STUDY TITLE

EPA FIBER PROJECT: TWO-WEEK RANGE FINDING STUDY – INHALATION EXPOSURE OF RATS TO AMPHIBOLE ASBESTOS

TEST MATERIAL

Libby Amphibole Fiber (LA2007)

STUDY INITIATION DATE

March 1, 2010

STUDY COMPLETION DATE

September 16, 2010

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LABORATORY PROTOCOL ID

Protocol Number 10002

SPONSOR

U.S. Environmental Protection Agency
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STATEMENT OF QUALITY ASSESSMENTS

The Hamner Institutes' Number and Title:	Protocol 10002: EPA/Fiber Project: Two-Week Range Finding Study - Inhalation Exposure of Rats to Amphibole Asbestos
The Hamner Institutes' Principal Investigator:	Earl Tewksbury
US EPA Contract No.:	EP-W-08-051

The above mentioned study was conducted under the Research Quality Standards of The Hamner Institutes for Health Sciences (The Hamner Institutes). These standards are designed to help assure the quality and integrity of studies. The study was subjected to Quality Assessments conducted by The Hamner Institutes' independent Quality Assurance personnel. The dates of the Quality Assessments, the phase reviewed and the date the Quality Assessment reports were sent to The Hamner Institutes' Principal Investigator, with subsequent circulation to The Hamner Institutes' Management are noted below.

Quality Assessment Date	Phase Reviewed	Date Report Sent to The Hamner Institutes' Principal Investigator with Subsequent Circulation to The Hamner Institutes' Management
03/16-17/2010	Necropsy (Component 1)	03/31/2010
03/22/2010	Necropsy (Component 2)	03/22/2010
04/08/2010	Inhalation Exposure	04/08/2010
08/04/2010	Draft Report Review	08/05/2010
08/11-12/2010 and 08/16/2010	Draft Report and Data Review	08/17/2010
08/17-18/2010	Draft Report Review	08/18/2010
08/12-14/2010, 08/20/2010 and 08/23/2010	Draft Report and Data Review	08/23/2010
08/23-24/2010	Draft Report Review	08/24/2010

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Introduction

The vermiculite mine near Libby, Montana was the world's leading source of vermiculite for 70 years until its closure in 1990. Vermiculite is used for insulation, as an absorbent material, and as a soil conditioner, and has applications in the construction, agricultural, horticultural and industrial markets. However, the Libby vermiculite ore coexists with a complex array of amphibole mineral types, primarily winchite, richterite, tremolite, and magnesioriebeckite with crystal forms (habits) ranging from asbestiform to acicular/prismatic.

Occupational exposure to Libby vermiculite has been (and continues to be) associated with significant increases in asbestosis, lung cancer, and pleural cancer compared to the rest of the U.S. population. For example, in addition to elevated rates of lung cancer and mesothelioma among Libby residents, 17.8% of 6,668 persons who lived or worked in the Libby area for at least 6 months before 1991 show (upon medical testing) pleural abnormalities (calcifications, thickenings, or plaques).

Furthermore, exposures to individuals outside of Libby have occurred, and are likely continuing; as asbestos-contaminated vermiculite ore from Libby was shipped to hundreds of locations around the nation for processing, and used as attic insulation in millions of homes throughout the United States. The health effects associated with former and current exposures from the asbestos contaminated vermiculite from the Libby mine continues to be a subject of intensive study and public health concern.

Study Objectives

The overall goal of this research was to improve the scientific basis for the risk assessment of asbestos-contaminated communities by conducting toxicology studies to help define key determinants of internal dose and provide critical insight on additional key health or pathologic endpoints. These objectives were to be met by a rodent inhalation study conducted with the amphibole asbestos that contaminates vermiculite from Libby, Montana (Libby amphibole or LA).

Study Design

This study consisted of two components:

1) The first component was a two-week range finder study designed to determine optimal fiber-aerosol concentrations to be used in the subsequent subchronic inhalation exposure study and to compare the potency of inhaled Libby amphibole fibers to the potency of inhaled amosite amphibole (AA), a known fibrogenic asbestos fiber. Component 1 consisted of 5 groups exposed to lofted LA or AA by nose-only inhalation for 6 hours a day, 5 days a week for two weeks at concentrations of 0, 0.5, 3.5, and 25 mg/m^3 LA or 3.5 mg/m^3 AA. After the completion of exposures, animals were euthanized and necropsied after the last 6-hr daily exposure, either immediately for inflammation evaluation or approximately 4-days later for histopathology/proliferation evaluation. Each exposure group consisted of 7 male F344 rats (Table 1).

2) The second component was a dosimetry study to determine initial fiber deposition and clearance/biopersistence. It was designed to provide a time course of fiber burden data in various regions of the respiratory tract (head, trachea, lung lobes) and GI tract. These data were to be used to derive LA-specific inhalability, deposition efficiency, and clearance rates for development of modifications to the Multi-path particle dosimetry (MPPD) model used to describe inhalation dosimetry. In Component 2 animals were divided into one of three exposure groups: 1-day, 5-days or 10 days (5 days/week for 2 weeks). Groups were exposed to lofted LA by nose-only inhalation for 6 hours a day at concentrations of 0.5, 3.5, and 25 mg/m^3 LA. After the completion of exposures, animals were euthanized and necropsied either immediately after exposure (“zero time”) for 1-day, 5-day, and 10-day groups or at 6-, 12-, or 24-hrs following the last daily exposure for 1-day and 5-day groups. During necropsies 14 tissues were collected from each animal, placed into vials and stored frozen at approximately -70^{o}c for future analysis. Each group consisted of 6 male F344 rats (Table 2).

Methods

Material

Libby and UICC (International Union against Cancer) Amosite Amphibole fibers were obtained from the United States Geological Survey (USGS) by the Sponsor. All identity, purity, composition, stability, method of synthesis, fabrication and/or derivation information for these test materials used in this study were documented by the Sponsor. This documentation is maintained by the Sponsor at the address indicated on the title page of this report. A MSDS or CAS number is not available for the Libby Amphibole at this time. Amosite amphibole CAS number is 12172-73-5.

The Libby Amphibole (LA) was hand delivered by the Sponsor to The Hamner Institutes in a one-gallon plastic container. The plastic container was stored under room temperature and humidity in a glove box located in the inhalation monitoring corridor 200U.

UICC Amosite Amphibole (AA) was shipped directly from the USGS (Denver, CO) by courier in an insulated cooler. The Amosite Amphibole was placed into a plastic bag within a metal container and stored under room temperature and humidity in the Amosite generation hood.

An archival sample was taken from each of the test materials and stored under ambient conditions.

Animals and Animal Husbandry.

The Hamner Institutes for Health Sciences is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). This study complies with all appropriate parts of the Animal Welfare Act regulations promulgated by the U.S. Department of Agriculture and in effect as of the start of this study.

A total of 256 male rats (Fischer [F344]) were obtained from Charles River Laboratories, Kingston, NY (Table 3). Animals were received in three shipments to The Hamner Institutes to maintain similar age and weight for each component of the study. All animals were approximately 8 weeks of age and appeared to be in good health. Weights ranged from 151.6 to 181.5 grams the day after receipt. Animals were acclimated to the facility for approximately two weeks.

During the acclimation period, animals were individually housed in stainless steel wire-mesh cages in an animal room (room 298/300). During the second week of acclimation rats were trained to the nose-only restraint tubes for 5 daily periods of increasing duration -- approximately 1, 2, 3, 4, and 6 hours. At the start of exposures, weights ranged from 167.5 to 233.1 grams. Weight gain after receipt at the testing facility indicated that animals maintained good health during the acclimation period.

Room conditions were maintained between 20 and 24 °C, 30-70 % humidity, with a 12 hour light/dark cycle. Animals were fed a certified rodent diet, NIH-07 (pellets); Zeigler Brothers., Gardners, PA and reverse osmosis purified municipal tap water, *ad libitum*, except during exposure, when food was withheld. Certification of analysis of feed batch was supplied by the manufacturer. There were no known contaminants in the feed that were expected to interfere with the results of this study. Drinking water analyses were conducted quarterly by an independent laboratory. There were no known contaminants in the drinking water that were expected to interfere with the results of this study. Documentation of these analyses is maintained on file at the Testing Facility and applicable copies are kept with the study files.

A total of 232 animals were selected for study prior to the start of exposures. The extra animals were ordered for methods training or to be used in case of pre-study death or moribundity. All animals were weighed and assigned randomized animal numbers using an Instem Provantis™ 8 protocol (Instem Provantis™, Conshohoken, PA). After selection for study, each animal was identified with transponders with a number assigned by the Testing Facility. Animals were also assigned to necropsy groups. A cage assignment chart indicated cage assignment by the animal identification number.

Animals were transferred to assigned animal holding rooms with 8-m^3 containment chambers (rooms 292 through 296) just prior to exposures. For protection of study personnel, RCC nose-only exposure systems were housed in each of the 8-m^3 chambers. For each exposure, animals were placed into nose-only tubes (Battelle Memorial Institute, WA or equivalent design) and attached to a RCC nose-only system. Animals were exposed 6 hours per day, for 1-day, 5-days, or 10-days to aerosolized LA, AA or hepa-filter house air. Animal exposures were staggered throughout a 7week schedule to accommodate labor-intensive post-exposure necropsy procedures (Table 3). After exposures and a least a 15-minute clearance period, animals were transferred from nose-only tubes on the exposure system to the individual wire mesh cages located in each holding room. Animals were provided with water ad libitum and food during non-exposure periods. Room conditions for both 8-m^3 and holding room were maintained between 20 and 24 °C, 30-70 % humidity, with a 12 hour light/dark cycle.

Component1 Core Group Histopathology/Cell Proliferation

One day following the last exposure, animals assigned to histopathology/cell proliferation core group (Table1) were administered Brdu via surgically implanted Alzet micro-osmotic pumps (Model 2 ML1: 7-day pump, 10 microliters/hr) with 5 mg/mL solution of BrdU in phosphate buffered saline. The surgery was performed aseptically under isoflurane anesthesia. The dorsal thoracic region was shaved and an incision large enough to accommodate the pump was made with a scalpel and scissors. The pump was inserted through the incision and pushed cranially into the subcutaneous pocket. The incision was closed with wound clips. Animals were provided thermal support (heating pad) and continually observed post surgery until ambulatory.

Three days post surgery animals were weighed, euthanized by an overdose of sodium pentobarbital, exsanguinated by transection of the abdominal aorta; and then necropsied. The necropsy included examination of the external surface and all orifices; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass. The pathology observations were conducted by Dr. Gabrielle A. Willson, B.V.M.S., MRCVS, F.R.C. Path, Experimental Pathology Laboratories, Inc., RTP, NC (EPL).

The lungs and trachea were removed for histopathology and cell proliferation evaluations. The trachea and lungs were fixed in situ with 10% neutral buffered formalin (NBF) at approximately 30 cm pressure. The nasal cavities were flushed with NBF. The head was removed, skinned, trimmed of excess tissue, and stored in NBF for approximately 3 days. The heads were then rinsed in running tap water, decalcified, and re-rinsed in water. Cross sectional blocks of the nasal cavity were prepared (at least 4 levels), embedded in paraffin wax, sectioned (approximately 5 micrometers), deparaffinized, and stained with hematoxylin and eosin (H&E). The lungs (2 sections - left pulmonary lobe, right cranial lobe), trachea (2 sections), and duodenum (1 section per lungs block and 1 section per trachea block) were fixed with NBF for 48 hours, rinsed, and stored in 70% ethanol, embedded in paraffin wax, sectioned (approximately 5 micrometers), deparaffinized, and stained with H&E. An additional set of slides from the lungs and trachea blocks were stained with a collagen specific stain (e.g., Masson-Goldner's trichrome stain). The rib cage (pleural tissues) was preserved in NBF for possible future analysis. Samples of the left ventricular tissue (cut longitudinally into 3 separate samples for storage) and thoracic aorta (trim off connective tissue and cut into 2 separate samples for storage) were removed and frozen in liquid nitrogen and stored at approximately -70°C for potential future analysis.

Preparation of histological slides and microscopic examinations were performed by Experimental Pathology Laboratories, Inc. (Durham, NC). Evaluations of the

lung and tracheal tissues included assessment of collagen deposition at the broncho-alveolar junction, pulmonary fibrosis, interstitial fibrosis, peribronchiolar lesions, and pleural changes. The severity of fibrotic response and the area of the lung showing fibrosis were quantified (graded with severity scores).

Two additional cross sections of the blocks of the lungs and trachea (each containing the duodenum for background reference) were immunostained with BrdU for cell proliferation evaluation. Cell proliferation was quantitated as labeling index or unit length labeling index (ULLI). The anatomical sites selected for evaluation were defined by the histopathology data collected above.

Component1 Core Group Inflammation Evaluation

Following the last exposure, animals assigned to the inflammation evaluation core group (Table1) were euthanized by an overdose of sodium pentobarbital, exsanguinated by transection of the abdominal aorta; and then necropsied. Lungs were lavaged for the collection of cells and BAL fluid. The trachea was cannulated and the lungs lavaged with a total of 25 ml phosphate-buffered saline (PBS). BAL cells were isolated and the total number of cells enumerated. Slide preparations of BAL cells were prepared and stained with Diff-Quik, and the cell differential percentages were determined, including the quantification of macrophages, lymphocytes, neutrophils, and eosinophils. Lavaged lungs were saved in liquid nitrogen, as separate lobes in separate tubes. Cell-free BAL fluid supernatant was assessed for total protein, lactate dehydrogenase, β-glucuronidase, N-acetyl-β-d-glucosaminidase, and alkaline phosphatase. Excess supernatant was saved in 2 separate tubes for possible future cytokine analysis. Serum and plasma (citrate was used as the anticoagulant) samples were also collected and stored at approximately -70°C for potential future analysis.

Component2 Clearance/Biopersistence Evaluation

Following the last exposure and assigned clearance time point (0, 6, 12 or 24 hours), animals assigned to the clearance/biopersistence (Table2) were euthanized by an overdose of sodium pentobarbital, exsanguinated by transection of the abdominal aorta and then necropsied. An agarose cast was made of the animal's pleural space by instilling liquid agarose into the pleural cavity and chilling the carcass on ice. After chilling for approximately 15 minutes the head was removed from the carcass, skinned, cleaned and the nasal cavity collected. After approximately 30 minutes, the carcass was removed from the ice and the trachea and larynx were collected followed by the entire solid agarose cast located in the pleural cavity. After the cast was removed the remaining tissues were collected: lungs (5 lobes), lung-associated lymph nodes, esophagus, stomach, small intestine (duodenum/jejunum/ileum), cecum, and

colon/rectum. A total of 14 tissues were collected from each animal into pre-weighed glass collection vials (VWR, Trace Clean Vials). All vials were stored at approximately -70^{o}C for future analysis.

Exposure System

Animals were exposed in five direct flow nose-only exposure systems (RCC, Geneva, Switzerland). Three towers were used for the LA target exposure concentrations, one for the AA target concentration, and one tower for the control group.

Each tower was located in a separate 8m^3 chamber for exposure containment. An air and vacuum rotameter with an exhaust fan controlled airflow through the exposure towers. The ball setting on the air supply rotameter was used to monitor the airflow. The rotameter was calibrated with a mass flow meter (MFM, Model 4040, TSI, Inc., Shoreview, MN). A 3-way valve was used to control airflow passing through either the generator or a bypass line directly to the exposure towers. Pressure was monitored with a magnehelic differential pressure gauge (Dwyer Instruments Inc., Michigan City, Indiana) at a tower inlet.

Temperature and relative humidity were measured near the top of each 8m^3 chamber and at a port on each exposure tower by a Rotronic Humidity Sensor (Series 200, Rotronic Instruments Corp., Huntington, NY) connected to the Continuum Building Automation System (Andover Controls Corporation, TAC, Carrollton, TX). Temperature was calibrated by comparing the ambient air temperature recorded by the probe to a certified mercury thermometer. The relative humidity sensor was calibrated by immersing the sensor probe in an atmosphere of known humidity generated from saturated salt solutions.

The 8m^3 chamber temperature and humidity readings represented the animal environment under which the exposures were conducted while the nose-only exposure tower readings represented the conditions of the atmosphere being inhaled by the animals. Relative humidity conditions in the nose-only exposure towers were intentionally kept below 10% to avoid aerosol agglomeration.

Generation System

The Libby and Amosite Amphibole exposure atmospheres were generated using rotating brush generators (Aerosol Generator, Model CR-3000 & CR-3020, CR Equipments SA, CH-1295 Tannay, Switzerland) to aerosolize the fiber test material. A piston pushed a column of the test material into a rotating brush, which swept material off the top of the column into the generator air stream. The test material was packed into a generator piston with minimal pressure using a

piston-packing tool. The air delivery pressure at the air supply rotameter for each tower was maintained at approximately 20 psi. The fibers were carried from a rotating brush generator into hepa-filtered house air at 50 liters per minute for the Libby towers and at 15 liters per minute for the control and Amosite towers. The fibers leaving a generator were delivered past a krypton-85 source (Kr85, 10mCi, Isotopes Products Inc., Valencia, CA) to reduce charges on the particles entering the exposure tower. The generator brush and piston speeds were adjusted to produce the required particle concentrations. A diagrammatic representation of the exposure system setup is seen in Figure 1.

Concentration Measurement

During the exposures, measurements of airborne aerosol concentrations were performed in the animals' breathing zone. Aerosol concentrations were measured using a gravimetric filter and were determined by taking a sample from a port on the exposure tower. A sample of the atmosphere was pulled through the filter at a known flow rate and time. The aerosol concentration was calculated from the mass of the fiber collected on the filter and the volume of atmosphere pulled through the filter.

Fiber concentrations were also continuously monitored using a light scatter aerosol monitor (Real-Time Aerosol Monitor (RAM), MIE [Monitoring Instruments for the Environment, Inc] Billerica, Massachusetts). This system was used to provide backup information to the gravimetric filter and a secondary means to monitor the operation of the generation system.

Tower Distribution

Using gravimetric filters the uniformity of distribution within the exposure tower was checked by measuring the concentration at 3 different locations within the tower (Top, Middle and Bottom tiers). It was determined that the variability in tower concentration was less than 7.1%, indicating that the distribution of the test material within the tower was uniform.

Fiber Size Distribution

The uniformity of the fiber size distribution within the exposure tower was checked by measuring the fiber size distribution at 3 different locations within the tower (Top, Middle and Bottom tiers) using an aerodynamic particle sizer (APS,

Model 3321, TSI, Inc., Shoreview, MN) and 0.2 µm polycarbonate filters collected for scanning electronic microscopy analysis. It was determined that the variability in tower fiber size distribution was less than 3% and 11% for APS samples and SEM filters, respectively, indicating that the distribution of the test compound within the chamber was uniform.

Statistics

Basic summary statistics, including means and standard deviations were compiled for most exposure and in-life data. For the BAL cytology report statistical analyses (e.g. mean, standard deviation, minimum, maximum) and analysis of differences between exposure groups were completed via application of statistical analysis software (STATISTIX for Windows, version 2.0; Analytical Software, Tallahassee, Florida). Using this software, a Kruskal-Wallis one-way analysis of variance with a Bonferonni-like comparison procedure was performed to determine treatment group (exposure level) differences in means of differential cell percentages. For the cell proliferation report, statistical analysis of the data was completed via application of statistical analysis software (JMP, version 8.0, Cary, NC). A Dunnett's or Kruskal-Wallis procedure was used to test for difference between control and fiber-exposed groups. JMP was also used for statistical analysis in the BAL fluid analysis. Variance was tested using the Levene's test, Welch's ANOVA and a standard ANOVA. The Tukey-Kramer test was used for pair-wise comparison of individual groups.

Results

Test Substance Characterization

There were no chemical analyses of the LA or AA conducted by the Hamner Institutes. All identity, purity, composition, stability, method of synthesis, fabrication and/or derivation information for test materials used in this study were documented by the Sponsor. This documentation is maintained by the Sponsor at the address indicated on the title page of this report.
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Exposure conditions

Over the course of the exposures, aerosol concentration, temperature, humidity, air flow, and static pressure readings were recorded for each dose group. The grand mean, standard deviation, maximum, minimum and the number of readings for all conditions were compiled for each group (Tables 4 - 8). During

the study, the mean temperature, relative humidity, and air flow remained at targets, and did not deviate outside of prescribed ranges. The study grand mean aerosol concentrations were 0.08 (± 0.18), 3.67 (± 1.58), 0.53 (± 0.11), 3.59 (± 0.91), and 26.76 (± 9.11) mg/m^3 for the target concentrations of 0 mg/m^3 control, 3.5 mg/m^3 AA, 0.5 mg/m^3 LA, 3.5 mg/m^3 LA, 25 mg/m^3 LA, respectively. Summary tables of exposure conditions were prepared for each individual necropsy group (Appendix II). On four occasions during the study (Study Days 9, 10, 11, and 49) the aerosol concentrations for the 25 mg/m^3 dose group reached above 45 mg/m^3. On these days it is believed that deposited fiber in the exposure system re-entrained in the air stream causing the spike in concentration. After study day 11, the exposure tower was vacuumed out regularly to help prevent fiber build up. Fiber deposition in exposure system lines between the generator and the tower are most likely the cause of the concentration spike on study day 49.

An aerodynamic particle sizer (APS) was used to measure the fiber size distribution. Measurements were made by sampling from each tower once a week, for a total of two samples from each of the control and AA towers and six samples from each of the three LA towers, over the course of the study. The average count median aerodynamic diameter and geometric standard deviation (CMAD (GSD)) of the aerosols were calculated as 1.54 (1.76), 1.22 (1.44), 1.19 (1.49), 1.21 (1.48), and 1.35 (1.50) µm for the control, AA, LA low, LA intermediate, and LA high concentration towers, respectively (Table 9). Aerosols with particle size distributions between 1 and 4 µm are generally considered as respirable by rodents. The CMAD and GSD standard deviation between weekly samples ranged from 0.01 to 0.13 and 0.01 to 0.02, respectively, indicating the aerosols were stable over the course of the exposure. The control tower aerosol size distribution was based on a very small particle concentration of 0.06 particles/cubic centimeter from background particles. All other tower size distributions were based on particle average concentrations between 35 and 616 particles/cubic centimeter.

Filter samples taken for scanning electron microscopy (SEM) analysis were also used to measure the fiber size distribution. SEM filters were taken from each tower once a week, for a total of two filters from the AA tower and six samples from each of the three LA towers, over the course of the exposure. For all SEM filter samples the average fiber diameter and length was between 0.329 and 0.399 µm and 4.379 and 7.235 µm, respectively, (Tables 10 through 13).

Animal Body Weights

Animals were weighed at time of randomization and weekly during this study (Appendix I) and average group weights were determined (Tables 14 through 23). Only necropsy group 2 animals were weighed at time of necropsy which

occurred three days post surgery and four days post last nose-only exposure. All other groups were necropsied 0 to 24 hours post nose-only exposure and final weights were not taken. A slight decrease was noted in 6 animals (3 controls, 1 LA low-dose, 1 LA high-dose, 1 AA dose) in necropsy groups 1 and 2 after a week of exposure with the greatest weight decrease at 2.4%. This slight decrease was most likely attributed to the stress of 6 hour restraint periods in the nose-only tubes. All animals in necropsy group 2 showed weight gain after a second week of exposures and surgery (implant of BrdU mini-pump) as well as at time of necropsy. Animals in necropsy groups 3, 6, and 7 showed weight gain after five, one, and four days of fiber exposure, respectively. All other group weights were pre-exposure weights.

Clinical Observations

Over the course of the study, no unscheduled deaths occurred, nor were any animals found in a moribund condition. Clinical observations were conducted when animals were weighed. All observations were normal (Appendix I).

Gross Pathology

No observations of adverse health effects were noted during the necropsy examination of the external surfaces and orifices; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and the neck; or the remainder of the carcass of animals assigned to the Component 1 Histopathology/Cell Proliferation Core group.

Histopathology/ Cell Proliferation

Lung and trachea slides from animals assigned to the Component 1 Histopathology/Cell Proliferation Core group were evaluated via light microscopic examination (Appendix III). There were no histopathological findings in the trachea. In the lungs of the 0.5 mg/m^3 LA and 3.5 mg/m^3 AA groups, minimal alveolus inflammation with foreign bodies were observed. In the 3.5 mg/m^3 LA group minimal to moderate alveolus inflammation, with minimal interstitial fibrosis and foreign bodies were observed in the lung. More alveolus inflammation was evident in the 3.5 mg/m^3 LA group than in the 3.5 mg/m^3 AA group. In the 25 mg/m^3 LA group, minimal inflammation and fibrosis were observed with bronchiole epithelial hyperplasia diagnosed in five of seven animals and foreign body (presumably fibers) present in all animals.

Cell replication analysis was performed on the terminal bronchiole of left lung samples from animals assigned to the Component 1 Histopathology/Cell Proliferation Core group (Appendix IV). A labeling index was completed for each animal. A first statistical analysis of the labeling index using all animal data did not show an exposure-related effect of the LA fiber. After a discussion with the pathologist five animals (two from the control group and three from the 0.5 mg/m^3 LA group) were censored from the data due to presumed non-treatment related inflammation in four animals and a low cell labeling in the terminal bronchiole region of one animal. After the exclusion of the specified animals, a dose-dependent increase in labeling indices was seen with increasing LA fiber concentration. Statistically significant increases in cell replication were observed in the terminal bronchiolar region of male rat lungs in the 25 mg/m^3 LA group (Dunnett's, $p < 0.05$).

Inflammation Evaluation

The BAL cell differential absolute counts and percentages for each animal assigned to the Component 1 Inflammation Evaluation core group were determined (Appendix V). There were no significant differences among exposure groups in the eosinophil or lymphocyte percentages. Neutrophil percentage means of the 3.5 mg/m^3 LA and 25 mg/m^3 LA groups were higher (α=0.05) than the neutrophil percentage mean of the control group. The neutrophil percentage mean of the 25 mg/m^3 LA group was also higher (α=0.05) than the neutrophil percentage mean of the 0.5 mg/m^3 LA group. Macrophage percentage means were lower for 3.5 mg/m^3 LA and 25 mg/m^3 LA groups (α=0.05) than that of the control group. Overall, there were no noteworthy differences from the control group BAL cell differential percentages values induced by exposures to Amosite at 3.5 mg/m^3 or Libby amphibole asbestos at 0.5 mg/m^3. The decrease in macrophages with simultaneous increases in neutrophils suggests a greater potential for 3.5 and 25 mg/m^3 LA fiber to elicit an acute inflammatory response during a 10-day exposure period.

Bronchoalveolar lavage (BAL) fluid was tested for elevated levels of alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein, N-acetyl-β-D-glucosaminidase (NAG) to evaluate lung inflammation and injury (Appendix V). The concentrations of all analytes were elevated in the 3.5 mg/m^3 AA (positive control) group when compared to the 0.0 mg/m^3 (air control) group, but none at a statistically significant level. No significant differences were seen for any of the analytes in comparisons between the air control and the 0.5 mg/m^3 LA groups, or between 3.5 mg/m^3 AA and 0.5 mg/m^3 LA groups. All analytes were significantly elevated in the 25 mg/m^3 LA group compared to either air control or the positive control group. LDH and total protein were significantly higher in the 3.5 mg/m^3 LA group than either the air or positive control groups indicating increase in

cellular toxicity and alveolar-capillary permeability. ALP levels were significantly higher in the 3.5 mg/m^3 LA compared to the air control, but not to the AA positive control, indicating increased cellular damage. NAG, an indicator of phagocytic cell activation was not significantly elevated in the 3.5 mg/m^3 LA group compared to either air or positive control group.

Clearance/Biopersistence

All tissue samples were collected from Component 2 animals and frozen at approximately -70^{o}C. All samples will remain frozen until a final method for tissue digestion has been developed at which point tissues will be analyzed and fiber clearance and biopersistence determined.

Conclusion

A two component study was conducted with male Fischer 344 rats exposed to AA and LA fiber aerosols by inhalation. The first component was a two-week range finder study designed to determine optimal fiber-aerosol concentrations to be used in the subsequent subchronic inhalation exposure study and to compare the potency of inhaled Libby amphibole fibers to the potency of inhaled amosite amphibole, a known fibrogenic asbestos fiber. Component 1 consisted of 5 groups (7 animals per group) exposed to lofted LA or AA by nose-only inhalation for 6 hours a day, 5 days a week for two weeks at concentrations of 0, 0.5, 3.5, and 25 mg/m^3 LA or 3.5 mg/m^3 AA. The second component was a dosimetry study to determine initial fiber deposition and clearance/biopersistence of LA fiber. Component 2 consisted of 9 groups of animals (6 animals per group) exposed for 6 hours a day for 1-day, 5-days or 10 days (5 days/week for 2 weeks) to concentrations of 0.5, 3.5, or 25 mg/m^3 LA with necropsy time points at 0, 6, 12, or 24 hours after the last exposure.

No effect on bodyweight was observed, with animals exposed to 25 mg/m^3 LA fiber for two weeks having a weight gain of approximately 14% between the start of exposures and necropsy. There were no abnormal clinical observations made during the study, and no fiber exposure-related lesions were observed during gross examination at necropsy. Target tissues of the trachea and lung were examined histopathologically. There were no fiber exposure-related microscopic changes observed in the trachea. In the lung, minimal to moderate inflammation was observed in the LA intermediate and high dose groups, with bronchiole epithelial hyperplasia seen in 5 of 7 animals in the high dose group. Foreign bodies (presumably fibers) were observed in all of the high dose animals. The alveolus inflammation in the LA intermediate dose group was graded slightly higher than that of the AA group. Minimal interstital fibrosis was observed in the lungs of rats of the 3.5 and 25 mg/m^3 LA groups. Cellular replication analysis

indicated an increase in replication with increase LA concentration with statistically significant increase in the high LA dose group. A statistically significant increase in the number of neutrophils, as well as the levels of LDH, ALP, and total protein, were observed in the BAL of the 3.5 and 25 mg/m^3 LA dose groups. Macrophage percentages were lower in the 3.5 and 25 mg/m^3 LA dose groups compared to the control group. Overall the study data indicates increased lung damage at dose groups of 3.5 mg/m^3 AA, 3.5 mg/m^3 LA and 25 mg/m^3 LA compared to the control group.

This study was performed to help select target exposure concentrations for a 90-day inhalation study. Based on effects observed in this two week study, and in consultation with the sponsor, proposed target concentrations are 10 mg/m^3 for the LA high concentration, 3.3 mg/m^3 for the LA intermediate and the AA concentrations, 1.0 mg/m^3 for the LA low concentration, and 0.0 mg/m^3 for the control concentration. These targets may change as discussions between The Hamner Institutes and the Sponsor continue.

This study suggests potency of inhaled Libby amphibole fibers in rats following two weeks of exposure is equal to or slightly greater than the potency of inhaled amosite amphibole at comparable exposure concentrations.

Protocol Deviations and Explanations

Four protocol deviations occurred during this study: 1) The BAL fluids were not analyzed for β-glucuronidase. The assay kit standards failed analysis, and a kit from a second manufacturer was discontinued. 2) Not all animals were weighed at necropsy. The animals were weighed at time of receipt and weekly during the study. Necropsy group 2 animals (Component 1 Core Group histopathology/ cell proliferation) were weighed at time of necropsy, 4 days post the last nose-only exposure. All other animals were necropsied within 0 to 24 hours post the last nose-only exposure. 3) The cell differential percentages were not determined for monocytes. This endpoint was inadvertently placed in the protocol and protocol amendment 1. Macrophages, which are from the monocyte lineage, were evaluated. 4) The daily test material concentration was inadvertently not obtained for the low dose concentration on two study days. On study day 36 the mass weight filter was connected to the incorrect vacuum sample line inside the chamber which resulted in a sample not being collected. On study day 47 the valve controlling the mass weight filter vacuum was not turned on which resulted in a sample not being collected. These deviations do not affect the quality or integrity of the study.

Archives

At the completion of the study, all reports, raw data, and retained samples will be maintained in the Testing Facility's Archives for a period of one year after submission of the signed final report. The Sponsor will then be contacted in order to determine the final disposition of these materials.

Tables

Table 1: Study Design – Animals for Component 1 Core Group

Component 1 Dose Groups	Inflammation Evaluation (BAL) Necropsy Group# 1	Histopathology/Cell Proliferation Evaluation (Brdu) Necropsy Group# 2
Air Control 0.0 mg/m^3	7	7
Amosite 3.5 mg/m^3	7	7
LA Low 0.5 mg/m^3	7	7
LA Mid 3.5 mg/m^3	7	7
LA High 25 mg/m^3	7	7
Necropsy:	35	35

Total: 70

Table 2: Study Design – Animals for Component 2 Clearance/Biopersistence

Component 2 Exposure Period (Days)	Necropsy Time Point After Last 6 Hour Exposure	Exposure Dose Groups	Number of Animals	Necropsy Group #
1	Immediately	LA 0.5 mg/m^3 LA 3.5 mg/m^3 LA 25 mg/m^3	6 6 6	8
5	Immediately	LA 0.5 mg/m^3 LA 3.5 mg/m^3 LA 25 mg/m^3	6 6 6	4
10	Immediately	LA 0.5 mg/m^3 LA 3.5 mg/m^3 LA 25 mg/m^3	6 6 6	3
1	6 hours	LA 0.5 mg/m^3 LA 3.5 mg/m^3 LA 25 mg/m^3	6 6 6	10
1	12 hours	LA 0.5 mg/m^3 LA 3.5 mg/m^3 LA 25 mg/m^3	6 6 6	9
1	24 hours	LA 0.5 mg/m^3 LA 3.5 mg/m^3 LA 25 mg/m^3	6 6 6	11
5	6 hours	LA 0.5 mg/m^3 LA 3.5 mg/m^3 LA 25 mg/m^3	6 6 6	6
5	12 hours	LA 0.5 mg/m^3 LA 3.5 mg/m^3 LA 25 mg/m^3	6 6 6	5
5	24 hours	LA 0.5 mg/m^3 LA 3.5 mg/m^3 LA 25 mg/m^3	6 6 6	7
		Necropsy:	162	
		Total:	162	

Table 3. Animal in-life timeline.

