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Office of Toxic Substances
U. S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

Re: EPA Document Control Number 8EHQ-0988-07525

A copy of the protocol for "The Acute Toxicity of Fibrous Materials" is attached as requested by Judy Leranger on January 4, 1989.

Neither the protocol nor the cover letter contains confidential information.

F. D. Griffith, Ph.D.
Manager, Toxicology Services
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FDG/bh (TS143 2.20)

Enclosure

VVV 000006875

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THE ACUTE PULMONARY TOXICITY OF FIBROUS MATERIALS

a Proposal

Submitted to
F.D. Griffith, Ph.D.
3M
Saint Paul, Minnesota

9-7-87

by
J. Wesley Clayton, Ph.D.
Department of Pharmacology and Toxicology
College of Pharmacy
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September 1987

Acute Pulmonary Toxicity of Fibrous Materials

Introduction

The objective of this proposal is to evaluate the acute pulmonary toxicity of three samples of fibrous materials which will be supplied by 3M. In this study, the test materials and an asbestos control will be suspended in physiological saline and injected intratracheally into the rats lungs at the bifurcation of the bronchi. Several markers of toxicity will be recorded at regular intervals throughout a post-injection period of six months, at which time, the surviving rats will be killed for necropsy and lung histology. Rats which succumb during the dosing or observation periods will, lacking significant post-mortem autolysis, be subjected to necropsy and histology of the lungs.

In the proposed study, the markers of toxicity that will be recorded are: changes in respiration, loss or gain in body weight, appearance, and general behavior throughout the observation period post-injection. The test procedure that is proposed is widely accepted in toxicology. A review which covers this area is Henderson (1984). A paper reporting specific results is Henderson, et al (1978, 1979). The results of the proposed study will be compared between and among control and test groups. Post-mortem autolysis potentially occurring from spontaneous deaths will be evaluated by Dr. Susan Wilson, DVM.

Test Materials

All samples will be sent to the Principal Investigator, J. Wesley Clayton, College of Pharmacy, the University of Arizona, Tucson, Arizona 85721. Although an adequate description of the test materials and the asbestos control has not yet been received, the list below shows the number of samples to be tested.

1. Saline control
2. Asbestos (positive control)
3. Fiber 1 chemical and physical properties will be supplied by 3M

4. Fiber 2 chemical and physical properties will be supplied by 3M

5. Fiber 3 chemical and physical properties will be supplied by 3M

One dose of control or test materials will be intratracheally injected. The study will not result in a dose-response curve; results will be compared between and among the various groups of rats.

Work Plan

Sprague-Dawley, male rats identified with ear tags will be obtained from Hilltop Farms. The intratracheal injections will begin after a two-week quarantine period for the animals. During this time the animals will be housed in the Division of Animal Resources of the Arizona Health Sciences Center. Light cycle 12 on 12 hr - off and humidity at 50% RH will be controlled. Rats will be housed 4 per cage. Feed (Wayne Lab. Blocs) and water will be available and libitum except immediately following dosing. Fasting will be 24 hours post-dosing. Cages will be changed twice weekly. The animals will be weighed regularly and examined by trained personnel. Dr. Wilson, DVM will advise as to the suitability of individual animals for the test. Rats will then be randomly assigned to three test and two control groups for each species. Each group will consist of ten male rats (175-200 grams). The intratracheal dosages could range from 0.5 mg per rat to 5 mg per rat. This dose range has been used in previous studies, Stoner et al 1985, Grimm et al 1986. The volume of the dose will be 0.5 ml of 0.15 M NaCl. To administer the dose of fibrous material, the animal will be anesthetized with methohexital. After anesthesia has been established a blunted, 19-gauge needle, attached to a syringe containing the fibers will be inserted into the trachea through the mouth and vocal folds. This procedure can be completed while the animal is fully anesthetized. Each rat will then be returned to its cage for observation. During the subsequent observation period of six months, the animals will be observed for survival, signs of toxicity (such as changed respiration) and body weights which will be measured three times weekly during the isolation period and the three weeks post-injection.

