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Evaluation of the deposition, translocation and pathological response of brake dust with and without added chrysotile in comparison to crocidolite asbestos following short-term inhalation: Interim results

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ABSTRACT

Chrysotile has been frequently used in the past in manufacturing brakes and continues to be used in brakes in many countries. This study was designed to provide an understanding of the biokinetics and potential toxicology following inhalation of brake dust following short term exposure in rats. The deposition, translocation and pathological response of brake dust derived from brake pads manufactured with chrysotile were evaluated in comparison to the amphibole, crocidolite asbestos. Rats were exposed by inhalation 6 h/day for 5 days to either brake dust obtained by sanding of brake-drums manufactured with chrysotile, a mixture of chrysotile and the brake dust or crocidolite asbestos. No significant pathological response was observed at any time point in either the brake dust or chrysotile/brake dust exposure groups. The long chrysotile fibers (>20 μm) cleared quickly with $T_{1/2}$ estimated as 30 and 33 days, respectively in the brake dust and the chrysotile/brake dust exposure groups. In contrast, the long crocidolite fibers had a $T_{1/2}$ > 1000 days and initiated a rapid inflammatory response in the lung following exposure resulting in a 5-fold increase in fibrotic response within 91 days. These results provide support that brake dust derived from chrysotile containing brake drums would not initiate a pathological response in the lung following short term inhalation.

Introduction

The use of braking systems for automobiles had evolved from the earliest automobiles. Initially, friction materials were used that consisted of materials like camel hair, cotton belting, elm wood and cotton based materials impregnated with different ingredients (Harper, 1998; Paustenbach et al., 2004). These initial materials, however, were limited in their ability to withstand heat and control speed. From the early 1900s chrysotile fibers were found to be an effective replacement for these earlier materials. The chrysotile fibers maintained their integrity under higher temperatures which allowed the driver to brake at increased vehicle speeds (Harper, 1998). Because of these unique characteristics, chrysotile became the material of choice for vehicle brakes.

With the use of chrysotile, researchers began to investigate the degree of exposure to the fibers experienced by mechanics servicing the brakes. Short duration activities, such as removal of brake-wear debris (e.g., brake dust) from brake assemblies (often using compressed air or a dry brush) and the machining of brake linings (often by grinding or bevelling the lining surfaces to provide a better fit with the drum) have been reported to produce occupational dust exposures (Richter et al., 2009).

While many publications have reported that brakes that have used chrysotile are not related to disease when taking into consideration con founders such as smoking and other exposures (Butnor et al., 2003; Finley et al., 2012; Laden et al., 2004; Marsh et al., 2011; Paustenbach et al., 2004); others continue to report a relationship with mesothelioma (Dodson and Hammer, 2012; Freeman and Kohles, 2012; Lemen, 2004). Although chrysotile containing brakes are not manufactured in the Unites States they can be imported, and they are still manufactured and used in many countries. The U.S. Census

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Bureau indicated that companies in the United States imported asbestos-containing brake pads and linings from Brazil, China, Colombia, India, and Mexico in 2012 (Harmonized Tariff Schedule code 6813.20.00.10 and 6813.20.00.20, www.census.gov/foreign-trade/). In addition, the United Nations Commodity Trade Database indicates that Indonesia, Kazakhstan, Malaysia, Russia, and Ukraine also may have exported asbestos-containing brakes in 2012 (Harmonized Tariff Schedule code 681320, comtrade.un.org/db/). The potential for exposure to chrysotile containing brake dust remains.

This study was designed to evaluate the hypothesis of whether brake dust from chrysotile containing brake drums will produce a pathological response following short term exposure in rats. Brake dust has not been previously evaluated in animal studies.

The exposure design was based upon a frequently used protocol for the evaluation of fiber biopersistence and short term toxicity (EUR, 18748 EN, 1999; ILSI, 2005) which has been used for evaluating a wide variety of synthetic and natural mineral fibers. This design is based upon the evaluation of fiber lung clearance using lung digestion procedures. In addition to criteria specified in these protocols, the current study includes histopathological examination of the lungs, the evaluation of fiber localization and number in the lung and pleura using confocal microscopy, and the quantification of fibrosis (collagen) in the lung and pleura through confocal microscopy (Antonini et al., 1999). This is the first such study in which the fibrotic response following fiber inhalation has been quantified using confocal microscopy.

The interim results presented here on the lung provide a basis for evaluating the biopersistence and pulmonary response of both brake dust alone and brake dust combined with added chrysotile in comparison to crocidolite asbestos following short term exposure through 91 days post exposure. The procedures used for evaluation of the pleural space included examination of the diaphragm as a parietal pleural tissue and the *in situ* examination of the lungs and pleural space obtained from freeze-substituted tissue in deep frozen rats, however, due to the large amount of data these results will be published separately.

