

# Lung Proliferative and Clearance Responses to Inhaled *para*-Aramid RFP in Exposed Hamsters and Rats: Comparisons with Chrysotile Asbestos Fibers

David B. Warheit, Suzanne I. Snajdr, Mark A. Hartsky, and Steven R. Frame

DuPont Haskell Laboratory, Newark, Delaware

This study compared pulmonary effects of *para*-aramid respirable-sized, fiber-shaped particles (RFP) (*p*-aramid fibrils) and chrysotile asbestos fiber exposures in rats. Additional *p*-aramid inhalation studies were conducted in hamsters to compare species responses. The hamster results are preliminary. The parameters studied were clearance/biopersistence of inhaled *p*-aramid RFP or size-separated asbestos fibers as well as pulmonary cell proliferation and inflammation indices after 2-week inhalation exposures. Rats were exposed nose only to chrysotile asbestos fibers at concentrations of 459 and 782 fibers/ml or to *p*-aramid RFP at 419 or 772 fibrils/ml. Hamsters were exposed whole body to *p*-aramid RFP at concentrations of 358 and 659 fibrils/ml. Subsequently, animals were assessed immediately (time 0) as well as 5 days (10 days for hamsters), 1, 3, 6, and 12 months postexposure. Lung burdens for the *p*-aramid-exposed rats were  $4.8 \times 10^7$  and  $7.6 \times 10^7$  fibrils/lung, with similar numbers of chrysotile fibers  $> 5 \mu\text{m}$  recovered from the lungs of asbestos-exposed rats. In comparison,  $1.4 \times 10^6$  fibrils/lung were recovered in the high-dose hamster group. Biopersistence studies in *p*-aramid-exposed rats and hamsters demonstrated an initial increase (relative to time 0) in retained *p*-aramid fibrils during the first month postexposure, which indicated breakage or shortening of inhaled fibrils. This result was associated with a progressive reduction, and increased residence time in the lung, in the mean lengths of the fibrils, which signified biodegradability of inhaled *p*-aramid fibrils in both species. In contrast, clearance of short chrysotile asbestos fibers was rapid, but clearance of the long chrysotile fibers was slow or insignificant, as evidenced by a progressive increase over time in the mean lengths of fibers recovered from the lungs of exposed rats. Two-week, high-dose exposures to *p*-aramid in both rats and hamsters produced transient increases in pulmonary inflammatory and cell proliferative responses. In contrast, inhalation of size-separated chrysotile asbestos fibers in rats produced persistent increases in cell labeling indices of airway, alveolar, and subpleural cells measured through a period of 1 to 3 months postexposure. These results suggest that inhaled *p*-aramid RFP are biodegradable in the lungs of exposed rats and hamsters. In contrast, exposures to chrysotile asbestos fibers in rats resulted in a selective pulmonary retention of long chrysotile fibers. — *Environ Health Perspect* 105(Suppl 5):1219–1222 (1997)

**Key words:** pulmonary cell proliferation, fiber toxicology, biodegradability of inhaled fibers, chrysotile asbestos fibers, *para*-aramid fibrils, organic fibers, biopersistence, fiber clearance, interspecies comparisons, pulmonary effects, inhaled fibers, inhalation toxicology, lung effects, hamsters, rats

## Introduction

*p*-Aramid fibrils have been used as asbestos fiber substitutes in commercial applications such as brake linings and gaskets. In the current studies, the pulmonary effects of *p*-aramid respirable-sized, fiber-shaped

particulates (RFP) have been compared to those of chrysotile asbestos after 2-week inhalation exposures in rats. Fiber dimension is an important factor that influences the pulmonary toxicity of inhaled fibers

when various fiber types are compared. It is generally accepted that longer fibers produce greater toxicity than shorter fibers with similar chemistry (1). Most sample preparations of Union Internationale Contre le Cancer (UICC) chrysotile asbestos fibers are very short, with average lengths of 2  $\mu\text{m}$ . The *p*-aramid samples used in this study had mean lengths in the range of 11 to 12  $\mu\text{m}$ . Therefore, to make reasonable comparisons of the effects of inhaled *p*-aramid with chrysotile asbestos fibers in rats, we attempted to enhance the proportion of longer fibers in the sample preparation of chrysotile asbestos fibers.

