolution or by building a leached layer easily removed by a physical process (7).

Processes important for fiber removal are listed in Figure 1. In a simplistic approach the clearance of fibers from lungs may be expressed by first-order kinetics. This approach may be too simple for the final evaluation of data, but it is

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Abbreviations used: *k*, clearance rate constant; *m*, retained fiber mass; MMVF, man-made vitreous fibers; *t*, time.

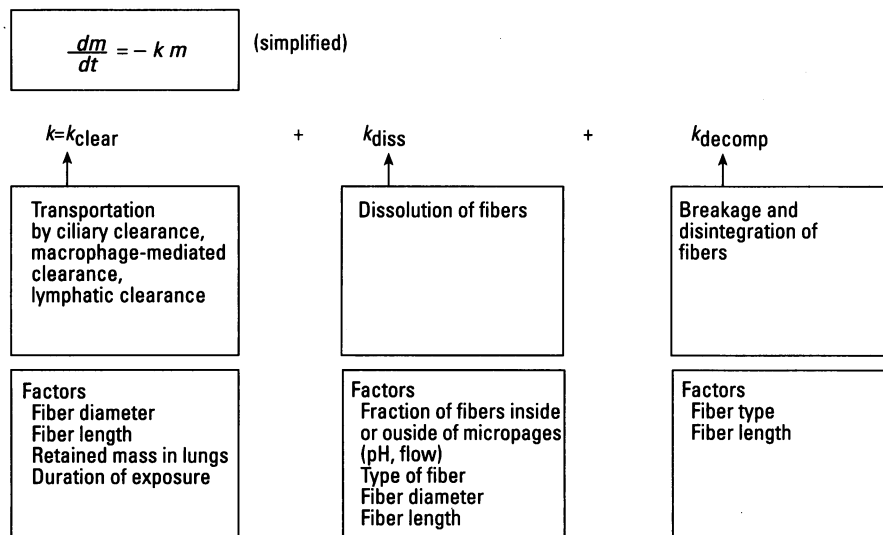


Figure 1. Principles of fiber removal from lungs and factors that influence each fiber clearance process. Abbreviations: m , retained fiber mass; k , clearance rate constant; t , time.

appropriate for the understanding of the principles this approach.

The overall clearance rate constant k can be separated into subfractions. k_{clear} describes the transportation of fibers by physical removal from lungs as an entire particle, by ciliary clearance, macrophage-mediated clearance, and lymphatic clearance. k_{diss} describes the fraction of clearance that is due to dissolution, and k_{decomp} is fiber removal by breakage and disintegration of fibers. These three processes are dependent on fiber characteristics and the exposure regimen. This clearly shows that conventions must be found for the measurement of biodurability.

The physical transportation of fibers may be species dependent. This distinction should be considered when comparing rats to humans.

Test Methods

There are two different principal approaches published for the analysis of biodurability: *a*) Short-term inhalation of fibers and a posttreatment observation period of 6 to 18 months, with serial sacrifices for analysis of fibers in lungs (8–12); *b*) Intratracheal instillation of sized fibers (rat respirable) of 0.5 to 2 mg per animal and subsequent analysis (13–15). The dose of 2 mg was chosen to achieve a lung burden in a chronic inhalation study.

The advantage of a short-term inhalation compared to intratracheal instillation is more even deposition of fibers in the respiratory tract. However, calculations of Yu and Asgharian (3) show that fibers longer than 20 μm have a relatively high

deposition in the tracheobronchial airways because of interception. Recent data on the clearance of isometric particles from human airways show that ciliary clearance does not remove all particles after 2 days (16). This effect may also occur in rats; therefore, clearance of fibers after short-term inhalation may be influenced by ciliary clearance. After a long-term exposure, fibers may accumulate in the lung compartment with the slowest clearance. It is not clear whether data obtained after short-term exposure in rats can be extrapolated to humans who are exposed chronically.