Component	Order Date	Arrival Date	Randomized Date	Necropsy Group#	Exposure start	Exposure finish	Necropsy Date
1	02/4/2010	02/17/2010	02/18/2010	1	3/1/2010	3/12/2010	3/12/2010
				2	3/1/2010	3/12/2010	3/16/2010
2	02/11/2010	03/03/2010	03/04/2010	3	3/15/2010	3/26/2010	3/26/2010
				7	3/17/2010	3/21/2010	3/22/2010
				10	3/18/2010	3/18/2010	3/18/2010
				6	3/20/2010	3/24/2010	3/24/2010
2	02/11/2010	03/24/2010	03/25/2010	5	4/5/2010	4/9/2010	4/10/2010
				9	4/7/2010	4/7/2010	4/8/2010
				4	4/12/2010	4/16/2010	4/16/2010
				8	4/12/2010	4/12/2010	4/12/2010
				11	4/13/2010	4/13/2010	4/14/2010

Table 4. Conditions for Air Control Exposures

Study Day	8m^3 Chamber Temp (^{o}F)	8m^3 Chamber Humidity (%)	Tower Temp (^{o}F)	Tower Humidity (%)	Tower Pressure (in of H_20)	Tower Air Flow Ball Setting	Aerosol Conc. (mg/m^3)
Grand Mean	**66.7**	**51.1**	**69.0**	**3.7**	**-0.10**	**15.7**	**0.077**
St. Dev.	0.3	1.2	0.4	1.0	0.02	0.4	0.177
Minimum	66.1	49.7	68.7	2.4	-0.13	15.0	-0.080
Maximum	67.2	53.9	70.0	5.7	-0.08	16.0	0.547
Count	10	10	10	10	10	10	10

Table 5. Conditions for Amosite 3.5 mg/m^3 Exposures

	8m^3 Chamber Temp (^{o}F)	8m^3 Chamber Humidity (%)	Tower Temp (^{o}F)	Tower Humidity (%)	Tower Pressure (in of H_20)	Tower Air Flow Ball Setting	Aerosol Conc. (mg/m^3)
Grand Mean	**66.5**	**58.7**	**71.1**	**9.4**	**-0.10**	**66.3**	**3.67**
St. Dev.	0.2	3.5	0.4	2.6	0.02	0.4	1.58
Minimum	66.2	53.7	70.7	5.1	-0.13	66.0	1.99
Maximum	66.8	66.8	72.0	14.5	-0.08	67.0	6.20
Count	10	10	10	10	10	10	10

Table 6. Conditions for Libby 0.5 mg/m^3 Exposures

	8m^3 Chamber Temp (^{o}F)	8m^3 Chamber Humidity (%)	Tower Temp (^{o}F)	Tower Humidity (%)	Tower Pressure (in of H_20)	Tower Air Flow Ball Setting	Aerosol Conc. (mg/m^3)
Grand Mean	**68.1**	**51.9**	**71.0**	**8.7**	**-0.15**	**84.9**	**0.534**
St. Dev.	0.2	2.8	0.6	5.8	0.04	0.4	0.110
Minimum	67.7	48.3	70.4	4.7	-0.28	83.0	0.296
Maximum	68.7	58.2	72.2	27.9	-0.08	85.3	0.764
Count	32	32	32	32	32	32	30

Table 7. Conditions for Libby 3.5 mg/m^3 Exposures

	8m^3 Chamber Temp (^{o}F)	8m^3 Chamber Humidity (%)	Tower Temp (^{o}F)	Tower Humidity (%)	Tower Pressure (in of H_20)	Tower Air Flow Ball Setting	Aerosol Conc. (mg/m^3)
Grand Mean	**67.3**	**52.6**	**64.3**	**4.1**	**-0.20**	**81.9**	**3.59**
St. Dev.	0.3	2.5	0.5	0.5	0.03	0.7	0.91
Minimum	66.6	47.4	63.6	3.0	-0.34	80.7	2.06
Maximum	68.1	57.2	65.3	4.9	-0.14	83.0	6.33
Count	32	32	32	32	32	32	32

Table 8. Conditions for Libby 25 mg/m^3 Exposures

	8m^3 Chamber Temp (^{o}F)	8m^3 Chamber Humidity (%)	Tower Temp (^{o}F)	Tower Humidity (%)	Tower Pressure (in of H_20)	Tower Air Flow Ball Setting	Aerosol Conc. (mg/m^3)
Grand Mean	**67.3**	**50.2**	**69.8**	**7.7**	**-0.21**	**81.6**	**26.8**
St. Dev.	0.3	1.8	1.1	3.6	0.04	0.8	9.1
Minimum	68.1	55.8	71.6	19.3	-0.11	82.7	49.1
Maximum	66.5	47.4	64.9	4.3	-0.37	78.0	12.5
Count	32	32	32	32	32	32	32

Table 9. APS Fiber Size Distributions

	Tower	Count Median Aerodynamic Diameter (CMAD) (µm)	Geometric Standard Deviation (Sigma G)	Particle Conc. (p/cc)
	Grand Mean	**1.22**	**1.44**	**616.3**
292	Std Dev	0.01	0.01	205.1
Amosite	Maximum daily mean	1.21	1.43	761.3
3.5 mg/m^3	Minimum daily mean	1.22	1.45	471.3
	No. of Days	2	2	2
	Grand Mean	**1.19**	**1.49**	**35.0**
293	Std Dev	0.05	0.02	20.2
Libby Fiber	Maximum daily mean	1.26	1.51	70.4
0.5 mg/m^3	Minimum daily mean	1.12	1.46	11.8
	No. of Days	6	6	6
	Grand Mean	**1.21**	**1.48**	**177.5**
294	Std Dev	0.04	0.02	123.7
Libby Fiber	Maximum daily mean	1.24	1.49	429.5
3.5 mg/m^3	Minimum daily mean	1.15	1.45	117.6
	No. of Days	6	6	6
	Grand Mean	**1.35**	**1.50**	**462.0**
295	Std Dev	0.04	0.01	192.0
Libby Fiber	Maximum daily mean	1.40	1.51	735.1
25 mg/m^3	Minimum daily mean	1.30	1.49	285.3
	No. of Days	6	6	6
	Grand Mean	**1.54**	**1.76**	**0.06**
296	Std Dev	0.13	0.02	0.05
Control	Maximum daily mean	1.63	1.77	0.09
0.0 mg/m^3	Minimum daily mean	1.44	1.74	0.02
	No. of Days	2	2	2

CMAD = Geometric diameter measurement of APS.

Table 10. SEM Fiber Size Distributions for Amosite 3.5 mg/m^3

Tower 292 Samples		
Sample:	1	2
Study Day:	5	12
Date:	03/05/10	03/12/10
Total Number of Objects Sized:	**356**	**248**
Objects with L/D Ratio < 3	138	108
Objects with L/D Ratio ≥ 3	218	140
Objects with L/D Ratio ≥ 5	152	98
Objects with L/D Ratio ≥ 20	25	27
Number of Fibers (L/D ≥ 3):	**218**	**140**
Average Fiber Diameter (µm):	0.352	0.329
Average Fiber Length (µm):	4.379	5.823

Table 11. SEM Fiber Size Distributions for Libby 0.5 mg/m^3

Tower 293 Samples						
Sample:	1	2	3	4	5	6
Study Day:	5	12	21	26	37	47
Date:	03/05/10	03/12/10	03/21/10	03/26/10	04/06/10	04/16/10
Total Number of Objects Sized:	**324**	**217**	**259**	**265**	**358**	**302**
Objects with L/D Ratio < 3	93	36	84	78	104	87
Objects with L/D Ratio ≥ 3	231	181	175	187	254	215
Objects with L/D Ratio ≥ 5	178	139	134	138	189	169
Objects with L/D Ratio ≥ 20	53	44	45	43	60	56
Number of Fibers (L/D ≥ 3):	**231**	**181**	**175**	**187**	**254**	**215**
Average Fiber Diameter (µm):	0.351	0.346	0.360	0.372	0.356	0.353
Average Fiber Length (µm):	5.330	5.476	6.194	5.907	5.473	5.808

Table 12. SEM Fiber Size Distributions for Libby 3.5 mg/m^3

Tower 294 Samples						
Sample:	1	2	3	4	5	6
Study Day:	5	12	21	26	37	47
Date:	03/05/10	03/12/10	03/21/10	03/26/10	04/06/10	04/16/10
Total Number of Objects Sized:	**202**	**254**	**339**	**392**	**306**	**250**
Objects with L/D Ratio < 3	58	74	84	96	75	55
Objects with L/D Ratio ≥ 3	144	180	255	296	231	195
Objects with L/D Ratio ≥ 5	113	142	197	243	173	157
Objects with L/D Ratio ≥ 20	32	37	58	69	55	46
Number of Fibers (L/D ≥ 3):	**144**	**180**	**255**	**296**	**231**	**195**
Average Fiber Diameter (µm):	0.337	0.339	0.372	0.348	0.359	0.322
Average Fiber Length (µm):	5.364	5.815	5.652	4.927	5.119	5.221

Table 13. SEM Fiber Size Distributions for Libby 25 mg/m^3

	Tower 295 Samples					
Sample:	1	2	3	4	5	6
Study Day:	5	12	21	26	37	47
Date:	03/05/10	03/12/10	03/21/10	03/26/10	04/06/10	04/16/10
Total Number of Objects Sized:	**231**	**175**	**307**	**273**	**230**	**196**
Objects with L/D Ratio < 3	45	30	69	59	45	47
Objects with L/D Ratio ≥ 3	186	145	238	214	185	149
Objects with L/D Ratio ≥ 5	155	136	197	174	150	122
Objects with L/D Ratio ≥ 20	57	54	67	53	55	38
Number of Fibers (L/D ≥ 3):	**186**	**145**	**238**	**214**	**185**	**149**
Average Fiber Diameter (µm):	0.399	0.368	0.339	0.349	0.386	0.378
Average Fiber Length (µm):	6.272	7.235	5.880	5.614	6.058	6.407

Table 14. Average Body Weights for Component 1, Necropsy Group 1

		Weight (grams)				
Weighing Date	Dose Group	Control 0.0 mg/m^3	Libby Low 0.5 mg/m^3	Libby Mid 3.5 mg/m^3	Libby High 25 mg/m^3	Amosite 3.5 mg/m^3
Pre-Exposure	**Average**	**165.7**	**165.8**	**166.9**	**166.4**	**168.9**
Study Day -10	Std. Dev.	7.1	4.8	6.2	4.7	5.7
02/18/10	Maximum	175.0	172.7	174.4	174.1	176.0
	Minimum	157.6	158.4	158.1	158.7	159.5
	# of Animals	7	7	7	7	7
Pre-Exposure	**Average**	**184.5**	**185.2**	**190.2**	**184.5**	**190.3**
Study Day 0	Std. Dev.	3.5	3.3	7.5	3.5	9.4
02/28/10	Maximum	187.3	188.7	199.4	187.3	201.8
	Minimum	177.0	178.6	177.9	177.0	177.3
	# of Animals	7	7	7	7	7
Post 5 Days of Exposure	**Average**	**196.4**	**189.6**	**195.8**	**190.8**	**198.1**
	Std. Dev.	9.3	3.9	9.0	5.3	9.6
Study Day 7	Maximum	214.4	194.3	204.6	197.6	212.4
03/07/10	Minimum	189.6	183.7	178.4	184.3	182.8
	# of Animals	7	7	7	7	7

Table 15. Average Body Weights for Component 1, Necropsy Group 2

Weighing Date	Dose Group	Weight (grams) Control 0.0 mg/m^3	Libby Low 0.5 mg/m^3	Libby Mid 3.5 mg/m^3	Libby High 25 mg/m^3	Amosite 3.5 mg/m^3
Pre-Exposure	**Average**	**167.6**	**162.5**	**167.5**	**166.6**	**169.1**
Study Day -10	Std. Dev.	7.1	4.6	5.8	5.6	3.4
02/18/10	Maximum	175.8	169.6	175.2	174.5	174.0
	Minimum	158.5	157.2	160.1	157.6	163.9
	# of Animals	7	7	7	7	7
Pre-Exposure	**Average**	**188.1**	**182.2**	**187.9**	**185.6**	**191.6**
Study Day 0	Std. Dev.	8.4	8.6	7.1	10.9	7.1
02/28/10	Maximum	198.6	199.8	201.1	202.0	202.6
	Minimum	175.5	174.6	179.1	167.5	184.2
	# of Animals	7	7	7	7	7
Post 5 Days of Exposure	**Average**	**192.9**	**191.3**	**194.9**	**193.9**	**200.3**
Study Day 7	Std. Dev.	9.9	11.8	10.0	14.3	11.4
03/07/10	Maximum	201.6	214.4	211.0	211.6	216.2
	Minimum	175.4	179.1	181.8	167.7	186.3
	# of Animals	7	7	7	7	7
Post 10 Days of Exposure	**Average**	**207.8**	**203.9**	**207.1**	**205.3**	**214.2**
Study Day14	Std. Dev.	12.6	15.7	11.6	16.4	15.0
03/14/10	Maximum	223.2	233.4	224.7	226.5	235.9
	Minimum	184.0	187.8	191.9	175.4	199.2
	# of Animals	7	7	7	7	7
Necropsy	**Average**	**213.8**	**210.4**	**213.5**	**211.5**	**222.2**
Study Day 16	Std. Dev.	12.2	16.4	11.0	16.7	16.0
03/16/10	Maximum	229.0	242.3	227.5	233.6	244.3
	Minimum	191.8	194.8	198.1	182.3	201.8
	# of Animals	7	7	7	7	7

Table 16. Average Body Weights for Component 2, Necropsy Group 3

Weighing Date	Dose Group	Weight (grams) Libby Low 0.5 mg/m^3	Libby Mid 3.5 mg/m^3	Libby High 25 mg/m^3
Pre-	**Average**	**172.9**	**172.5**	**166.0**
Exposure	Std. Dev.	4.1	4.9	7.4
Study Day 04	Maximum	178.2	180.4	179.0
03/04/10	Minimum	167.3	167.3	159.1
	# of Animals	6	6	6
Pre-	**Average**	**184.1**	**181.8**	**177.3**
Exposure	Std. Dev.	3.9	4.6	6.8
Study Day 08	Maximum	189.2	185.8	187.7
03/08/10	Minimum	178.8	174.1	172.0
	# of Animals	6	6	6
Pre-	**Average**	**189.3**	**187.4**	**181.5**
Exposure	Std. Dev.	6.1	5.8	5.7
Study Day 14	Maximum	197.4	194.4	189.3
03/14/10	Minimum	180.8	179.2	174.1
	# of Animals	6	6	6
Post 5 Days	**Average**	**199.7**	**195.0**	**190.6**
Of Exposure	Std. Dev.	10.7	9.2	5.7
Study Day 21	Maximum	215.5	205.9	195.2
03/21/10	Minimum	184.1	181.0	181.0
	# of Animals	6	6	6

Table 17. Average Body Weights for Component 2, Necropsy Group 4

Weighing Date	Dose Group	Weight (grams) Libby Low 0.5 mg/m^3	Libby Mid 3.5 mg/m^3	Libby High 25 mg/m^3
Pre-	**Average**	**164.7**	**165.3**	**164.5**
Exposure	Std. Dev.	7.0	6.9	6.3
Study Day 25	Maximum	173.5	175.7	171.3
03/25/10	Minimum	152.9	157.8	154.8
	# of Animals	6	6	6
Pre-	**Average**	**190.0**	**194.0**	**194.0**
Exposure	Std. Dev.	6.9	9.7	7.9
Study Day 32	Maximum	200.1	209.8	205.9
04/01/10	Minimum	178.7	185.7	181.6
	# of Animals	6	6	6
Pre-	**Average**	**191.4**	**195.6**	**192.7**
Exposure	Std. Dev.	7.8	8.9	7.2
Study Day 35	Maximum	203.8	209.2	201.8
04/04/10	Minimum	180.1	189.6	180.2
	# of Animals	6	6	6
Pre-	**Average**	**212.4**	**218.2**	**214.0**
Exposure	Std. Dev.	9.9	8.6	7.2
Study Day42	Maximum	226.6	233.1	223.1
04/11/10	Minimum	198.4	209.3	201.8
	# of Animals	6	6	6

Table 18. Average Body Weights for Component 2, Necropsy Group 5

Weighing Date	Dose Group	Weight (grams) Libby Low 0.5 mg/m^3	Libby Mid 3.5 mg/m^3	Libby High 25 mg/m^3
Pre-	**Average**	**168.4**	**162.8**	**163.3**
Exposure	Std. Dev.	5.6	5.9	5.6
Study Day 25	Maximum	174.9	170.6	172.1
03/25/10	Minimum	158.2	153.2	156.7
	# of Animals	6	6	6
Pre-	**Average**	**194.8**	**187.4**	**188.2**
Exposure	Std. Dev.	5.0	8.7	7.4
Study Day 32	Maximum	201.8	197.6	201.6
04/01/10	Minimum	186.7	175.5	180.4
	# of Animals	6	6	6
Pre-	**Average**	**195.6**	**187.2**	**188.2**
Exposure	Std. Dev.	5.9	8.7	7.1
Study Day 35	Maximum	204.8	197.7	200.8
04/04/10	Minimum	186.2	176.6	181.5
	# of Animals	6	6	6

Table 19. Average Body Weights for Component 2, Necropsy Group 6

Weighing Date	Dose Group	Weight (grams) Libby Low 0.5 mg/m^3	Libby Mid 3.5 mg/m^3	Libby High 25 mg/m^3
Pre-	**Average**	**170.9**	**172.1**	**174.1**
Exposure	Std. Dev.	4.6	6.3	5.2
Study Day 04	Maximum	178.8	180.2	180.2
03/04/10	Minimum	165.6	163.4	166.2
	# of Animals	6	6	6
Pre-	**Average**	**180.9**	**184.0**	**183.5**
Exposure	Std. Dev.	3.7	4.1	6.4
Study Day 08	Maximum	185.4	189.6	190.1
03/08/10	Minimum	175.1	179.3	174.6
	# of Animals	6	6	6
Pre-	**Average**	**185.7**	**187.8**	**185.5**
Exposure	Std. Dev.	4.1	3.9	6.5
Study Day 14	Maximum	190.1	193.6	193.0
03/14/10	Minimum	180.5	182.9	176.9
	# of Animals	6	6	6
Post 1 Day	**Average**	**205.7**	**206.8**	**201.2**
Of Exposure	Std. Dev.	6.7	4.9	7.7
Study Day21	Maximum	213.1	212.6	214.0
03/21/10	Minimum	194.6	201.2	191.2
	# of Animals	6	6	6

Table 20. Average Body Weights for Component 2, Necropsy Group 7

Weighing Date	Dose Group	Weight (grams) Libby Low 0.5 mg/m^3	Libby Mid 3.5 mg/m^3	Libby High 25 mg/m^3
Pre-	**Average**	**169.0**	**169.2**	**169.2**
Exposure	Std. Dev.	7.7	5.8	6.5
Study Day 4	Maximum	176.8	176.8	178.5
03/04/10	Minimum	157.9	159.3	161.9
	# of Animals	6	6	6
Pre-	**Average**	**179.4**	**178.2**	**181.4**
Exposure	Std. Dev.	6.7	6.0	7.6
Study Day 8	Maximum	186.1	185.6	192.0
03/08/10	Minimum	170.2	171.1	172.8
	# of Animals	6	6	6
Pre-	**Average**	**183.4**	**182.4**	**184.1**
Exposure	Std. Dev.	8.4	5.8	7.4
Study Day 14	Maximum	193.0	188.7	192.2
03/14/10	Minimum	174.2	174.2	173.3
	# of Animals	6	6	6
Post 4 Days	**Average**	**197.5**	**194.2**	**199.1**
Of Exposure	Std. Dev.	11.6	6.0	8.4
Study Day21	Maximum	213.9	200.7	212.8
03/21/10	Minimum	184.0	183.6	190.2
	# of Animals	6	6	6

Table 21. Average Body Weights for Component 2, Necropsy Group 8

Weighing Date	Dose Group	Weight (grams) Libby Low 0.5 mg/m^3	Libby Mid 3.5 mg/m^3	Libby High 25 mg/m^3
Pre-Exposure Study Day 25 03/25/10	**Average**	**166.3**	**165.6**	**162.7**
	Std. Dev.	7.7	5.4	4.3
	Maximum	175.0	172.3	168.7
	Minimum	154.0	156.5	158.7
	# of Animals	6	6	6
Pre-Exposure Study Day 32 04/01/10	**Average**	**195.6**	**190.1**	**187.4**
	Std. Dev.	6.4	11.3	6.0
	Maximum	202.1	209.3	194.6
	Minimum	184.1	177.0	180.8
	# of Animals	6	6	6
Pre-Exposure Study Day 35 04/04/10	**Average**	**195.7**	**189.2**	**186.0**
	Std. Dev.	5.8	11.8	8.0
	Maximum	203.7	209.1	196.5
	Minimum	187.3	175.4	176.6
	# of Animals	6	6	6
Pre-Exposure Study Day42 04/11/10	**Average**	**216.6**	**211.3**	**205.8**
	Std. Dev.	4.8	15.2	8.5
	Maximum	221.7	232.8	216.2
	Minimum	210.0	191.4	192.7
	# of Animals	6	6	6

Table 22. Average Body Weights for Component 2, Necropsy Group 9

Weighing Date	Dose Group	Weight (grams) Libby Low 0.5 mg/m^3	Libby Mid 3.5 mg/m^3	Libby High 25 mg/m^3
Pre-	**Average**	**166.5**	**161.8**	**166.9**
Exposure	Std. Dev.	6.9	7.4	5.1
Study Day 25	Maximum	178.7	170.2	172.8
03/25/10	Minimum	159.5	151.6	160.6
	# of Animals	6	6	6
Pre-	**Average**	**188.3**	**186.8**	**191.3**
Exposure	Std. Dev.	7.4	8.1	6.6
Study Day 32	Maximum	200.4	193.5	198.3
04/01/10	Minimum	179.3	175.4	184.0
	# of Animals	6	6	6
Pre-	**Average**	**188.0**	**187.3**	**190.0**
Exposure	Std. Dev.	7.3	8.4	6.1
Study Day 35	Maximum	200.1	194.1	199.0
04/04/10	Minimum	178.0	175.7	183.5
	# of Animals	6	6	6

Table 23. Average Body Weights for Component 2, Necropsy Group 10

Weighing Date	Dose Group	Weight (grams) Libby Low 0.5 mg/m^3	Libby Mid 3.5 mg/m^3	Libby High 25 mg/m^3
Pre-	**Average**	**172.3**	**172.3**	**167.0**
Exposure	Std. Dev.	6.1	6.0	7.6
Study Day 4	Maximum	181.5	179.5	175.8
03/04/10	Minimum	164.0	164.7	155.5
	# of Animals	6	6	6
Pre-	**Average**	**184.0**	**183.8**	**180.5**
Exposure	Std. Dev.	3.8	6.1	7.7
Study Day 8	Maximum	189.0	192.6	189.8
03/08/10	Minimum	178.6	175.4	172.0
	# of Animals	6	6	6
Pre-	**Average**	**187.1**	**187.2**	**185.0**
Exposure	Std. Dev.	2.9	5.2	7.6
Study Day 14	Maximum	189.3	194.1	193.4
03/14/10	Minimum	181.9	182.5	173.2
	# of Animals	6	6	6

Table 24. Average Body Weights for Component 2, Necropsy Group 11

Weighing Date	Dose Group	Weight (grams) Libby Low 0.5 mg/m^3	Libby Mid 3.5 mg/m^3	Libby High 25 mg/m^3
Pre-Exposure Study Day 25 03/25/10	**Average**	**166.3**	**165.6**	**162.7**
	Std. Dev.	7.7	5.4	4.3
	Maximum	175.0	172.3	168.7
	Minimum	154.0	156.5	158.7
	# of Animals	6	6	6
Pre-Exposure Study Day 32 04/01/10	**Average**	**195.6**	**190.1**	**187.4**
	Std. Dev.	6.4	11.3	6.0
	Maximum	202.1	209.3	194.6
	Minimum	184.1	177.0	180.8
	# of Animals	6	6	6
Pre-Exposure Study Day 35 04/04/10	**Average**	**195.7**	**189.2**	**186.0**
	Std. Dev.	5.8	11.8	8.0
	Maximum	203.7	209.1	196.5
	Minimum	187.3	175.4	176.6
	# of Animals	6	6	6
Pre-Exposure Study Day42 04/11/10	**Average**	**216.6**	**211.3**	**205.8**
	Std. Dev.	4.8	15.2	8.5
	Maximum	221.7	232.8	216.2
	Minimum	210.0	191.4	192.7
	# of Animals	6	6	6

Figures

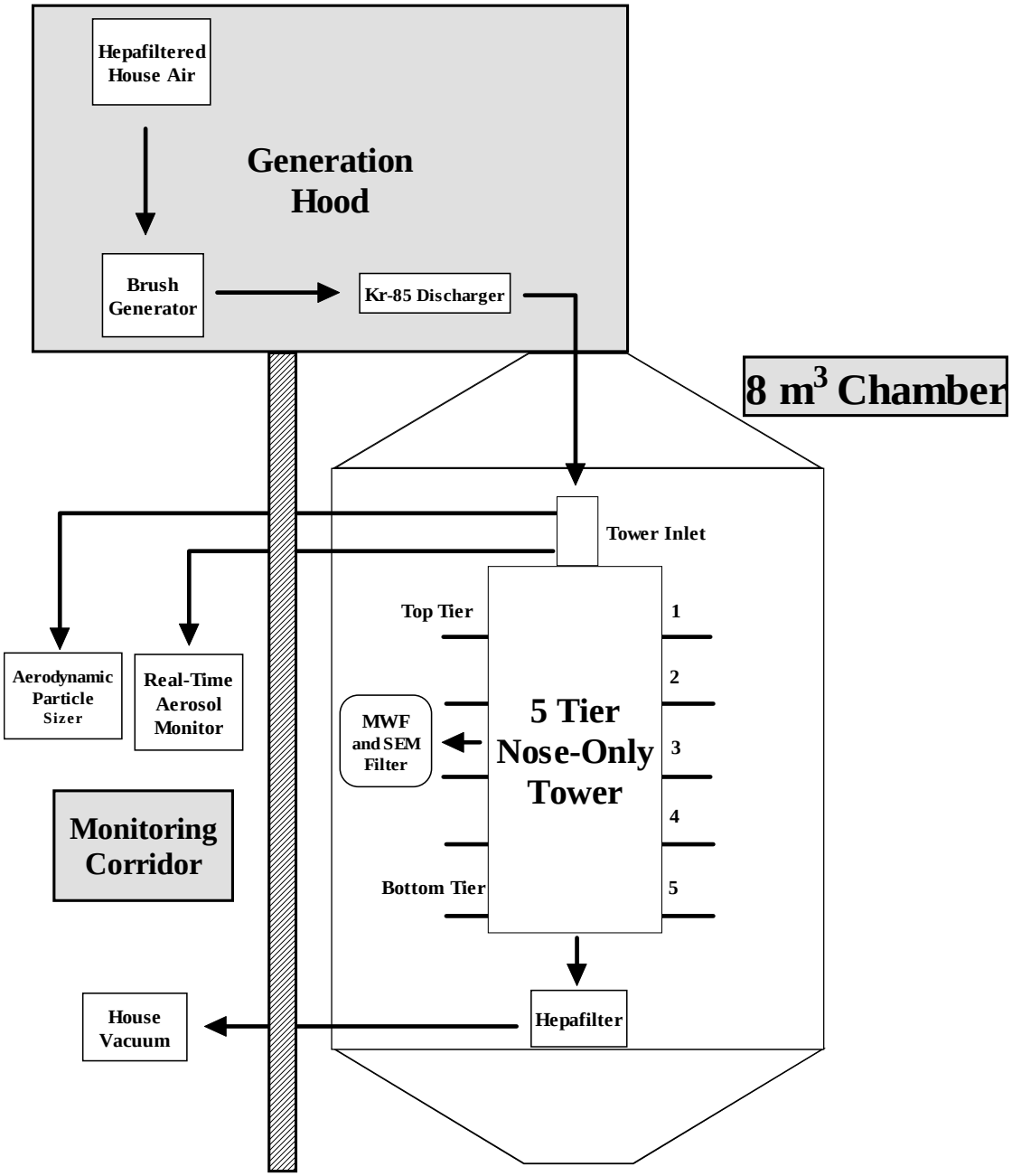

Figure 1. Fiber generation and exposure system schematic diagram.

APPENDIX 1:
Inlife Data - Animal body weights and clinical observations

Contents

Table 1. Individual body weights for Necropsy Group 1 Rats.

		Animal Body Weights (grams)		
Group 1 Component 1 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 02/18/10	Pre-Exposure 02/28/10	Post 5 Days of Exposure 03/07/10
101	Control	161.0	187.1	192.4
102	Control	172.3	196.2	191.4
103	Control	157.6	190.4	189.7
104	Control	159.2	188.6	193.4
105	Control	171.9	198.9	204.1
106	Control	162.9	189.6	189.6
107	Control	175.0	206.3	214.4
108	Amosite	168.0	190.6	203.2
109	Amosite	169.5	193.2	197.3
110	Amosite	167.8	181.6	182.8
111	Amosite	176.0	201.8	204.5
112	Amosite	165.8	177.3	192.1
113	Amosite	159.5	185.9	194.1
114	Amosite	175.4	201.5	212.4
115	Libby Low	158.4	178.6	184.8
116	Libby Low	162.5	186.6	191.4
117	Libby Low	170.1	188.7	190.8
118	Libby Low	163.6	184.0	183.7
119	Libby Low	166.3	187.4	194.3
120	Libby Low	166.8	184.6	189.7
121	Libby Low	172.7	186.4	192.4
122	Libby Mid	174.0	199.4	204.6
123	Libby Mid	158.1	192.2	199.7
124	Libby Mid	165.2	190.0	197.2
125	Libby Mid	174.4	195.4	203.8
126	Libby Mid	169.6	193.5	195.8
127	Libby Mid	161.1	177.9	178.4
128	Libby Mid	165.8	182.7	191.0
129	Libby High	165.5	185.1	192.2
130	Libby High	158.7	177.0	185.1
131	Libby High	167.0	186.9	191.2
132	Libby High	169.0	184.5	188.2
133	Libby High	164.2	187.3	196.8
134	Libby High	174.1	186.2	184.3
135	Libby High	166.1	184.2	197.6

Table 2. Individual body weights for Necropsy Group 2 Rats.

		Animal Body Weights (grams)				
Group 2 Component 1 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 02/18/10	Pre-Exposure 02/28/10	Post 5 Days of Exposure 03/07/10	Post 10 Days of Exposure 03/14/10	Necropsy 03/16/10
201	Control	158.5	183.2	185.4	199.8	206.3
202	Control	175.8	194.5	199.3	213.9	220.9
203	Control	172.9	195.9	198.0	211.7	219.5
204	Control	158.9	175.5	175.4	184.0	191.8
205	Control	174.4	198.6	201.2	213.7	218.8
206	Control	166.0	182.9	201.6	223.2	229.0
207	Control	166.5	185.9	189.1	208.5	210.0
208	Amosite	166.5	185.6	198.1	211.3	222.3
209	Amosite	172.1	202.6	216.2	235.9	244.3
210	Amosite	169.7	187.4	186.3	199.2	209.5
211	Amosite	169.5	189.6	191.4	202.3	212.4
212	Amosite	174.0	200.1	213.4	233.1	241.6
213	Amosite	163.9	184.2	192.7	202.0	201.8
214	Amosite	167.7	191.9	203.8	215.9	223.4
215	Libby Low	161.2	174.6	179.1	187.8	194.8
216	Libby Low	159.3	175.9	183.1	195.1	203.0
217	Libby Low	165.4	183.6	192.6	205.2	212.6
218	Libby Low	169.6	199.8	214.4	233.4	242.3
219	Libby Low	158.5	177.8	186.8	196.7	199.2
220	Libby Low	166.1	185.1	197.5	215.1	219.6
221	Libby Low	157.2	178.9	185.7	194.1	201.2
222	Libby Mid	164.2	184.0	192.3	201.5	209.7
223	Libby Mid	167.0	189.3	198.4	213.8	223.3
224	Libby Mid	175.2	201.1	211.0	224.7	227.5
225	Libby Mid	174.1	191.8	202.7	215.7	220.8
226	Libby Mid	162.4	185.5	192.4	206.1	213.0
227	Libby Mid	169.5	184.7	185.6	196.1	202.3
228	Libby Mid	160.1	179.1	181.8	191.9	198.1
229	Libby High	157.6	167.5	167.7	175.4	182.3
230	Libby High	165.7	186.4	201.5	211.3	220.6
231	Libby High	171.7	202.0	211.6	226.5	233.6
232	Libby High	164.8	180.0	192.6	203.6	212.3
233	Libby High	174.5	194.7	205.1	218.7	223.3
234	Libby High	163.1	183.2	190.6	203.4	206.1
235	Libby High	168.8	185.7	188.5	198.4	202.6

Table 3. Individual body weights for Necropsy Group 3 Rats.

		Animal Body Weights (grams)			
Group 3 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/04/10	Pre-Exposure 03/08/10	Pre-Exposure 03/14/10	Post 5 Days of Exposure 03/2110
313	Libby Low	174.5	187.0	197.4	215.5
314	Libby Low	168.7	178.8	180.8	184.1
315	Libby Low	178.2	189.2	195.3	207.0
316	Libby Low	167.3	180.8	188.0	198.2
317	Libby Low	175.1	185.8	188.1	196.1
318	Libby Low	173.7	182.8	186.0	197.1
331	Libby Mid	180.4	183.4	187.7	194.5
332	Libby Mid	167.3	178.1	182.5	188.8
333	Libby Mid	171.1	174.1	179.2	181.0
334	Libby Mid	176.5	185.8	187.8	196.2
335	Libby Mid	170.0	184.8	192.8	203.3
336	Libby Mid	169.7	184.3	194.4	205.9
349	Libby High	169.3	176.1	181.4	192.9
350	Libby High	159.1	172.0	180.4	195.2
351	Libby High	166.1	183.5	186.4	192.7
352	Libby High	179.0	187.7	189.3	195.2
353	Libby High	162.9	172.0	177.1	181.0
354	Libby High	159.7	172.4	174.1	186.5

Table 4. Individual body weights for Necropsy Group 4 Rats.

		Animal Body Weights (grams)			
Group 4 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/25/10	Pre-Exposure 04/01/10	Pre-Exposure 04/04/10	Pre-Exposure 04/11/10
307	Libby Low	168.3	188.3	191.9	209.6
308	Libby Low	173.5	200.1	203.8	226.6
309	Libby Low	161.9	190.1	191.0	213.8
310	Libby Low	164.3	191.9	194.4	219.4
311	Libby Low	167.1	190.6	187.3	206.5
312	Libby Low	152.9	178.7	180.1	198.4
325	Libby Mid	166.8	191.3	190.1	209.3
326	Libby Mid	159.4	189.6	190.2	214.4
327	Libby Mid	175.7	209.8	209.2	233.1
328	Libby Mid	170.0	201.5	204.5	222.3
329	Libby Mid	157.8	185.7	189.6	218.1
330	Libby Mid	161.9	185.9	189.7	211.8
343	Libby High	170.8	197.2	196.6	219.1
344	Libby High	154.8	181.6	180.2	201.8
345	Libby High	171.3	205.9	201.8	223.1
346	Libby High	160.2	193.1	194.1	214.1
347	Libby High	164.7	193.8	192.8	213.0
348	Libby High	165.3	192.2	190.6	212.8

Table 5. Individual body weights for Necropsy Group 5 Rats.

		Animal Body Weights (grams)		
Group 5 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/25/10	Pre-Exposure 04/01/10	Pre-Exposure 04/05/10
507	Libby Low	166.9	194.0	195.1
508	Libby Low	170.3	195.2	195.4
509	Libby Low	174.9	201.8	204.8
510	Libby Low	171.1	197.4	196.0
511	Libby Low	158.2	186.7	186.2
512	Libby Low	168.8	193.9	196.0
525	Libby Mid	160.4	184.4	184.1
526	Libby Mid	164.7	188.2	186.2
527	Libby Mid	170.6	196.9	197.5
528	Libby Mid	165.8	197.6	197.7
529	Libby Mid	153.2	175.5	176.6
530	Libby Mid	162.1	181.8	181.3
543	Libby High	156.7	184.8	187.2
544	Libby High	167.4	190.9	191.1
545	Libby High	172.1	201.6	200.8
546	Libby High	160.8	180.4	181.5
547	Libby High	159.6	184.7	182.7
548	Libby High	163.2	186.5	185.6

Table 6. Individual body weights for Necropsy Group 6 Rats.

