Chronic Inhalation and Biopersistence of Refractory Ceramic Fiber in Rats and Hamsters

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Lifetime “nose-only” inhalation studies were conducted in rats using four types of refractory ceramic fibers (FCF), 1 μm in diameter x 22 to 26 μm length: High Purity, Kaolin, Zirconia, and After-Service; and on hamsters using Kaolin RCF. For comparison, animals also were exposed to chrysotile fibers. Rats were exposed 6 hr/day, 5 days/week for 24 months to concentrations ranging between 3 and 30 mg/m³. Time- and dose-dependent lesions in the rat included the development of interstitial fibrosis, pleural fibrosis, pulmonary tumors, and mesothelioma. Exposure to 3, 9, or 16 mg/m³ produced no excess lung tumors; no fibrosis was seen at 3 mg/m³. A significant increase in lung tumors and interstitial fibrosis was observed at 30 mg/m³. A single mesothelioma was observed in rats exposed to 9 mg/m³, while two occurred at 30 mg/m³. Hamsters were similarly exposed to 30 mg/m³ Kaolin RCF for 18 months; no lung tumors were induced, but pulmonary and pleural fibrosis were observed and there was a 42% incidence of mesothelioma. Multiple interim sacrifices together with recovery animals allowed detailed assessment of the lung burden of RCF, which was found to be dose related and, at the high doses, exceeded 10³ fibers/mg of dry lung. During the various recovery periods there was a clear reduction in fiber burden. Mathematical modeling of these data for deposition, clearance, and retention and for species is currently underway. — Environ Health Perspect 102(Suppl 5):207–209 (1994)

Key words: refractory ceramic fiber, RCF, biopersistence, chronic disease

Introduction

Kaolin-based refractory ceramic fiber (RCF) is a man-made mineral fiber that has a wide industrial application. It is the smallest category of man-made vitreous fibers (MMVF), representing about 1 to 2% of the total world-wide MMVF production. RCF is a white fibrous material made by melting a combination of Al₂O₃ and SiO₂ (50:50) or by melting kaolin clay. Other oxides sometimes are added to change the fiber properties (1). The principal applications are related to resistance to high temperatures, insulation properties, light weight, and low cost.

Toxicologic studies to date produced conflicting data in terms of evaluating potential carcinogenic hazard (2, 3). Given the increasing use and potential for human exposure, there was a clear need to better characterize the potential hazards of chronic exposure to RCF fibers. The purpose of these investigations, which are part of a larger program consisting of both toxicologic and epidemiologic investigations, was to conduct state-of-the-art studies to ascertain any potential health hazard of RCF.

Methods and Materials

Exposure

Weanling Syrian golden hamsters and Fischer 344 rats were exposed to the experimentally determined maximum tolerated dose of 30 mg/m³ of presized fiber, 1 μm in diameter and 22 to 26 μm in length. The animals were exposed 6 hr/day, 5 days/week, rats for 24 months and hamsters for 18 months. The terminal sacrifice occurred at 29 months for rats and 20 months for hamsters, by which time survival in the population of exposed animals had been reduced to 20%. Groups of three or six animals were removed periodically for histopathological examination, scanning electron microscopic (SEM) examination, and determination of lung burden, during and at termination of the exposure.

Rats were exposed to four types of RCFs. These included three RCFs—Kaolin, Zirconia, and High-Purity of different chemical compositions—and an “After-service” RCF, which is a kaolin-based ceramic fiber previously exposed to high temperatures. The After-Service fiber contained 27% cristobalite, a form of crystalsilica, that had shorter fibers with a mean length of 12.7 μm, but an increased mean diameter of 1.38 μm. Hamsters were exposed only to the Kaolin RCF. For comparison the animals were also exposed to chrysotile. Properties of the aerosolized fiber are given in Table 1.