For regulatory purposes Bernstein et al. (9) suggested using only clearance data derived from rat short-term inhalation studies of fibers longer than 20 μm . The argument for this suggestion is that macrophage-mediated clearance will not affect these results, as the fibers are too long to be removed by macrophages. This is important to consider in order to measure biodurability (i.e., removal only by dissolution and disintegration) and to avoid the fraction of removal by physical clearance. However, fibers longer than 20 μm show high deposition in the ciliated airways. Lehnert and Oberdörster (17) noted that the mucous layer in the conducting airways is absent in some areas. This also indicates that the results of biodurability after short-term inhalation exposure may be compromised by slow physical removal processes.

Intratracheal instillation of rat respirable fibers may lead to a less even distribution in the lungs compared to inhalation. The fraction that is deposited in the pulmonary

region is probably higher because the deposition by interception of long fibers is less. In addition, fibers longer than 40 μm can contribute to the formation of aggregates in lungs. The technique of intratracheal instillation should be validated by lung examination using scanning electron microscopy. One technical advantage of the intratracheal instillation method is that the amount of sized fibers to be used is less (by a factor of 50), compared to the short-term inhalation assay. Macrophage-mediated clearance may be significantly reduced at a lung burden of about 1 mg of fibers (11). However, the instillation of 1 or 2 mg of fibers induces inflammatory reactions in the lung, which may change the flow of fluids responsible for fiber dissolution.

Further key issues that must be addressed for regulatory purposes are

- The removal of fibers from the lung depends on fiber diameter. Rat inhalable fibers are thinner compared to human inhalable fibers. In rat studies, different diameters of various fibers were used although they were sized to achieve a similar diameter. It is an open question whether a correction factor should be applied for the different diameters.
- The retained dose may influence macrophage-mediated clearance (Figure 1). Although in intratracheal instillation studies, the same dose (e.g., 2 mg) is given to rats, the retained dose may vary at the first sacrifice date for fibers with relatively fast dissolution. A correction factor for differences in the retained dose is under discussion but currently no satisfactory approach is available.
- For comparing *in vitro* dissolution results with biodurability data, the ciliated clearance and macrophage-mediated clearance should be minimal.
- Information on carcinogenicity (if available) must be correlated with results of biodurability to define critical values for regulation. A critical step is to define half-times of fiber clearance below which no carcinogenicity can be assumed.

In summary, in short-term inhalation studies a significant fraction of fibers is cleared by physical removal of fibers. Completeness of short-term clearance of long fibers deposited in the tracheobronchial region has not been confirmed. In the observed overall clearance, there may be considerable interference with the dissolution of fibers. Dissolution of fibers in lungs is an important factor in the potential accumulation of MMVF in human lungs. If biodurability data are used for regulation of MMVF, this issue should be considered.

Data from Research and Consulting Company, Hingen, Switzerland and Fraunhofer Institute, Hannover, Germany) are currently being reviewed for use in standardizing biodurability tests. Important areas of consideration include *a*) methods of application of fibers, *b*) role of dose, *c*) influence of fiber dimensions on results, *d*) counting procedures of fibers, and *e*) mathematical evaluation of data (e.g., how to calculate a half-time for regulation).

For the 5-day inhalation test for biodurability, a biphasic kinetic may be the best approach, leading to two half-times for the fast and the slow phase.

Although from a scientific point of view, there are ongoing questions about quantifying the influence of fiber dimensions, dose, and exposure route, consistent data on the biodurability of vitreous glass fibers are available that may be used for regulatory purposes.

In Germany, MMVF currently are regulated partly on the basis of the chemical composition of fibers. This approach is based primarily on correlation between carcinogenicity data after ip injection and chemical composition (18).

In the European Union, both methods of biodurability studies, short-term inhalation and intratracheal instillation, are currently considered to be used for regulatory purposes.