		Animal Body Weights (grams)			
Group 6 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/04/10	Pre-Exposure 03/08/10	Pre-Exposure 03/14/10	Post 1 Day of Exposure 03/21/10
501	Libby Low	178.8	175.1	180.5	194.6
502	Libby Low	172.6	183.2	188.9	209.2
503	Libby Low	168.0	182.4	186.6	205.7
504	Libby Low	169.0	178.4	180.7	201.9
505	Libby Low	171.1	185.4	190.1	213.1
506	Libby Low	165.6	180.7	187.5	209.9
519	Libby Mid	168.8	179.3	182.9	201.2
520	Libby Mid	180.2	189.6	193.6	210.9
521	Libby Mid	163.4	181.3	187.8	210.0
522	Libby Mid	169.7	183.7	185.9	202.8
523	Libby Mid	171.9	181.8	185.7	203.1
524	Libby Mid	178.7	188.3	191.1	212.6
537	Libby High	174.2	185.8	184.7	200.2
538	Libby High	174.7	190.1	193.0	214.0
539	Libby High	170.5	174.6	176.9	191.2
540	Libby High	180.2	187.5	190.7	205.0
541	Libby High	166.2	176.3	178.9	199.4
542	Libby High	178.8	186.4	188.9	197.6

Table 7. Individual body weights for Necropsy Group 7 Rats.

		Animal Body Weights (grams)			
Group 7 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/04/10	Pre-Exposure 03/08/10	Pre-Exposure 03/14/10	Post 4 Days of Exposure 03/2110
313	Libby Low	174.3	185.2	192.4	213.9
314	Libby Low	162.7	173.2	174.2	184.0
315	Libby Low	157.9	170.2	175.1	189.4
316	Libby Low	176.8	184.0	186.5	196.3
317	Libby Low	166.9	177.4	179.2	192.5
318	Libby Low	175.6	186.1	193.0	209.1
331	Libby Mid	172.5	185.6	188.6	198.8
332	Libby Mid	168.2	171.1	174.2	183.6
333	Libby Mid	168.9	178.3	184.2	195.6
334	Libby Mid	176.8	179.9	179.9	192.1
335	Libby Mid	159.3	171.4	178.6	194.5
336	Libby Mid	169.6	183.1	188.7	200.7
349	Libby High	164.7	178.4	183.6	199.3
350	Libby High	169.2	172.8	177.3	190.6
351	Libby High	165.3	182.6	188.4	212.8
352	Libby High	161.9	174.5	173.3	190.2
353	Libby High	178.5	192.0	192.2	202.3
354	Libby High	175.4	188.1	189.6	199.4

Table 8. Individual body weights for Necropsy Group 8 Rats.

		Animal Body Weights (grams)			
Group 8 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/25/10	Pre-Exposure 04/01/10	Pre-Exposure 04/04/10	Pre-Exposure 04/11/10
301	Libby Low	161.8	192.4	193.0	216.3
302	Libby Low	175.0	198.6	198.8	221.7
303	Libby Low	154.0	184.1	187.3	210.0
304	Libby Low	170.9	197.5	193.0	212.3
305	Libby Low	171.1	202.1	203.7	221.7
306	Libby Low	164.7	198.6	198.3	217.3
319	Libby Mid	163.3	181.2	179.1	197.0
320	Libby Mid	166.7	187.9	188.5	211.9
321	Libby Mid	156.5	177.0	175.4	191.4
322	Libby Mid	165.6	191.5	191.0	220.1
323	Libby Mid	172.3	209.3	209.1	232.8
324	Libby Mid	169.3	193.4	192.1	214.3
337	Libby High	159.7	180.8	176.6	192.7
338	Libby High	162.7	186.3	183.5	204.5
339	Libby High	167.0	194.6	196.5	216.2
340	Libby High	158.7	181.1	180.2	201.4
341	Libby High	159.4	187.3	184.5	206.4
342	Libby High	168.7	194.0	194.7	213.4

Table 9. Individual body weights for Necropsy Group 9 Rats.

		Animal Body Weights (grams)		
Group 9 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/25/10	Pre-Exposure 04/01/10	Pre-Exposure 04/04/10
407	Libby Low	178.7	200.4	200.1
408	Libby Low	167.6	192.4	189.9
409	Libby Low	159.5	183.9	184.6
410	Libby Low	166.6	188.7	188.5
411	Libby Low	166.4	179.3	178.0
412	Libby Low	160.3	184.9	187.0
425	Libby Mid	151.6	175.4	175.7
426	Libby Mid	157.6	188.3	191.0
427	Libby Mid	165.1	193.5	193.9
428	Libby Mid	168.9	192.7	191.3
429	Libby Mid	157.3	177.9	177.5
430	Libby Mid	170.2	192.9	194.1
443	Libby High	163.4	184.5	183.8
444	Libby High	172.8	197.4	193.9
445	Libby High	170.4	198.3	199.0
446	Libby High	163.0	187.9	187.8
447	Libby High	170.9	195.8	192.0
448	Libby High	160.6	184.0	183.5

Table 10. Individual body weights for Necropsy Group 10 Rats.

		Animal Body Weights (grams)		
Group 10 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/04/10	Pre-Exposure 03/08/10	Pre-Exposure 03/14/10
401	Libby Low	175.7	186.9	189.0
402	Libby Low	168.2	178.6	181.9
403	Libby Low	164.0	181.3	187.2
404	Libby Low	173.4	185.4	185.8
405	Libby Low	181.5	189.0	189.2
406	Libby Low	170.9	182.8	189.3
419	Libby Mid	177.9	188.7	193.0
420	Libby Mid	164.7	175.4	182.5
421	Libby Mid	169.6	183.3	183.5
422	Libby Mid	174.8	182.6	186.8
423	Libby Mid	167.2	180.4	183.2
424	Libby Mid	179.5	192.6	194.1
437	Libby High	155.5	172.0	173.2
438	Libby High	168.7	175.5	181.9
439	Libby High	169.1	183.8	190.0
440	Libby High	175.8	189.8	193.4
441	Libby High	172.1	187.9	190.6
442	Libby High	160.5	173.9	180.9

Table 11. Individual body weights for Necropsy Group 11 Rats.

		Animal Body Weights (grams)			
Group 11 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/25/10	Pre-Exposure 04/01/10	Pre-Exposure 04/04/10	Pre-Exposure 04/11/10
413	Libby Low	168.2	197.1	195.3	217.5
414	Libby Low	164.7	190.0	188.2	207.0
415	Libby Low	161.9	186.3	185.1	202.9
416	Libby Low	175.0	197.5	196.4	219.4
417	Libby Low	166.5	189.8	192.0	215.0
418	Libby Low	163.1	189.2	190.2	213.3
431	Libby Mid	171.1	194.2	192.8	217.8
432	Libby Mid	170.5	197.4	195.7	214.6
433	Libby Mid	172.5	190.5	189.5	214.8
434	Libby Mid	169.3	193.1	193.2	214.2
435	Libby Mid	157.2	166.6	164.1	174.7
436	Libby Mid	159.9	182.3	182.3	204.9
449	Libby High	168.3	182.0	179.6	197.2
450	Libby High	152.4	179.6	179.0	197.6
451	Libby High	163.6	187.0	184.7	210.7
452	Libby High	158.7	187.6	185.9	205.3
453	Libby High	166.1	188.7	187.9	204.7
454	Libby High	159.1	181.1	184.2	204.2

Table 12. Individual clinical observations for Necropsy Group 1 Rats.

		Clinical Observations	
Group 1 Component 1 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 02/28/10	Post 5 Days of Exposure 03/07/10
101	Control	Normal	Normal
102	Control	Normal	Normal
103	Control	Normal	Normal
104	Control	Normal	Normal
105	Control	Normal	Normal
106	Control	Normal	Normal
107	Control	Normal	Normal
108	Amosite	Normal	Normal
109	Amosite	Normal	Normal
110	Amosite	Normal	Normal
111	Amosite	Normal	Normal
112	Amosite	Normal	Normal
113	Amosite	Normal	Normal
114	Amosite	Normal	Normal
115	Libby Low	Normal	Normal
116	Libby Low	Normal	Normal
117	Libby Low	Normal	Normal
118	Libby Low	Normal	Normal
119	Libby Low	Normal	Normal
120	Libby Low	Normal	Normal
121	Libby Low	Normal	Normal
122	Libby Mid	Normal	Normal
123	Libby Mid	Normal	Normal
124	Libby Mid	Normal	Normal
125	Libby Mid	Normal	Normal
126	Libby Mid	Normal	Normal
127	Libby Mid	Normal	Normal
128	Libby Mid	Normal	Normal
129	Libby High	Normal	Normal
130	Libby High	Normal	Normal
131	Libby High	Normal	Normal
132	Libby High	Normal	Normal
133	Libby High	Normal	Normal
134	Libby High	Normal	Normal
135	Libby High	Normal	Normal

Table 13. Individual clinical observations for Necropsy Group 2 Rats.

		Clinical Observations		
Group 2 Component 1 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 02/28/10	Post 5 Days of Exposure 03/07/10	Post 10 Days of Exposure 03/14/10
201	Control	Normal	Normal	Normal
202	Control	Normal	Normal	Normal
203	Control	Normal	Normal	Normal
204	Control	Normal	Normal	Normal
205	Control	Normal	Normal	Normal
206	Control	Normal	Normal	Normal
207	Control	Normal	Normal	Normal
208	Amosite	Normal	Normal	Normal
209	Amosite	Normal	Normal	Normal
210	Amosite	Normal	Normal	Normal
211	Amosite	Normal	Normal	Normal
212	Amosite	Normal	Normal	Normal
213	Amosite	Normal	Normal	Normal
214	Amosite	Normal	Normal	Normal
215	Libby Low	Normal	Normal	Normal
216	Libby Low	Normal	Normal	Normal
217	Libby Low	Normal	Normal	Normal
218	Libby Low	Normal	Normal	Normal
219	Libby Low	Normal	Normal	Normal
220	Libby Low	Normal	Normal	Normal
221	Libby Low	Normal	Normal	Normal
222	Libby Mid	Normal	Normal	Normal
223	Libby Mid	Normal	Normal	Normal
224	Libby Mid	Normal	Normal	Normal
225	Libby Mid	Normal	Normal	Normal
226	Libby Mid	Normal	Normal	Normal
227	Libby Mid	Normal	Normal	Normal
228	Libby Mid	Normal	Normal	Normal
229	Libby High	Normal	Normal	Normal
230	Libby High	Normal	Normal	Normal
231	Libby High	Normal	Normal	Normal
232	Libby High	Normal	Normal	Normal
233	Libby High	Normal	Normal	Normal
234	Libby High	Normal	Normal	Normal
235	Libby High	Normal	Normal	Normal

Table 14. Individual clinical observations for Necropsy Group 3 Rats.

		Clinical Observations			
Group 3 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/04/10	Pre-Exposure 03/08/10	Pre-Exposure 03/14/10	Post 5 Days of Exposure 03/21/10
313	Libby Low	Normal	Normal	Normal	Normal
314	Libby Low	Normal	Normal	Normal	Normal
315	Libby Low	Normal	Normal	Normal	Normal
316	Libby Low	Normal	Normal	Normal	Normal
317	Libby Low	Normal	Normal	Normal	Normal
318	Libby Low	Normal	Normal	Normal	Normal
331	Libby Mid	Normal	Normal	Normal	Normal
332	Libby Mid	Normal	Normal	Normal	Normal
333	Libby Mid	Normal	Normal	Normal	Normal
334	Libby Mid	Normal	Normal	Normal	Normal
335	Libby Mid	Normal	Normal	Normal	Normal
336	Libby Mid	Normal	Normal	Normal	Normal
349	Libby High	Normal	Normal	Normal	Normal
350	Libby High	Normal	Normal	Normal	Normal
351	Libby High	Normal	Normal	Normal	Normal
352	Libby High	Normal	Normal	Normal	Normal
353	Libby High	Normal	Normal	Normal	Normal
354	Libby High	Normal	Normal	Normal	Normal

Table 15. Individual clinical observations for Necropsy Group 4 Rats.

		Clinical Observations			
Group 4 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/25/10	Pre-Exposure 04/01/10	Pre-Exposure 04/04/10	Pre-Exposure 04/11/10
307	Libby Low	Normal	Normal	Normal	Normal
308	Libby Low	Normal	Normal	Normal	Normal
309	Libby Low	Normal	Normal	Normal	Normal
310	Libby Low	Normal	Normal	Normal	Normal
311	Libby Low	Normal	Normal	Normal	Normal
312	Libby Low	Normal	Normal	Normal	Normal
325	Libby Mid	Normal	Normal	Normal	Normal
326	Libby Mid	Normal	Normal	Normal	Normal
327	Libby Mid	Normal	Normal	Normal	Normal
328	Libby Mid	Normal	Normal	Normal	Normal
329	Libby Mid	Normal	Normal	Normal	Normal
330	Libby Mid	Normal	Normal	Normal	Normal
343	Libby High	Normal	Normal	Normal	Normal
344	Libby High	Normal	Normal	Normal	Normal
345	Libby High	Normal	Normal	Normal	Normal
346	Libby High	Normal	Normal	Normal	Normal
347	Libby High	Normal	Normal	Normal	Normal
348	Libby High	Normal	Normal	Normal	Normal

Table 16. Individual clinical observations for Necropsy Group 5 Rats.

		Clinical Observations		
Group 5 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/25/10	Pre-Exposure 04/01/10	Pre-Exposure 04/05/10
507	Libby Low	Normal	Normal	Normal
508	Libby Low	Normal	Normal	Normal
509	Libby Low	Normal	Normal	Normal
510	Libby Low	Normal	Normal	Normal
511	Libby Low	Normal	Normal	Normal
512	Libby Low	Normal	Normal	Normal
525	Libby Mid	Normal	Normal	Normal
526	Libby Mid	Normal	Normal	Normal
527	Libby Mid	Normal	Normal	Normal
528	Libby Mid	Normal	Normal	Normal
529	Libby Mid	Normal	Normal	Normal
530	Libby Mid	Normal	Normal	Normal
543	Libby High	Normal	Normal	Normal
544	Libby High	Normal	Normal	Normal
545	Libby High	Normal	Normal	Normal
546	Libby High	Normal	Normal	Normal
547	Libby High	Normal	Normal	Normal
548	Libby High	Normal	Normal	Normal

Table 17. Individual clinical observations for Necropsy Group 6 Rats.

		Clinical Observations			
Group 6 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/04/10	Pre-Exposure 03/08/10	Pre-Exposure 03/14/10	Post 1 Day of Exposure 03/21/10
501	Libby Low	Normal	Normal	Normal	Normal
502	Libby Low	Normal	Normal	Normal	Normal
503	Libby Low	Normal	Normal	Normal	Normal
504	Libby Low	Normal	Normal	Normal	Normal
505	Libby Low	Normal	Normal	Normal	Normal
506	Libby Low	Normal	Normal	Normal	Normal
519	Libby Mid	Normal	Normal	Normal	Normal
520	Libby Mid	Normal	Normal	Normal	Normal
521	Libby Mid	Normal	Normal	Normal	Normal
522	Libby Mid	Normal	Normal	Normal	Normal
523	Libby Mid	Normal	Normal	Normal	Normal
524	Libby Mid	Normal	Normal	Normal	Normal
537	Libby High	Normal	Normal	Normal	Normal
538	Libby High	Normal	Normal	Normal	Normal
539	Libby High	Normal	Normal	Normal	Normal
540	Libby High	Normal	Normal	Normal	Normal
541	Libby High	Normal	Normal	Normal	Normal
542	Libby High	Normal	Normal	Normal	Normal

Table 18. Individual clinical observations for Necropsy Group 7 Rats.

		Clinical Observations			
Group 7 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/04/10	Pre-Exposure 03/08/10	Pre-Exposure 03/14/10	Post 4 Days of Exposure 03/2110
313	Libby Low	Normal	Normal	Normal	Normal
314	Libby Low	Normal	Normal	Normal	Normal
315	Libby Low	Normal	Normal	Normal	Normal
316	Libby Low	Normal	Normal	Normal	Normal
317	Libby Low	Normal	Normal	Normal	Normal
318	Libby Low	Normal	Normal	Normal	Normal
331	Libby Mid	Normal	Normal	Normal	Normal
332	Libby Mid	Normal	Normal	Normal	Normal
333	Libby Mid	Normal	Normal	Normal	Normal
334	Libby Mid	Normal	Normal	Normal	Normal
335	Libby Mid	Normal	Normal	Normal	Normal
336	Libby Mid	Normal	Normal	Normal	Normal
349	Libby High	Normal	Normal	Normal	Normal
350	Libby High	Normal	Normal	Normal	Normal
351	Libby High	Normal	Normal	Normal	Normal
352	Libby High	Normal	Normal	Normal	Normal
353	Libby High	Normal	Normal	Normal	Normal
354	Libby High	Normal	Normal	Normal	Normal

Table 19. Individual clinical observations for Necropsy Group 8 Rats.

		Clinical Observations		
Group 8 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 04/01/10	Pre-Exposure 04/04/10	Pre-Exposure 04/11/10
301	Libby Low	Normal	Normal	Normal
302	Libby Low	Normal	Normal	Normal
303	Libby Low	Normal	Normal	Normal
304	Libby Low	Normal	Normal	Normal
305	Libby Low	Normal	Normal	Normal
306	Libby Low	Normal	Normal	Normal
319	Libby Mid	Normal	Normal	Normal
320	Libby Mid	Normal	Normal	Normal
321	Libby Mid	Normal	Normal	Normal
322	Libby Mid	Normal	Normal	Normal
323	Libby Mid	Normal	Normal	Normal
324	Libby Mid	Normal	Normal	Normal
337	Libby High	Normal	Normal	Normal
338	Libby High	Normal	Normal	Normal
339	Libby High	Normal	Normal	Normal
340	Libby High	Normal	Normal	Normal
341	Libby High	Normal	Normal	Normal
342	Libby High	Normal	Normal	Normal

Table 20. Individual clinical observations for Necropsy Group 9 Rats.

		Clinical Observations		
Group 9 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/25/10	Pre-Exposure 04/01/10	Pre-Exposure 04/04/10
407	Libby Low	Normal	Normal	Normal
408	Libby Low	Normal	Normal	Normal
409	Libby Low	Normal	Normal	Normal
410	Libby Low	Normal	Normal	Normal
411	Libby Low	Normal	Normal	Normal
412	Libby Low	Normal	Normal	Normal
425	Libby Mid	Normal	Normal	Normal
426	Libby Mid	Normal	Normal	Normal
427	Libby Mid	Normal	Normal	Normal
428	Libby Mid	Normal	Normal	Normal
429	Libby Mid	Normal	Normal	Normal
430	Libby Mid	Normal	Normal	Normal
443	Libby High	Normal	Normal	Normal
444	Libby High	Normal	Normal	Normal
445	Libby High	Normal	Normal	Normal
446	Libby High	Normal	Normal	Normal
447	Libby High	Normal	Normal	Normal
448	Libby High	Normal	Normal	Normal

Table 21. Individual clinical observations for Necropsy Group 10 Rats.

		Clinical Observations		
Group 10 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/04/10	Pre-Exposure 03/08/10	Pre-Exposure 03/14/10
401	Libby Low	Normal	Normal	Normal
402	Libby Low	Normal	Normal	Normal
403	Libby Low	Normal	Normal	Normal
404	Libby Low	Normal	Normal	Normal
405	Libby Low	Normal	Normal	Normal
406	Libby Low	Normal	Normal	Normal
419	Libby Mid	Normal	Normal	Normal
420	Libby Mid	Normal	Normal	Normal
421	Libby Mid	Normal	Normal	Normal
422	Libby Mid	Normal	Normal	Normal
423	Libby Mid	Normal	Normal	Normal
424	Libby Mid	Normal	Normal	Normal
437	Libby High	Normal	Normal	Normal
438	Libby High	Normal	Normal	Normal
439	Libby High	Normal	Normal	Normal
440	Libby High	Normal	Normal	Normal
441	Libby High	Normal	Normal	Normal
442	Libby High	Normal	Normal	Normal

Table 22. Individual clinical observations for Necropsy Group 11 Rats.

		Clinical Observations			
Group 11 Component 2 Animal Number (10002-)	Dose Group Assignment	Pre-Exposure 03/25/10	Pre-Exposure 04/01/10	Pre-Exposure 04/04/10	Pre-Exposure 04/11/10
413	Libby Low	Normal	Normal	Normal	Normal
414	Libby Low	Normal	Normal	Normal	Normal
415	Libby Low	Normal	Normal	Normal	Normal
416	Libby Low	Normal	Normal	Normal	Normal
417	Libby Low	Normal	Normal	Normal	Normal
418	Libby Low	Normal	Normal	Normal	Normal
431	Libby Mid	Normal	Normal	Normal	Normal
432	Libby Mid	Normal	Normal	Normal	Normal
433	Libby Mid	Normal	Normal	Normal	Normal
434	Libby Mid	Normal	Normal	Normal	Normal
435	Libby Mid	Normal	Normal	Normal	Normal
436	Libby Mid	Normal	Normal	Normal	Normal
449	Libby High	Normal	Normal	Normal	Normal
450	Libby High	Normal	Normal	Normal	Normal
451	Libby High	Normal	Normal	Normal	Normal
452	Libby High	Normal	Normal	Normal	Normal
453	Libby High	Normal	Normal	Normal	Normal
454	Libby High	Normal	Normal	Normal	Normal

APPENDIX II: Inhalation Summary Exposure Report

Appendix Title

Inhalation Exposure Summary Report: Inhalation Exposure to Libby Amphibole.

Study Title

EPA Fiber Project: Two-week Range Finding Study – Inhalation Exposure of Rats to Amphibole Asbestos.

Study Protocol

10002

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Introduction

The vermiculite mine near Libby, Montana was the world's leading source of vermiculite for 70 years until its closure in 1990. Vermiculite is used for insulation, as an absorbent material, and as a soil conditioner, and has applications in the construction, agricultural, horticultural and industrial markets. However, the Libby vermiculite ore coexists with a complex array of amphibole mineral types, primarily winchite, richterite, tremolite, and magnesioriebeckite with crystal forms (habits) ranging from asbestiform to acicular/prismatic. Occupational exposure to Libby vermiculite has been (and continues to be) associated with significant increases in asbestosis, lung cancer, and pleural cancer compared to the rest of the U.S. population. Furthermore, exposures to individuals outside of Libby have occurred, and are likely continuing; as asbestos-contaminated vermiculite ore from Libby was shipped to hundreds of locations around the nation for processing, and used as attic insulation in millions of homes throughout the United States. The health effects associated with former and current exposures from the asbestos contaminated vermiculite from the Libby mine continues to be a subject of intensive study and public health concern.

The overall goal of this research is to improve the scientific basis for the risk assessment of asbestos-contaminated communities by conducting toxicology studies to help define key determinants of internal dose and provide critical insight on additional key health or pathologic endpoints. There are two (2) components to the study. The first component is a 2-week range-finder study designed to determine optimal fiber-aerosol concentrations to be used in the subsequent subchronic inhalation exposure study and to compare the potency of inhaled Libby amphibole fibers to the potency of inhaled Amosite, a known fibrogenic amphibole asbestos fiber. The second component is a dosimetry study to determine initial fiber deposition and clearance/biopersistence. This study is designed to provide a time course of fiber burden data in various regions of the respiratory tract (head, trachea, lung lobes) and GI tract. These data will be used to derive LA-specific inhalability, deposition efficiency, and clearance rates for development of modifications to the Multi-path particle dosimetry (MPPD) model used to describe inhalation dosimetry.

This report describes the methods for Libby Amphibole (LA) and Amosite Amphibole (AA) fiber generation, analysis and exposure to facilitate the objectives.

Summary

Aerosols of LA and AA were generated by the lofting of fiber into the breathing air of F344 rats and administered by nose-only inhalation. The concentration of the fiber at each exposure tower was monitored using a light scatter aerosol instrument and measured by gravimetric filter. LA exposures were conducted for 6 hours per day for a total of 32 days. The aerosol concentration average daily means (±standard deviations) for these exposures were 0.53 (± 0.11), 3.59 (± 0.91), and 26.76 (± 9.11) mg/m^3 for the target concentrations of 0.5, 3.5 and 25 mg/m^3 of fiber in air, respectively. Control group and AA exposures were conducted for 6 hours per day for a total of 10 days with an aerosol concentration average daily mean (±standard deviations) of 0.08 (± 0.18) and 3.67 (± 1.58) mg/m^3 of fiber in air, respectively.

The environmental parameters specified in the protocol for temperature, relative humidity and airflow were maintained at or near the target set points of 68^{o}F, 50%, and 0.375 L/min/port, respectively, throughout the entire study.

The exposures were conducted from March 1st, 2010 through April 16th, 2010.

Materials and Methods

Chemical

Libby and UICC (International Union against Cancer) Amosite Amphibole fibers were obtained from the United States Geological Survey (USGS) by the Sponsor. All identity, purity, composition, stability, method of synthesis, fabrication and/or derivation information for these test materials used in this study are documented by the Sponsor (or his designee). This documentation is maintained by the Sponsor at the address indicated on the title page of this report. A MSDS or CAS number is not available for the Libby Amphibole at this time. The physical properties of Amosite (CAS# 12172-73-5) are listed in Table 1.

The Libby Amphibole was hand delivered by the Sponsor to The Hamner Institutes in a one-gallon plastic container. The plastic container was stored under room temperature and humidity in a glove box located in the inhalation monitoring corridor 200U. The contents of the container were used for the pilot (Protocol 09003) and this study.

UICC Amosite Amphibole was shipped directly from the USGS (Denver, CO) by courier in an insulated cooler. The Amosite Amphibole was placed into a plastic bag within a metal container and stored under room temperature and humidity in the Amosite generation hood. Test material from this container was used for this study.

An archival sample was taken from each of the test materials and stored under ambient conditions.

Exposure System

Animals (Rats) were exposed in five direct flow nose-only exposure systems (RCC, Geneva, Switzerland). Three towers were used for the LA target exposure concentrations, one for the AA target concentration, and one tower for the control group.

Each tower was located in a separate $8m^3$ chamber for exposure containment. An air and vacuum rotameter with an exhaust fan controlled airflow thru the exposure towers. The ball setting on the air supply rotameter was used to monitor the airflow. The rotameter was calibrated with a mass flow meter (MFM, Model 4040, TSI, Inc., Shoreview, MN). A 3-way valve was used to control airflow passing through either the generator or a bypass line directly to the exposure towers. Pressure was monitored with a magnehelic differential pressure gauge (Dwyer Instruments Inc., Michigan City, Indiana) at a tower inlet.

Temperature and relative humidity were measured near the top of each $8m^3$ chamber and at a port on each exposure tower by a Rotronic Humidity Sensor (Series 200, Rotronic Instruments Corp., Huntington, NY) connected to the Continuum Building Automation System (Andover Controls Corporation, TAC, Carrollton, TX). Temperature was calibrated by comparing the ambient air temperature recorded by the probe to a certified mercury thermometer. The relative humidity sensor was calibrated by immersing the sensor probe in an atmosphere of known humidity generated from saturated salt solutions.

The $8m^3$ chamber temperature and humidity readings represented the animal environment under which the exposures were conducted while the nose-only exposure tower readings represented the conditions of the atmosphere being inhaled.

Generation System

The Libby and Amosite Amphibole exposure atmospheres were generated using rotating brush generators (Aerosol Generator, Model CR-3000 & CR-3020, CR Equipements SA, CH-1295 Tannay, Switzerland) to aerosolize the fiber test material. A piston pushed a column of the test material into a rotating brush, which swept material off the top of the column into the generator air stream. The test material was packed into a generator piston with minimal pressure using a piston-packing tool. The air delivery pressure at the air supply rotameters for each tower was maintained at approximately 20 psi. The fibers were carried from a rotating brush generator into hepa-filtered house air at 50 liters per minute for the Libby towers and at 15 liters per minute for the control and Amosite towers. The fibers leaving a generator were delivered past a krypton-85 source (Kr^{85}, 10mCi, Isotopes Products Inc., Valencia, CA) to reduce charges on the particles at the exposure tower. The generator brush and piston speeds were adjusted to produce the required particle concentrations. A diagrammatic representation of the exposure system setup is seen in Figure 1.

Analytical System

Exposure atmosphere concentrations on each nose-only tower were measured daily using mass weight (gravimetric) filters at a port on the tower. A microbalance (Model C31, ATI CAHN Instruments, Boston, MA) was used to weigh filter samples twice before and after each sampling. Concentrations on each tower were continuously monitored during exposures using a light scatter aerosol monitor (Real-Time Aerosol Monitor (RAM), MIE Inc., Billerica, MA) from a port at the tower inlet.

Particle Size Distribution Measurements

Once per week particle size distributions were conducted using an optical particle sizing spectrometer (Aerodynamic Particle Sizer (APS), Model 3321, TSI, Inc. St. Paul, MN). The instrument was connected to a port at the tower inlet and hepa-filtered air was added in order to keep the aerosol concentration below overload conditions. Overload conditions are reached at approximately 1000 particles / cubic centimeter and indicated by a light on the front panel of the instrument. The reported results from each week were the average of data gathered from 5 one-minute samples.

Aerosol samples were collected on polycarbonate filters once a week from each of the towers during the exposures. Following collection, filters were transferred to a 25 mm aluminum pin mounts, adhered with conductive lubricant (Neolube No.20, Huron, IN) and coated with gold-palladium using a sputter coater (Sputter Coater, Model Hummer V, Technics, Dublin, CA). Samples were imaged using the scanning electron microscopy (JEOL, Model JSM-840A, Tokyo, Japan) and the images analyzed with Image-Pro Plus (V5.0.1.11 for Windows/XP, Media Cybernetics Inc., Bethesda, MD) to obtain fiber diameter, fiber length and the number of fibers from the captured images.

The gold-palladium coating, angstroms thick, was used to stop fiber loss during electron microscopy analysis by reducing electrostatic charge on the fibers

The APS samples and filter analysis were used to establish the particle size distribution and that the distribution was consistent at the start and end of the exposures.

Tower Distribution

Each Libby exposure tower was checked for uniformity of distribution of the test material by measuring the aerosol mass concentration, APS size distributions, and SEM size distributions at top, middle, and bottom ports for each tower. Tower distribution measurements for these exposures were performed prior to the start of Protocol 10002.

Study Design and Study Day Numbering

Due to the necessity to distribute various biological time points, this study was conducted over a seven-week period. The study design placed animals into two components (see Table 2). Component 1 consisted of two core groups, necropsy group 1 (N1) for inflammation evaluation and necropsy group 2 (N2) for histopathology and cell proliferation evaluation. Each group consisted of 35 animals. Seven animals from each group were placed on each of the five exposure towers (control, Libby low dose, Libby middle dose, Libby high dose, Amosite dose). Both groups were exposed to the test material for 6 hours per day for 5 days a week for 2 weeks. Group N1 was necropsied immediately following the last exposure. Group N2 was treated with Brdu the day following the last exposure and necropsied 3 days later. Component 2 consisted of nine groups, necropsy groups N3 thru N11, for clearance/biopersistence evaluation. Each group consisted of 18 animals with 6 animals from each group placed on each of the three Libby Amphibole exposure towers (low dose, middle dose, high dose). Each group was exposed to test material for 6 hours per day for 1, 5 or 10 days followed by a necropsy time point of 0, 6, 12, or 24 hours after the last exposure.

Study day numbering started from the first day of exposure and continued sequentially up to and including the day when the last animal was necropsied.

An exposure day was defined as a 6-hour exposure period. The daily start and finish times for exposures varied depending on the animal group end point and followed one of three schedules: 7am – 1pm, 8am - 2pm or 12pm - 6pm. Each exposure was followed by at least a 15-minute clearance period for all concentrations prior to the opening of the $8m^3$ chambers for animal care procedures. The exposures were performed from March 1, 2010 through April 16th, 2010.

Environmental Parameters

The temperature and relative humidity from each of the $8m^3$ chambers and exposure towers were hand-recorded three times daily for each exposure period. The exposure tower air flow rotameter ball setting and pressure were similarly hand recorded three times daily for each exposure period.

Domiciliary Housing

Animals were housed during non-exposure periods in individual wire mesh cages. After the exposure and clearance period, animals were transferred from nose-only tubes on the exposure towers to the housing cages located in each $8m^3$ chamber anteroom. Animals were provided with water *ad libitum* and food during non-exposure periods. The lighting cycle for the animals was 12 hours of light followed by 12 hours of darkness and was controlled by the Continuum Building Automation System.

Project Personnel

Inhalation Facility

Exposure Facility Manager	Brian Wong, Ph.D.
Lead Research Associate	Earl W. Tewksbury, B. A.
Research Associate	Carl U. Parkinson, LATg
Research Associate	Kay C. Roberts, A. S.

Results

Chemical

There were no chemical analyses of the LA or AA conducted.

Tower Distribution

The results from the tower distribution measurements are in tables 3a – 3c. The coefficient of variation calculated from mass weight filters (Table 3a) for the top, middle and bottom tier was less than 7.0% for each exposure tower. The coefficient of variation obtained from the size distribution data of APS samples (Table 3b) was less that 2.7% between tiers for each of the exposure towers. Both coefficients indicate that the test material was uniformly distributed throughout the exposure towers.

The fiber diameter and length measurements obtained from SEM filter samples (Table 3c) were similar for each exposure tower and had an average size range of 0.313 µm to 0.383 µm and 4.9 µm to 6.2 µm, respectively.

Generation and Nose Only Exposure Concentration

Fiber size distribution was sampled once a week during the exposure period by APS (Table 4a – 4f) and SEM filter samples (Table 5a – 5d). The APS average count median aerodynamic diameter (CMAD) was 1.22 µm, 1.19 µm, 1.21 µm, 1.35 µm, 1.54 µm for the 3.5 mg/m^3 Amosite tower, the 0.5 mg/m^3 Libby tower, 3.5 mg/m^3 Libby tower, 25 mg/m^3 Libby tower, and the control tower, respectively. The control tower size distribution was based on average particle concentration of 0.09 particle/cc. Samples from the other towers were based on average concentrations between 12 and 763 particles/cc. For all SEM filter samples the average fiber diameter and length was between 0.329 and 0.399 µm and 4.379 and 7.235 µm, respectively. The APS and SEM data indicate that fiber size distributions were relatively stable during the six week exposure period.

Tables 6a – 6e show the summary and individual daily data for fiber generation and characterization for each exposure group. The grand daily mean and standard deviation of the average daily mean values for MWF fiber concentration, 8m^3 temperature, 8m^3 humidity, exposure tower temperature, exposure tower humidity, tower static pressure, and tower airflow ball setting are shown. The smallest minimum daily mean and the largest maximum daily mean are also shown. Groups N8 thru N11 were one day exposures and have only 1 daily value to report.

Fiber Concentration:
The grand daily means (± standard deviations) for fiber concentration based on mass weight filter data for Groups N1 and N2 were 0.077 (± 0.177), 3.67 (± 1.58), 0.564 (± 0.090), 3.52 (± 0.72), and 28.2 (± 13.8) mg/m^3 for the target concentrations of 0.0 mg/m^3 (control), 3.5 mg/m^3 AA, 0.5 mg/m^3 LA, 3.5 mg/m^3 LA and 25 mg/m^3 LA, respectively.