## Methods

Groups of male Crl:CDBR rats or male Syrian golden hamsters (Charles River Breeding Laboratories, Kingston, NY), 7 to 8 weeks of age, were exposed 6 hr/day, 5 days/week for 2 weeks to aerosols of UICC chrysotile asbestos fibers (Canadian chrysotile) or to *p*-aramid RFP. For this study, Kevlar was a representative *p*-aramid RFP (2). After exposure, the lungs of *p*-aramid- or chrysotile-exposed animals and aged-matched sham controls were evaluated for cell proliferation on airway, parenchymal, and subpleural surfaces using bromodeoxyuridine (BrdU) immunostaining techniques. Evaluations were conducted for pulmonary clearance and retention at time 0, 5 days, and 1, 3, 6, and 12 months postexposure. Similarly, the lungs of *p*-aramid-exposed hamsters were evaluated immediately after exposure as well as at 10 days and 1 and 3 months postexposure.

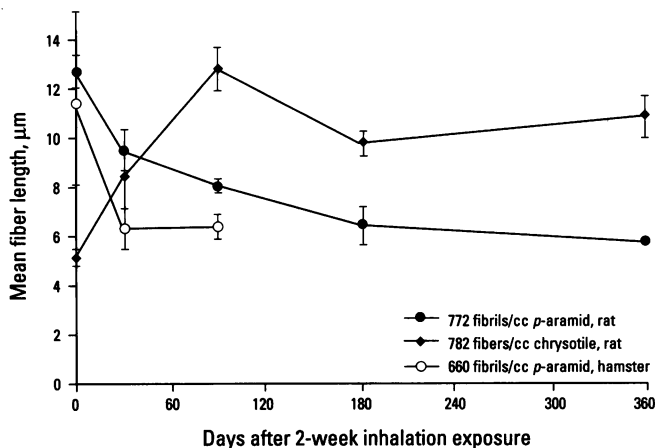
The general experimental design, RFP preparation, inhalation exposure techniques, methods for pulmonary lavage, biochemical assays, cell proliferation, and lung digestion and biopersistence studies were similar to those previously conducted in *p*-aramid- and chrysotile asbestos-exposed rats. The exception was that hamsters had whole-body exposure to reduce stress-related effects. Deposition patterns of inhaled fibrils for the two exposure methods were similar. Methods used for inhalation studies in rats were previously reported (3,4). In the current study, four rats or hamsters per exposure group per time period were used in cell proliferation and lung tissue studies. Fixed lungs of *p*-aramid-exposed rats or hamsters were digested using a 10-min incubation with 1.3% Clorox; fixed lungs of chrysotile-exposed rats were digested using a 3-hr

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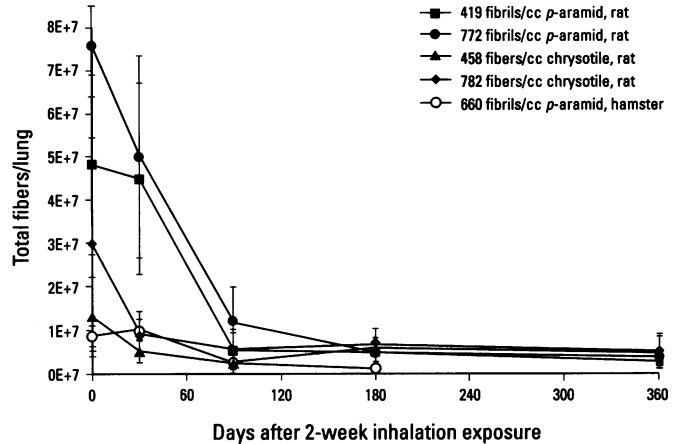
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Address correspondence to Dr. D.B. Warheit, DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, DE 19714. Telephone: (302) 366-5322. Fax: (302) 366-5207. E-mail: david.b.warheit@usa.dupont.com

Abbreviations used: BrdU, bromodeoxyuridine; RFP, respirable-sized, fiber-shaped particulate(s); UICC, Union Internationale Contre le Cancer.