Fiber Aerosol Generation and Atmospheric Characterization

An innovative inhalation exposure system that allowed for the exposure of single animals was specifically designed for this study (4). This system allowed for a flow-past, nose-only exposure to each animal. Fiber was lofted using a nondestructive system

| Test fibers | Mean Fr/cc fibers (mg/m³) | Mean diameter (μm) | Mean length (μm) |
|-------------|--------------------------|-------------------|-----------------|
| Kaolin      | 30                       | 215               | 0.9             | 22.1 |
| Kaolin      | 29                       | 191               | 0.9             | 22.2 |
| Kaolin      | 16                       | 115.1             | 1.0             | 20.8 |
| Kaolin      | 9                        | 73.6              | 1.0             | 20.7 |
| Kaolin      | 3                        | 26.0              | 1.0             | 20.8 |
| Zirconia    | 29                       | 224               | 1.0             | 18.6 |
| High purity | 29                       | 172               | 1.0             | 24.2 |
| After service | 30                   | 1060              | 1.3             | 12.7 |
| Chrysotile  | 11                       | 1000              | 0.98            | 2.17 |

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Environmental Health Perspectives 207
Table 2. Pathology and lung burden following exposure to RCFs (24-month exposure).

| Test fibers | Lung burden, fibers/ml dry lung | Total Wagner score | Total lung tumorsa | Mesotheliomas |
|-------------|---------------------------------|-------------------|-------------------|--------------|
| Kaolin, hamsters | 30 2.39E+05 | 4 | 0 | 43 (42%) |
| Kaolin, rats | 29 3.70E+05 | 4 | 16 (14.6%) | 2 (1.6%) |
| Kaolin | 16 2.70E+05 | 4 | 2 (1.8%) | 0 |
| Kaolin | 9 1.80E+05 | 4 | 5 (4.3%) | 1 (0.8%) |
| Kaolin | 3 5.50E+04 | 3.2 | 3 (2.7%) | 0 |
| Zirconia | 29 9.60E+05 | 4 | 11 (8.0%) | 3 (2.5%) |
| High Purity | 29 2.70E+05 | 4.3 | 18 (15%) | 2 (1.7%) |
| After Service | 30 5.90E+07 | 3.8 | 4 (4%) | 1 (0.8%) |
| Chrysotile | 11 1.00E+07 | 4 | 13 (21%) | 1 (1.4%) |

aAdenoma and carcinoma.

that delivered the fiber to the animals' noses at a positive pressure and in laminar flow.

Chamber concentrations of the RCF were monitored by gravimetry for fiber mass (mg/m³) and by optical microscopy for total fiber count (fibers/cc). Fiber length was measured using optical microscopy, and SEM was used to determine fiber diameter. To assure the uniformity of the exposure over each 6-hr exposure, the fiber concentrations were monitored continuously using a RAS (CGA Corporation) light-scattering device.

Clinicopathology

Rats were observed twice daily throughout the study for clinical signs, morbidity, and mortality. Lungs were removed in toto, weighed, examined for gross pathology under a dissecting microscope, and then perfused with Karnovsky’s fixative via the trachea at a pressure of 30 cm for 2 hr. After fixation, a consistently uniform transverse section of the left lung, 2-mm diameter, was obtained from each animal for routine histopathology. Sections were stained with hematoxylin and eosin (H&E) and Masson-Goldner's trichrome stain for collagen deposition. Lungs were examined and classified histopathologically according to the established guidelines (5). In this system a grade of 1.0 is considered to be normal, grades 2 and 3 are evidence of focal cellular changes and are considered to be reversible, while grades 4 to 8 represent the former lesions plus increasing degrees of fibrosis and are irreversible.

Lung Fiber Determinations

The right intermediate lobe was removed prior to fixation, weighed, and frozen to determine lung burden. These samples were dried to constant weight, ashed using a low-temperature plasma asher (LFE LTA 504 multiple chamber plasma unit), and examined at ×400 using a phase contrast microscope. The results were expressed as numbers of the World Health Organization (WHO) fibers per milligram dry lung. The distribution of fiber size, by diameter and length, was also recorded (6,7).