The grand daily means (± standard deviations) for fiber concentration based on mass weight filter data for Group N3 were 0.544 (± 0.125), 3.49 (± 0.78), and 24.5 (± 3.6) mg/m^3 for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for fiber concentration based on mass weight filter data for Group N4 were 0.485 (± 0.089), 4.18 (± 1.41), and 24.1 (± 4.4) mg/m^3 for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for fiber concentration based on mass weight filter data for Group N5 were 0.469 (± 0.146), 3.43 (± 0.77), and 30.4 (± 11.0) mg/m^3 for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for fiber concentration based on mass weight filter data for Group N6 were 0.508 (± 0.082), 3.62 (± 1.07), and 26.1 (± 3.2) mg/m^3 for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for fiber concentration based on mass weight filter data for Group N7 were 0.627 (± 0.098), 3.73 (± 1.01), and 26.0 (± 3.3) mg/m^3 for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for fiber concentration based on mass weight filter data for Group N8 were 0.354, 3.38, and 24.1 mg/m^3 for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for fiber concentration based on mass weight filter data for Group N9 were 0.653, 3.06, and 49.1 mg/m^3 for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for fiber concentration based on mass weight filter data for Group N10 were 0.658, 3.86, and 25.3 mg/m^3 for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for fiber concentration based on mass weight filter data for Group N11 were 0.520, 4.02, and 30.3 mg/m^3 for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

8m^3 Chamber Temperature:
The grand daily means (± standard deviations) for 8m^3 temperature for Groups N1 and N2 were 66.7 (± 0.3), 66.5 (± 0.2), 68.2 (± 0.2), 67.1 (± 0.3), and 67.3 (± 0.2) ^{o}F for the target concentrations of 0.0 mg/m^3 (control), 3.5 mg/m^3 AA, 0.5 mg/m^3 LA, 3.5 mg/m^3 LA and 25 mg/m^3 LA, respectively.

The grand daily means (± standard deviations) for 8m^3 temperature for Group N3 were 68.1 (± 0.2), 67.4 (± 0.3), and 67.3 (± 0.3) ^{o}F for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for 8m^3 temperature for Group N4 were 68.1 (± 0.4), 67.4 (± 0.4), and 67.1 (± 0.5) ^{o}F for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for 8m^3 temperature for Group N5 were 68.1 (± 0.1), 67.5 (± 0.3), and 67.3 (± 0.4) ^{o}F for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for $8m^3$ temperature for Group N6 were 68.2 (± 0.2), 67.5 (± 0.4), and 67.4 (± 0.4) ^oF for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for $8m^3$ temperature for Group N7 were 68.2 (± 0.2), 67.3 (± 0.2), and 67.3 (± 0.1) ^oF for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for $8m^3$ temperature for Group N8 were 68.1, 67.4, and 67.4 ^oF for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for $8m^3$ temperature for Group N9 were 68.3, 67.5, and 67.4 ^oF for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for $8m^3$ temperature for Group N10 were 68.3, 67.2, and 67.6 ^oF for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for $8m^3$ temperature for Group N11 were 68.7, 67.4, and 67.3 ^oF for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

$8m^3$ Chamber Relative Humidity:
The grand daily means (± standard deviations) for $8m^3$ relative humidity for Groups N1 and N2 were 51.1 (± 1.2), 58.7 (± 3.5), 50.8 (± 1.9), 52.7 (± 2.3), and 50.1 (± 1.2) %RH for the target concentrations of 0.0 mg/m^3 (control), 3.5 mg/m^3 AA, 0.5 mg/m^3 LA, 3.5 mg/m^3 LA and 25 mg/m^3 LA, respectively.

The grand daily means (± standard deviations) for $8m^3$ relative humidity for Group N3 were 54.4 (± 2.9), 54.0 (± 2.3), and 51.1 (± 2.5) %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for $8m^3$ relative humidity for Group N4 were 52.3 (± 2.7), 52.7 (± 2.0), and 49.5 (± 1.4) %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for $8m^3$ relative humidity for Group N5 were 49.7 (± 1.2), 49.5 (± 1.5), and 49.3 (± 1.5) %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for $8m^3$ relative humidity for Group N6 were 53.3 (± 3.7), 54.0 (± 2.4), and 50.9 (± 2.7) %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for $8m^3$ relative humidity for Group N7 were 53.2 (± 3.8), 53.2 (± 2.0), and 49.8 (± 0.5) %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for $8m^3$ relative humidity for Group N8 were 51.7, 50.8, and 48.8 %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for $8m^3$ relative humidity for Group N9 were 51.7, 47.4, and 47.4 %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for $8m^3$ relative humidity for Group N10 were 55.1, 51.5, and 49.4 %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for 8m^3 relative humidity for Group N11 were 56.4, 55.8, and 51.9 %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

Exposure Tower Temperature:
The grand daily means (± standard deviations) for exposure tower temperature for Groups N1 and N2 were 69.0 (± 0.4), 71.1 (± 0.4), 71.5 (± 0.7), 64.8 (± 0.6), and 69.8 (± 1.8) ^{o}F for the target concentrations of 0.0 mg/m^3 (control), 3.5 mg/m^3 AA, 0.5 mg/m^3 LA, 3.5 mg/m^3 LA and 25 mg/m^3 LA, respectively.

The grand daily means (± standard deviations) for exposure tower temperature for Group N3 were 70.8 (± 0.4), 64.1 (± 0.3), and 70.0 (± 0.8) ^{o}F for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower temperature for Group N4 were 70.7 (± 0.2), 64.1 (± 0.4), and 69.5 (± 0.4) ^{o}F for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower temperature for Group N5 were 70.5 (± 0.2), 64.1 (± 0.1), and 69.6 (± 0.2) ^{o}F for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower temperature for Group N6 were 70.6 (± 0.2), 64.0 (± 0.3), and 69.5 (± 0.3) ^{o}F for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower temperature for Group N7 were 71.0 (± 0.4), 64.2 (± 0.3), and 70.2 (± 0.9) ^{o}F for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower temperature for Group N8 were 70.9, 64.1, and 69.8 %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower temperature for Group N9 were 70.9, 64.2, and 69.6 %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower temperature for Group N10 were 71.1, 64.2, and 71.6 %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower temperature for Group N11 were 70.7, 64.0, and 69.4 %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

Exposure Tower Humidity:
The grand daily means (± standard deviations) for exposure tower relative humidity for Groups N1 and N2 were 3.7 (± 1.0), 9.4 (± 2.6), 9.6 (± 7.1), 4.3 (± 0.3), and 9.2 (± 4.3) %RH for the target concentrations of 0.0 mg/m^3 (control), 3.5 mg/m^3 AA, 0.5 mg/m^3 LA, 3.5 mg/m^3 LA and 25 mg/m^3 LA, respectively.

The grand daily means (± standard deviations) for exposure tower relative humidity for Group N3 were 10.2 (± 5.7), 4.0 (± 0.4), and 8.3 (± 4.2) %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower relative humidity for Group N4 were 5.1 (± 0.3), 3.5 (± 0.5), and 5.7 (± 0.7) %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower relative humidity for Group N5 were 5.9 (± 0.2), 4.5 (± 0.4), and 6.8 (± 0.6) %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower relative humidity for Group N6 were 8.9 (± 7.1), 3.8 (± 0.2), and 6.6 (± 2.4) %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower relative humidity for Group N7 were 15.9 (± 6.8), 3.9 (± 0.3), and 6.5 (± 1.4) %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower relative humidity for Group N8 were 4.7, 4.3, and 6.1 %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower relative humidity for Group N9 were 4.7, 4.2, and 6.4 %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower relative humidity for Group N10 were 16.2, 4.4, and 7.0 %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower relative humidity for Group N11 were 5.4, 3.8, and 6.7 %RH for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

Exposure Tower Static Pressure:
The grand daily means (± standard deviations) for exposure tower static pressure for Groups N1 and N2 were -0.10 (± 0.02), -0.10 (± 0.02), -0.12 (± 0.04), -0.19 (± 0.01), and -0.21 (± 0.03) inches of water for the target concentrations of 0.0 mg/m^3 (control), 3.5 mg/m^3 AA, 0.5 mg/m^3 LA, 3.5 mg/m^3 LA and 25 mg/m^3 LA, respectively.

The grand daily means (± standard deviations) for exposure tower static pressure for Group N3 were -0.14 (± 0.03), -0.20 (± 0.02), and -0.19 (± 0.05) inches of water for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower static pressure for Group N4 were -0.17 (± 0.06), -0.23 (± 0.06), and -0.24 (± 0.07) inches of water for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower static pressure for Group N5 were -0.19 (± 0.02), -0.20 (± 0.03), and -0.19 (± 0.02) inches of water for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower static pressure for Group N6 were -0.14 (± 0.03), -0.18 (± 0.02), and -0.22 (± 0.04) inches of water for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower static pressure for Group N7 were -0.14 (± 0.03), -0.18 (± 0.03), and -0.20 (± 0.02) inches of water for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower static pressure for Group N8 were –0.15, -0.20, and –0.21 inches of water for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower static pressure for Group N9 were –0.15, -0.18, and –0.21 inches of water for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower static pressure for Group N10 were –0.17, -0.20, and –0.19 inches of water for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower static pressure for Group N11 were –0.16, -0.20, and –0.23 inches of water for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

Exposure Tower Air Flow Ball Setting:
Air rotameters used to control the exposure tower air flow were calibrated during pre-study setup. A rotameter ball setting of 16.0 and 66.0 were equivalent to an approximate air flow rate of 15.0 L/min for the control and Amosite towers, respectively. Rotameter ball settings of 85.0, 82.0, 81.0 were equivalent to an approximate air flow of 50.0 L/min for the 0.5 mg/m^3, 3.5 mg/m^3, 25 mg/m^3 Libby towers, respectively.

The grand daily means (± standard deviations) for exposure tower airflow ball setting for Groups N1 and N2 were 15.7 (± 0.4), 66.3 (± 0.4), 84.8 (± 0.7), 82.1 (± 0.7), and 81.7 (± 0.4) for the target concentrations of 0.0 mg/m^3 (control), 3.5 mg/m^3 AA, 0.5 mg/m^3 LA, 3.5 mg/m^3 LA and 25 mg/m^3 LA, respectively.

The grand daily means (± standard deviations) for exposure tower airflow ball setting for Group N3 were 85.0 (± 0.2), 82.2 (± 0.7), and 81.7 (± 0.4) for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower airflow ball setting for Group N4 were 85.0 (± 0.0), 81.9 (± 0.6), and 81.9 (± 0.2) for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower airflow ball setting for Group N5 were 85.0 (± 0.0), 81.5 (± 0.8), and 81.1 (± 1.9) for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower airflow ball setting for Group N6 were 85.0 (± 0.0), 81.3 (± 0.4), and 81.5 (± 0.4) for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The grand daily means (± standard deviations) for exposure tower airflow ball setting for Group N7 were 84.9 (± 0.1), 81.9 (± 0.9), and 81.1 (± 0.3) for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower airflow ball setting for Group N8 were 85.0, 81.3, and 82.0 for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower airflow ball setting for Group N9 were 85.0, 82.7, and 82.7 for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower airflow ball setting for Group N10 were 85.0, 82.0, and 81.0 for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

The daily results for exposure tower airflow ball setting for Group N11 were 85.0, 81.7, and 81.7 for the target concentrations of 0.5, 3.5 and 25 mg/m^3 Libby Amphibole, respectively.

Deviations

Mass weight filters were not collected on two occasions, Study Day 36 and 47, from the 0.5 mg/m^3 Libby Amphibole tower as specified in the protocol. On Study Day 36 the mass weight filter holder was inadvertently connected to the SEM filter sample line, so there was no airflow through the filter. Afterwards the sample lines were more visibly labeled to prevent such an incident from recurring. On Study Day 47 the MWF filter was recorded as started however the on/off valve controlling the sample flow had not been turned on so no sample was taken. There was no impact to the exposures as a result of these deviations. Each deviation was documented and reported to the Principal Investigator as a Protocol Deviation.

Conclusions

AA and LA exposures were conducted by nose-only inhalation for 6 hours a day for 10 and 32 days, respectively. The concentration of fiber aerosol at each exposure tower was monitored using a light scatter aerosol instrument and measured by gravimetric filter. The aerosol concentration average daily means (±standard deviations) for these exposures were 0.53 (± 0.11), 3.59 (± 0.91), 26.76 (± 9.11) and 3.67 (± 1.58) mg/m^3 for the target concentrations of 0.5 mg/m^3 of LA fiber, 3.5 mg/m^3 of LA fiber, 25 mg/m^3 of LA fiber, and 3.5 mg/m^3 of AA fiber in air, respectively. A control group exposure was conducted for 6 hours per day for a total of 10 days with an aerosol concentration average daily mean (±standard deviations) of 0.08 (± 0.18) mg/m^3.

The environmental parameters specified in the protocol for temperature, relative humidity and airflow were maintained at or near the target set points of 68^{o}F, 50%, and 0.375 L/min/port, respectively, throughout the entire study.

The exposures were conducted from March 1st, 2010 through April 16th, 2010.

References

Hinds WC. Aerosol Technology – Properties, Behavior, and Measurements of Airborne Particles. New York: Wiley, 1999, p.92.

Table 1. Physical and Chemical Properties of UICC Amosite

Name:	Amosite
Synonyms:	Amosite, UICC Amosite,
CAS No.:	12172-73-5[1]
Molecular Formula:	$(Mg,Fe)_7Si_8O_{22}(OH)_2$[1]
Molecular Weight:	1171.51[1]
Specific Gravity:	3.43 g/cc[1]
Flash Point:	No Data
Vapor Pressure:	No Data
Stability:	Stable under ordinary conditions of use and storage[1]
Lot/Batch Number:	No Data
Appearance:	Fibrous Solid, Color – Brown, Gray or Green[1]
Melting Point/Range:	Decomposes below melting point of 950°C[1]
Identity and Purity:	Documentation maintained by Sponsor
Storage Conditions:	Ambient conditions, in hood
Container:	Plastic Bag, inside metal container
Manufacturer:	International Union Against Cancer (UICC)
Supplied by:	Sponsor

[1]Source: MSDS from Structure Probe Inc (SPI) for Product# 02703AB - UICC Amosite Asbestos Standard

Libby Amphibole: No MSDS or Certificate of Analysis was available.

Table 2. Necropsy Group Identification

Necropsy Group Number	Group Identification	Exposure Start Date	Exposure Stop Date	Necropsy Date
N1	Component 1 – Inflammation Evaluation	01-Mar-10	12-Mar-10	12-Mar-10
N2	Component 1 – Histopathology/Cell Proliferation	01-Mar-10	12-Mar-10	16-Mar-10
N3	Component 2 – 10 day exposure, 0 hour Necropsy	15-Mar-10	26-Mar-10	26-Mar-10
N4	Component 2 – 5 day exposure, 0 hour Necropsy	12-Apr-10	16-Apr-10	16-Apr-10
N5	Component 2 – 5 day exposure, 12 hour Necropsy	05-Apr-10	09-Apr-10	10-Apr-10
N6	Component 2 – 5 day exposure, 6 hour Necropsy	20-Mar-10	24-Mar-10	24-Mar-10
N7	Component 2 – 5 day exposure, 24 hour Necropsy	17-Mar-10	21-Mar-10	22-Mar-10
N8	Component 2 – 1 day exposure, 0 hour Necropsy	12-Apr-10	12-Apr-10	12-Apr-10
N9	Component 2 – 1 day exposure, 12 hour Necropsy	07-Apr-10	07-Apr-10	08-Apr-10
N10	Component 2 – 1 day exposure, 6 hour Necropsy	18-Mar-10	18-Mar-10	18-Mar-10
N11	Component 2 – 1 day exposure, 24 hour Necropsy	13-Apr-10	13-Apr-10	14-Apr-10

Table 3a. Fiber Distribution Uniformity in Exposure Towers - Filter Data

Tower	Date	Sample Location	Mass Concentration (mg/m^3)	Average (mg/m^3)	Standard Deviation	Coefficient of Variance
293	02/08/10	Top Tier	0.568	0.561	0.030	5.2
		Middle Tier	0.587			
		Bottom Tier	0.529			
294	02/11/10	Top Tier	3.604	3.89	0.27	7.0
		Middle Tier	4.141			
		Bottom Tier	3.928			
295	06/24/09	Top Tier	20.9	20.7	0.4	2.1
		Middle Tier	21.0			
		Bottom Tier	20.2			

Table 3b. Fiber Distribution Uniformity in Exposure Towers - APS Particle Size Distribution

Tower	Date	Sample Location	Count Median Aerodynamic Diameter µm (standard deviation) [1][4]	Geometric Standard Deviation Sigma G (standard deviation) [1]
293	02/28/10	Inlet	1.19 (0.01)	1.48 (0.01)
		Top Tier	1.14 (0.01)	1.47 (0.00)
		Middle Tier	1.15 (0.02)	1.48 (0.01)
		Bottom Tier	1.12 (0.02)	1.48 (0.01)
		Mean	**1.15**	**1.48**
		Std. Dev.[2]	**0.03**	**0.00**
		CV.[3]	**2.60**	**0.00**
294	02/28/10	Inlet	1.21 (0.02)	1.47 (0.01)
		Top Tier	1.18 (0.01)	1.46 (0.00)
		Middle Tier	1.18 (0.01)	1.46 (0.01)
		Bottom Tier	1.20 (0.01)	1.47 (0.00)
		Mean	**1.19**	**1.47**
		Std. Dev. [2]	**0.01**	**0.00**
		CV.[3]	**1.10**	**0.00**
295	08/07/09	Inlet	1.26 (0.02)	1.48 (0.01)
		Top Tier	1.19 (0.01)	1.46 (0.01)
		Middle Tier	1.26 (0.02)	1.47 (0.01)
		Bottom Tier	1.24 (0.01)	1.46 (0.01)
		Mean	**1.24**	**1.47**
		Std. Dev. [2]	**0.03**	**0.01**
		CV.[3]	**2.61**	**0.66**

[1] Each aerosol diameter and geometric standard deviation reading is the mean of 5 APS samples taken from each location for towers 293 and 294 and the mean of 7 APS samples taken from each location for tower 295. The standard deviation is for each of these means within each port.
[2] Std. Dev. - Standard Deviation of the Mean between ports.
[3] CV. - Coefficient of Variation = (Std. Dev. / Average) x 100
[2] The CMAD (Count Median Aerodynamic Diameter) is equivalent to the geometric mean as measured by an Aerodynamic Particle Sizer. (Hinds, 1999).

Table 3c. Fiber Distribution Uniformity in Exposure Towers - SEM Fiber Size Distribution

Tower 293 - 02/27/10	Top Tier	Middle Tier	Bottom Tier
Total Number of Objects Sized:	**394**	**400**	**333**
Objects with L/D Ratio < 3	121	120	118
Objects with L/D Ratio ≥ 3	273	280	215
Objects with L/D Ratio ≥ 5	221	211	170
Objects with L/D Ratio ≥ 20	62	69	54
Number of Fibers (L/D ≥ 3):	**273**	**280**	**215**
Average Fiber Diameter (µm):	0.342	0.324	0.313
Average Fiber Length (µm):	5.233	5.529	4.902

Tower 294 - 02/28/10	Top Tier	Middle Tier	Bottom Tier
Total Number of Objects Sized:	**496**	**459**	**531**
Objects with L/D Ratio < 3	133	159	184
Objects with L/D Ratio ≥ 3	363	300	347
Objects with L/D Ratio ≥ 5	287	223	282
Objects with L/D Ratio ≥ 20	78	73	71
Number of Fibers (L/D ≥ 3):	**363**	**300**	**347**
Average Fiber Diameter (µm):	0.351	0.380	0.346
Average Fiber Length (µm):	5.238	6.164	5.092

Tower 295 - 08/0709	Top Tier	Middle Tier	Bottom Tier
Total Number of Objects Sized:	**487**	**357**	**312**
Objects with L/D Ratio < 3	123	91	82
Objects with L/D Ratio ≥ 3	364	266	230
Objects with L/D Ratio ≥ 5	285	218	190
Objects with L/D Ratio ≥ 20	78	64	48
Number of Fibers (L/D ≥ 3):	**364**	**266**	**230**
Average Fiber Diameter (µm):	0.355	0.359	0.383
Average Fiber Length (µm):	4.930	5.498	5.468

Tower	Average Fiber	Top Tier	Middle Tier	Bottom Tier	Average	Std. Dev.[1]	CV[2]
293	Diameter (µm):	0.342	0.324	0.313	0.326	0.015	4.5
	Length (µm):	5.233	5.529	4.902	5.221	0.314	6.0
294	Diameter (µm):	0.351	0.380	0.346	0.359	0.018	5.1
	Length (µm):	5.238	6.164	5.092	5.498	0.581	10.6
295	Diameter (µm):	0.355	0.359	0.383	0.366	0.015	4.1
	Length (µm):	4.930	5.498	5.468	5.299	0.320	6.0

[1] Std. Dev. - Standard Deviation of the Mean between ports.
[2] CV. - Coefficient of Variation = (Std. Dev. / Average) x 100

Table 4a APS Summary Fiber Size Distributions During Exposures [1]

Tower		Count Median Aerodynamic Diameter (CMAD) (μm)[2]	Geometric Standard Deviation (Sigma G)	Particle Conc. (p/cc)
	Grand Mean	**1.22**	**1.44**	**616.3**
292	Std Dev	0.01	0.01	205.1
Amosite	Maximum daily mean	1.21	1.43	761.3
3.5 mg/m^3	Minimum daily mean	1.22	1.45	471.3
	No. of Days	2	2	2
	Grand Mean	**1.19**	**1.49**	**35.0**
293	Std Dev	0.05	0.02	20.2
Libby Fiber	Maximum daily mean	1.26	1.51	70.4
0.5 mg/m^3	Minimum daily mean	1.12	1.46	11.8
	No. of Days	6	6	6
	Grand Mean	**1.21**	**1.48**	**177**
294	Std Dev	0.04	0.02	124
Libby Fiber	Maximum daily mean	1.24	1.49	429
3.5 mg/m^3	Minimum daily mean	1.15	1.45	118
	No. of Days	6	6	6
	Grand Mean	**1.35**	**1.50**	**462.0**
295	Std Dev	0.04	0.01	192.0
Libby Fiber	Maximum daily mean	1.40	1.51	735.1
25 mg/m^3	Minimum daily mean	1.30	1.49	285.3
	No. of Days	6	6	6
	Grand Mean	**1.54**	**1.76**	**0.06**
296	Std Dev	0.13	0.02	0.05
Control	Maximum daily mean	1.63	1.77	0.09
0.0 mg/m^3	Minimum daily mean	1.44	1.74	0.02
	No. of Days	2	2	2

[1] See tables 4b – 4f for individual daily values.
[2] The CMAD (Count Median Aerodynamic Diameter) is equivalent to the geometric mean as measured by an Aerodynamic Particle Sizer. (Hinds, 1999).

Table 4b APS Fiber Size Distribution: 3.5 mg/m^3 Amosite Amphibole Tower 292.

Date	Location	Count Median Aerodynamic Diameter (CMAD) (µm) [1][3]	Geometric Standard Deviation (Sigma G) [1]	Tower Particle Conc. (p/cc) [1][2]
03/05/2010	Inlet	1.22	1.45	761.3
03/12/2010	Inlet	1.21	1.43	471.3
Grand Mean		**1.22**	**1.44**	**616.3**
Std Dev		0.01	0.01	205.1
Maximum daily mean		1.21	1.43	761.3
Minimum daily mean		1.22	1.45	471.3
No. of Days		2	2	2

[1] Average of 5 readings.
[2] Dilution factor was 1.6.
[3] The CMAD (Count Median Aerodynamic Diameter) is equivalent to the geometric mean as measured by an Aerodynamic Particle Sizer. (Hinds, 1999).

Table 4c APS Fiber Size Distributions: 0.5 mg/m^3 Libby Amphibole Tower 293.

Date	Location	Count Median Aerodynamic Diameter (CMAD) (µm) [1][3]	Geometric Standard Deviation (Sigma G) [1]	Tower Particle Conc. (p/cc) [1][2]
03/05/2010	Inlet	1.18	1.49	70.4
03/12/2010	Inlet	1.26	1.51	32.9
03/18/2010	Inlet	1.24	1.49	43.2
03/25/2010	Inlet	1.12	1.47	24.0
04/05/2010	Inlet	1.16	1.46	27.9
04/13/2010	Inlet	1.20	1.50	11.8
Grand Mean		**1.19**	**1.49**	**35.0**
Std Dev		0.05	0.02	20.2
Maximum daily mean		1.26	1.51	70.4
Minimum daily mean		1.12	1.46	11.8
No. of Days		6	6	6

[1] Average of 5 readings.
[2] Dilution factor was 1.6.
[3] The CMAD (Count Median Aerodynamic Diameter) is equivalent to the geometric mean as measured by an Aerodynamic Particle Sizer. (Hinds, 1999).

Table 4d APS Fiber Size Distributions: 3.5 mg/m^3 Libby Amphibole Tower 294.

Date	Location	Count Median Aerodynamic Diameter (CMAD) (µm) [1][3]	Geometric Standard Deviation (Sigma G) [1]	Tower Particle Conc. (p/cc) [1][2]
03/05/2010	Inlet	1.22	1.48	117.5
03/12/2010	Inlet	1.17	1.45	131.2
03/18/2010	Inlet	1.24	1.48	429.5
03/25/2010	Inlet	1.15	1.47	140.3
04/05/2010	Inlet	1.24	1.49	118.6
04/13/2010	Inlet	1.22	1.49	127.6
Grand Mean		**1.21**	**1.48**	**177**
Std Dev		0.04	0.02	124
Maximum daily mean		1.24	1.49	429
Minimum daily mean		1.15	1.45	118
No. of Days		6	6	6

[1] Average of 5 readings.
[2] Dilution factor was 1.6.
[3] The CMAD (Count Median Aerodynamic Diameter) is equivalent to the geometric mean as measured by an Aerodynamic Particle Sizer. (Hinds,1999).

Table 4e APS Fiber Size Distributions: 25 mg/m^3 Libby Amphibole Tower 295.

Date	Location	Count Median Aerodynamic Diameter (CMAD) (µm) [1][3]	Geometric Standard Deviation (Sigma G) [1]	Tower Particle Conc. (p/cc) [1][2]
03/05/2010	Inlet	1.30	1.49	735.1
03/12/2010	Inlet	1.34	1.49	370.7
03/18/2010	Inlet	1.36	1.50	661.6
03/25/2010	Inlet	1.31	1.49	428.1
04/05/2010	Inlet	1.37	1.49	291.4
04/13/2010	Inlet	1.40	1.51	285.3
Grand Mean		**1.35**	**1.50**	**462.0**
Std Dev		0.04	0.01	192.0
Maximum daily mean		1.40	1.51	735.1
Minimum daily mean		1.30	1.49	285.3
No. of Days		6	6	6

[1] Average of 5 readings.
[2] Dilution factor was 2.1.
[3] The CMAD (Count Median Aerodynamic Diameter) is equivalent to the geometric mean as measured by an Aerodynamic Particle Sizer. (Hinds,1999).

Table 4f APS Fiber Size Distributions: 0 mg/m^3 Control Tower 296.

Date	Location	Count Median Aerodynamic Diameter (CMAD) (µm) [1][3]	Geometric Standard Deviation (Sigma G) [1]	Tower Particle Conc. (p/cc) [1][2]
03/05/2010	Inlet	1.44	1.74	0.02
03/12/2010	Inlet	1.63	1.77	0.09
Grand Mean		**1.54**	**1.76**	**0.06**
Std Dev		0.13	0.02	0.05
Maximum daily mean		1.63	1.77	0.09
Minimum daily mean		1.44	1.74	0.02
No. of Days		2	2	2

[1] Average of 5 readings.
[2] Dilution factor was 1.6.
[3] The CMAD (Count Median Aerodynamic Diameter) is equivalent to the geometric mean as measured by an Aerodynamic Particle Sizer. (Hinds,1999).

Table 5a SEM Fiber Size Distribution– 3.5 mg/m^3 Amosite Amphibole Tower 292

Tower 292 Samples		
Sample:	1	2
Study Day:	5	12
Date:	03/05/10	03/12/10
Total Number of Objects Sized:	356	248
Objects with L/D Ratio < 3	138	108
Objects with L/D Ratio ≥ 3	218	140
Objects with L/D Ratio ≥ 5	152	98
Objects with L/D Ratio ≥ 20	25	27
Number of Fibers (L/D ≥ 3):	218	140
Average Fiber Diameter (µm):	0.352	0.329
Average Fiber Length (µm):	4.379	5.823

Table 5b SEM Fiber Size Distribution – 0.5 mg/m^3 Libby Amphibole Tower 293

Tower 293 Samples

Sample:	1	2	3	4	5	6
Study Day:	5	12	21	26	37	47
Date:	03/05/10	03/12/10	03/21/10	03/26/10	04/06/10	04/16/10
Total Number of Objects Sized:	**324**	**217**	**259**	**265**	**358**	**302**
Objects with L/D Ratio < 3	93	36	84	78	104	87
Objects with L/D Ratio ≥ 3	231	181	175	187	254	215
Objects with L/D Ratio ≥ 5	178	139	134	138	189	169
Objects with L/D Ratio ≥ 20	53	44	45	43	60	56
Number of Fibers (L/D ≥ 3):	**231**	**181**	**175**	**187**	**254**	**215**
Average Fiber Diameter (µm):	0.351	0.346	0.360	0.372	0.356	0.353
Average Fiber Length (µm):	5.330	5.476	6.194	5.907	5.473	5.808

Table 5c SEM Fiber Size Distribution – 3.5 mg/m^3 Libby Amphibole Tower 294

	Tower 294 Samples					
Sample:	1	2	3	4	5	6
Study Day:	5	12	21	26	37	47
Date:	03/05/10	03/12/10	03/21/10	03/26/10	04/06/10	04/16/10
Total Number of Objects Sized:	**202**	**254**	**339**	**392**	**306**	**250**
Objects with L/D Ratio < 3	58	74	84	96	75	55
Objects with L/D Ratio ≥ 3	144	180	255	296	231	195
Objects with L/D Ratio ≥ 5	113	142	197	243	173	157
Objects with L/D Ratio ≥ 20	32	37	58	69	55	46
Number of Fibers (L/D ≥ 3):	**144**	**180**	**255**	**296**	**231**	**195**
Average Fiber Diameter (µm):	0.337	0.339	0.372	0.348	0.359	0.322
Average Fiber Length (µm):	5.364	5.815	5.652	4.927	5.119	5.221

Table 5d SEM Fiber Size Distribution – 25 mg/m^3 Libby Amphibole Tower 295

	Tower 295 Samples					
Sample:	1	2	3	4	5	6
Study Day:	5	12	21	26	37	47
Date:	03/05/10	03/12/10	03/21/10	03/26/10	04/06/10	04/16/10
Total Number of Objects Sized:	**231**	**175**	**307**	**273**	**230**	**196**
Objects with L/D Ratio < 3	45	30	69	59	45	47
Objects with L/D Ratio ≥ 3	186	145	238	214	185	149
Objects with L/D Ratio ≥ 5	155	136	197	174	150	122
Objects with L/D Ratio ≥ 20	57	54	67	53	55	38
Number of Fibers (L/D ≥ 3):	**186**	**145**	**238**	**214**	**185**	**149**
Average Fiber Diameter (µm):	0.399	0.368	0.339	0.349	0.386	0.378
Average Fiber Length (µm):	6.272	7.235	5.880	5.614	6.058	6.407

Table 6a Inhalation Data for Air Control – Group N1 and N2

8m^3 Chamber	8m^3 Chamber	Tower	Tower	Tower	Tower	Aerosol

Date	Study Day	Temp (^oF)	Humidity (%)	Temp (^oF)	Humidity (%)	Pressure (in of H_20)	Air Flow Ball Setting	Conc. (mg/m^3)
01-Mar-10	1	67.0	50.4	69.2	3.9	-0.10	15.0	-0.056
02-Mar-10	2	66.5	49.7	68.7	3.6	-0.08	15.2	0.547
03-Mar-10	3	67.2	50.8	70.0	2.7	-0.13	15.7	-0.080
04-Mar-10	4	66.9	51.0	69.5	4.7	-0.12	15.0	0.048
05-Mar-10	5	66.4	51.5	69.0	5.7	-0.10	15.7	-0.003
08-Mar-10	8	66.8	51.5	68.9	3.7	-0.10	16.0	0.085
09-Mar-10	9	66.1	51.4	68.7	3.4	-0.09	16.0	0.003
10-Mar-10	10	66.3	51.5	68.7	3.0	-0.09	16.0	0.136
11-Mar-10	11	67.0	49.8	69.1	2.4	-0.10	16.0	0.064
12-Mar-10	12	66.5	53.9	68.7	4.4	-0.13	16.0	0.025
	Grand Mean	66.7	51.1	69.0	3.7	-0.10	15.7	0.077
	St. Dev.	0.3	1.2	0.4	1.0	0.02	0.4	0.177
	Minimum	66.1	49.7	68.7	2.4	-0.13	15.0	-0.080
	Maximum	67.2	53.9	70.0	5.7	-0.08	16.0	0.547
	Count	10	10	10	10	10	10	10

Table 6b Inhalation Data for Amosite 3.5 mg/m^3 Exposure Dose – Group N1 and N2

Date	Study Day	$8m^3$ Chamber Temp (^oF)	$8m^3$ Chamber Humidity (%)	Tower Temp (^oF)	Tower Humidity (%)	Tower Pressure (in of H_20)	Tower Air Flow Ball Setting	Aerosol Conc. (mg/m^3)
01-Mar-10	1	66.6	56.9	71.4	14.5	-0.08	67.0	4.680
02-Mar-10	2	66.5	59.3	72.0	10.2	-0.13	66.3	5.730
03-Mar-10	3	66.5	59.1	70.8	5.1	-0.08	66.0	3.850
04-Mar-10	4	66.5	57.5	70.7	6.3	-0.08	66.0	6.201
05-Mar-10	5	66.2	60.1	71.0	9.1	-0.09	66.7	4.578
08-Mar-10	8	66.5	56.9	70.9	8.3	-0.12	66.0	3.084
09-Mar-10	9	66.7	56.6	70.7	11.1	-0.12	66.0	2.131
10-Mar-10	10	66.4	60.1	71.0	9.1	-0.12	67.0	2.134
11-Mar-10	11	66.5	66.8	71.1	9.4	-0.10	66.3	1.988
12-Mar-10	12	66.8	53.7	71.0	10.8	-0.10	66.0	2.283
	Grand Mean	**66.5**	**58.7**	**71.1**	**9.4**	**-0.10**	**66.3**	**3.67**
	St. Dev.	0.2	3.5	0.4	2.6	0.02	0.4	1.58
	Minimum	66.2	53.7	70.7	5.1	-0.13	66.0	1.99
	Maximum	66.8	66.8	72.0	14.5	-0.08	67.0	6.20
	Count	10	10	10	10	10	10	10

Table 6c Inhalation Data for Libby 0.5 mg/m^3 Exposure Dose – All Groups

Study	$8m^3$ Chamber Temp	$8m^3$ Chamber Humidity	Tower Temp	Tower Humidity	Tower Pressure	Tower Air Flow	Aerosol Conc.