The brake dust used in this study was obtained from commercial brake pads that were produced using chrysotile as one of the components by sanding the surfaces of the brake pads using a commercial brake pad sanding machine with the dust collected on filters. The sanded brake dust consists largely of binders with some chrysotile. To achieve the recommended aerosol concentration referred to in the above protocol (>100 f/cm^3 longer than 20 μm; this length category being related to pathogenesis) two brake dust exposure groups were included, one with brake dust alone and the other with brake dust with added chrysotile. Also included in this study was a comparative positive control group using crocidolite asbestos exposed at a similar concentration of fibers longer than 20 μm. This group was unique in that the crocidolite asbestos was obtained directly from South Africa without prior selection or milling as has been performed for most previously studied samples.

Methods

The aerosol generation/exposure, in-life and pathology phases of this study were performed by the Fraunhofer Institute for Toxicology and Experimental Medicine (Hannover, Germany) in compliance with the Principles of Good Laboratory Practice (German Chemicals Act §19a, Appendix 1, July 02, 2008, Federal Law Gazette I, No. 28, p. 1146) and the German animal protection law (Tierschutzgesetz of May 18, 2006, German Federal Law Gazette I, page 1206, 1313). The fiber counting and sizing was performed by Gesellschaft für Schadstoffanalytik mbH (Ratingen, Germany). The confocal microscopy was performed by Rogers Imaging (Needham, Massachusetts, USA).

Brake dust preparation

The brake dust was produced directly from asbestos-containing friction products (automotive drum brake shoes) by the RJ LeeGroup Ltd. (Monroeville, PA, USA). The brake shoes were obtained from Davies McFarland & Carroll (Pittsburgh, PA). The shoes were designed to fit the drum brakes of mid-1960's Chevrolet Impala model cars. These shoes were labeled either “BX MN FF” or “BX MG FF” (manufactured by Bendix). Two of the shoes had never been installed in vehicles; the other shoes that were used in this project were installed and operated in vehicles for a two week period. The friction material was evaluated and found to contain approximately 30% (by area) chrysotile asbestos (analyzed in accordance with EPA 600/R-93/116). No amphibole asbestos minerals have been observed in any of the aerosol or lung samples from these brake shoes or in the added chrysotile used in this study.

The brake dust was produced by grinding the brake shoes using a commercial AMMCO arc grinder (Model 8000, S/N 24788) with a modified dust collection system. The arc grinder is a motorized sander that is swept across the surface of the brake shoe with the dust collected on an attached 8 × 10 inch quartz micro-fiber filter that was used in place of a collection bag. A Tisch high volume air sampler sampling pump (Tisch Environmental Inc., Ohio, USA) was used following the filter to provide uniform sampling suction over the course of the grinding operation. All brake dust preparation took place at the RJ LeeGroup facility in a room equipped with an Aramsco Comanche® HEPA ventilation unit (Model 55011) with a nominal flowrate of 1800 cfm (50 m^3/min).

The composition of the brake dust was determined quantitatively using inductively coupled plasma mass spectrometry (ICP-MS) following the German norm DIN EN ISO 17294-2 (INDIKATOR GmbH, Wuppertal, Germany). The results are presented in the Supplementary data, Tables S-1 and S-2.

Chrysotile

The chrysotile fiber used in this study had the mineralogical grade of 5R04 according to the Canadian chrysotile asbestos classification (Cossette and Delvaux, 1979). The chrysotile sample was chosen based upon an evaluation of which chrysotile grade was ordered or supplied for use in brake manufacturing in a random search of 67 formulations dating from 1964 to 1986. Chrysotile grade 5R04 was used most frequently (25% of the samples) and was chosen for use in the study. All of the grade 5R04 chrysotile in these samples was supplied by Johns-Manville. The sample used in this study was obtained directly from Mine Jeffery Canada (formerly the Johns-Manville Mine).

The 5R04 sample received had some large bundles of fibers. To separate these bundles into respirable fibers without significantly reducing the fiber length, the bulk material was passed one time for 60 s through a table top rotating blade mill to break up the large bundles and then was passed once to separate the fibers through the Cyclotec Sample Mill (FOSS Tecator, Denmark) which rolls the sample against the inner circumference and then separates the fibrils through a fine mesh screen.

Crocidolite asbestos

The crocidolite asbestos used previously in animal studies has been largely either the Union for International Cancer Control (UICC) or US National Institute of Environmental Health Sciences (NIEHS) prepared crocidolite. Both of these samples were ground extensively more than 30 years ago using large scale industrial mills resulting in size distribution not typical of the commercial product (Bernstein et al., 2013). In this study, a crocidolite asbestos sample from the Voorspoed mine in South Africa was obtained from the National Institute of Occupational Health — NIOH, South Africa. This mine is located in Limpopo Province which at the time when mining took place was called Transvaal Province.