REFERENCES

- McClellan RO, Hesterberg TW. Role of biopersistence in the pathogenicity of man-made fibers and methods for evaluating biopersistence: a summary of two round-table discussions. *Environ Health Perspect* 102(Suppl 5):277-283 (1994).
- Lippmann M. Biophysical factors affecting fiber toxicity. In: *Fiber Toxicology* (Warheit DB, ed) San Diego:Academic Press, 1993;259-303.
- Yu CP, Asgharian B. Mathematical models of fiber deposition in the lung. In: *Fiber Toxicology* (Warheit DB, ed.). San Diego, CA:Academic Press, 1993;73-98.
- Morgan A. Effect of length on the clearance of fibres from the lung and on body formation. In: *Biological Effects of Mineral Fibres* (Wagner, JC, ed). IARC Scientific Publ No 30. Lyon: International Agency for Research on Cancer, 1980;329-335.
- Bolton RE, Vincent JH, Jones AD, Addison J, Beckett ST. An overload hypothesis for pulmonary clearance of UICC amosite fibres inhaled by rats. *Br J Ind Med* 40:264-272 (1983).
- Scholze H, Conradt R. An *in vitro* study of the chemical durability of siliceous fibres. *Ann Occup Hyg* 31:639-692 (1987).
- Potter RM, Mattson SM. Glass fiber dissolution in a physiological saline solution. *Glastech Ber* 64:16-28 (1991).
- Musselman RP, Miiller W, Easters W, Hadley J, Kampstrup O, Thévenaz P, Hesterberg T. Biopersistence of crocidolite versus man-made vitreous fibers in rat lungs after brief exposures. In: *Toxic and Carcinogenic Effects of Solid Particles in the Respiratory Tract*. Washington:ILSI Press, 1994;451-454.
- Bernstein DM, Morscheidt C, Grimm HG, Thévenaz P, Teichert U. Evaluation of soluble fibers using the inhalation biopersistence model, a nine-fiber comparison. *Inhal Toxicol* 8: 345-385 (1996).
- Bernstein DM, Morscheidt C, Tiesler H, Grimm H-G, Thévenaz P, Teichert U. Evaluation of the biopersistence of commercial and experimental fibers following inhalation. *Inhal Toxicol* 7:131-158 (1995).
- Creutzenberg O, Bellmann B, Muhle H. Biopersistence and bronchoalveolar lavage fluids analysis after a subacute inhalation of rats to various man-made mineral fibres. *Inhaled Particles, VIII. Ann Occup Hyg* 41:213-218 (1997).
- Hesterberg TW, Miiller WC, Musselman RP, Kamstrup O, Hamilton RD, Thevenaz P. Biopersistence of man-made vitreous fibers and crocidolite asbestos in the rat lung following inhalation. *Fundam Appl Toxicol* 29:267-279 (1996).
- Muhle H, Bellmann B, Pott F. Comparative investigations of the biodurability of mineral fibers in the rat lung. *Environ Health Perspect* 102(Suppl 5):163-168 (1994).
- Bellmann B, Muhle H, Kamstrup O, Draeger UF. Investigations of the biodurability of man-made vitreous fibers in rat lungs. *Environ Health Perspect* 102(Suppl 5):185-189 (1994).
- Bellmann B, Muhle H, Kamstrup O, Draeger UF. Investigation of the biodurability of chemically different stone wool fibres. *Exp Toxicol Pathol* 47:195-201 (1995).
- Scheuch G, Stahlhofen W, Heyder J. An approach to deposition and clearance measurements in human airways. *J Aerosol Med* 9: 35-41 (1996).
- Lehnert BE, Oberdörster G. Fate of fibers in the lower respiratory tract and fiber-induced lung diseases. In: *Fiber Toxicology* (Warheit DB, ed). San Diego, CA:Academic Press, 1993; 349-394.
- Germany, Federal Minister of Labor. TRGS 906 (Technische Regel für Gefahrstoffe). Begründung für die Einstufung von anorganischen Faserstäuben (ausgenommen Asbest). *Bundesarbeitsblatt* 10:46 (1995).