Date	Day	(°F)	(%)	(°F)	(%)	(in of H_20)	Ball Setting	(mg/m^3)
01-Mar-10	1	68.1	50.2	71.8	5.1	-0.22	85.0	0.426
02-Mar-10	2	68.3	54.5	71.8	5.2	-0.08	85.3	0.642
03-Mar-10	3	67.7	51.6	70.7	7.6	-0.17	85.0	0.432
04-Mar-10	4	68.3	49.3	72.2	27.9	-0.10	83.0	0.514
05-Mar-10	5	68.1	53.8	71.8	8.2	-0.10	85.0	0.595
08-Mar-10	8	68.3	48.7	72.0	15.3	-0.11	85.0	0.589
09-Mar-10	9	68.2	49.5	71.9	6.5	-0.10	84.7	0.579
10-Mar-10	10	68.0	50.0	72.2	5.9	-0.10	85.0	0.534
11-Mar-10	11	68.2	50.2	70.4	7.7	-0.15	85.0	0.718
12-Mar-10	12	68.5	50.5	70.6	6.5	-0.10	85.0	0.611
15-Mar-10	15	67.8	54.0	70.6	9.3	-0.16	85.3	0.488
16-Mar-10	16	68.3	48.7	71.4	11.1	-0.17	85.0	0.650
17-Mar-10	17	68.2	58.2	71.7	21.9	-0.13	84.7	0.764
18-Mar-10	18	68.3	55.1	71.1	16.2	-0.17	85.0	0.658
19-Mar-10	19	68.0	53.8	70.5	14.7	-0.10	85.0	0.589
20-Mar-10	20	68.1	48.8	70.7	21.5	-0.15	85.0	0.627
21-Mar-10	21	68.4	50.2	71.0	5.0	-0.13	85.0	0.497
22-Mar-10	22	68.3	56.3	70.5	6.1	-0.18	85.0	0.546
23-Mar-10	23	67.9	57.2	70.4	5.4	-0.12	85.0	0.448
24-Mar-10	24	68.1	54.2	70.6	6.6	-0.13	85.0	0.420
25-Mar-10	25	67.9	50.7	70.4	5.3	-0.14	85.0	0.523
26-Mar-10	26	67.8	55.4	70.5	5.6	-0.15	85.0	0.352
05-Apr-10	36	68.2	49.4	70.5	6.0	-0.22	85.0	[1]
06-Apr-10	37	68.0	49.9	70.5	6.2	-0.20	85.0	0.476
07-Apr-10	38	68.3	48.3	70.8	5.6	-0.16	85.0	0.653
08-Apr-10	39	68.1	51.7	70.4	5.8	-0.19	85.0	0.450
09-Apr-10	40	68.1	49.4	70.4	5.7	-0.17	85.0	0.296
12-Apr-10	43	68.1	51.7	70.9	4.7	-0.15	85.0	0.354
13-Apr-10	44	68.7	56.4	70.7	5.4	-0.16	85.0	0.520
14-Apr-10	45	67.8	53.5	70.6	5.3	-0.28	85.0	0.554
15-Apr-10	46	67.9	50.0	70.5	5.3	-0.12	85.0	0.511
16-Apr-10	47	67.9	49.9	70.7	4.8	-0.15	85.0	[2]
	Grand Mean	**68.1**	**51.9**	**71.0**	**8.7**	**-0.15**	**84.9**	**0.534**
	St. Dev.	0.2	2.8	0.6	5.8	0.04	0.4	0.110
	Minimum	67.7	48.3	70.4	4.7	-0.28	83.0	0.296
	Maximum	68.7	58.2	72.2	27.9	-0.08	85.3	0.764
	Count	32	32	32	32	32	32	30
Necropsy Group 1 and 2 (Study Day 1 to Study Day 12)								
	Mean	**68.2**	**50.8**	**71.5**	**9.6**	**-0.12**	**84.8**	**0.564**
	St. Dev.	0.2	1.9	0.7	7.1	0.04	0.7	0.090
	Minimum	67.7	48.7	70.4	5.1	-0.22	83.0	0.426
	Maximum	68.5	54.5	72.2	27.9	-0.08	85.3	0.718
	Count	10	10	10	10	10	10	10
Necropsy Group 3 (Study Day 15 to Study Day 26)								

Mean	**68.1**	**54.4**	**70.8**	**10.2**	**-0.14**	**85.0**	**0.544**
St. Dev.	0.2	2.9	0.4	5.7	0.03	0.2	0.125
Minimum	67.8	48.7	70.4	5.3	-0.18	84.7	0.352
Maximum	68.3	58.2	71.7	21.9	-0.10	85.3	0.764
Count	10	10	10	10	10	10	10

Necropsy Group 4 (Study Day 43 to Study Day 47)

Mean	**68.1**	**52.3**	**70.7**	**5.1**	**-0.17**	**85.0**	**0.485**
St. Dev.	0.4	2.7	0.2	0.3	0.06	0.0	0.089
Minimum	67.8	49.9	70.5	4.7	-0.28	85.0	0.354
Maximum	68.7	56.4	70.9	5.4	-0.12	85.0	0.554
Count	5	5	5	5	5	5	4

Necropsy Group 5 (Study Day 36 to Study Day 40)

Mean	**68.1**	**49.7**	**70.5**	**5.9**	**-0.19**	**85.0**	**0.469**
St. Dev.	0.1	1.2	0.2	0.2	0.02	0.0	0.146
Minimum	68.0	48.3	70.4	5.6	-0.22	85.0	0.296
Maximum	68.3	51.7	70.8	6.2	-0.16	85.0	0.653
Count	5	5	5	5	5	5	4

Necropsy Group 6 (Study Day 20 to Study Day 24)

Mean	**68.2**	**53.3**	**70.6**	**8.9**	**-0.14**	**85.0**	**0.508**
St. Dev.	0.2	3.7	0.2	7.1	0.03	0.0	0.082
Minimum	67.9	48.8	70.4	5.0	-0.18	85.0	0.420
Maximum	68.4	57.2	71.0	21.5	-0.12	85.0	0.627
Count	5	5	5	5	5	5	5

Necropsy Group 7 (Study Day 17 to Study Day 21)

Mean	**68.2**	**53.2**	**71.0**	**15.9**	**-0.14**	**84.9**	**0.627**
St. Dev.	0.2	3.8	0.4	6.8	0.03	0.1	0.098
Minimum	68.0	48.8	70.5	5.0	-0.17	84.7	0.497
Maximum	68.4	58.2	71.7	21.9	-0.10	85.0	0.764
Count	5	5	5	5	5	5	5

Necropsy Group 8 (Study Day 43 to Study Day 43)

Daily Result	**68.1**	**51.7**	**70.9**	**4.7**	**-0.15**	**85.0**	**0.354**
Count	1	1	1	1	1	1	1

Necropsy Group 9 (Study Day 38 to Study Day 38)

Daily Result	**68.3**	**48.3**	**70.8**	**5.6**	**-0.16**	**85.0**	**0.653**
Count	1	1	1	1	1	1	1

Necropsy Group 10 (Study Day 18 to Study Day 18)

Daily Result	**68.3**	**55.1**	**71.1**	**16.2**	**-0.17**	**85.0**	**0.658**
Count	1	1	1	1	1	1	1
Necropsy Group 11 (Study Day 44 to Study Day 44)							
Daily Result	**68.7**	**56.4**	**70.7**	**5.4**	**-0.16**	**85.0**	**0.520**
Count	1	1	1	1	1	1	1

[1] and [2] MWF inadvertently were not taken, see Deviations.

Table 6d Inhalation Data for Libby 3.5 mg/m^3 Exposure Dose – All Groups

Date	Study Day	8m^3 Chamber Temp (^{o}F)	8m^3 Chamber Humidity (%)	Tower Temp (^{o}F)	Tower Humidity (%)	Tower Pressure (in of H_20)	Tower Air Flow Ball Setting	Aerosol Conc. (mg/m^3)
01-Mar-10	1	67.2	52.7	65.3	4.8	-0.20	81.0	3.711
02-Mar-10	2	67.1	50.6	65.3	3.9	-0.20	81.3	4.728
03-Mar-10	3	66.6	50.7	63.8	4.1	-0.22	82.0	4.068
04-Mar-10	4	66.8	49.4	65.3	4.6	-0.17	83.0	3.387
05-Mar-10	5	67.1	54.2	64.9	4.4	-0.20	82.0	2.673
08-Mar-10	8	67.2	52.9	65.0	4.5	-0.18	82.0	2.440
09-Mar-10	9	67.1	50.7	64.9	4.3	-0.20	82.7	3.378
10-Mar-10	10	67.3	53.7	65.2	3.9	-0.19	82.0	3.824
11-Mar-10	11	67.2	54.7	64.0	4.0	-0.20	81.7	4.155
12-Mar-10	12	67.6	56.9	64.0	4.5	-0.18	83.0	2.826
15-Mar-10	15	67.5	55.1	64.2	4.2	-0.19	82.7	2.725
16-Mar-10	16	67.5	56.4	64.4	4.1	-0.17	82.7	3.800
17-Mar-10	17	67.1	54.4	64.7	3.5	-0.20	83.0	4.470
18-Mar-10	18	67.2	51.5	64.2	4.4	-0.20	82.0	3.859
19-Mar-10	19	67.1	53.7	64.0	4.0	-0.20	82.3	3.672
20-Mar-10	20	67.5	55.5	63.7	4.1	-0.14	81.0	2.056
21-Mar-10	21	67.6	50.8	64.2	3.8	-0.17	81.0	4.576
22-Mar-10	22	68.1	57.2	64.4	3.7	-0.20	81.0	3.175
23-Mar-10	23	67.1	53.5	63.7	4.0	-0.18	81.7	3.642
24-Mar-10	24	67.1	53.2	63.8	3.5	-0.20	81.7	4.639
25-Mar-10	25	67.3	49.3	63.9	4.2	-0.25	82.0	2.135
26-Mar-10	26	67.6	55.9	63.9	4.6	-0.23	83.0	2.823
05-Apr-10	36	68.1	49.7	64.3	4.4	-0.25	81.7	2.701
06-Apr-10	37	67.1	48.9	64.0	4.9	-0.20	81.7	4.463
07-Apr-10	38	67.5	47.4	64.2	4.2	-0.18	82.7	3.056
08-Apr-10	39	67.6	51.2	64.0	4.9	-0.19	81.0	2.882
09-Apr-10	40	67.5	50.4	64.0	4.3	-0.18	80.7	4.029
12-Apr-10	43	67.4	50.8	64.1	4.3	-0.20	81.3	3.383
13-Apr-10	44	67.4	55.8	64.0	3.8	-0.20	81.7	4.015
14-Apr-10	45	67.9	53.0	64.6	3.0	-0.34	82.0	4.572

15-Apr-10	46	66.9	52.4	63.6	3.4	-0.20	81.7	2.584
16-Apr-10	47	67.3	51.3	64.0	3.0	-0.20	83.0	6.331
	Grand Mean	**67.3**	**52.6**	**64.3**	**4.1**	**-0.20**	**81.9**	**3.59**
	St. Dev.	0.3	2.5	0.5	0.5	0.03	0.7	0.91
	Minimum	66.6	47.4	63.6	3.0	-0.34	80.7	2.06
	Maximum	68.1	57.2	65.3	4.9	-0.14	83.0	6.33
	Count	32	32	32	32	32	32	32
Necropsy Group 1 and 2 (Study Day 1 to Study Day 12)								
	Mean	**67.1**	**52.7**	**64.8**	**4.3**	**-0.19**	**82.1**	**3.52**
	St. Dev.	0.3	2.3	0.6	0.3	0.01	0.7	0.72
	Minimum	66.6	49.4	63.8	3.9	-0.22	81.0	2.44
	Maximum	67.6	56.9	65.3	4.8	-0.17	83.0	4.73
	Count	10	10	10	10	10	10	10
Necropsy Group 3 (Study Day 15 to Study Day 16)								
	Mean	**67.4**	**54.0**	**64.1**	**4.0**	**-0.20**	**82.2**	**3.49**
	St. Dev.	0.3	2.3	0.3	0.4	0.02	0.7	0.78
	Minimum	67.1	49.3	63.7	3.5	-0.25	81.0	2.14
	Maximum	68.1	57.2	64.7	4.6	-0.17	83.0	4.64
	Count	10	10	10	10	10	10	10
Necropsy Group 4 (Study Day 43 to Study Day 47)								
	Mean	**67.4**	**52.7**	**64.1**	**3.5**	**-0.23**	**81.9**	**4.18**
	St. Dev.	0.4	2.0	0.4	0.5	0.06	0.6	1.41
	Minimum	66.9	50.8	63.6	3.0	-0.34	81.3	2.58
	Maximum	67.9	55.8	64.6	4.3	-0.20	83.0	6.33
	Count	5	5	5	5	5	5	5
Necropsy Group 5 (Study Day 36 to Study Day 40)								
	Mean	**67.5**	**49.5**	**64.1**	**4.5**	**-0.20**	**81.5**	**3.43**
	St. Dev.	0.3	1.5	0.1	0.4	0.03	0.8	0.77
	Minimum	67.1	47.4	64.0	4.2	-0.25	80.7	2.70
	Maximum	68.1	51.2	64.3	4.9	-0.18	82.7	4.46
	Count	5	5	5	5	5	5	5
Necropsy Group 6 (Study Day 20 to Study Day 24)								
	Mean	**67.5**	**54.0**	**64.0**	**3.8**	**-0.18**	**81.3**	**3.62**
	St. Dev.	0.4	2.4	0.3	0.2	0.02	0.4	1.07
	Minimum	67.1	50.8	63.7	3.5	-0.20	81.0	2.06
	Maximum	68.1	57.2	64.4	4.1	-0.14	81.7	4.64
	Count	5	5	5	5	5	5	5
Necropsy Group 7 (Study Day 17 to Study Day 21)								

Mean	**67.3**	**53.2**	**64.2**	**3.9**	**-0.18**	**81.9**	**3.73**
St. Dev.	0.2	2.0	0.3	0.3	0.03	0.9	1.01
Minimum	67.1	50.8	63.7	3.5	-0.20	81.0	2.06
Maximum	67.6	55.5	64.7	4.4	-0.14	83.0	4.58
Count	5	5	5	5	5	5	5
Necropsy Group 8 (Study Day 43 to Study Day 43)							
Daily Result	**67.4**	**50.8**	**64.1**	**4.3**	**-0.20**	**81.3**	**3.38**
Count	1	1	1	1	1	1	1
Necropsy Group 9 (Study Day 38 to Study Day 38)							
Daily Result	**67.5**	**47.4**	**64.2**	**4.2**	**-0.18**	**82.7**	**3.06**
Count	1	1	1	1	1	1	1
Necropsy Group 10 (Study Day 18 to Study Day 18)							
Daily Result	**67.2**	**51.5**	**64.2**	**4.4**	**-0.20**	**82.0**	**3.86**
Count	1	1	1	1	1	1	1
Necropsy Group 11 (Study Day 44 to Study Day 44)							
Daily Result	**67.4**	**55.8**	**64.0**	**3.8**	**-0.20**	**81.7**	**4.02**
Count	1	1	1	1	1	1	1

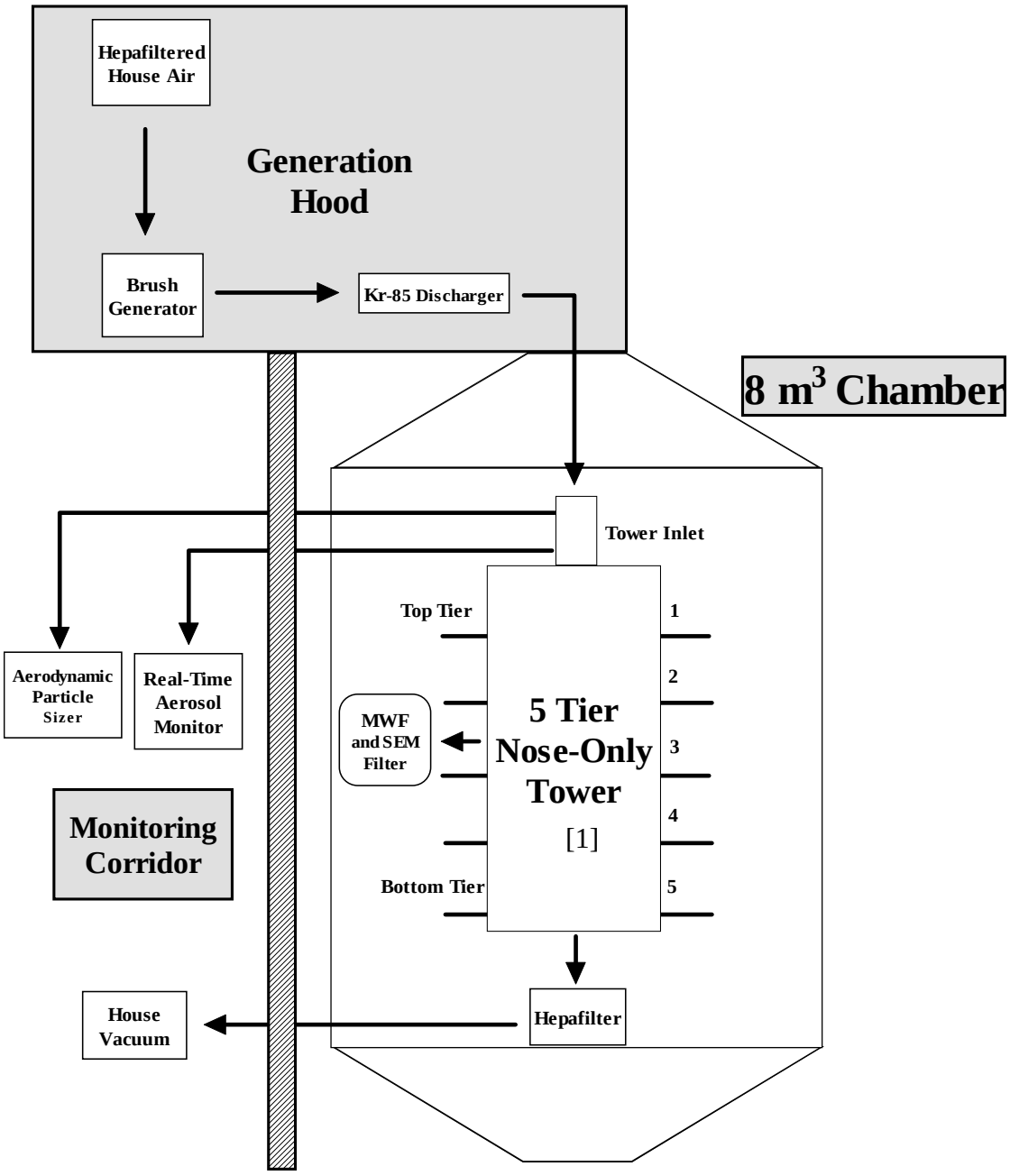
Table 6e Inhalation Data for Libby Amphibole 25 mg/m3 Exposure Dose – All Groups

Date	Study Day	$8m^3$ Chamber Temp (^oF)	$8m^3$ Chamber Humidity (%)	Tower Temp (^oF)	Tower Humidity (%)	Tower Pressure (in of H_20)	Tower Air Flow Ball Setting	Aerosol Conc. (mg/m^3)
01-Mar-10	1	67.2	49.7	70.7	7.4	-0.20	81.7	17.459
02-Mar-10	2	67.1	48.8	71.1	6.4	-0.26	81.3	12.538
03-Mar-10	3	67.3	49.1	69.7	6.9	-0.24	81.7	25.771
04-Mar-10	4	67.2	48.9	70.5	16.4	-0.19	81.0	20.500
05-Mar-10	5	67.2	49.4	70.8	10.9	-0.21	81.7	24.756
08-Mar-10	8	67.4	50.8	70.5	17.0	-0.17	81.7	24.539
09-Mar-10	9	67.1	50.7	64.9	4.3	-0.20	82.7	46.153
10-Mar-10	10	67.6	50.7	70.7	8.2	-0.22	82.0	48.973
11-Mar-10	11	67.0	50.6	69.6	8.5	-0.23	81.7	46.433
12-Mar-10	12	67.5	52.8	69.7	6.4	-0.21	82.0	15.224
15-Mar-10	15	67.1	50.7	70.6	19.3	-0.24	82.0	17.599
16-Mar-10	16	67.2	50.8	70.5	8.9	-0.18	82.0	24.133
17-Mar-10	17	67.2	50.4	69.5	7.3	-0.18	81.7	26.714
18-Mar-10	18	67.6	49.4	71.6	7.0	-0.19	81.0	25.317

19-Mar-10	19	67.2	49.9	70.7	8.0	-0.20	81.0	20.879
20-Mar-10	20	67.3	49.1	69.5	5.6	-0.22	81.0	27.253
21-Mar-10	21	67.3	50.0	69.7	4.4	-0.23	81.0	29.698
22-Mar-10	22	68.1	55.6	70.0	6.4	-0.20	82.0	26.520
23-Mar-10	23	67.1	50.5	69.3	5.8	-0.17	81.7	20.857
24-Mar-10	24	67.0	49.2	69.3	10.7	-0.28	81.7	26.042
25-Mar-10	25	67.0	48.7	69.3	5.0	-0.13	82.0	29.490
26-Mar-10	26	67.5	55.8	69.6	4.9	-0.11	82.0	26.984
05-Apr-10	36	67.8	49.0	69.9	6.6	-0.18	81.7	26.341
06-Apr-10	37	66.7	50.2	69.3	6.0	-0.20	82.3	20.286
07-Apr-10	38	67.4	47.4	69.6	6.4	-0.21	82.7	49.147
08-Apr-10	39	67.2	51.3	69.6	7.2	-0.17	81.0	29.256
09-Apr-10	40	67.1	48.8	69.5	7.6	-0.20	78.0	27.078
12-Apr-10	43	67.4	48.8	69.8	6.1	-0.21	82.0	24.105
13-Apr-10	44	67.3	51.9	69.4	6.7	-0.23	81.7	30.330
14-Apr-10	45	67.6	48.5	69.9	5.5	-0.37	82.0	22.452
15-Apr-10	46	66.5	49.0	69.1	4.7	-0.20	81.7	25.154
16-Apr-10	47	66.8	49.2	69.2	5.5	-0.18	82.0	18.236
	Grand Mean	**67.3**	**50.2**	**69.8**	**7.7**	**-0.21**	**81.6**	**26.8**
	St. Dev.	0.3	1.8	1.1	3.6	0.04	0.8	9.1
	Minimum	68.1	55.8	71.6	19.3	-0.11	82.7	49.1
	Maximum	66.5	47.4	64.9	4.3	-0.37	78.0	12.5
	Count	32	32	32	32	32	32	32
Necropsy Group 1 and 2 (Study Day 1 to Study Day 12)								
	Mean	**67.3**	**50.1**	**69.8**	**9.2**	**-0.21**	**81.7**	**28.2**
	St. Dev.	0.2	1.2	1.8	4.3	0.03	0.4	13.8
	Minimum	67.0	48.8	64.9	4.3	-0.26	81.0	12.5
	Maximum	67.6	52.8	71.1	17.0	-0.17	82.7	49.0
	Count	10	10	10	10	10	10	10
Necropsy Group 3 (Study Day 15 to Study Day 16)								
	Mean	**67.3**	**51.1**	**70.0**	**8.3**	**-0.19**	**81.7**	**24.5**
	St. Dev.	0.3	2.5	0.8	4.2	0.05	0.4	3.6
	Minimum	67.0	48.7	69.3	4.9	-0.28	81.0	17.6
	Maximum	68.1	55.8	71.6	19.3	-0.11	82.0	29.5
	Count	10	10	10	10	10	10	10
Necropsy Group 4 (Study Day 43 to Study Day 47)								
	Mean	**67.1**	**49.5**	**69.5**	**5.7**	**-0.24**	**81.9**	**24.1**
	St. Dev.	0.5	1.4	0.4	0.7	0.07	0.2	4.4
	Minimum	66.5	48.5	69.1	4.7	-0.37	81.7	18.2
	Maximum	67.6	51.9	69.9	6.7	-0.18	82.0	30.3
	Count	5	5	5	5	5	5	5
Necropsy Group 5 (Study Day 36 to Study Day 40)								

Mean	**67.3**	**49.3**	**69.6**	**6.8**	**-0.19**	**81.1**	**30.4**
St. Dev.	0.4	1.5	0.2	0.6	0.02	1.9	11.0
Minimum	66.7	47.4	69.3	6.0	-0.21	78.0	20.3
Maximum	67.8	51.3	69.9	7.6	-0.17	82.7	49.1
Count	5	5	5	5	5	5	5
Necropsy Group 6 (Study Day 20 to Study Day 24)							
Mean	**67.4**	**50.9**	**69.5**	**6.6**	**-0.22**	**81.5**	**26.1**
St. Dev.	0.4	2.7	0.3	2.4	0.04	0.4	3.2
Minimum	67.0	49.1	69.3	4.4	-0.28	81.0	20.9
Maximum	68.1	55.6	70.0	10.7	-0.17	82.0	29.7
Count	5	5	5	5	5	5	5
Necropsy Group 7 (Study Day 17 to Study Day 21)							
Mean	**67.3**	**49.8**	**70.2**	**6.5**	**-0.20**	**81.1**	**26.0**
St. Dev.	0.1	0.5	0.9	1.4	0.02	0.3	3.3
Minimum	67.2	49.1	69.5	4.4	-0.23	81.0	20.9
Maximum	67.6	50.4	71.6	8.0	-0.18	81.7	29.7
Count	5	5	5	5	5	5	5
Necropsy Group 8 (Study Day 43 to Study Day 43)							
Daily Result	**67.4**	**48.8**	**69.8**	**6.1**	**-0.21**	**82.0**	**24.1**
Count	1	1	1	1	1	1	1
Necropsy Group 9 (Study Day 38 to Study Day 38)							
Daily Result	**67.4**	**47.4**	**69.6**	**6.4**	**-0.21**	**82.7**	**49.1**
Count	1	1	1	1	1	1	1
Necropsy Group 10 (Study Day 18 to Study Day 18)							
Daily Result	**67.6**	**49.4**	**71.6**	**7.0**	**-0.19**	**81.0**	**25.3**
Count	1	1	1	1	1	1	1
Necropsy Group 11 (Study Day 44 to Study Day 44)							
Daily Result	**67.3**	**51.9**	**69.4**	**6.7**	**-0.23**	**81.7**	**30.3**
Count	1	1	1	1	1	1	1

Figure 1. Diagram of Libby Fiber Exposure System.



[1] The control and Amosite exposure systems were similar in design however consisted of only one tier.

APPENDIX III: Pathology Report

Experimental Pathology Laboratories, Inc.

Hamner Protocol No.: 10002
EPL Project No.: 304-447
Final Report
September 9, 2010

EPA FIBER PROJECT: TWO-WEEK RANGE-FINDING STUDY – INHALATION EXPOSURE OF RATS TO AMPHIBOLE ASBESTOS

THE HAMNER INSTITUTES FOR HEALTH SCIENCES PROTOCOL NO. 10002

EPL PROJECT NO.: 304-447
EPL PATHDATA NO. 90037

FINAL PATHOLOGY REPORT

Submitted to:

The Hamner Institutes for Health Sciences
P.O. Box 12137
6 Davis Drive
Research Triangle Park, NC 27709

Submitted by:

Experimental Pathology Laboratories, Inc.
P.O. Box 12766
Research Triangle Park, NC 27709

September 9, 2010

EPL® Experimental Pathology Laboratories, Inc.

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EPL PROJECT NO.: 304-447
EPL PATHDATA NO. 90037

FINAL PATHOLOGY REPORT

BACKGROUND

The vermiculite mine near Libby, Montana was the world's leading source of vermiculite for 70 years until its closure in 1990. Vermiculite is used for insulation, as an absorbent material, and as a soil conditioner, and has applications in the construction, agricultural, horticultural and industrial markets. However, the Libby vermiculite ore coexists with a complex array of amphibole mineral types, primarily winchite, richterite, tremolite, and magnesioriebeckite with crystal forms (habits) ranging from asbestiform to acicular/prismatic.

Occupational exposure to Libby vermiculite has been (and continues to be) associated with significant increases in asbestosis, lung cancer, and pleural cancer compared to the rest of the U.S. population. For example, in addition to elevated rates of lung cancer and mesothelioma among Libby residents, 17.8% of 6,668 persons who lived or worked in the Libby area for at least 6 months before 1991 show (upon medical testing) pleural abnormalities (calcifications, thickenings, or plaques).

Furthermore, exposures to individuals outside of Libby have occurred, and are likely continuing; as asbestos-contaminated vermiculite ore from Libby was shipped to hundreds of locations around the nation for processing, and used as attic insulation in millions of homes throughout the United States. The health effects associated with former and current exposures from the asbestos contaminated vermiculite from the Libby mine continues to be a subject of intensive study and public health concern.

OBJECTIVE

The overall goal of this research is to improve the scientific basis for the risk assessment of asbestos-contaminated communities by conducting toxicology studies to help define key determinants of internal dose and provide critical insight on additional key health or pathologic endpoints. These types of toxicology studies can only be done in animals and to date, rodent inhalation studies have not been conducted with the amphibole asbestos that contaminates vermiculite from Libby, Montana (Libby amphibole or LA).

There were two (2) components to the study. The first component was a 2-week range-finder study designed to determine optimal fiber-aerosol concentrations to be used in the subsequent subchronic inhalation exposure study and to compare the potency of inhaled Libby amphibole fibers to the potency of inhaled amosite, a known fibrogenic amphibole asbestos fiber. The second component was a dosimetry study to determine initial fiber deposition and clearance/biopersistence. This study was designed to provide a time course of fiber burden data in various regions of the respiratory tract (head, trachea, lung lobes) and GI tract. These data will be used to derive LA-specific inhalability, deposition efficiency, and clearance rates for development of modifications to the Multi-path particle dosimetry (MPPD) model used to describe inhalation dosimetry.

INTRODUCTION

This Hamner Institutes for Health Sciences Study 10002 was conducted in male F344 rats. The study design is shown in (Table 1).

Table 1. Study Design

Component 1	Exposure Level (mg/m^3)	Animal Numbers
Core (10-day exposure)	Control	201-207
	Amosite 3.5	208-214
	LA 0.5	215-221
	LA 3.5	222-228
	LA 25	229-235
	Total	35

HISTOPATHOLOGY PROCEDURES

Necropsies were performed at The Hamner Institutes. Animals to be euthanized were deeply anesthetized with sodium pentobarbital (intraperitoneal injection, approximately 50 mg/kg) and exsanguinated by transection of the abdominal aorta.

The lungs (left lobe, right cranial lobe), trachea and duodenum (control tissue for Brdu), were fixed with NBF for 48 hours, rinsed, and stored in 70% ethanol, embedded in paraffin wax, sectioned (approximately 5 micrometers), deparaffinized, and stained with H&E. An additional set of slides from the lung and trachea blocks were stained with a collagen specific stain (Masson's trichrome). Nasal cavity was fixed in NBF, decalcified and processed to H&E slides.

Lung and trachea were evaluated via light microscopy by an EPL pathologist and the results are presented in Table 2.

During the light microscopic examination histopathologic diagnoses were recorded. Microscopic findings were graded using a subjective grading scale (1=minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe). This severity scoring system was also applied to quantify the lung fibrotic response. The presence of foreign body was recorded as present (P). This report contains the evaluation of the Brdu-treated animals of Component 1.

Definition of Histological Terms:

Bronchiole epithelial hyperplasia was only diagnosed at the high dose (25 mg/m^3) of Libby amphibole and characterized by increase in the number of cells in the epithelium of the terminal bronchioles.

Alveolus inflammation was characterized by an infiltration of macrophages, mononuclear cells and occasional neutrophils. A constituent of this process were occasional granulomas which are aggregates of macrophages with rare giant cells. The location of this inflammation was mainly in pericentral alveoli around alveolar ducts. This inflammation was associated with exposure to fibers.

Interstitial fibrosis was characterized by fibroblasts and collagen deposition in alveolar walls and within granulomas. The diagnosis of fibrosis was reinforced by positive staining with Masson's trichrome.

Foreign body was diagnosed when fibers were evident in sections. These were mostly contained in macrophages some of which were completely engulfed while others were partially engulfed.

Chronic inflammation focal used to describe discrete lesions which are seen as part of the background in control animals and characterized by a focal lesion with alveolar macrophages with or without fibrosis of interstitium frequently, but not exclusively, in a subpleural location.

RESULTS

A summary of the histological findings is given in Table 2. There were no histological findings in trachea.

Table 2. Summary of Incidence and Severity [ ] of Findings in the Lung (number of animals = 7 per group)

Exposure	Bronchiole Epithelial Hyperplasia	Alveolus Inflammation	Interstitial Fibrosis	Inflammation Chronic Focal	Foreign Body*
Control	0	0	0	3 [0.4]	0
Amosite 3.5 mg/m^3	0	7 [1]	4 [0.6]	3 [0.4]	6
Libby amphibole 0.5 mg/m^3	0	6 [0.9]	0	5 [0.7]	2
Libby amphibole 3.5 mg/m^3	0	7 [2]	3 [0.4]	1 [0.1]	6
Libby amphibole 25 mg/m^3	5 (0.7)	7 [2]	7 [1]	0	7

*Not graded

Control

Focal chronic inflammation was seen in the lungs of three control animals.

Amosite 3.5 mg/m^3

Minimal alveolus inflammation, interstitial fibrosis and foreign body (fiber) were evident in animals from this treatment group.

Libby amphibole 0.5 mg/m^3

Minimal inflammation was evident in the lungs of six animals in the group. In two animals foreign body (fibers) were evident.

Libby amphibole 3.5 mg/m^3

Minimal to moderate alveolus inflammation, minimal interstitial fibrosis and foreign body (fiber) were evident in animals from this treatment group. There was more alveolus inflammation evident than with Amosite 3.5 mg/m^3.

Libby amphibole 25 mg/m^3

The most intense response was seen in the lungs of the animals exposed to 25 mg/m^3 Libby amphibole with mild alveolus inflammation and minimal fibrosis evident in all animals. Additionally, bronchiole epithelial hyperplasia was diagnosed in 5 animals and foreign body (fibers) was present in all animals in the group.

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CONCLUSIONS

Exposure of rats to 3.5 mg/m^3 Libby amphibole induced changes which were slightly more severe than those induced in rats exposed to 3.5 mg/m^3 Amosite. Fibrosis was not seen in rats exposed to 0.5 mg/m^3 Libby amphibole.

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F.R.C. Path.
Pathologist

9 Sept 2010
Date

GAW/dc

QUALITY ASSURANCE FINAL CERTIFICATION

EPL®

Experimental Pathology Laboratories, Inc.

QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: EPA Fiber Project: Two-Week Range-Finding Study – Inhalation Exposure of Rats to Amphibole Asbestos

Client Study: Protocol No. 10002

EPL Principal Investigator: Dr. Gabrielle Willson

EPL Project Number: 304-447

EPL Pathologist: Dr. Gabrielle Willson

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the EPL Principal Investigator and Management are indicated below.