**Figure 1.** Lung retention and clearance of inhaled *p*-aramid RFP in exposed rats and hamsters and inhaled chrysotile asbestos fibers in exposed rats. The numbers of retained RFP recovered from lungs of exposed hamsters was increased relative to the fibril burden of animals immediately after exposure. This was attributed to a decrease in mean fibril lengths (Figure 2), which indicated breakage or shortening of retained RFP in the lungs of exposed hamsters. Mean values are given for four hamsters or rats per dose at time 0 and 1, 3, 6, and 12 months postexposure.



**Figure 2.** Mean lengths of chrysotile fibers or *p*-aramid fibrils recovered from the lungs of exposed rats or hamsters following 2-week inhalation exposure. Values given are mean lengths  $\pm$  SD immediately after exposure, and 1 and 3 months postexposure. These results indicate a shortening of inhaled *p*-aramid fibrils over time, which suggests biodegradability of inhaled fibrils. In contrast, a progressive increase in mean lengths of chrysotile-exposed animals signifies clearance of short asbestos fibers concomitant with insignificant clearance of long asbestos fibers.

incubation with 5.25% Clorox solution. The Microsoft Excel software program (Redmond, WA) with a two-tailed Student's *t*-test was used for statistical analysis.

## Results

Figure 1 summarizes pulmonary clearance and retention data from rats exposed to chrysotile asbestos fibers and from rats and hamsters exposed to *p*-aramid RFP. Clearance of inhaled *p*-aramid fibrils in rats and hamsters was initially slower when compared with that for inhaled chrysotile asbestos fibers. This slower pattern was also associated with a corresponding shortening of retained fibrils over time (Figure 2), which indicates a biodegradation of inhaled *p*-aramid fibrils. Subsequently, at the 1- to 3-month postexposure time periods, there was rapid clearance of inhaled *p*-aramid RFP (Figure 1). In contrast to *p*-42aramid RFP, clearance of short chrysotile asbestos fibers was rapid; however, a subpopulation of longer fibers was cleared at a slower or insignificant rate. Compared to *p*-aramid fibrils, which appeared to be biodegradable in the lungs of exposed rats, the mean lengths of chrysotile asbestos fibers recovered from digested lung tissue were enhanced with increasing residence time in the lung. This suggests that the subpopulation of short asbestos fibers was selectively cleared from the lungs with apparent insignificant pulmonary clearance of the subpopulation of long chrysotile asbestos fibers (Figure 2).

In *p*-aramid-exposed rats, increased pulmonary cell labeling effects relative to controls were measured on terminal bronchiolar, pulmonary parenchymal, and subpleural surfaces immediately after 2-week exposures to RFP. The effects were no different from those of controls following a 5-day postexposure period (Figures 3, 4, 5). In contrast, significant increases compared to controls were measured on airway, parenchymal, and subpleural surfaces of asbestos-exposed rats (Figures 3, 4, 5). These effects persisted for a period of 1 to 3 months postexposure and were correlated with slow or insignificant clearance of long chrysotile asbestos fibers (see Figure 2).

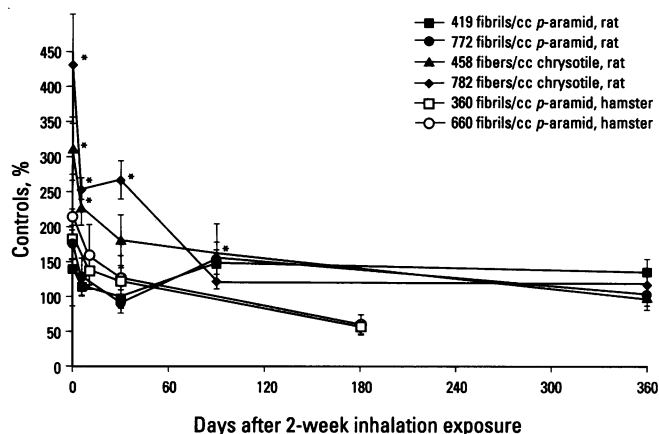
## Discussion

The progressive reduction in length of retained *p*-aramid fibrils with increasing residence time in the lung signifies a reduction in length of retained RFP, and is likely to account for the enhanced numbers of fibrils relative to the retained lung burden immediately after the end of the 2-week exposure, measured 1-month postexposure. These data are consistent with results of earlier studies in *p*-aramid-exposed rats in which the mean and median lengths of retained fibrils were progressively reduced with increasing residence time in the lung (3-7).