Thereafter, until termination of the study, the rats will be weighed weekly and at euthanasia, which will be done by intraperitoneal injection of pentabarbital. The inflated lungs will be dissected free from the heart lung bloc, weighed and fixed in 10% formalin instilled through the trachea, filling the lungs. The lung tissue will be stained with hematoxylin and eosin for microscopic examination. The data resulting from this study will be tabulated, graphs made of body weights plotted against time, and the pathologist's report. Animal remains will be incinerated.

The data from the proposed project will be recorded in bound laboratory notebooks along with SOPs. These items, along with correspondence and other applicable documents, tissues, blocks, slides and photomicrographs will be retained in the archives of the Quality Assurance Office, Room 252 in the College of Pharmacy, Dr. Clayton's laboratory. All reports will be prepared on a Xerox 860 Word Processor and stored on "write-protected" disks. All statistical computations (ANOVA, standard method, Grimm, et al., 1986) will be filed and accessible for review. The Quality Assurance Unit will audit and validate data entries and reports.

Facilities

The study proposed will be conducted in the laboratories located on the second floor of the College of Pharmacy at the Arizona Health Sciences Center, Tucson, Arizona 85721, which is the area occupied by the Department of Pharmacology and Toxicology. Animal work consisting of intratracheal injections will be carried out in the inhalation toxicology laboratory. Animals will be housed in the Division of Animal Resources connected by a limited access tunnel to the second floor of the College of Pharmacy.

(1) Animals, Necropsy, and Storage. The Division of Animal Resources is accredited by the American Association for Accreditation of Laboratory Animal Care. It is under the direction of Susan Wilson, D.V.M. This division has provided us with a double room that contains a high velocity hood with filtering systems. Histopathology services,

including interpretation by a veterinary pathologist, are available through the Division of Animal Resources. Prior to the conduct of animal studies at the University of Arizona, the proposal, written in lay language, must be approved by the Committee on Animal Care. This process has often required three weeks.

(2) Safety Procedures. The College of Pharmacy is a part of the University of Arizona Risk Management and Radioactive Safety programs. All hazardous materials are managed by this division, including receipt, inventory while on the premises, and disposal at approved sites maintained by the University of Arizona. In addition, this division provides classes for employees on safe handling of radioactive and toxic chemicals. They also monitor the laboratories to ensure that personnel are adequately protected.

(3) Good Laboratory Practice. The University of Arizona, Department of Pharmacology and Toxicology, has instituted procedures for compliance with GLP regulations. A departmental Quality Assurance Unit has been established to monitor toxicity studies conducted under grant or contract. DEC Rainbow computer with a printer will be used to store and retrieve data generated in this study in order to assure proper auditing and validation of laboratory data.

(4) Biostatistics. The University provides a resource center to aid in experimental design and statistical analysis of the data. In addition Dr. Thomas Moon of the Cancer Center and John Gaines of the Medical Center Biostatistics group are available for consultation.

The criteria used to evaluate the results and accomplishments of the proposed project will be the deviations of the test groups from the controls. The significance of the deviations will be based on a confidence level of $p<0.05$ to assist in the judgment of effect.

(5) Personnel Responsibilities

All aspects of the project will be conducted in the laboratories of inhalation toxicology and biochemical toxicology located on the second floor of the College of Pharmacy and the adjacent Division of Animal Resources. The Principal Investigator, J. Wesley Clayton, will have the overall responsibility for the management of the project. This includes preparation of the proposal to the Committee on Animal Care; hiring of personnel, setting the Quality Assurance plan; developing Standard Operating Procedures with the Research Technician, preparing and assembling the requisite reports. The Principal Investigator will monitor the accounting and approve all orders for the purchase of equipment and supplies.

A Research Technician will see to it that SOP's are prepared and followed, check animals for suitability for the experiments, check solutions, prepare orders for reagents, maintain the stock of laboratory supplies, make data entries into the laboratory notebooks, and into the statistical program on the DEC Rainbow computer.

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