Area Inspected	Dates Inspection	Dates Reporting
EPL Project Sheets	March 8, 2010; March 17, 2010; March 18, 2010; June 15, 2010	March 8, 2010; March 17, 2010; March 18, 2010; June 15, 2010
Necropsy Records Review	March 11, 2010; March 18, 2010	March 11, 2010; March 18, 2010
Project Setup	March 12, 2010; March 19, 2010; March 22, 2010; April 6, 2010	March 12, 2010; March 19, 2010; March 22, 2010; April 6, 2010
Data Review	March 12, 2010; April 2, 2010; April 27, 2010	March 12, 2010; April 5, 2010; April 27, 2010
Draft Pathology Report	May 4 & 5, 2010	May 5, 2010
Final Pathology Report	September 9, 2010	September 9, 2010

Date of last quarterly facility inspection: July 2010

Jane J. Hollingsworth
EPL Quality Assurance Unit

September 9, 2010
Date

Form No. 6-2 (June 22, 2009)

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATA

PAGE : 1/ 5
PRO 10002 (304447)

TEST ITEM : Libby Amphibole (LA), Amosite
TEST SYSTEM : RAT, Two-Weeks, Inhalation
SPONSOR : The Hamner

PATHOL. NO.: 90037 GAW
DATE : 08-SEP-10
PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : C, +BrdU

ANIMAL NUMBER :	201 MK0	202 MK0	203 MK0	204 MK0	205 MK0	206 MK0	207 MK0
TRACHEA	-	-	-	-	-	-	-
LUNG	+	+	-	-	-	+	-
- Inflammation Chronic Focal	1.	1.	.	.	.	1.	.

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATA

TEST ITEM : Libby Amphibole (LA), Amosite
TEST SYSTEM : RAT, Two-Weeks, Inhalation
SPONSOR : The Hamner

PATHOL. NO.: 90037 GAW
DATE : 08-SEP-10
PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : AM, +BrdU

ANIMAL NUMBER :	208 MK0	209 MK0	210 MK0	211 MK0	212 MK0	213 MK0	214 MK0
TRACHEA	-	-	-	-	-	-	-
LUNG	+	+	+	+	+	+	+
- Alveolus Inflammation.	1.	1.	1.	1.	1.	1.	1.
- Interstitium Fibrosis.	1.	1.	.	1.	1.	.	.
- Foreign Body.	P.	.	P.	P.	P.	P.	P.
- Inflammation Chronic Focal	.	.	1.	1.	.	.	1.

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATA

TEST ITEM : Libby Amphibole (LA), Amosite
TEST SYSTEM : RAT, Two-Weeks, Inhalation
SPONSOR : The Hamner

PATHOL. NO.: 90037 GAW
DATE : 08-SEP-10
PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : LA, +BrdU

ANIMAL NUMBER :	215 MK0	216 MK0	217 MK0	218 MK0	219 MK0	220 MK0	221 MK0
TRACHEA	-	-	-	-	-	-	-
LUNG	+	+	+	+	+	+	+
- Alveolus Inflammation.	1.	1.	1.	1.	1.	1.	.
- Foreign Body.	.	.	.	P.	P.	.	.
- Inflammation Chronic Focal	.	1.	1.	1.	.	1.	1.

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATA

TEST ITEM : Libby Amphibole (LA), Amosite
TEST SYSTEM : RAT, Two-Weeks, Inhalation
SPONSOR : The Hamner

PATHOL. NO.: 90037 GAW
DATE : 08-SEP-10
PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : LA, +BrdU

ANIMAL NUMBER :	222 MK0	223 MK0	224 MK0	225 MK0	226 MK0	227 MK0	228 MK0
TRACHEA	-	-	-	-	-	-	-
LUNG	+	+	+	+	+	+	+
- Alveolus Inflammation.	2.	2.	2.	2.	1.	2.	3.
- Interstitium Fibrosis.	.	1.	1.	1.	.	.	.
- Foreign Body.	P.	P.	P.	P.	P.	P.	.
- Inflammation Chronic Focal	.	.	.	1.	.	.	.

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATA

PRO 10002 (304447)

TEST ITEM : Libby Amphibole (LA), Amosite
TEST SYSTEM : RAT, Two-Weeks, Inhalation
SPONSOR : The Hamner

PATHOL. NO.: 90037 GAW
DATE : 08-SEP-10
PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : LA, +BrdU

ANIMAL NUMBER :	229 MK0	230 MK0	231 MK0	232 MK0	233 MK0	234 MK0	235 MK0
TRACHEA	-	-	-	-	-	-	-
LUNG	+	+	+	+	+	+	+
- Alveolus Inflammation.	2.	2.	2.	2.	2.	2.	2.
- Interstitium Fibrosis.	1.	1.	1.	1.	1.	1.	1.
- Foreign Body.	P.	P.	P.	P.	P.	P.	P.
- Bronchiole Epithelial Hyperplasia.	.	1.	.	1.	1.	1.	1.

EXPLANATION OF CODES

EXPLANATION OF CODES AND SYMBOLS

CODES AND SYMBOLS USED AT TABLE LEVEL:

AOFT = Animal Organ Finding Table

CODES AND SYMBOLS USED AT ANIMAL LEVEL:

M = Male Animal
F = Female Animal
K0 = Terminal Sacrifice Group
K1 . . . K9 = Interim Sacrifice Group 1 . . . 9
R1 . . . R9 = Recovery / Post-Treatment Group 1 . . . 9
+ = Intercurrent Death / Sacrificed Moribund

CODES AND SYMBOLS USED AT ORGAN LEVEL:

A = Organ autolytic, evaluation not possible
G = Gross finding evaluated histologically
0 = Tissue not present for histologic examination
‘ = Histologic examination not required
+ = Organ examined, findings present
- = Organ examined, no pathologic findings noted (AOFT only)
(= Only one of paired organs examined/present
! = No corresponding microscopic finding required
NAD = No abnormalities detected

CODES AND SYMBOLS USED AT FINDING LEVEL:

GRADE 1 = Minimal / very few / very small
GRADE 2 = Slight / mild / few / small
GRADE 3 = Moderate / moderate number / moderate size
GRADE 4 = Marked / many / large / moderately severe
GRADE 5 = Massive / extensive number / extensive size / severe
P = Finding present, severity not scored
B0 = Benign neoplasm
N0 = Malignant neoplasm
M = Metastasis
(= Finding unilateral in paired organs

CORRELATION TABLE: NECROPSY-MICROSCOPY:

Evaluation not Required = No corresponding microscopic finding required

APPENDIX IV: Cell Replication in Rat Lung

The Hamner Institutes for Health Sciences Study # 10002

EPA Fiber Project: Two-Week Range-Finding Study – Inhalation

Exposure of Rats to Amphibole Asbestos

Cell Replication in Rat Lung

Final

Elizabeth Gross Bermudez

August 11, 2010

The Hamner Institutes for Health Sciences Study # 10002
Cell Replication Report – August 11, 2010

Report prepared by:

Elizabeth Gross Bermudez

8-11-10

Date

Objective

This study (Component 1 (Core) of The Hamner Institutes for Health Sciences Study 10002, EPA Fiber Project: Two-week range-finding study – Inhalation exposure of rats to amphibole asbestos) was designed to determine optimal fiber-aerosol concentrations to be used in a subsequent sub chronic inhalation exposure study and to compare the potency of inhaled Libby amphibole (LA) fibers to the potency of inhaled Amosite, a known fibrogenic amphibole asbestos fiber.

This report presents data on cell replication analyses conducted as a portion of The Hamner study.

Introduction

This Hamner Institutes for Health Sciences Study Number 10002 was conducted in male F344 rats. The study design is shown in Table 1.

Table 1.

	Exposure Level (mg/mm^3)	Animal Numbers
Core (10 day exposure)	Control	201-207
	Amosite 3.5	208-214
	LA 0.5	215-221
	LA 3.5	222-228
	LA 25	229-235
	Total	35

Methods

Cell Replication Analysis

Thirty-five male F344 rats mice (7/concentration group) were assigned to the cell replication portion of the study (Table 1). Seven animals per group were killed after 10 days of air or fiber exposure. Only the lower respiratory tract (left lung lobe and trachea) of rats was processed for cell replication, and only the left lung lobe was evaluated. The BrdU labeled cells were immunostained in paraffin embedded lung tissue sections using standard operating procedures for immunostaining (EPL Inc., Research Triangle Park,

NC). A section of duodenum was placed on each slide and was utilized as control tissue for verification of BrdU incorporation. Figure 1 shows a typical BrdU immuno-stained section of a control rat lung.

Following review of the histopathology report, the BrdU stained sections, and discussion with the study pathologist; the terminal bronchiole was selected for cell replication analysis. This was designed to compliment the bronchiole epithelial hyperplasia finding seen in the LA 25 group and perhaps provide a more sensitive indicator of bronchiole epithelial changes.

The lung-labeling index for each rat was determined in a blinded manner by counting a minimum of 400 cells/lung (or all that could be identified on the slide) for the target area, terminal bronchiole. This is indicated as TB in Figure 1. Labeling index is calculated as number of labeled cells divided by the total number of cells and is expressed as a percent.

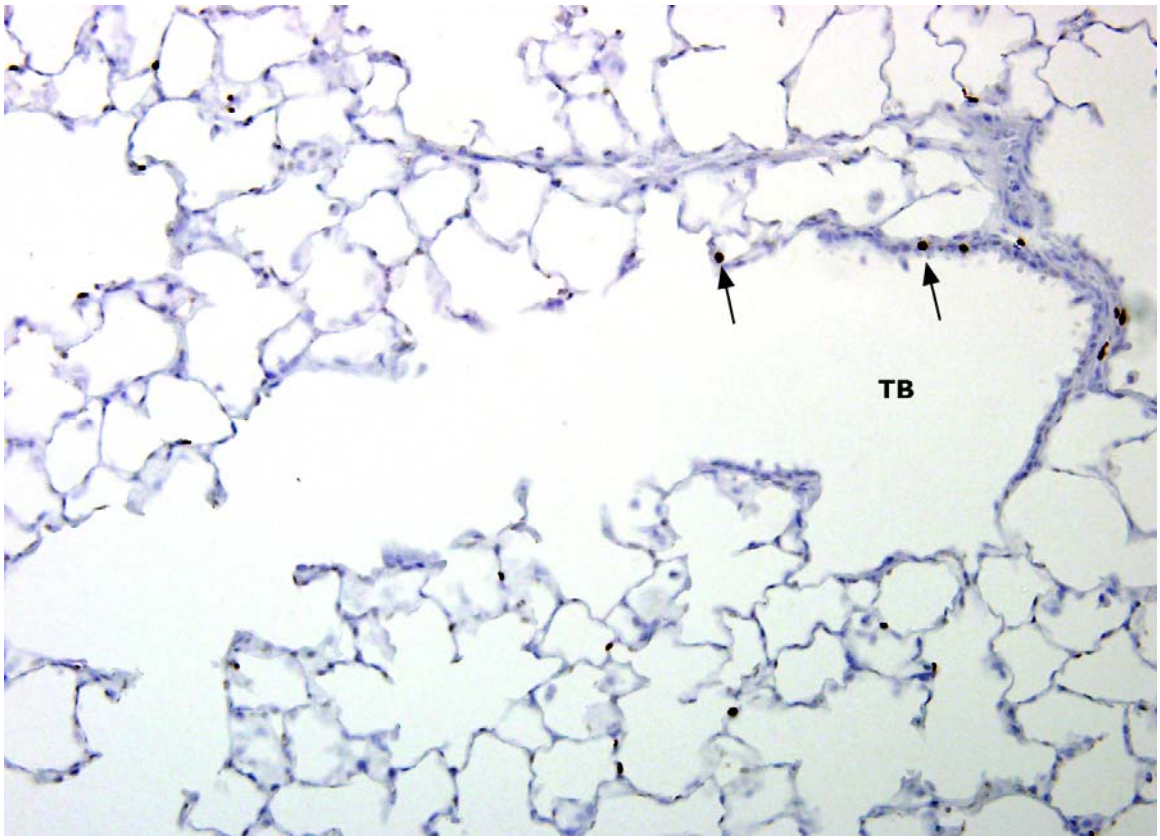

Figure 1. Photomicrograph of a terminal bronchiole (TB) from control rat lung (animal 201) (20X) is shown to demonstrate typical areas evaluated for labeling index. Red/brown nuclei are BrdU positive cells (arrows).

Cell Replication Data Analysis

Cell replication data were analyzed using JMP software. Because assumptions of normality and homogeneity of variance were rejected ($p < 0.01$) for the large data set, an arcsine (square root) transformation was applied to the data sets. If the transformed data met normality and homogeneity of variance criteria, a Dunnett's procedure was then used to test for differences between control and fiber-exposed groups. If the transformed data did not meet normality and homogeneity of variance criteria, nonparametric testing (Kruskal–Wallis) was employed to test for differences between control and fiber-exposed groups. A probability value of 0.05 was used as the critical level of significance. An ANOVA was employed to examine the groups for a treatment effect. A T-test was used to test for differences between control and the Amosite group because there was only one concentration of Amosite used in the study. Regression analysis was performed on the data set, to determine trends in the data using the data analysis tools in Excel.

Results

Figure 1 shows a typical BrdU-stained section of a control rat lung (Animal 201). Of interest are the labeled cells (examples marked with arrows) located in the bronchiolar epithelium of terminal bronchioles marked TB in the figure.

Tables 2 and 3, Figures 2 and 3, and Appendix 1 (individual animal data) present the cell replication results for this study. For purposes of this report, two statistical analyses were conducted (Appendix 2. JMP print out). All data were used for the first analysis. The second analysis censored data from five animals. One animal (215) was censored because too few terminal bronchioles could be located in the lung section, and only 43 cells could be counted. This is far less than the 400 cells targeted. Animals with 200+ cells counted remained in the analysis. Additionally, following discussion with the pathologist (Dr. Wall standing in for Dr. Willson who was unavailable), four additional animals (204, 205, 217, and 218) were censored from the data set due to presumed non-treatment related pathology (inflammation).

Results Using the Entire Data Set

The data (including all animals) for the LA fiber did not show a treatment effect. The LA 0.5 animals showed a twofold increase over controls. However, animals exposed at higher concentrations showed smaller increases over controls (Table 2, Figure 2). Transformed data did not meet normality and homogeneity of variance criteria, and nonparametric testing (Kruskal–Wallis) was employed to test for differences between control and fiber-exposed (LA) groups. None of the treatment groups were statistically significantly different from the control ($p < 0.05$).

Table 2.

Male F344 Rat Lung Labeling Indices Following Inhalation Exposure to Fibers
Uncensored Data

Dose Group (mg/mm^3)	Bronchiolar	
	Labeling Index[a,b]	% Control
0	10.27 ± 7.61	100
Amosite 3.5	11.62 ± 3.82	113
LA 0.5	20.75 ± 21.27	202
LA 3.5	12.31 ± 6.27	120
LA 25	15.84 ± 5.66	154

a n= 7 rats per group
b Mean ± standard deviation
* Significantly different from control ($p \leq 0.05$, Dunnett's test on arcsine [sqrt] transformed data)

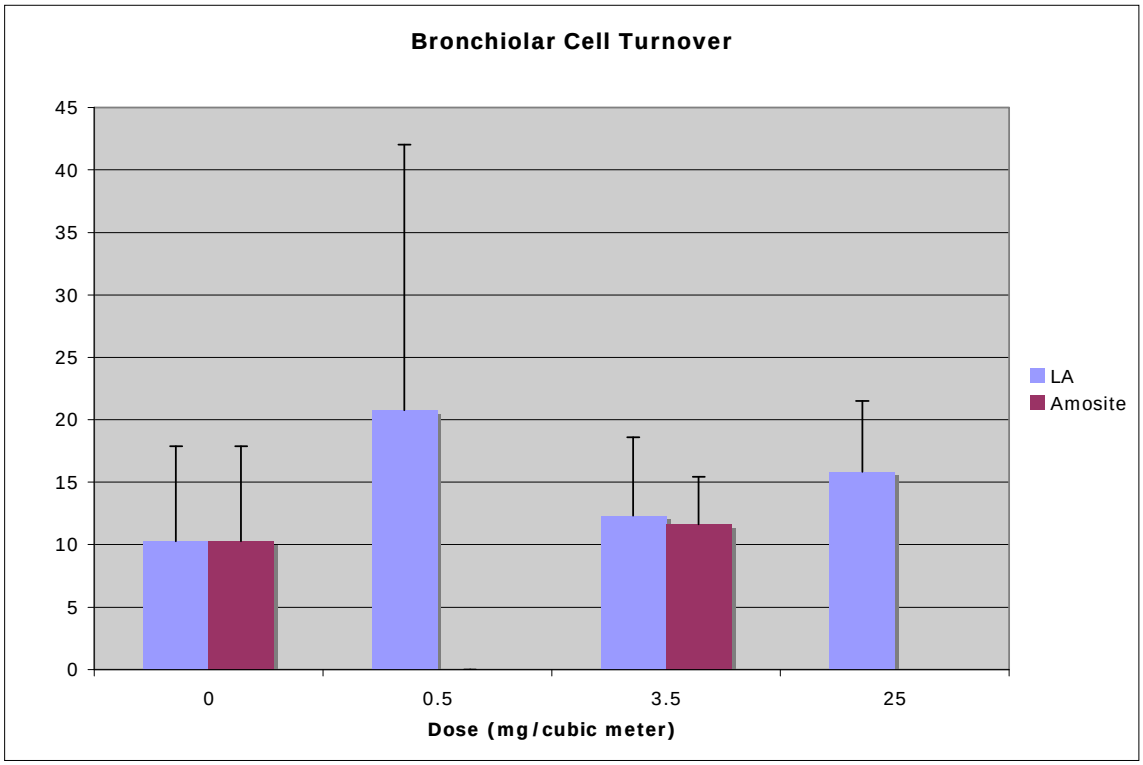

Figure 2. Labeling indices for left lung of rats treated with air, LA, or Amosite. Data are expressed as mean for the group. Error bars are standard deviation. All data was included in this figure.

Results Using the Censored Data Set

Ten days of exposure resulted in dose-dependant increases in labeling indices with increasing LA concentration when specified animals were excluded (Table 3, Figure 3) (regression, $p < 0.05$, $R^2 = 0.4$). Statistically significant increases in cell replication were observed in the terminal bronchiolar region of male rat lungs in the LA 25 group (Dunnett's, $p < 0.05$). The Amosite 3.5 group did not have labeling indices that were different from the control group (T-test).

Table 3.

Male F344 Rat Lung Labeling Indices Following Inhalation Exposure to Fibers

Censored Data

Dose Group (mg/mm^3)	Bronchiolar Labeling Index[a]	Bronchiolar % Control
0	6.35[b] ± 3.20	100
Amosite 3.5	11.62[c] ± 3.82	183
LA 0.5	8.92[d] ± 4.57	141
LA 3.5	12.31[c] ± 6.27	194
LA 25	15.84[c]* ± 5.66	250

a Mean ± standard deviation
b n= 5 rats per group
c n= 7 rats per group
d n= 4 rats per group
* Significantly different from control ($p \leq 0.05$, Dunnett's test on arcsine [sqrt] transformed data)

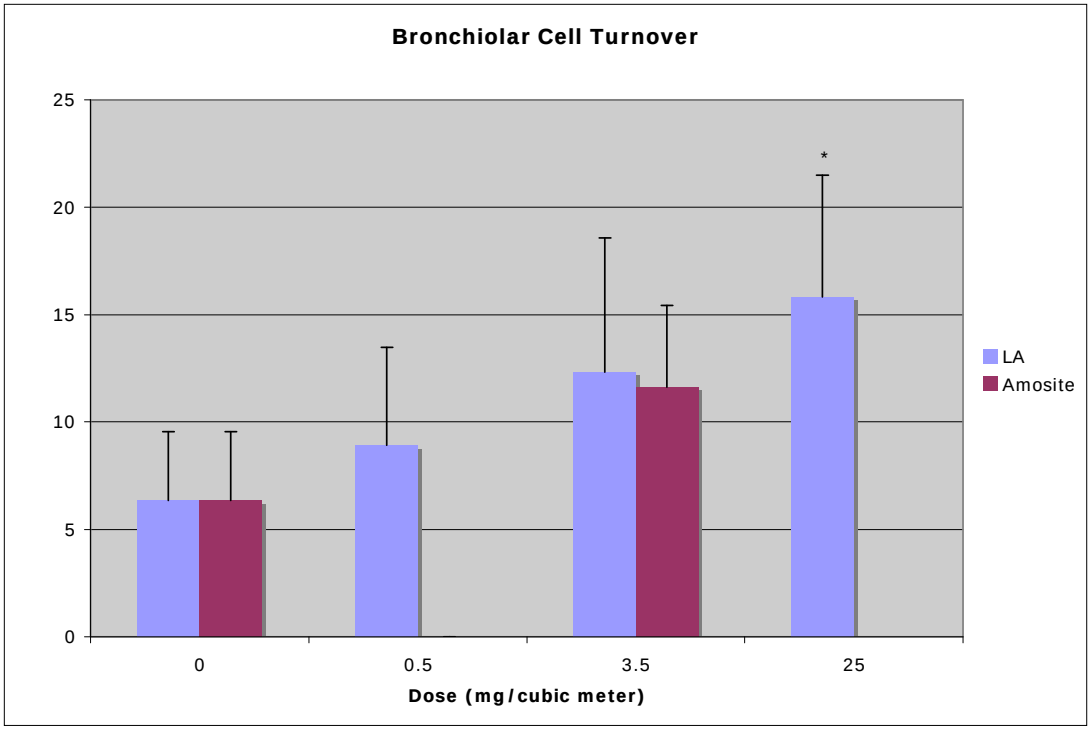

Figure 3. Labeling indices for left lung of rats treated with air, LA, or Amosite. Data are expressed as mean for the group. An asterisk (*) indicates significantly different from control (Dunnett's, $p < 0.05$). Error bars are standard deviation. Five animals were censored from the data set presented in this figure.

Conclusion and Recommendations

Labeling index provides a sensitive measure of bronchiole epithelial changes in rat lung following even 10 days exposure to asbestos fibers. While only the LA25 group had a significant increase in labeling index for the terminal bronchioles, a dose response was seen in the censored data set. Focal inflammatory responses may lead to a more organ-wide increase in cell replication within the lung. This was seen in the rats that were censored from the data set (204, 205, 217, and 218) and may explain the rather large standard deviations seen in some of the data. It is recommended that the pathology and cell replication data sets be compared to address this issue.

It is further recommended that cross sections of lung, rather than the longitudinal sections prepared for this study, be used when terminal bronchioles are being evaluated. The cross section provides a better chance of seeing terminal bronchioles and would allow for 400 cells to be counted for each rat. In this study, many sections (5/35) did not have enough terminal bronchioles to count 400 cells.

The Hamner Institutes for Health Sciences Study # 10002
Cell Replication Report – August 11, 2010

Appendix 1

Raw Data for Cell Replication in Terminal Bronchioles

		BRONCHIOLAR				Group mean	SD	% of control
Dose	ID	labeled	unlabeled	total	LI			
0	201	13	63	481	8.523908524	10.26546239	7.60971237	100
0	201	7	118					
0	201	6	61					
0	201	5	88					
0	201	10	110					
0	202	5	84	430	4.418604651			
0	202	9	149					
0	202	2	80					
0	202	3	98					
0	203	10	155	433	5.311778291			
0	203	5	75					
0	203	4	77					
0	203	4	103					
0	204	45	100	414	24.39613527			
0	204	28	78					
0	204	13	30					
0	204	8	57					
0	204	7	48					
0	205	24	144	413	15.73849879			
0	205	14	78					
0	205	11	57					
0	205	16	69					
0	206	6	91	412	10.67961165			
0	206	6	80					
0	206	9	59					
0	206	5	56					
0	206	18	82					
0	207	5	128	466	2.789699571			
0	207	4	110					

0	207	1	57					
0	207	0	72					
0	207	3	86					
0.5	221	7	53	403	9.677419355		20.74897413	21.26500774	202.1241064
0.5	221	6	42					
0.5	221	7	68					
0.5	221	2	68					
0.5	221	4	48					
0.5	221	13	85					
0.5	220	4	51	369	5.691056911			
0.5	220	8	101					
0.5	220	1	48					
0.5	220	3	90					
0.5	220	5	58					
0.5	219	4	51	441	5.215419501			
0.5	219	2	61					
0.5	219	2	41					
0.5	219	2	110					
0.5	219	10	42					
0.5	219	2	75					
0.5	219	1	38					
0.5	218	44	25	462	47.18614719			
0.5	218	44	30					
0.5	218	14	52					
0.5	218	28	37					
0.5	218	42	29					
0.5	218	13	30					
0.5	218	33	41					
0.5	217	60	16	480	55.41666667			
0.5	217	48	37					
0.5	217	22	27					
0.5	217	15	33					
0.5	217	34	43					
0.5	217	37	24					
0.5	217	50	34					
0.5	216	14	34	378	15.07936508			

0.5	216	2	34					
0.5	216	10	65					
0.5	216	13	66					
0.5	216	6	50					
0.5	216	8	36					
0.5	216	4	36					
0.5	215	3	40	43	6.976744186			
3.5	228	18	55	322	18.32298137	12.31247342	6.265697077	119.9407581
3.5	228	15	85					
3.5	228	26	123					
3.5	227	10	83	482	6.22406639			
3.5	227	2	64					
3.5	227	3	84					
3.5	227	5	77					
3.5	227	10	144					
3.5	226	3	48	447	10.29082774			
3.5	226	5	47					
3.5	226	6	47					
3.5	226	7	73					
3.5	226	9	46					
3.5	226	6	34					
3.5	226	5	59					
3.5	226	5	47					
3.5	225	9	52	444	11.93693694			
3.5	225	5	43					
3.5	225	17	42					
3.5	225	5	40					
3.5	225	9	73					
3.5	225	6	90					
3.5	225	2	51					
3.5	224	16	48	412	23.30097087			
3.5	224	16	52					
3.5	224	12	50					
3.5	224	22	40					
3.5	224	21	72					
3.5	224	9	54					

3.5	223	7	52	411	7.299270073			
3.5	223	2	64					
3.5	223	1	36					
3.5	223	5	57					
3.5	223	7	53					
3.5	223	5	47					
3.5	223	3	72					
3.5	222	1	42	261	8.812260536			
3.5	222	9	61					
3.5	222	8	49					
3.5	222	5	86					
25	235	6	56	407	10.56511057	15.83903792	5.661056203	154.2944421
25	235	7	62					
25	235	4	74					
25	235	18	113					
25	235	8	59					
25	234	11	63	469	15.7782516			
25	234	4	39					
25	234	4	33					
25	234	15	99					
25	234	5	30					
25	234	13	56					
25	234	6	19					
25	234	16	56					
25	233	28	86	486	15.43209877			
25	233	11	68					
25	233	13	53					
25	233	6	63					
25	233	7	58					
25	233	10	83					
25	232	8	46	426	12.67605634			
25	232	7	51					
25	232	11	93					
25	232	9	61					
25	232	13	65					
25	232	6	56					

	25	231	40	69	446	28.02690583			
	25	231	45	123					
	25	231	21	62					
	25	231	19	67					
	25	230	17	97	465	14.62365591			
	25	230	12	51					
	25	230	16	84					
	25	230	13	72					
	25	230	10	93					
	25	229	30	175	472	13.77118644			
	25	229	21	116					
	25	229	5	25					
	25	229	9	91					
A		214	9	70	451	10.42128603	11.61734204	3.817909226	113.1692037
A		214	11	66					
A		214	4	59					
A		214	2	30					
A		214	10	104					
A		214	11	75					
A		213	3	50	416	7.211538462			
A		213	9	66					
A		213	3	48					
A		213	10	144					
A		213	5	78					
A		212	13	184	441	7.936507937			
A		212	5	70					
A		212	8	79					
A		212	9	73					
A		211	11	64	348	10.34482759			
A		211	1	53					
A		211	7	62					
A		211	7	52					
A		211	7	45					
A		211	3	36					
A		210	8	44	409	17.84841076			
A		210	13	47					

A	210	11	63		
A	210	7	23		
A	210	11	58		
A	210	6	36		
A	210	17	65		
A	209	15	67	440	12.5
A	209	12	69		
A	209	11	32		
A	209	8	62		
A	209	1	48		
A	209	3	35		
A	209	5	72		
A	208	8	68	425	15.05882353
A	208	14	84		
A	208	14	85		
A	208	8	66		
A	208	20	58		

Appendix 2
JMP Printouts

Distributions fiber cell rep data, transformed
transform

Normal(0.37524,0.15251)

Quantiles

100.0%	maximum	0.83967
99.5%		0.83967
97.5%		0.83967
90.0%		0.57783
75.0%	quartile	0.40835
50.0%	median	0.34284
25.0%	quartile	0.26887
10.0%		0.22854
2.5%		0.16781
0.5%		0.16781
0.0%	minimum	0.16781

Moments

Mean	0.375244
Std Dev	0.152512
Std Err Mean	0.028822
Upper 95% Mean	0.434382
Lower 95% Mean	0.3161061
N	28

Fitted Normal
Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	0.375244	0.3161061	0.434382
Dispersion	σ	0.152512	0.1205789	0.2075896

-2log(Likelihood) = -26.8481314127568

Goodness-of-Fit Test
Shapiro-Wilk W Test

W		Prob<W
0.865564		0.0020*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Oneway Analysis of transform By Dose all fiber data

Excluded Rows
7

Tests that the Variances are Equal

Level	Count	Std Dev	MeanAbsDif to Mean	MeanAbsDif to Median
0	7	0.1216867	0.0940612	0.0974184
0.5	7	0.2551628	0.2072286	0.1865940
25	7	0.0722406	0.0444552	0.0437352
3.5	7	0.0917422	0.0707912	0.0709876

Test	F Ratio	DFNum	DFDen	Prob > F
O'Brien[.5]	3.6807	3	24	0.0260*
Brown-Forsythe	2.0579	3	24	0.1325
Levene	5.7561	3	24	0.0041*
Bartlett	3.6565	3	.	0.0119*

Welch's Test

Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
1.2577	3	12.714	0.3303

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
0	7	76.000	10.8571	-1.326
0.5	7	100.000	14.2857	-0.053
25	7	132.000	18.8571	1.592
3.5	7	98.000	14.0000	-0.159

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
3.3673	3	0.3384

Oneway Analysis of transform censored By Dose (some data removed)

Missing Rows
5Excluded Rows
7

Oneway Anova
Summary of Fit

Rsquare	0.404263
Adj Rsquare	0.3102
Root Mean Square Error	0.078691
Mean of Response	0.335568
Observations (or Sum Wgts)	23

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Dose	3	0.07983969	0.026613	4.2978	0.0179*
Error	19	0.11765459	0.006192		
C. Total	22	0.19749427			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
0	5	0.248269	0.03519	0.17461	0.32193

0.5	4	0.296608	0.03935	0.21426	0.37896
25	7	0.405395	0.02974	0.34314	0.46765
3.5	7	0.350362	0.02974	0.28811	0.41261

Std Error uses a pooled estimate of error variance

Tests that the Variances are Equal

Level	Count	Std Dev	MeanAbsDif to Mean	MeanAbsDif to Median
0	5	0.0661819	0.0530590	0.0540739
0.5	4	0.0781502	0.0609641	0.0609641
25	7	0.0722406	0.0444552	0.0437352
3.5	7	0.0917422	0.0707912	0.0709876

Test	F Ratio	DFNum	DFDen	Prob > F
O'Brien[.5]	0.2285	3	19	0.8754
Brown-Forsythe	0.3542	3	19	0.7866
Levene	0.4094	3	19	0.7481
Bartlett	0.1860	3	.	0.9060

Warning: Small sample sizes. Use Caution.

Welch's Test
Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
4.7600	3	9.3196	0.0284*

Means Comparisons
Comparisons with a control using Dunnett's Method
Control Group =
0

|d|	Alpha
2.54204	0.05

Level	Abs(Dif)-LSD	p-Value
25	0.04	0.0078*
3.5	-0.02	0.0950
0.5	-0.09	0.6809
0	-0.13	1.0000

Positive values show pairs of means that are significantly different.

Oneway Analysis of transform censored By Dose Amosite vs. control

Missing Rows
2Excluded Rows
21
Tests that the Variances are Equal

Level	Count	Std Dev	MeanAbsDif to Mean	MeanAbsDif to Median
0	5	0.0661819	0.0530590	0.0540739

A	7	0.0590071	0.0466682	0.0446281

Test	F Ratio	DFNum	DFDen	p-Value
O'Brien[.5]	0.1328	1	10	0.7231
Brown-Forsythe	0.1971	1	10	0.6666
Levene	0.1311	1	10	0.7249
Bartlett	0.0579	1	.	0.8098
F Test 2-sided	1.2580	4	6	0.7621

Warning: Small sample sizes. Use Caution.

Welch's Test

Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
6.7065	1	8.0927	0.0318*

t Test
2.5897

Means Comparisons

Comparisons for each pair using Student's t

t	Alpha
2.22814	0.05

Abs(Dif)-LSD	A	0
A	-0.07381	0.015114
0	0.015114	-0.08734

Positive values show pairs of means that are significantly different.

APPENDIX V: Bronchoalveolar Lavage Cytology Report

EPA FIBER PROJECT: TWO-WEEK RANGE-FINDING STUDY – INHALATION EXPOSURE OF RATS TO AMPHIBOLE ASBESTOS: BRONCHOALVEOLAR LAVAGE CYTOLOGY

PROTOCOL NUMBER: 10002
EPL PROJECT NO.: 304-447

"FINAL"
BRONCHOALVEOLAR LAVAGE CYTOLOGY REPORT

Submitted to:

The Hamner Institutes for Health Sciences
6 Davis Drive
P.O. Box 12137
Research Triangle Park, NC 27709-2137

Submitted by:

Experimental Pathology Laboratories, Inc.
P.O. Box 12766
Research Triangle Park, NC 27709

August 31, 2010

EPA FIBER PROJECT: TWO-WEEK
RANGE-FINDING STUDY – INHALATION EXPOSURE
OF RATS TO AMPHIBOLE ASBESTOS:
BRONCHOALVEOLAR LAVAGE CYTOLOGY

PROTOCOL NUMBER: 10002
EPL PROJECT NO.: 304-447

"FINAL"
BRONCHOALVEOLAR LAVAGE CYTOLOGY REPORT

TABLE OF CONTENTS

EPA FIBER PROJECT: TWO-WEEK RANGE-FINDING STUDY – INHALATION EXPOSURE OF RATS TO AMPHIBOLE ASBESTOS: BRONCHOALVEOLAR LAVAGE CYTOLOGY

PROTOCOL NUMBER: 10002
EPL PROJECT NO.: 304-447

"FINAL"
BRONCHOALVEOLAR LAVAGE CYTOLOGY REPORT

INTRODUCTION

This two-week range-finding study was conducted to determine optimal fiber-aerosol concentrations to be used in a subsequent subchronic inhalation exposure study and to compare the potency of inhaled Libby amphibole (LA) fibers to the potency of inhaled amosite, a known fibrogenic amphibole asbestos fiber. As part of this study, Experimental Pathology Laboratories, Inc. (EPL®) was requested to perform the quantitative bronchoalveolar lavage (BAL) cytological evaluation of prepared cytological slides provided by the Hamner Institutes for Health Sciences.

The experimental design pertinent to the BAL cytology analysis is summarized in Table 1 (below). Only the 35 animals assigned to the Component 1 Core (10-day Exposure) group were sampled for BAL cytology.

