50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	PB90-194572

4. Title and Subtitle Pulmonary Response to Inhaled Fibrogenic Minerals. Final Report	5. Report Date 00/00/00
	6.
7. Author(s) Anonymous	8. Performing Organization Rept. No.
9. Performing Organization Name and Address	10. Project/Task/Work Unit No.
	11. Contract (C) or Grant(G) No. (C) (G)
12. Sponsoring Organization Name and Address	13. Type of Report & Period Covered
	14.

15. Supplementary Notes

16. Abstract (Limit: 200 words) The fibrogenicity of silica (7631869), bentonite (1302789), kaolin (1332587), talc (14807966), vermiculite (1318009), feldspar, and coal in animals in nose only exposures was investigated, and the cytotoxicity of the minerals was studied in in-vitro tests. Data were evaluated to provide comparative cytotoxic correlations on fibrogenicity and information for interpreting human exposure effects. Enzymes were measured as indicators of adverse activity of minerals on macrophages. Results of physical and chemical analysis of the minerals showed that all minerals tested were smaller than 7 micrometers and were within the respirable size range. In in-vitro hemolysis studies, bentonite, kaolin, silica, and vermiculite showed the greatest hemolysis, and talc, coal, and feldspar showed the least. Release of cytosolic enzyme lactate-dehydrogenase, indicative of membrane damage, was significant with kaolin, silica, and vermiculite and least with talc, coal and bentonite. The studies suggested that silica, kaolin, bentonite, vermiculite and feldspar all induced an initial acute pulmonary response, but that there appeared to be no correlation between this acute response in-vivo and their chronic pulmonary response. These studies also indicated that the initial pulmonary response reflected in-vitro was not correlated with the chronic response to the dust.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms Respiratory-system-disorders, Inhalation-studies, Laboratory-animals, Mineral-dusts, Dust-inhalation, Airborne-particles, Pulmonary-function, Lung-disease

c. COSATI Field/Group

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages 5
	22. Security Class (This Page)	22. Price

(See ANSI-Z39.18) ...ns on Reverse

OPTIONAL FORM 272(4-77)
(Formerly NTIS-35)
Department of Commerce

R0801

PROJECT TITLE: Pulmonary Response to Inhaled Fibrogenic Minerals (CAN # 114)

FINAL REPORT

The initial goals of this study were: to evaluate the fibrogenicity of silica, bentonite, kaolin, talc, vermiculite, feldspar, and coal in animals by "nose only" exposures; to determine the cytotoxicity of same minerals in in vitro tests; to evaluate the data and provide comparative cytotoxic correlations on fibrogenicity and provide information for interpreting human exposure effects.

This project was generated in response to the concern generated by occasional case reports and Health Hazard Evaluation reports on minerals suspected to have a potential to induce fibrogenicity in humans. Therefore, the project was initially designed to evaluate the mechanisms of pathogenicity of minerals by acute inhalation exposures. However, due to technical problems in the inhalation experiments the project design was changed to intratracheal instillation experiments without any major changes in the experimental protocol or goals.

From the results obtained from these studies it is anticipated to tailor a series of in vitro tests to predict the toxicity of a potential toxic mineral dust of health concern. Availability of such a test for screening occupational exposures is of great value in the prevention of mineral dust pneumoconiosis.

All the minerals used in the study were obtained from the primary producers, (bentonite, kaolin, Georgia Kaolin Co., Augusta, GA; talc, Cliff Mine Ore Co., GA; vermiculite, Sansun Mines, Charlottesville, VA; feldspar, Pacer Corp., Cluster, SD; coal, Bureau of Mines, Pittsburgh, PA). Crystalline silica (Min-U-Sil) used as a positive toxic and fibrogenic control was

obtained from the Pennsylvania Sand and Glass Corporation, Pittsburgh, PA. Barite used as a negative non-toxic and non-fibrogenic control was obtained from Georgia Barite Co., Augusta, GA. All the minerals were size fractionated to less than 5 μm diameter using an accucut particle classifier (Donaldson Majal Division, St. Paul, MN). Size fractionated samples were subjected to qualitative and quantitative elemental analysis by proton-induced x-ray emission (PIXE), atomic absorption spectrometry, and automated x-ray energy spectrometric (XES) analysis and image analysis to determine and verify mineral purity, elemental composition, morphology, size dimensions and concentration of minerals by number percentage. Each mineral sample prepared on Nuclepore filters was analyzed in a scanning electron microscope interfaced with XES and a LeMont DA-10 image analyzer. A minimum of 1000 particles in all mineral samples were analyzed for elemental composition and circular area equivalent diameters. Mass median aerodynamic diameters were of minerals made in duplicate samples using a particle sizer (TSI Model APS3000). Surface area measurements were made on a minimum of 4 samples using the conventional nitrogen adsorption technique.