Results

Animals were exposed to varying quantities of rodent-respirable RCF aerosols. Lofting did not alter the dimensions of the fibers. RCF lung burdens were found to be dose related and, at the high doses, exceeded 10⁶ fibers/mg of dry lung. A substantial reduction in fiber burden, fiber clearance, was observed when animals were removed (recovery animals) from the exposure (Figure 1). These data suggest a half-life of approximately 200 days for Kaolin RCF in the rat.

After 6 months of exposure to 30 mg/m³ of Kaolin RCF, hamsters developed interstitial fibrosis (grade 4), which did not progress further. Chrysotile induced a more extensive fibrosis (grade 5) than RCF. At termination, microscopic examination revealed a 42% incidence of pleural mesotheliomas in hamsters exposed to Kaolin RCF, but no lung tumors whatsoever. Neither lung nor pleural tumors were observed in the animals exposed to the chrysotile.

In rats, chrysotile induced a fibrosis that was qualitatively different than the fibrosis observed in the rats exposed to the RCF. While both fibers produced alterations that occurred in the same locations and were characterized as Wagner grade 4, the deposition of collagen following exposure to the asbestos was more severe than that in any of the groups exposed to the RCF.

Rats exposed to 30 mg/m³ of RCF had a noticeably different tumor response than the hamsters, with a statistically significant incidence of 15% lung tumors in the group exposed to Kaolin RCF, 9% in the Zirconia RCF-exposed group, and 15% in the group exposed to High-Purity RCF. Mesotheliomas also were observed, which appear to be biologically significant; but the incidence was not statistically significant. Rats exposed to the "After-service" fiber had fibrosis and "silicosis," but the 3.2% incidence of tumors was comparable to the incidence of 1.6% in the control group. Rats exposed to the lower doses of Kaolin RCF experienced a dose-dependent reduction in the observed responses. No fibrosis was observed at 3 mg/m³. There

![Graph](https://via.placeholder.com/150)

Figure 1. Lung retention of kaolin in rats. Lung burdens expressed in WHO fibers per milligram dry lung tissue in rats exposed and sacrificed immediately (24 months, 0 months recovery) or removed from the exposure and held for observation until the end of the exposure period (18-months exposure, 6-months recovery, etc.).
was no excess of lung tumors at 3, 9 or 16 mg/m³, and a single mesothelioma was observed at 9 mg/m³ (Table 2).

As expected from prior studies (5,8), 21% of the rats exposed to the chrysotile developed lung tumors. All other lesions were considered consistent with the strains and ages of the animals in this study.

Discussion

The results from these studies indicate that repeated exposure to extremely high concentrations approximately 200 fibers per cubic centimeter of specially processed rodent-respirable RCF can produce progressive lung damage (fibrosis), lung tumors, and pleural tumors (mesothelioma). The absence of lung tumors in the hamsters exposed to the chrysotile was surprising and may indicate that the hamster is resistant to the development of lung tumors. Further, the lack of lung tumors and surprisingly high incidence of mesotheliomas in the hamster, as compared to the rat, suggests a difference in either species sensitivity, pulmonary dynamics (deposition, translocation, or clearance) or a combination of both.

Lung disease has been demonstrated to be far more significant than pleural disease in humans exposed to natural fibers. Hence, these data raise doubts about the usefulness and validity of the hamster as a surrogate for humans for the qualitative or quantitative evaluation of the hazard posed by exposure to fibers. The results observed with the After-Service fiber are also striking. The data suggest that fiber length may be a significant factor in the tumorigenicity of a durable fiber. Further, it appears from the dose-response study of Kaolin RCF that the dose-response curve is exceedingly steep. The data suggest that a no-effect level was observed for both fibrosis and tumor production.

A very large number of data points were obtained on the lung burdens and biopersistence of fibers in the exposed animals. These dose-to-target-organ data are being modeled to evaluate the deposition, clearance, and retention of the inhaled fibers. Preliminary modeling data suggest impaired clearance occurred at 30 mg/m³ with a half-life of approximately 200 days as compared to the lower doses with half-lives of about 140 days. It is expected that this phase of the investigation will be very useful in understanding the observed differences between rats and hamsters and will contribute to a quantitative summary for descriptive toxicology studies.

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