Table 1. Experimental Design

Component	Group	Exposure Level (mg/m^3)	Animal Identification Number 10002-xxx	
		Treatment	BAL	+ BrdU
Component 1	Core (10-day Exposure)	Control	101-107	201-207
		Amosite 3.5	108-114	208-214
		LA 0.5	115-121	215-221
		LA 3.5	122-128	222-228
		LA 25	129-135	229-235
		Total	35	35

BRONCHOALVEOLAR LAVAGE CYTOLOGY PROCEDURES

Slide preparations for 35 rat BAL cell samples that were stained with Hema 3 stain (Fisher Diagnostics, Middletown, VA) [similar to Diff-Quik stain] were provided to EPL for determination of the cell differential percentages. Three hundred (300) cells per

slide were enumerated and differentiated (Gao et al., 2006; Palmans et al., 2000) via a manual tagging method using digital photographic images of cells in photographic fields (10x total magnification). The fields for photography were selected using a random pattern that prevented duplicate photography of the same fields.

The method of enumeration was performed via the application of manual tagging procedures available in the image analysis software, ImagePro Plus, v.5.0.2.9 (Media Cybernetics, Inc., Silver Spring, Maryland). Briefly, the cells enumerated included: macrophages, lymphocytes, neutrophils, and eosinophils. For each cell type a unique color code was assigned as the class color identifier. Using the appropriate class color each type of cell in each photograph was counted. As each cell was counted it was tagged with a unique number that had the color code for its class. The evaluation of photographic fields and counting was continued until a count of 300 cells was accumulated. The absolute differential cell count for each photographic field was manually recorded.

Using an EXCEL spreadsheet, the absolute counts for each cell type observed in all photographic fields counted were totaled for each animal. The absolute count of each cell type for each animal was divided by 300 and the result multiplied by 100 to obtain the cell differential percentage for each cell type enumerated. The individual photomicrographic image absolute counts, the compilation of absolute cell counts for each cell type for each animal, and the calculated cell differential percentages for each animal were tabulated for inclusion in this report and further statistical analysis. Statistical analyses including the calculation of descriptive statistics (e.g. mean, standard deviation, minimum, maximum) and analysis of differences between exposure groups were completed via application of statistical analysis software (STATISTIX for Windows, version 2.0; Analytical Software, Tallahassee, Florida). Using this software, a Kruskal-Wallis one-way analysis of variance with a Bonferonni-like comparison procedure (α= 0.05) was performed to determine treatment group (exposure level) differences in differential cell percentages means. To facilitate the statistical analysis, the exposure level (treatment) groups were numbered as follows: 1 – control, 2 – amosite 3.5 mg/m^3, 3 – LA 0.5 mg/m^3, 4 – LA 3.5 mg/m^3, and 5 – LA 25 mg/m^3.

RESULTS AND DISCUSSION

The BAL cell differential absolute counts and percentages for each animal are presented in Appendix A. The BAL cell differential absolute counts recorded for each 20X digital photographic field evaluated are recorded in Appendix B for all animals that had samples evaluated. Detailed descriptive statistics for the differential cell counts and differential cell percentages are presented in Appendix C. Table 2 below summarizes the BAL cytology cell differential percentages for each treatment group.

Table 2. BAL Cell Differential Percentages Means Analysis Summary

Exposure Level (mg/m^3)	Number of Animals Evaluated	Eosinophil Percentage	Neutrophil Percentage	Lymphocyte Percentage	Macrophage Percentage
Control (0)	7	0 ± 0.00[a]	0.81 ± 0.635	1.00 ± 0.981	98.19 ± 0.573
Amosite 3.5	7	0 ± 0.00	7.43 ± 2.506	0.76 ± 0.937	91.81 ± 2.267
LA 0.5	7	0.05 ± 0.125	2.10 ± 1.424	0.33± 0.508	97.52 ± 1.152
LA 3.5	7	0 ± 0.00	10.48 ± 4.520[b]	1.00 ± 1.952	88.52 ± 4.012[b]
LA 25	7	0 ± 0.00	47.24 ± 16.161[b,c]	0.52 ± 0.938	52.24 ± 16.569[b,c]

[a] The group mean cell percentage and one standard deviation is listed for eosinophil, neutrophil, lymphocyte, and macrophage percentages.
[b] Different from the Control group (α=0.05).
[c] Different from the LA 0.5 group (α=0.05).

There were no exposure group differences in the eosinophil percentages means (Appendix D). Only one animal in the LA 0.5 group (Animal 115) had an eosinophil present in its BAL cytology images. Neutrophil percentages means of the LA 3.5 and LA 25 mg/m^3 groups were higher (α=0.05) than the neutrophil percentage mean of the Control group (Appendix E). The neutrophil percentage mean of the LA 25 mg/m^3 group was also higher (α=0.05) than the neutrophil percentage mean of the LA 0.5 mg/m^3 group.

There were no exposure group differences in the lymphocyte percentages means (Appendix F). The macrophage percentage mean for the LA 25 mg/m^3 group was lower (α=0.05) than those of the Control and LA 0.5 mg/m^3 groups (Appendix G). The macrophage percentage mean for the LA 3.5 mg/m^3 group was lower (α=0.05) than that

of the Control group. Among affected groups, the proportion of neutrophils was increased when the percentage of macrophages was statistically decreased. Qualitatively, the reductions in macrophages in the LA 3.5 and LA 25 mg/m^3 groups with concurrent increases in the percentages of neutrophils suggest a greater potential for those concentrations of LA to elicit an acute inflammatory response during a 10-day exposure period. The similarity of the cell differential percentages in the Control, Amosite 3.5 mg/m^3, and LA 0.5 mg/m^3 groups indicate that Amosite and LA at those levels may be well-tolerated over a 10-day exposure period. However, the differential cytology does not provide a clear indication of the relationship of the differential cytology to the quantity or density of cells in the BAL fluid.

CONCLUSIONS

The BAL cell differential cytology disclosed that Libby amphibole (LA) asbestos exposure of rats at 3.5 or 25 mg/m^3 for 10 days resulted in reduced percentages of macrophages and increased percentages of neutrophils. Overall, there were no noteworthy differences from the Control group BAL cell differential percentages values induced by exposures to Amosite at 3.5 mg/m^3 or Libby amphibole asbestos at 0.5 mg/m^3. Eosinophils were very rare in any animals and the numbers of lymphocytes were very small in animals in all exposure groups, and neither eosinophils nor lymphocytes appeared to be involved in the lung cellular response to the inhaled materials or in normal physiologic activities in the alveolar space.

Henry G. Wall

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31 August 2010
DATE

HGW/dc

REFERENCES

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EPL®

Experimental Pathology Laboratories, Inc.

QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: EPA Fiber Project: Two-Week Range-Finding Study – Inhalation Exposure of Rats to Amphibole Asbestos **(BAL report)**

Client Study: Protocol No. 10002

EPL Principal Investigator: Dr. Gabrielle Willson

EPL Project Number: 304-447

EPL Pathologist: Dr. Henry G. Wall

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the EPL Principal Investigator and Management are indicated below.

Area Inspected	Dates of Inspection	Dates of Reporting
EPL Project Sheets	March 8, 2010; March 17, 2010; March 18, 2010; June 15, 2010	March 8, 2010; March 17, 2010; March 18, 2010; June 15, 2010
Draft Pathology Report	June 17, 2010; July 6, 2010	June 17, 2010; July 6, 2010
Final Pathology Report	August 31, 2010	August 31, 2010

Date of last quarterly facility inspection: July 2010

Jane J. Hollingsworth
EPL Quality Assurance Unit

August 31, 2010
Date

APPENDIX A

INDIVIDUAL ANIMAL BAL CELLS DIFFERENTIAL COUNTS PER PHOTOGRAPHIC FIELD

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix A

Individual Animal BAL Cells Differential Counts per Photographic Field

Component	Group	Exposure Level (mg/m^3) Treatment	Animal I.D.	Field #	Eosinophils	Neutrophils	Lymphocytes	Macrophages	Total Cells per Animal
1	Core	Control	101	10				38	
1	Core	Control	101	9				79	
1	Core	Control	101	8			1	27	
1	Core	Control	101	7				37	
1	Core	Control	101	6			6	38	
1	Core	Control	101	5			2	33	
1	Core	Control	101	4				37	
1	Core	Control	101	3				2	
							9	**291**	**300**
1	Core	Control	102	10				56	
1	Core	Control	102	9		1	1	163	
1	Core	Control	102	6		3		76	
						4	**1**	**295**	**300**
1	Core	Control	103	11				106	
1	Core	Control	103	8		2		73	
1	Core	Control	103	7		2		40	
1	Core	Control	103	6		1		36	
1	Core	Control	103	5				40	
						5		**295**	**300**

Hamner Institutes for Health Sciences Protocol Number 10002

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Appendix A

Individual Animal BAL Cells Differential Counts per Photographic Field

Component	Group	Exposure Level (mg/m^3) Treatment	Animal I.D.	Field #	Eosinophils	Neutrophils	Lymphocytes	Macrophages	Total Cells per Animal
1	Core	Control	104	7		1	3	105	
1	Core	Control	104	5				107	
1	Core	Control	104	4				35	
1	Core	Control	104	3				49	
						1	**3**	**296**	**300**
1	Core	Control	105	10		1		159	
1	Core	Control	105	9				38	
1	Core	Control	105	7		1	2	99	
						2	**2**	**296**	**300**
1	Core	Control	106	10			4	178	
1	Core	Control	106	5		1		61	
1	Core	Control	106	4				56	
						1	**4**	**295**	**300**
1	Core	Control	107	10		2		97	
1	Core	Control	107	8		1	2	166	
1	Core	Control	107	7		1		31	
						4	**2**	**294**	**300**

Appendix A

Individual Animal BAL Cells Differential Counts per Photographic Field

Component	Group	Exposure Level (mg/m^3) Treatment	Animal I.D.	Field #	Eosinophils	Neutrophils	Lymphocytes	Macrophages	Total Cells per Animal
1	Core	Amosite 3.5	108	11		10		201	
1	Core	Amosite 3.5	108	10		8		37	
1	Core	Amosite 3.5	108	9		2		42	
						20		**280**	**300**
1	Core	Amosite 3.5	109	9		5		14	
1	Core	Amosite 3.5	109	7		5		47	
1	Core	Amosite 3.5	109	5		12	3	189	
1	Core	Amosite 3.5	109	4		9		16	
						31	**3**	**266**	**300**
1	Core	Amosite 3.5	110	9		6	1	100	
1	Core	Amosite 3.5	110	8		6	2	83	
1	Core	Amosite 3.5	110	7		5	2	18	
1	Core	Amosite 3.5	110	6		6		71	
						23	**5**	**272**	**300**
1	Core	Amosite 3.5	111	10		3	7	142	
1	Core	Amosite 3.5	111	9		6		96	
1	Core	Amosite 3.5	111	6				46	
						9	**7**	**284**	**300**
1	Core	Amosite 3.5	112	10		9		141	
1	Core	Amosite 3.5	112	9		10		57	
1	Core	Amosite 3.5	112	7		3		80	
						22		**278**	**300**

Appendix A

Individual Animal BAL Cells Differential Counts per Photographic Field

Component	Group	Exposure Level (mg/m^3) Treatment	Animal I.D.	Field #	Eosinophils	Neutrophils	Lymphocytes	Macrophages	Total Cells per Animal
1	Core	Amosite 3.5	113	10		4		163	
1	Core	Amosite 3.5	113	9		11		68	
1	Core	Amosite 3.5	113	8		5		49	
						20		**280**	**300**
1	Core	Amosite 3.5	114	3		9		23	
1	Core	Amosite 3.5	114	9		1		7	
1	Core	Amosite 3.5	114	8		3	1	10	
1	Core	Amosite 3.5	114	7		2		60	
1	Core	Amosite 3.5	114	10		1		23	
1	Core	Amosite 3.5	114	6		3		50	
1	Core	Amosite 3.5	114	5		7		30	
1	Core	Amosite 3.5	114	4		2		16	
1	Core	Amosite 3.5	114	2		2		24	
1	Core	Amosite 3.5	114	1		1		25	
						31	**1**	**268**	**300**
1	Core	LA 0.5	115	9		1		109	
1	Core	LA 0.5	115	8	1	7	1	121	
1	Core	LA 0.5	115	7				60	
					1	**8**	**1**	**290**	**300**
1	Core	LA 0.5	116	10		7		241	
1	Core	LA 0.5	116	9		1		51	
						8		**292**	**300**

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix A

Individual Animal BAL Cells Differential Counts per Photographic Field

Component	Group	Exposure Level (mg/m^3) Treatment	Animal I.D.	Field #	Eosinophils	Neutrophils	Lymphocytes	Macrophages	Total Cells per Animal
1	Core	LA 0.5	117	10				87	
1	Core	LA 0.5	117	8		1		97	
1	Core	LA 0.5	117	7				52	
1	Core	LA 0.5	117	5		2		61	
						3		**297**	**300**
1	Core	LA 0.5	118	10			4	133	
1	Core	LA 0.5	118	9				103	
1	Core	LA 0.5	118	8				60	
							4	**296**	**300**
1	Core	LA 0.5	119	10		4		137	
1	Core	LA 0.5	119	9		3		105	
1	Core	LA 0.5	119	8		1		50	
						8		**292**	**300**
1	Core	LA 0.5	120	10			2	45	
1	Core	LA 0.5	120	6		1		88	
1	Core	LA 0.5	120	2		2		62	
1	Core	LA 0.5	120	1		1		30	
1	Core	LA 0.5	120	7				9	
1	Core	LA 0.5	120	5				60	
						4	**2**	**294**	**300**

Appendix A

Individual Animal BAL Cells Differential Counts per Photographic Field

Component	Group	Exposure Level (mg/m^3) Treatment	Animal I.D.	Field #	Eosinophils	Neutrophils	Lymphocytes	Macrophages	Total Cells per Animal
1	Core	LA 0.5	121	10		4		111	
1	Core	LA 0.5	121	6		4		57	
1	Core	LA 0.5	121	5		4		23	
1	Core	LA 0.5	121	9		1		96	
						13		**287**	**300**
1	Core	LA 3.5	122	9		4	2	32	
1	Core	LA 3.5	122	8		3		56	
1	Core	LA 3.5	122	7		4		44	
1	Core	LA 3.5	122	6		7		58	
1	Core	LA 3.5	122	5		3		47	
1	Core	LA 3.5	122	4		8		32	
						29	**2**	**269**	**300**
1	Core	LA 3.5	123	10		2	13	10	
1	Core	LA 3.5	123	9		4		50	
1	Core	LA 3.5	123	8		5		81	
1	Core	LA 3.5	123	7		3		42	
1	Core	LA 3.5	123	6		5	3	82	
						19	**16**	**265**	**300**

Appendix A

Individual Animal BAL Cells Differential Counts per Photographic Field

Component	Group	Exposure Level (mg/m^3) Treatment	Animal I.D.	Field #	Eosinophils	Neutrophils	Lymphocytes	Macrophages	Total Cells per Animal
1	Core	LA 3.5	124	10		13		63	
1	Core	LA 3.5	124	9		12		46	
1	Core	LA 3.5	124	8		16		37	
1	Core	LA 3.5	124	7		7		57	
1	Core	LA 3.5	124	6		13		36	
						61		**239**	**300**
1	Core	LA 3.5	125	10		7		54	
1	Core	LA 3.5	125	9		8		79	
1	Core	LA 3.5	125	3		7		93	
1	Core	LA 3.5	125	2		5		47	
						27		**273**	**300**
1	Core	LA 3.5	126	10		4		61	
1	Core	LA 3.5	126	9		5		52	
1	Core	LA 3.5	126	8		6		85	
1	Core	LA 3.5	126	7		12		75	
						27		**273**	**300**
1	Core	LA 3.5	127	10		6		45	
1	Core	LA 3.5	127	9		7		47	
1	Core	LA 3.5	127	8		7		46	
1	Core	LA 3.5	127	7		5		50	
1	Core	LA 3.5	127	6		6		81	
						31		**269**	**300**

Appendix A

Individual Animal BAL Cells Differential Counts per Photographic Field

Component	Group	Exposure Level (mg/m^3) Treatment	Animal I.D.	Field #	Eosinophils	Neutrophils	Lymphocytes	Macrophages	Total Cells per Animal
1	Core	LA 3.5	128	10		5		89	
1	Core	LA 3.5	128	9		10	3	138	
1	Core	LA 3.5	128	8		11		44	
						26	**3**	**271**	**300**
1	Core	LA 25	129	14		1		8	
1	Core	LA 25	129	13		9		17	
1	Core	LA 25	129	12		6		33	
1	Core	LA 25	129	11		7		14	
1	Core	LA 25	129	10		3		22	
1	Core	LA 25	129	9		7		13	
1	Core	LA 25	129	8		2		20	
1	Core	LA 25	129	7		9		20	
1	Core	LA 25	129	6		8		24	
1	Core	LA 25	129	5		5		6	
1	Core	LA 25	129	4		5		32	
1	Core	LA 25	129	3		5		13	
1	Core	LA 25	129	2		7		4	
						74		**226**	**300**

Appendix A

Individual Animal BAL Cells Differential Counts per Photographic Field

Component	Group	Exposure Level (mg/m^3) Treatment	Animal I.D.	Field #	Eosinophils	Neutrophils	Lymphocytes	Macrophages	Total Cells per Animal
1	Core	LA 25	130	10		13		26	
1	Core	LA 25	130	9		6		17	
1	Core	LA 25	130	8		3		38	
1	Core	LA 25	130	7		9		38	
1	Core	LA 25	130	6		13		14	
1	Core	LA 25	130	5		9		18	
1	Core	LA 25	130	4		6		43	
1	Core	LA 25	130	3		10		37	
						69		**231**	**300**
1	Core	LA 25	131	10		24	2	12	
1	Core	LA 25	131	8		25		20	
1	Core	LA 25	131	7		30	5	11	
1	Core	LA 25	131	6		26		30	
1	Core	LA 25	131	5		39		25	
1	Core	LA 25	131	3		24		27	
						168	**7**	**125**	**300**
1	Core	LA 25	132	5		38		30	
1	Core	LA 25	132	4		54		46	
1	Core	LA 25	132	3		35		46	
1	Core	LA 25	132	2		50		1	
						177		**123**	**300**

Appendix A

Individual Animal BAL Cells Differential Counts per Photographic Field

Component	Group	Exposure Level (mg/m^3) Treatment	Animal I.D.	Field #	Eosinophils	Neutrophils	Lymphocytes	Macrophages	Total Cells per Animal
1	Core	LA 25	133	1		30		18	
1	Core	LA 25	133	2		21		37	
1	Core	LA 25	133	3		19		24	
1	Core	LA 25	133	4		32		34	
1	Core	LA 25	133	10		30		12	
1	Core	LA 25	133	9		27		11	
1	Core	LA 25	133	8		5			
						164		136	300
1	Core	LA 25	134	8		7			
1	Core	LA 25	134	9		68		82	
1	Core	LA 25	134	15		63		51	
1	Core	LA 25	134	19		22		7	
						160		140	300
1	Core	LA 25	135	9		42	2	27	
1	Core	LA 25	135	8		31		21	
1	Core	LA 25	135	3		40		20	
1	Core	LA 25	135	2		11			
1	Core	LA 25	135	10		56	2	48	
						180	4	116	300

APPENDIX B

BAL CELL DIFFERENTIAL COUNTS AND PERCENTAGES PER ANIMAL

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix B

BAL Cell Differential Counts
and Percentages Per Animal

Component	Group	Exposure Level (mg/m^3)	Group Number (GRP)	Animal I.D.	Eosinophils per 300 cells (count)	Eosinophils per 300 cells (%)	Neutrophils per 300 cells (count)
1	Core	Control	1	101	0	0	0
1	Core	Control	1	102	0	0	4
1	Core	Control	1	103	0	0	5
1	Core	Control	1	104	0	0	1
1	Core	Control	1	105	0	0	2
1	Core	Control	1	106	0	0	1
1	Core	Control	1	107	0	0	4
1	Core	Amosite 3.5	2	108	0	0	20
1	Core	Amosite 3.5	2	109	0	0	31
1	Core	Amosite 3.5	2	110	0	0	23
1	Core	Amosite 3.5	2	111	0	0	9
1	Core	Amosite 3.5	2	112	0	0	22
1	Core	Amosite 3.5	2	113	0	0	20
1	Core	Amosite 3.5	2	114	0	0	31
1	Core	LA 0.5	3	115	1	0.33	8
1	Core	LA 0.5	3	116	0	0	8
1	Core	LA 0.5	3	117	0	0	3
1	Core	LA 0.5	3	118	0	0	0
1	Core	LA 0.5	3	119	0	0	8
1	Core	LA 0.5	3	120	0	0	4
1	Core	LA 0.5	3	121	0	0	13
1	Core	LA 3.5	4	122	0	0	29
1	Core	LA 3.5	4	123	0	0	19
1	Core	LA 3.5	4	124	0	0	61
1	Core	LA 3.5	4	125	0	0	27
1	Core	LA 3.5	4	126	0	0	27
1	Core	LA 3.5	4	127	0	0	31
1	Core	LA 3.5	4	128	0	0	26

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix B

Bronchoalveolar Lavage Cell Differential Counts
and Percentages per Animal
(Continuation)

Component	Group	Exposure Level (mg/m^3)	Group Number (GRP)	Animal I.D.	Eosinophils per 300 cells (count)	Eosinophils per 300 cells (%)	Neutrophils per 300 cells (count)
1	Core	LA 25	5	129	0	0	74
1	Core	LA 25	5	130	0	0	69
1	Core	LA 25	5	131	0	0	168
1	Core	LA 25	5	132	0	0	177
1	Core	LA 25	5	133	0	0	164
1	Core	LA 25	5	134	0	0	160
1	Core	LA 25	5	135	0	0	180

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix B

Bronchoalveolar Lavage Cell Differential Counts
and Percentages per Animal
(Continuation)

Component	Group	Exposure Level (mg/m^3)	Group Number (GRP)	Animal I.D.	Neutrophils per 300 cells (%)	Lymphocytes per 300 cells (count)	Lymphocytes per 300 cells (%)
1	Core	Control	1	101	0.00	9	3.00
1	Core	Control	1	102	1.33	1	0.33
1	Core	Control	1	103	1.67	0	0.00
1	Core	Control	1	104	0.33	3	1.00
1	Core	Control	1	105	0.67	2	0.67
1	Core	Control	1	106	0.33	4	1.33
1	Core	Control	1	107	1.33	2	0.67
1	Core	Amosite 3.5	2	108	6.67	0	0.00
1	Core	Amosite 3.5	2	109	10.33	3	1.00
1	Core	Amosite 3.5	2	110	7.67	5	1.67
1	Core	Amosite 3.5	2	111	3.00	7	2.33
1	Core	Amosite 3.5	2	112	7.33	0	0.00
1	Core	Amosite 3.5	2	113	6.67	0	0.00
1	Core	Amosite 3.5	2	114	10.33	1	0.33
1	Core	LA 0.5	3	115	2.67	1	0.33
1	Core	LA 0.5	3	116	2.67	0	0.00
1	Core	LA 0.5	3	117	1.00	0	0.00
1	Core	LA 0.5	3	118	0.00	4	1.33
1	Core	LA 0.5	3	119	2.67	0	0.00
1	Core	LA 0.5	3	120	1.33	2	0.67
1	Core	LA 0.5	3	121	4.33	0	0.00
1	Core	LA 3.5	4	122	9.67	2	0.67
1	Core	LA 3.5	4	123	6.33	16	5.33
1	Core	LA 3.5	4	124	20.33	0	0.00
1	Core	LA 3.5	4	125	9.00	0	0.00
1	Core	LA 3.5	4	126	9.00	0	0.00
1	Core	LA 3.5	4	127	10.33	0	0.00
1	Core	LA 3.5	4	128	8.67	3	1.00

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix B

Bronchoalveolar Lavage Cell Differential Counts
and Percentages per Animal
(Continuation)

Component	Group	Exposure Level (mg/m^3)	Group Number (GRP)	Animal I.D.	Neutrophils per 300 cells (%)	Lymphocytes per 300 cells (count)	Lymphocytes per 300 cells (%)
1	Core	LA 25	5	129	24.67	0	0.00
1	Core	LA 25	5	130	23.00	0	0.00
1	Core	LA 25	5	131	56.00	7	2.33
1	Core	LA 25	5	132	59.00	0	0.00
1	Core	LA 25	5	133	54.67	0	0.00
1	Core	LA 25	5	134	53.33	0	0.00
1	Core	LA 25	5	135	60.00	4	1.33

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix B

Bronchoalveolar Lavage Cell Differential Counts
and Percentages per Animal
(Continuation)

Component	Group	Exposure Level (mg/m^3)	Group Number (GRP)	Animal I.D.	Macrophages per 300 cells (count)	Macrophages per 300 cells (%)	Total Cells per Animal
1	Core	Control	1	101	291	97.00	300
1	Core	Control	1	102	295	98.33	300
1	Core	Control	1	103	295	98.33	300
1	Core	Control	1	104	296	98.67	300
1	Core	Control	1	105	296	98.67	300
1	Core	Control	1	106	295	98.33	300
1	Core	Control	1	107	294	98.00	300
1	Core	Amosite 3.5	2	108	280	93.33	300
1	Core	Amosite 3.5	2	109	266	88.67	300
1	Core	Amosite 3.5	2	110	272	90.67	300
1	Core	Amosite 3.5	2	111	284	94.67	300
1	Core	Amosite 3.5	2	112	278	92.67	300
1	Core	Amosite 3.5	2	113	280	93.33	300
1	Core	Amosite 3.5	2	114	268	89.33	300
1	Core	LA 0.5	3	115	290	96.67	300
1	Core	LA 0.5	3	116	292	97.33	300
1	Core	LA 0.5	3	117	297	99.00	300
1	Core	LA 0.5	3	118	296	98.67	300
1	Core	LA 0.5	3	119	292	97.33	300
1	Core	LA 0.5	3	120	294	98.00	300
1	Core	LA 0.5	3	121	287	95.67	300
1	Core	LA 3.5	4	122	269	89.67	300
1	Core	LA 3.5	4	123	265	88.33	300
1	Core	LA 3.5	4	124	239	79.67	300
1	Core	LA 3.5	4	125	273	91.00	300
1	Core	LA 3.5	4	126	273	91.00	300
1	Core	LA 3.5	4	127	269	89.67	300
1	Core	LA 3.5	4	128	271	90.33	300

Hamner Institutes for Health Sciences Protocol Number 10002

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Appendix B

Bronchoalveolar Lavage Cell Differential Counts
and Percentages per Animal
(Continuation)

Component	Group	Exposure Level (mg/m^3)	Group Number (GRP)	Animal I.D.	Macrophages per 300 cells (count)	Macrophages per 300 cells (%)	Total Cells per Animal
1	Core	LA 25	5	129	226	75.33	300
1	Core	LA 25	5	130	231	77.00	300
1	Core	LA 25	5	131	125	41.67	300
1	Core	LA 25	5	132	123	41.00	300
1	Core	LA 25	5	133	136	45.33	300
1	Core	LA 25	5	134	140	46.67	300
1	Core	LA 25	5	135	116	38.67	300

APPENDIX C

BAL CELL COUNTS AND PERCENTAGES DESCRIPTIVE STATISTICS

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix C

BAL Cell Counts and Percentages Descriptive Statistics

STATISTIX FOR WINDOWS BAL DIFF, 6/17/2010, 2:55:23 PM

DESCRIPTIVE STATISTICS FOR GRP = 1

VARIABLE	N	MEAN	SD	MINIMUM	MAXIMUM
EOA	7	0.0000	0.0000	0.0000	0.0000
EOP	7	0.0000	0.0000	0.0000	0.0000
NEA	7	2.4286	1.9024	0.0000	5.0000
NEP	7	0.8086	0.6347	0.0000	1.6700
LYA	7	3.0000	2.9439	0.0000	9.0000
LYP	7	1.0000	0.9811	0.0000	3.0000
MAA	7	294.57	1.7182	291.00	296.00
MAP	7	98.190	0.5733	97.000	98.670

DESCRIPTIVE STATISTICS FOR GRP = 2

VARIABLE	N	MEAN	SD	MINIMUM	MAXIMUM
EOA	7	0.0000	0.0000	0.0000	0.0000
EOP	7	0.0000	0.0000	0.0000	0.0000
NEA	7	22.286	7.5214	9.0000	31.000
NEP	7	7.4286	2.5056	3.0000	10.330
LYA	7	2.2857	2.8115	0.0000	7.0000
LYP	7	0.7614	0.9370	0.0000	2.3300
MAA	7	275.43	6.8034	266.00	284.00
MAP	7	91.810	2.2675	88.670	94.670

DESCRIPTIVE STATISTICS FOR GRP = 3

VARIABLE	N	MEAN	SD	MINIMUM	MAXIMUM
EOA	7	0.1429	0.3780	0.0000	1.0000
EOP	7	0.0471	0.1247	0.0000	0.3300
NEA	7	6.2857	4.2706	0.0000	13.000
NEP	7	2.0957	1.4236	0.0000	4.3300
LYA	7	1.0000	1.5275	0.0000	4.0000
LYP	7	0.3329	0.5085	0.0000	1.3300
MAA	7	292.57	3.4572	287.00	297.00
MAP	7	97.524	1.1518	95.670	99.000

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Appendix C

BAL Cell Counts and Percentages Descriptive Statistics (Continuation)

DESCRIPTIVE STATISTICS FOR GRP = 4

VARIABLE	N	MEAN	SD	MINIMUM	MAXIMUM
EOA	7	0.0000	0.0000	0.0000	0.0000
EOP	7	0.0000	0.0000	0.0000	0.0000
NEA	7	31.429	13.563	19.000	61.000
NEP	7	10.476	4.5200	6.3300	20.330
LYA	7	3.0000	5.8595	0.0000	16.000
LYP	7	1.0000	1.9518	0.0000	5.3300
MAA	7	265.57	12.040	239.00	273.00
MAP	7	88.524	4.0121	79.670	91.000

DESCRIPTIVE STATISTICS FOR GRP = 5

VARIABLE	N	MEAN	SD	MINIMUM	MAXIMUM
EOA	7	0.0000	0.0000	0.0000	0.0000
EOP	7	0.0000	0.0000	0.0000	0.0000
NEA	7	141.71	48.486	69.000	180.00
NEP	7	47.239	16.161	23.000	60.000
LYA	7	1.5714	2.8200	0.0000	7.0000
LYP	7	0.5229	0.9385	0.0000	2.3300
MAA	7	156.71	49.712	116.00	231.00
MAP	7	52.239	16.569	38.670	77.000

Groups and Variable Designations in Statistical Data:

Due to limitations on the number of characters per field numerical designations that could be used in the statistical analysis software numbers were assigned to each different "Exposure Level" group (GRP) as follows:

1 - Control (0 mg/m^3)
2 - Amosite 3.5 mg/m^3
3 - LA 0.5 mg/m^3
4 - LA 3.5 mg/m^3
5 - LA 25 mg/m^3

Additionally, the BAL count and percentage variables were abbreviated as follows:

EOA - eosinophil count, EOP - eosinophil percentage, NEA - neutrophil count, NEP - neutrophil percentage, LYA - lymphocyte count, LYP - lymphocyte percentage, MAA - macrophage count, MAP - macrophage percentage

APPENDIX D

EOSINOPHIL PERCENTAGES STATISTICAL ANALYSIS

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix D

Eosinophil Percentages Statistical Analysis

STATISTIX FOR WINDOWS BAL DIFF, 6/17/2010, 2:57:18 PM

KRUSKAL-WALLIS ONE-WAY NONPARAMETRIC AOV FOR EOP BY GRP

GRP	MEAN RANK	SAMPLE SIZE
1	17.5	7
2	17.5	7
3	20.0	7
4	17.5	7
5	17.5	7
TOTAL	18.0	35

KRUSKAL-WALLIS STATISTIC 4.0000
P-VALUE, USING CHI-SQUARED APPROXIMATION 0.4060

PARAMETRIC AOV APPLIED to RANKS

SOURCE	DF	SS	MS	F	P
BETWEEN	4	35.0000	8.75000	1.00	0.4229
WITHIN	30	262.500	8.75000		
TOTAL	34	297.500			

TOTAL NUMBER OF VALUES THAT WERE TIED 34
MAX. DIFF. ALLOWED BETWEEN TIES 0.00001

CASES INCLUDED 35 MISSING CASES 0

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix D

Eosinophil Percentages Statistical Analysis(Continuation)

STATISTIX FOR WINDOWS BAL DIFF , 6/17/2010, 2:58:14 PM

COMPARISONS OF MEAN RANKS OF EOP BY GRP

GRP	MEAN RANK	HOMOGENEOUS GROUPS
3	20.000	I
1	17.500	I
2	17.500	I
4	17.500	I
5	17.500	I

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

REJECTION LEVEL	0.050
CRITICAL Z VALUE	2.81
CRITICAL VALUE FOR COMPARISON	15.375

APPENDIX E

NEUTROPHIL PERCENTAGES STATISTICAL ANALYSIS

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix E

Neutrophil Percentages Statistical Analysis

STATISTIX FOR WINDOWS BAL DIFF, 6/17/2010, 2:58:52 PM

KRUSKAL-WALLIS ONE-WAY NONPARAMETRIC AOV FOR NEP BY GRP

GRP	MEAN RANK	SAMPLE SIZE
1	5.6	7
2	20.0	7
3	9.5	7
4	22.9	7
5	32.0	7
TOTAL	18.0	35

KRUSKAL-WALLIS STATISTIC 29.9699
P-VALUE, USING CHI-SQUARED APPROXIMATION 0.0000

PARAMETRIC AOV APPLIED to RANKS

SOURCE	DF	SS	MS	F	P
BETWEEN	4	3139.79	784.946	55.77	0.0000
WITHIN	30	422.214	14.0738		
TOTAL	34	3562.00			

TOTAL NUMBER OF VALUES THAT WERE TIED 17
MAX. DIFF. ALLOWED BETWEEN TIES 0.00001

CASES INCLUDED 35 MISSING CASES 0

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix E

Neutrophil Percentages Statistical Analysis(Continuation)

STATISTIX FOR WINDOWS BAL DIFF , 6/17/2010, 2:59:37 PM

COMPARISONS OF MEAN RANKS OF NEP BY GRP

GRP	MEAN RANK	HOMOGENEOUS GROUPS
5	32.000	I
4	22.857	I I
2	20.000	I I I
3	9.5000	.. I I
1	5.6429	 I

THERE ARE 3 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

REJECTION LEVEL	0.050
CRITICAL Z VALUE	2.81
CRITICAL VALUE FOR COMPARISON	15.375

APPENDIX F

LYMPHOCYTE PERCENTAGES STATISTICAL ANALYSIS

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix F

Lymphocyte Percentages Statistical Analysis

STATISTIX FOR WINDOWS BAL DIFF, 6/17/2010, 3:00:26 PM

KRUSKAL-WALLIS ONE-WAY NONPARAMETRIC AOV FOR LYP BY GRP

GRP	MEAN RANK	SAMPLE SIZE
1	23.1	7
2	19.4	7
3	15.2	7
4	17.1	7
5	15.2	7
TOTAL	18.0	35

KRUSKAL-WALLIS STATISTIC 3.3748
P-VALUE, USING CHI-SQUARED APPROXIMATION 0.4972

PARAMETRIC AOV APPLIED to RANKS

SOURCE	DF	SS	MS	F	P
BETWEEN	4	312.714	78.1786	0.83	0.5188
WITHIN	30	2837.79	94.5929		
TOTAL	34	3150.50			

TOTAL NUMBER OF VALUES THAT WERE TIED 32
MAX. DIFF. ALLOWED BETWEEN TIES 0.00001

CASES INCLUDED 35 MISSING CASES 0

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix F

Lymphocyte Percentages Statistical Analysis(Continuation)

STATISTIX FOR WINDOWS BAL DIFF , 6/17/2010, 3:01:02 PM

COMPARISONS OF MEAN RANKS OF LYP BY GRP

GRP	MEAN RANK	HOMOGENEOUS GROUPS
1	23.143	I
2	19.357	I
4	17.071	I
3	15.214	I
5	15.214	I

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

REJECTION LEVEL	0.050
CRITICAL Z VALUE	2.81
CRITICAL VALUE FOR COMPARISON	15.375

APPENDIX G

MACROPHAGE PERCENTAGES STATISTICAL ANALYSIS

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix G

Macrophage Percentages Statistical Analysis

STATISTIX FOR WINDOWS BAL DIFF, 6/17/2010, 3:01:44 PM

KRUSKAL-WALLIS ONE-WAY NONPARAMETRIC AOV FOR MAP BY GRP

GRP	MEAN RANK	SAMPLE SIZE
1	29.6	7
2	16.3	7
3	27.4	7
4	12.7	7
5	4.0	7
TOTAL	18.0	35

KRUSKAL-WALLIS STATISTIC 30.0540
P-VALUE, USING CHI-SQUARED APPROXIMATION 0.0000

PARAMETRIC AOV APPLIED to RANKS

SOURCE	DF	SS	MS	F	P
BETWEEN	4	3149.93	787.482	57.12	0.0000
WITHIN	30	413.571	13.7857		
TOTAL	34	3563.50			

TOTAL NUMBER OF VALUES THAT WERE TIED 16
MAX. DIFF. ALLOWED BETWEEN TIES 0.00001

CASES INCLUDED 35 MISSING CASES 0

Hamner Institutes for Health Sciences Protocol Number 10002

EPL PROJECT NO. 304-447

Appendix G

Macrophage Percentages Statistical Analysis (Continuation)

STATISTIX FOR WINDOWS BAL DIFF , 6/17/2010, 3:02:23 PM

COMPARISONS OF MEAN RANKS OF MAP BY GRP

GRP	MEAN RANK	HOMOGENEOUS GROUPS
1	29.643	I
3	27.357	I I
2	16.286	I I I
4	12.714	.. I I
5	4.0000	 I

THERE ARE 3 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

REJECTION LEVEL	0.050
CRITICAL Z VALUE	2.81
CRITICAL VALUE FOR COMPARISON	15.375

APPENDIX VI: Bronchoalveolar Lavage Fluid Analysis Report

Appendix Title

EP-W-08-051 – Results of BAL Fluid Analysis

Study Title

EPA Fiber Project: Two-week Range Finding Study – Inhalation Exposure of Rats to Amphibole Asbestos.