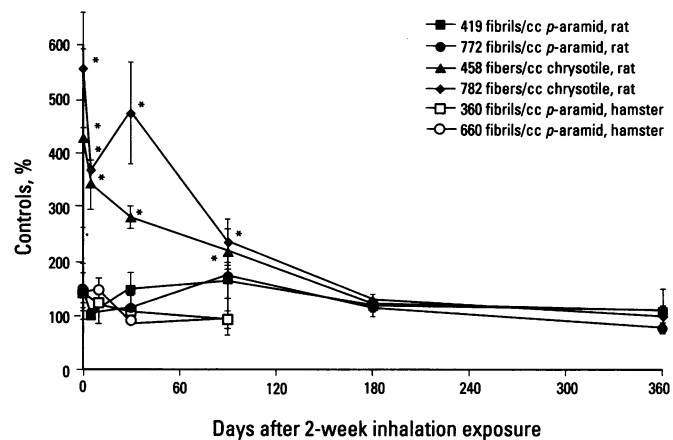
The biopersistence data demonstrating an increase in mean lengths of retained chrysotile fibers is consistent with reduced clearance of long chrysotile asbestos fibers.

This finding has been reported in previous studies by Roggli and Brody (8), who exposed rats to aerosols of chrysotile asbestos fibers for brief periods and assessed lung fiber clearance and dimensional changes as a function of time. Their results demonstrated a progressive increase in mean fiber length of retained chrysotile fibers in the lung.

The results of BrdU pulmonary cell proliferation in chrysotile-exposed rats presented here are consistent with data from a number of studies reported by Brody and colleagues (9-13). In those studies, brief exposures to chrysotile asbestos fibers produced a biphasic labeling response in the lungs of exposed rats and mice. This was characterized by dramatic increases in epithelial cell DNA synthesis and followed several days later by enhanced labeling of interstitial cells (9-13). In subsequent studies, a 3-day exposure prolonged the duration of increased cell labeling (12). In another study, it was reported that a 5-hr exposure to chrysotile fibers in mice produced substantial increases in mesothelial and subpleural cell-labeling indices at 2 and 8 days postexposure (13). The findings of sustained cell proliferative effects and biodegradability of inhaled chrysotile fibers (i.e., resulting in the selective retention of long fibers  $> 5 \mu\text{m}$ ) correlate with the fibrogenic and lung tumorigenic effects of asbestos that have been reported in exposed humans and experimental animals.



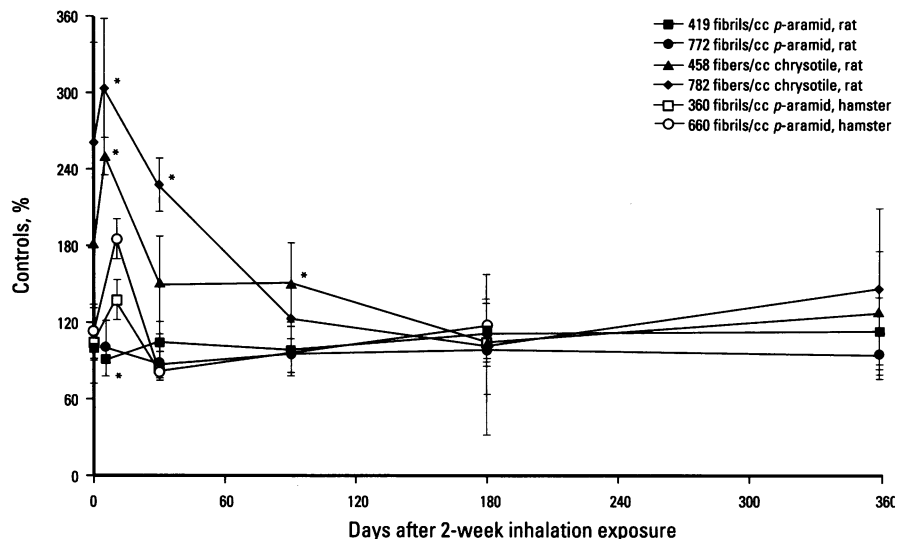
**Figure 3.** BrdU-labeling index of terminal bronchiolar cells in rats exposed to chrysotile asbestos fibers or *p*-aramid RFP or hamsters exposed to *p*-aramid RFP for 2 weeks. Values are expressed as percent response of sham control-labeled cells  $\pm$  SD. Substantial increases in BrdU immunostaining compared to controls were measured in airway regions of asbestos-exposed rats. Significant increases in labeling were measured in high-dose groups immediately after exposure, and 5 days, 1 month, and 3 months postexposure before returning to control levels at 6 months postexposure. In contrast, transient increases in cell-labeling indices were measured in *p*-aramid-exposed rats or hamsters relative to controls immediately after exposure, but were not measured 5 days later ( $*p < 0.05$ ).