Because it is widely accepted that the pathogenesis of dust induced pulmonary fibrosis involves the lysis of alveolar macrophages resulting in the discharge of lysosomal enzymes and other substances that promote inflammatory processes, fibroblast proliferation, we measured a number of enzymes as indicators of adverse activity of minerals on macrophages. In addition, irritation of cell membrane by minerals was compared using hemolysis as a measure of toxicity. All the <u>in vitro</u> studies were compared with results obtained for a positive toxic-fibrogenic mineral (crystalline silica) and a non-toxic non-fibrogenic mineral (barite).

Alveolar macrophages were obtained by bronchopulmonary lavage and used in assays measuring the release of a cytosolic enzyme lactate dehydrogenase (LDH) and lysosomal enzymes ß-glucuronidase (ß-GLUC) and ß-N-acetylglucosaminidase (ß-NAG). Male Sprague-Dawley rats 14-16 weeks old and weighing approximately 260-300 g were anesthetized with sodium pentobarbital and exsanquinated by cutting the abdominal aorta. The animals were then lavaged 10-12 times with 6-8 ml of warm (37^{o}C) Hank's balanced salt solution and a total 80 ml lavage was collected from each animal. Lavages were centrifuged at 500 xg for 10 min and the sedimented alveolar macrophages were suspended in HEPES medium containing 5 mM glucose. Cell counts and cell viability were obtained from preparation with trypan blue. Approximately 96% of the cells were alveolar macrophages.

Alveolar macrophages (2 x 10^{6} cells/ml) were incubated with or without the mineral dusts (1 mg/ml) in HEPES medium for 2 hours. At the end of the incubation period the supernatant fluid was separated by centrifugation and the enzymes released during incubation with minerals were determined. The enzyme released from cells was compared as a percentage of total enzyme present in the cell.

Hemolytic activity of minerals was determined using sheep erythrocyte preparations. Mineral samples were incubated at 37^{o}C with a 2% erythrocyte preparation and the percent of lysis was determined compared to 100% lysed samples.

Acute pulmonary response and fibrogenic potential of the minerals were evaluated by the intratracheal instillations of 5 mg of minerals in Fischer 344 SPF rats. Animals were anesthetized with sodium pentobarbitol and 5 mg mineral in sterile saline was instilled aseptically with a 20 gauge blunt

syringe inserted to the tracheal bifurcation level. Control animals received 0.5 ml sterile saline and animals were sacrificed at 1, 3, 7 day and 3 and 6 month intervals. Lungs were inflated to normal body pressure and infused with 10% buffered formalin. Lung tissues were processed and sections stained with hematoxylin-eosin and studied by light microscopy. Pathologic changes in the lungs were studied and recorded by photomicrography.

Results of the physical and chemical analysis of the minerals showed that all the minerals tested were smaller than 7 μm and were within the respirable size range. In all the mineral samples approximately 98% of the particles were less than 5 μm in diameter. Chemical analysis showed there were no appreciable contaminations in any of the minerals tested. Surface area measurements showed wide variability between the various minerals reflecting their size variability and the probable differences in particle distribution.

In the <u>in vitro</u> hemolysis studies, bentonite, kaolin, silica, and vermiculite showed the greatest hemolysis, and talc, coal, and feldspar showed the least. Bentonite and kaolin were more hemolytic than silica at all concentrations. All the minerals showed a linear dose dependent hemolytic response up to 50% and thereafter the hemolysis declined.

Release of cytosolic enzyme lactate dehydrogenase indicative of membrane damage was significant with kaolin, silica, and vermiculite and least with talc, coal, and bentonite. Similarly the two lysosomal enzymes ß-glucuronidase (ß-GLUC) and ß-N-acetylglucosaminidase (ß-NAG) were released selectively in greater proportions in response to kaolin, silica, vermiculite, and talc whereas other minerals induced only an insignificant amount of these enzymes.

Correlative in vivo studies with same minerals showed comparable in vivo responses. Positive controls with crystalline silica showed acute alveolitis reaction associated with lipoproteinosis, type II cell hyperplasia, and the development of well circumscribed interstitial granulomas. In contrast to this, bentonite, kaolin, vermiculite, and feldspar showed an initial acute inflammatory response and development of granulomas and partial or complete resolving of fibrosis at later stages.

In this study we compared the pulmonary response of intratracheally instilled minerals to their cytotoxicity to assess their potential to induce fibrogenicity. Although silica, kaolin, bentonite, vermiculite, and feldspar all induced an initial acute pulmonary response there appears to be no correlation between this acute response in vivo and their chronic pulmonary response. These studies also indicate the initial pulmonary response reflected in in vitro studies is not correlated with the chronic response of the dust.

A preliminary report of this study was published in the Annals of Occupational Hygiene, 32:279-289, 1988. The results of the comprehensive data are now being statistically evaluated and prepared for a scientific publication.

This project has been very productive and has contributed respirable size minerals to several other research projects. Several mineral dust samples developed for this project were made available to a large number of investigators in several institutions as a standard for in vitro and in vivo studies.