**Figure 4.** BrdU-labeling index of lung parenchymal cells in rats exposed to chrysotile asbestos fibers or *p*-aramid RFP, or hamsters exposed to *p*-aramid RFP for 2 weeks. Values are expressed as percent response of sham control-labeled cells  $\pm$  SD. Substantial increases in BrdU immunostaining compared to controls were measured in airway regions of asbestos-exposed rats. Significant increases in labeling were measured in high-dose groups immediately after exposure, and 5 days, 1 month, and 3 months postexposure before they returned to control levels at 6 months postexposure. In contrast, transient increases in cell-labeling indices were measured in *p*-aramid-exposed rats or hamsters relative to controls immediately after exposure but were not measured 5 days later ( $*p < 0.05$ ).

In contrast to the findings in chrysotile-asbestos-exposed rats, *p*-aramid inhalation exposure produced transient increases in cell-labeling responses on terminal bronchiolar, pulmonary parenchymal, and subpleural surfaces. The differences in pulmonary cell labeling results between *p*-aramid- and chrysotile-exposed rats might be accounted for, in part, by the biodegradation of the inhaled *p*-aramid RFP and the biopersistence of long chrysotile asbestos fibers.

In summary, 2-week high-dose exposures to *p*-aramid RFP in rats produces only transient increases in BrdU cell-labeling indices of terminal bronchiolar and subpleural regions. Similar results were observed in *p*-aramid-exposed hamsters. In contrast, 2-week, high-dose exposures to chrysotile asbestos fibers in rats produced sustained increases in cell-labeling indices of lung parenchymal, airway, and subpleural cells, which suggests that chrysotile produces a potent cell proliferative response. In addition, fiber clearance and biopersistence studies demonstrated that short chrysotile fibers were cleared rapidly, but longer chrysotile fibers were retained in the lung or cleared at an insignificant rate. In contrast, *p*-aramid RFP have low biopersistence in the lungs of exposed rats and hamsters. Based on these comparisons, we conclude



**Figure 5.** BrdU-labeling index of subpleural cells in rats exposed to chrysotile asbestos fibers or *p*-aramid RFP, or hamsters exposed to *p*-aramid RFP for 2 weeks. Values are expressed as percent response of sham control-labeled cells  $\pm$  SD. Substantial increases in BrdU immunostaining compared to controls were measured in subpleural regions of asbestos-exposed rats. Significant increases in labeling were measured in high-dose groups immediately after exposure, and 5 days, 1 month, and 3 months postexposure before they returned to control levels at 6 months postexposure. In contrast, transient increases in cell-labeling indices were measured in *p*-aramid-exposed rats or hamsters relative to controls immediately after exposure ( $*p < 0.05$ ).

that the proliferative effects and enhanced biodurability of chrysotile associated with the induction of chronic disease do not occur with inhaled *p*-aramid fibrils. Moreover, the finding that inhaled

*p*-aramid fibrils are biodegradable in the lungs of two rodent species lends support to the theory that inhaled *p*-aramid RFP are probably biodegradable in the lungs of exposed humans.

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