Study Protocol

10002

Author

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Signatures

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Objective: The goal of biochemical analysis of bronchoalveolar lavage (BAL) fluid is to identify changes in male F344 rats following two weeks of exposure to Libby amphibole asbestos. This is in comparison to changes seen following exposure to air (negative control) or amosite asbestos (positive control).

Introduction: Elevated levels of alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein, N-acetyl-β-D-glucosaminidase (NAG) and B-glucuronidase in BAL fluid are indicators of lung inflammation and injury.

Materials and Methods: Analytes in bronchoalveolar lavage fluid were determined using a Cobas Fara II Clinical Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). Kits for alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) and for control sera were obtained from Pointe Scientific (Canton, MI). A kit for N-acetyl-β-D-glucosaminidase (NAG) and NAG standard were obtained from Roche Diagnostics. Coomassie Plus – The Better Bradford Assay reagent and albumin standard for total protein analysis were obtained from Pierce Biochemicals (Rockford, IL).

B-glucuronidase was not assayed due to product unavailability. The most commonly cited assay kit is manufactured by Bio-Rad, the Fluor Ace β-glucuronidase Reporter Assay Kit. There were problems with the standard curve that Bio-Rad was unwilling to address. A similar Sigma product has been discontinued.

All assays were run according to kit instructions with modifications to accommodate automated analysis by the Cobas Fara II.

Results of BAL Fluid Analysis:

Group†	ALP U/l*	Std. Dev.	LDH U/l*	Std. Dev.	Tot. Prot. µg/ml*	Std. Dev.	NAG U/l*	Std. Dev.
Air control	51	4	18	3	74	11	1.69	0.23
Amosite 3.5	56	6	31	11	95	15	1.90	0.79
LA 0.5	49	4	29	9	80	9	1.93	0.28
LA 3.5	63	5	50	11	121	18	2.14	0.28
LA 25	109	12	71	8	160	16	3.57	0.55

†N=7 for each group
*Values are reported as means

Statistics: Statistical analysis of the data was performed (JMP (v 8.0), Cary, NC). Levene's test for inequality of variance was applied to the means. ALP data showed inequality of variance, so Welch's ANOVA, which weighs means of groups of unequal variance was used and showed significance at $p<.0001$. Remaining analytes showed no inequality of variance, so the standard ANOVA was used. All ANOVAs showed significant differences between means at $p<.0001$. The Tukey-Kramer test was used for pair-wise comparison of individual groups and is summarized in the table below.

Statistical Significance Table

Group A	Group B	ALP	LDH	Tot. Protein	NAG
Air control	**Amosite 3.5**	–	–	–	–
Air control	**LA 0.5**	–	–	–	–
Air control	**LA 3.5**	+	+	+	–

Air control	**LA 25**	+	+	+	+
Amosite 3.5	**LA 0.5**	–	–	–	–
Amosite 3.5	**LA 3.5**	–	+	+	–
Amosite 3.5	**LA 25**	+	+	+	+

- = no significance
+ = significant at a p value < 0.05

Conclusions: Concentrations of all analytes were elevated in the amosite 3.5 (positive control) group compared to the air control group, but none at a statistically significant level. No significant differences were seen for any analytes in comparisons between air control and LA 0.5, or between amosite positive control and LA 0.5. All analytes were significantly elevated in the LA 25 group compared to either air control or amosite positive control.

Analytes in the LA 3.5 group were significantly higher than either air or positive control group for LDH, an indicator of general cellular toxicity, and for total protein, an indicator of increased alveolar-capillary permeability. NAG, an indicator of phagocytic cell activation was not significantly elevated in the LA 3.5 group compared to either air or positive control group. ALP is normally secreted by Type II cells with elevated levels indicating damage to those cells. ALP was significantly elevated in the LA 3.5 group compared to the air control, but not to the amosite positive control.

APPENDIX VII: Protocol and Protocol Amendments

PROTOCOL

EPA FIBER PROJECT: TWO-WEEK RANGE FINDING STUDY – INHALATION EXPOSURE OF RATS TO AMPHIBOLE ASBESTOS

Protocol Number 10002

Sponsor: U.S. Environmental Protection Agency
Contract No. EP-W-08-051

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Date Issued: 02/04/2010

Protocol Title: **EPA FIBER PROJECT: TWO-WEEK RANGE FINDING STUDY - INHALATION EXPOSURE OF RATS TO AMPHIBOLE ASBESTOS**

Study Protocol No.: **10002**

REVIEWED AND APPROVED BY:

PRINCIPAL INVESTIGATOR

Earl Tewksbury, B.A. — Date: 02/03/2010

CO-INVESTIGATOR

Brian Wong, Ph.D. — Date: 2/03/2010

CO-INVESTIGATOR

Ed Bermudez, M.S., DABT — Date: 2/3/10

HEALTH, SAFETY and ENVIRONMENT

Richard Cravener, Jr. — Date: 03Feb10

MANAGEMENT for FUNDING

Darol E. Dodd, Ph.D., DABT — Date: 2/3/10

QUALITY ASSURANCE

Patricia O. Pomerleau — Date: 02/03/10

SPONSOR'S REPRESENTATIVE

Stephen Gavett, Ph.D. — Date

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SPONSOR'S REPRESENTATIVE

Stephen Gavett, Ph.D. — Date: 2/4/10

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1 INTRODUCTION

1.1 Background

The vermiculite mine near Libby, Montana was the world's leading source of vermiculite for 70 years until its closure in 1990. Vermiculite is used for insulation, as an absorbent material, and as a soil conditioner, and has applications in the construction, agricultural, horticultural and industrial markets. However, the Libby vermiculite ore coexists with a complex array of amphibole mineral types, primarily winchite, richterite, tremolite, and magnesioriebeckite with crystal forms (habits) ranging from asbestiform to acicular/prismatic.

Occupational exposure to Libby vermiculite has been (and continues to be) associated with significant increases in asbestosis, lung cancer, and pleural cancer compared to the rest of the U.S. population. For example, in addition to elevated rates of lung cancer and mesothelioma among Libby residents, 17.8% of 6,668 persons who lived or worked in the Libby area for at least 6 months before 1991 show (upon medical testing) pleural abnormalities (calcifications, thickenings, or plaques).

Furthermore, exposures to individuals outside of Libby have occurred, and are likely continuing; as asbestos-contaminated vermiculite ore from Libby was shipped to hundreds of locations around the nation for processing, and used as attic insulation in millions of homes throughout the United States. The health effects associated with former and current exposures from the asbestos contaminated vermiculite from the Libby mine continues to be a subject of intensive study and public health concern.

1.2 Objective

The overall goal of this research is to improve the scientific basis for the risk assessment of asbestos-contaminated communities by conducting toxicology studies to help define key determinants of internal dose and provide critical insight on additional key health or

pathologic endpoints. These types of toxicology studies can only be done in animals and to date, rodent inhalation studies have not been conducted with the amphibole asbestos that contaminates vermiculite from Libby, Montana (Libby amphibole or LA).

There are two (2) components to the study. The first component is a 2-week range-finder study designed to determine optimal fiber-aerosol concentrations to be used in the subsequent subchronic inhalation exposure study and to compare the potency of inhaled Libby amphibole fibers to the potency of inhaled amosite, a known fibrogenic amphibole asbestos fiber. The second component is a dosimetry study to determine initial fiber deposition and clearance/biopersistence. This study is designed to provide a time course of fiber burden data in various regions of the respiratory tract (head, trachea, lung lobes) and GI tract. These data will be used to derive LA-specific inhalability, deposition efficiency, and clearance rates for development of modifications to the Multi-path particle dosimetry (MPPD) model used to describe inhalation dosimetry.

2 EXPERIMENTAL DESIGN

2.1 Study Design Components

The study will have two animal in-life components (Table 5). Component 1 will consist of exposure of male F344 rats to LA and amosite in a two-week (5 days/week) range finding inhalation study. Animals will be evaluated for histopathology/proliferation and inflammation. In component 2, male F 344 rats will be exposed to LA for 1-day, 5-days or 10 days (5 days/week for 2 weeks) and will be evaluated for clearance/biopersistence (via tissue fiber burden analysis).

2.2 Justifications

2.2.1 Test Animal Selection

The rat is used as a surrogate for humans in the detection of toxicity and is a species in which known toxicants have been detected. This rodent species is commonly used in the conduct of toxicity studies and is recommended by several health effects testing guidelines. Historical control data are also available with this strain of rat for comparative evaluation, if necessary.

2.2.2 Number of Animals

The animal numbers proposed for this research are based on The Hamner Institutes' previous investigations on the inhalation toxicology of refractory ceramic fibers, glass fibers, and amosite asbestos fibers. The numbers were compared against the extensive literature on fiber toxicology studies performed over the past 30 years in Europe and the United States. The animal numbers were also discussed with three expert consultants (Drs. McConnell, Hesterberg, and Bernstein) and a statistician (D. House) who have been involved in the design and conduct of numerous fiber toxicology studies. A recent analytical pilot study conducted by The Hamner Institutes (Protocol Number 09003) focused on animal and tissue variability associated with fiber burden analysis. The results of this study were also used to determine an appropriate number of animals needed per dose and per time point to achieve the appropriate statistical power.

2.2.3 Route, Duration and Frequency of Administration

The test material, Libby amphibole (LA) fibers, will be lofted and administered by nose-only inhalation. The inhalation route is one of the potential routes of human exposure to this test material. The test subjects will be exposed for 6 hours

per day for either 1 day, 5 days or 10 days (5 days per week for two weeks).

2.2.4 *Exposure Levels*

Animals will be exposed to three concentrations of LA fibers (high, intermediate and low groups), to amosite fibers (positive control group) or to clean air (negative control group). LA fiber exposure will occur at target exposure concentrations of 0.5, 3.5 and 25 mg/m3 and amosite fiber exposure will be at a target concentration of 3.5 mg/m3. Exposure concentrations for this study were selected based on results from previous studies with amosite fibers.

2.3 Study Design Details

The in-life portion of this research will consist of two components: (1) Two-week (5 days/week) range-finding inhalation exposures of rats to LA and amosite asbestos samples for the evaluation of histopathology/proliferation and inflammation (bronchoalveolar lavage; BAL) (Table 1). Animals will be necropsied either immediately following the last daily (6-hr) exposure (BAL evaluation) or approximately 4 days following the last daily exposure (histopathology/proliferation evaluation).

(2) Two-week range-finding inhalation exposures of rats to LA fibers for the evaluation of clearance/biopersistence (via tissue fiber burden analysis) (Tables 2, 3, and 4). In this part of the study, animals will be divided into one of three exposure groups: 1-day, 5-day, or 10-day (2-weeks). Animals will be necropsied either immediately after exposure ("zero time") (1-day, 5-day, and 10-day groups; Table 2) or at 6-, 12-, or 24-hr following the last daily (6-hr) exposure (1-day and 5-day groups; Tables 3 and 4).

Table 1: Number of rats and exposure levels for histopathology/cell proliferation and inflammation endpoints.

Test Material	Nominal Concentration (mg/m^3)*	Number of rats necropsied immediately (inflammation evaluation) or approximately 4 days (histopathology/cell proliferation evaluation) after last daily (6-hr) exposure period		
		1 day	5 days	10 days (2 weeks)
Air Control	0	0	0	7 Non-BrdU 7 BrdU
Amosite	3.5	0	0	7 Non-BrdU 7 BrdU
LA	0.5	0	0	7 Non-BrdU 7 BrdU
LA	3.5	0	0	7 Non-BrdU 7 BrdU
LA	25	0	0	7 Non-BrdU 7 BrdU
Necropsy:	# of rats:	0	0	70
	Summation:	TOTAL	# of rats:	70

Table 2: Number of rats and exposure levels for deposition and clearance/biopersistence (1-day, 5-day, or 10-day exposures).

Test Material	Nominal Concentration (mg/m^3)	Number of rats necropsied immediately after last daily (6-hr) exposure period		
		1 day	5 days	10 days (2 weeks)
Air Control	0	0	0	0
Amosite	3.5	0	0	0
LA	0.5	6	6	6
LA	3.5	6	6	6
LA	25	6	6	6
Necropsy:	# of rats:	18	18	18
	Summation:	TOTAL	# of rats:	54

Table 3: Number of rats and exposure levels for deposition and clearance/biopersistence (1-day exposure)

Test Material	Nominal Concentration (mg/m^3)	Number of rats necropsied and time of necropsy following a 1-day (6-hr) exposure		
		6 hours	12 hours	24 hours
Air Control	0	0	0	0
Amosite	3.5	0	0	0
LA	0.5	6	6	6
LA	3.5	6	6	6
LA	25	6	6	6
Necropsy:	# of rats:	18	18	18
	Summation:	TOTAL	# of rats:	54

Table 4: Number of rats and exposure levels for deposition and clearance/biopersistence (5-day exposure)

Test Material	Nominal Concentration (mg/m^3)	Number of rats necropsied and time of necropsy following the last daily (6-hr) exposure of a 5-day exposure regimen		
		6 hours	12 hours	24 hours
Air Control	0	0	0	0
Amosite	3.5	0	0	0
LA	0.5	6	6	6
LA	3.5	6	6	6
LA	25	6	6	6
Necropsy:	# of rats:	18	18	18
	Summation:	TOTAL	# of rats:	54

3 STUDY TIMELINE

Study Initiation Date: Date Principal Investigator signs protocol.
Initial Pathology Report: Within 2-3 months of completion of exposure
Draft Report: Within 2-3 months of completion of exposure
(histopathology, cell proliferation, and inflammation evaluations)
Experimental Start Date: 1 March 2010 (Component 1)
15 March 2010 (Component 2)
Experimental Termination Date (In-life phase):
17 March 2010 (Component 1)
16 April 2010 (Component 2)

4 TEST MATERIALS

4.1 Test Materials

Name:	Libby Amphibole fiber preparation (LA 2007) and Amosite fiber (positive control test material)
CAS #, Formula, and Molecular Weight	N.A.*
Storage:	Store at room temperature. Keep container closed tightly.
Supplier:	U.S. Environmental Protection Agency
Lot Number:	N.A.

*N.A. = not applicable

4.2 Identity, Purity, Composition, Stability, and Method of Synthesis

If known, any identity, purity, composition, stability, method of synthesis, fabrication and/or derivation information of the test materials used in this study will be documented by the Sponsor (or sponsor designee). This documentation will be maintained by the Sponsor at the address indicated on the signature page of this protocol. Any work done by the Testing Facility to characterize the test material will be documented in the study file.

A useful website from the USGS Open File Report for very similar test materials, e.g., the water-elutriated LA2000 fiber preparation and the RTI amosite preparation follows:
http://pubs.usgs.gov/of/2009/1242/

There are no MSDS or other references for these test materials, except for Meeker et al.'s original paper on the raw LA2000 fiber material, as follows.

Meeker, G.P., A.M. Bern, I.K. Brownfield, H.A. Lowers, S.J. Sutley, T.M. Hoefen, and J.S. Vance. (2003). The composition and morphology of amphiboles from the Rainy Creek Complex, near Libby, Montana. *Am. Minerologist* 88:1955-1969.

4.3 Archival Sample

An archival sample from each lot of test material will be taken and stored in the Archives of the Testing Facility. If multiple studies are conducted with the same material, a common archival sample may be taken and appropriately labeled.

4.4 Unused Test Material

The unused portion of the test material, as well as any empty test material containers, will be returned to the Sponsor following completion of the final report.

5 TEST ANIMALS

5.1 Species and Strains

Rats: Fischer (CDF®) [F344/DuCrl]

5.2 Supplier

Source: Charles River Laboratories, Kingston, NY

5.3 Animal Requirements/Specifications

5.3.1 Gender and Number

	Total	Males
Number ordered	256	256
Number on Test	232	232

5.3.2 Age

Age at Receipt (weeks)	Age at Start of First Exposure (weeks)
~7-8	~9-10

5.3.3 Weight

Approximately 225-275 grams at first exposure.

5.4 Acclimation Period

Seven to eight-week old rats (approximately 150-175 grams) will be delivered approximately 2 weeks prior to the first exposure for both components of the study. [Note: A stagger exposure regimen will have to be used to accommodate daily necropsy capacity. Thus, animals will have to be ordered to arrive on different dates to keep ages/sizes consistent for dosing.] Shortly after their arrival at the laboratory, the animals will be removed from the shipping cartons and examined. All animals with evidence of disease or physical abnormalities will be euthanized and necropsied. If an unusually large number of animals show evidence of disease or physical abnormalities, the entire shipment of animals will be rejected for use in the study. The animals will be transported to an acclimation and training/habituation room prior to assignment to specific exposure animal rooms (292 through 296). From the time of delivery to the beginning of exposures, the rats will pass through two phases of animal care and training: animal room acclimation (1 to 2 weeks), and training/habituation to restraint (nose-only inhalation exposure tubes). The habituation schedule will maintain the rats in tubes for 5 daily periods of increasing duration -- approximately 1, 2, 3, 4, and 6 hours.

5.5 Selection for Study

More animals than required for the study will be purchased and acclimated. Animals considered suitable for study on the basis of pretest physical examinations, body weight, and any other pretest evaluations will be assigned to exposure groups. Body weights will be measured the day after arrival and at randomization for group assignment prior to the beginning of test material exposure. Individual weights of animals placed on test will be within ± 20% of the mean weight, if possible. An Instem Provantis™ 8 protocol (Provantis, Conshohoken, PA) will be used to randomize animals (during acclimation) and to collect body weight data. Disposition of animals not utilized in the study will be maintained in the study file.

5.6 Animal Identification

Each animal will be assigned a temporary identification number and cage location upon receipt. After selection for study, each animal will be identified with transponders with a number assigned by the Testing Facility (see Table 5). The study protocol number plus the number assigned by the Testing Facility will comprise the unique animal number for each animal. A cage assignment chart will indicate cage assignment by the animal identification number.

Table 5. Component, Group, Exposure Level, and Animal Identification Number

Component	Group	Exposure Level (mg/m^3)	Animal Identification Number 10002-xxx		
Component 1	Core (10-day exposure)	**Treatment**	**BAL**	**+ BrdU**	
		Control	101-107	201-207	
		Amosite 3.5	108-114	208-214	
		LA 0.5	115-121	215-221	
		LA 3.5	122-128	222-228	
		LA 25	129-135	229-235	
		Total	**35**	**35**	
Component 2	Clearance 1 (necropsy immediate post exposure)	**Exposure**	**1-Day**	**5-Days**	**10-Days**
		LA 0.5	301-306	307-312	313-318
		LA 3.5	319-324	325-330	331-336
		LA 25	337-342	343-348	349-354
		Total	**18**	**18**	**18**
	Clearance 2 (1-day exposure)	**Necropsy time post exposure**	**6 hours**	**12 hours**	**24 hours**
		LA 0.5	401-406	407-412	413-418
		LA 3.5	419-424	425-430	431-436
		LA 25	437-442	443-448	449-454
		Total	**18**	**18**	**18**
	Clearance 3 (5-day exposure)	**Necropsy time post exposure**	**6 hours**	**12 hours**	**24 hours**
		LA 0.5	501-506	507-512	513-518
		LA 3.5	519-524	525-530	531-536
		LA 25	537-542	543-548	549-554
		Total	**18**	**18**	**18**
	Total	**232**			

5.7 Animal Husbandry during Non-exposure Periods

5.7.1 Housing

Animals will be housed in individual wire mesh cages during the facility acclimation and pre- and post-exposure periods.

5.7.2 Environmental Conditions

5.7.2.1 Light Dark Cycle

Animal housing areas will be on a twelve hour light/dark cycle, controlled via the Andover Continuum Building Automation System. The timing of the cycle will be 0600 to 1800 hours, but may be adjusted prior to the start of the study to accommodate exposure schedules. Any changes will be made prior to the start of the study and will be documented in the study file.

5.7.2.2 Temperature

Temperature in the housing area will be monitored in accordance with Testing Facility SOPs to ensure that the desired range of 20-24°C is maintained to the maximum extent possible.

5.7.2.3 Humidity

Humidity in the housing area will be monitored in accordance with Testing Facility SOPs to ensure that the desired range of 30 to 70% is maintained to the maximum extent possible.

5.7.2.4 Feed and Feed Analysis

Animals will be fed a Certified Rodent Diet, NIH-07 pellets; Zeigler Bros., Gardners, PA, *ad libitum.*

Certification of batches of feed will be maintained on file by the manufacturer and the testing facility. There are no known contaminants in the feed that are expected to interfere with the results of this study.

5.7.2.5 Water and Water Analysis

Drinking water from the Durham, NC municipal water system will be filtered through an in-house reverse osmosis system; water will be available *ad libitum*, via automated watering system.

Water analyses are conducted quarterly by National Testing Laboratories, WaterCheck, Cleveland, OH, or equivalent, to assure that water meets standards specified under the EPA Federal Safe Drinking Water Act Regulations (40 CFR Part 141). Results of water analysis will be maintained on file at the Testing Facility.

5.7.3 Veterinary Care

Animals will be monitored by the technical staff for any conditions requiring possible veterinary care. If any such conditions are identified, the Attending Veterinarian will be notified for an examination and evaluation. Animals will be treated as outlined in the Animal Welfare Act Compliance section of this protocol.

5.8 Animal Husbandry during Exposure Periods

5.8.1 Housing

Animals will be placed in nose-only exposure tubes (Battelle Memorial Institute, WA or equivalent design) that attach to

the RCC nose-only inhalation exposure system. Two different sizes of exposure tubes are available depending on the size of the rat. For protection of personnel, the RCC exposure systems will be placed inside 8-m^3 containment chambers that are located inside assigned animal holding rooms (292 through 296).

5.8.2 Environmental Conditions

Temperature inside the 8-m^3 containment chamber and inside the animal holding room may be reduced to approximately 66 to 68°F or as low as practical for the facility cooling system, to reduce the stress from heat inside the exposure tube.

5.8.3 Feed and Water

Animals will not have access to food and water during the exposure period.

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6 TEST MATERIAL ADMINISTRATION

6.1 Route of Administration

The test material will be administered by nose-only inhalation using directed flow nose-only exposure systems (RCC, Geneva, Switzerland). These systems consist of modular tiers based on the Cannon nose-only exposure design. Each tier is capable of holding up to 16 rats and up to 10 tiers can be configured into the RCC exposure system. The number of tiers to be used for each exposure level will be documented in the study file.

6.2 Frequency and Duration of Administration

The LA test material will be administered for approximately 6 hours per exposure day for 1 day, 5 days or 10 days (5 days per week over a two week period). The amosite test material will be administered for approximately 6 hours per exposure day for 10 days (5 days per week over a two week period). Control animals

will be exposed to HEPA-filtered air only for 6 hours/day, 5 days/wk for two weeks.

6.3 Generation of Test Material

The test materials will be administered as an aerosol fiber in the breathing air of the animals. The test atmosphere will be generated using a rotating brush aerosol generator using procedures developed during pre-study trials. The method will be described in the raw data of the study and in the report.

The exposure system will be operated at a minimum air flow rate of 1.5 times the approximate minute ventilation of a rat (250 ml/min) for each animal or each open port. A HEPA filter will be placed in the exhaust line from the chamber to collect fibers that have passed through the system.

6.4 Monitoring of Test Material

6.4.1 Concentration

During the exposure, measurements of airborne concentrations will be performed in the animals' breathing zone. Aerosol concentration will be measured using a gravimetric filter or equivalent method. The analytical method will be developed in the pre-study trials and documented in the study file.

Fiber concentrations will be continuously monitored using a light scatter aerosol monitor (Real-Time Aerosol Monitor (RAM), MIE [Monitoring Instruments for the Environment, Inc] Billerica, Massachusetts or equivalent). This system will provide back up information to the gravimetric filter and will provide a secondary means to monitor the operation of the generation system.

6.4.2 Fiber Size distribution

Samples for fiber size distribution measurement will be collected directly from the animal exposure ports using a 0.2 μm polycarbonate filter and analyzed by scanning electron microscopy (JOEL Model JSM 840A, Tokyo, Japan, or equivalent).

6.4.3 Monitoring of Exposure Tower Conditions

Nose-only exposure tower temperature, relative humidity, airflow rate and static pressure will be monitored continuously and recorded at least three times during the exposure for all exposure groups, including the control group. Chamber temperature will be maintained, to the maximum extent possible, between 19 to 24°C. As dry air is being used in the generation system, relative humidity during the exposure is expected to be low (e.g., 3 to 10%).

6.5 Summary of Chamber Activity

The minimum frequency of chamber activity for all exposure chambers, including the control chamber, is summarized below:

Activity	Minimum Frequency per chamber
Measured Test Material Concentration	Once daily
Particle Size	Once weekly
Temperature	3 times daily
Relative Humidity	3 times daily
Airflow Rate	3 times daily
Static Pressure	3 times daily

7 EXPERIMENTAL EVALUATION

7.1 Observations

7.1.1 Viability Checks

Animals will be observed for morbidity, mortality, general appearance and signs of severe toxic effects before and after exposures during exposure days. Animals will be observed at least once during the day during non-exposure periods. Animals in extremely poor health or in a possible moribund condition will be identified for further monitoring and possible euthanasia.

7.1.2 Clinical Observations

Each animal will be examined at least twice pre-exposure, on the day of first exposure, and weekly during the exposure period. These examinations will include observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluations of respiration, autonomic and central nervous system effects, and reactivity to handling. Observations will be recorded in the Provantis™ 8 system.

7.2 Body Weight

Body weights will be recorded within two days after arrival, at randomization, weekly during acclimation, one day prior or on the first day of first exposure, weekly, and at necropsy. Body weights will be recorded in the Provantis™ 8 system.

7.3 5-Bromo-2'-deoxyuridine (BrdU) Implants for Cell Proliferation Evaluation

BrdU will be administered to 7 animals/concentration (Table 1) the afternoon following their last daily exposure (approximately 3.5 days prior to their scheduled sacrifice) via surgically implanted Alzet micro-osmotic pumps (Model 2 ML1: 7-day pump, 10 microliters/hr, or equivalent) filled with 5 mg/mL solution of BrdU in phosphate

buffered saline. The surgery will be performed aseptically under isoflurane anesthesia. The dorsal thoracic region will be shaved and an incision large enough to accommodate the pump will be made with scalpel or scissors. The pump will be inserted through the incision and pushed cranially into the subcutaneous pocket. The incision will be closed with wound clips. Further details are provided in Hamner SOP ANH-001-03.

8 POST MORTEM

8.1 Method of Euthanasia

Animals to be euthanized will be deeply anesthetized with sodium pentobarbital (intraperitoneal injection, approximately 50 mg/kg) and exsanguinated by transection of the abdominal aorta.

8.2 Necropsy Schedule

8.2.1 Moribund and Humane Euthanasia

Animals showing signs of severe debility, particularly if death appears imminent, will be euthanatized to prevent loss of tissues through autolysis. Necropsy should be performed immediately. If a necropsy cannot be performed on a euthanized animal or an animal found dead, the animal will be appropriately disposed due to tissue autolysis.

8.2.2 Terminal Necropsy

Necropsy of all surviving animals will be performed at the times shown in Tables 1 through 4.

8.3 Necropsy

8.3.1 Gross Necropsy

A complete macroscopic examination will be performed on all Component 1 histopathology animals and all unscheduled deaths (Components 1 and 2). All abnormal observations will be recorded. The necropsy will include examination of the

external surface of the body and all orifices; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass.

Postmortem examinations of Component 1 histopathology animals at terminal necropsy shall be conducted by a pathologist. The pathologist will be provided under contract with Experimental Pathology Laboratories, Inc. (Durham, NC).

8.3.2 *Component 1 Core Group Histopathology and Cell Proliferation (BrdU-Treated)*

For animals assigned to histopathology and cell proliferation (Tables 1 and 5), seven BrdU-treated rats per group will have lungs and trachea removed for histopathology and cell proliferation evaluations. The trachea and lungs will be fixed *in situ* with 10% neutral buffered formalin (NBF) at approximately 30 cm pressure. The nasal cavities will be flushed with NBF. The head will be removed, skinned, trimmed of excess tissue, and stored in NBF for approximately 3 days. The heads will then be rinsed in running tap water, decalcified, and re-rinsed in water. Cross sectional blocks of the nasal cavity will be prepared (at least 4 levels), embedded in paraffin wax, sectioned (approximately 5 micrometers), deparaffinized, and stained with hematoxylin and eosin (H&E). The lungs (left pulmonary lobe, right cranial lobe, and additional parts, if indicated), trachea, duodenum, and relevant gross organ/tissue lesions will be fixed with NBF for 48 hours, rinsed, and stored in 70% ethanol, embedded in paraffin wax, sectioned (approximately 5 micrometers), deparaffinized, and stained with H&E (except duodenum). Multiple sections of the tissues shall be taken. An additional set of slides from the lungs shall be stained with a collagen specific stain (e.g., Masson-Goldner's trichrome stain).

The rib cage (pleural tissues) will also be removed and preserved in NBF for possible future analysis. Samples of the left ventricular tissue (cut longitudinally into 3 separate samples for storage) and thoracic aorta (trim off connective tissue and cut into 2 separate samples for storage) will be removed and frozen in liquid nitrogen and stored at approximately -70°C (or colder) for potential future analysis.

Preparation of histological slides and microscopic examinations will be performed by Experimental Pathology Laboratories, Inc. (Durham, NC). Evaluations of the lung and tracheal tissues will include assessment of collagen deposition at the broncho-alveolar junction, pulmonary fibrosis, interstitial fibrosis, peribronchiolar lesions, and pleural changes. The severity of fibrotic response and the area of the lung showing fibrosis will be quantified.

Additional cross sections of the lungs, tracheal, and duodenal (for background reference on each slide) tissues will be immunostained with BrdU for cell proliferation evaluation. Cell proliferation will be quantitated as labeling index (LI) or unit length labeling index (ULLI). The anatomical sites selected for evaluation will be defined by the histopathology data collected above.

8.3.3 Component 1 Core Group Inflammation Evaluation (non-BrdU-treated)

For animals assigned for inflammation evaluation (Tables 1 and 5), seven (non-BrdU-treated) rats per group will have their lungs lavaged for the collection of cells and BAL fluid. The trachea will be cannulated and the lungs lavaged with a total of 25 ml phosphate-buffered saline (PBS). BAL cells will be isolated and the total number of cells enumerated. Slide preparations of BAL cells will be prepared and stained with Diff-Quik or similar stain, and the cell differential percentages determined, including the quantification of

macrophages, monocytes, lymphocytes, neutrophils, and eosinophils. Lavaged lungs will be saved in liquid nitrogen, as separate lobes in separate tubes.

Cell-free BAL fluid supernatant will be assessed for total protein, lactate dehydrogenase, β-glucuronidase, N-acetyl-β-d-glucosaminidase, and alkaline phosphatase. Excess supernatant will be saved in 3 separate tubes for possible future cytokine analysis.

Serum and plasma (citrate will be the anticoagulant used) samples will also be collected and stored at approximately -70°C (or colder) for potential future analysis.

8.3.4 Component 2 Clearance/Biopersistence Evaluation

For animals assigned to clearance/biopersistence evaluation (Tables 2, 3, 4, and 5), the following tissues will be collected for fiber burden analysis: URT, larynx, trachea, lungs (5 lobes), pleural cast, lung-associated lymph nodes, esophagus, stomach, small intestine (duodenum/jejunum/ileum), cecum, and colon/rectum. Further information about the necropsy and casting procedure is available in SOP EPT-125. Details of the digestions and preparation of GI tissue samples will be provided in a protocol amendment. Tissues will be stored frozen at approximately -70°C (or colder) until analysis.

Frozen tissue samples will be freeze dried using a Freezone® freeze drying system (Labconco, Kansas City, MO). Samples will be weighed before and after 6 hours of freeze drying and then again following overnight freeze drying to ensure complete drying.

Freeze dried samples will be either digested or ashed. Tissue digestion procedures may include acid or basic solutions. Options for ashing include low temperature or high temperature plasma asher (LTA-504, Anatech, Springfield, VA). Samples will be weighed before and after 24 hours of ashing and then again following another 24 hours to ensure complete ashing. Samples that do not readily reach a stable weight will continue to be ashed for no longer than 96 hours. Specific information on the procedures used will be documented in the data file.

8.3.5 Counting of Fibers (option to be considered by the Sponsor)

Ashed samples will be suspended in water and spotted on a filter. Filters will be sputter-coated (Hummer V, Anatech, Springfield, VA) and examined for fibers using a scanning electron microscope (SEM) (JOEL, Tokyo, Japan). Images of fibers will be captured by SEM and analyzed using Image Pro-Plus (Version 5.0 for Windows 2000/XP, Media Cybernetics Inc, MD). Measurements of fiber diameter, fiber length and the number of fibers will be obtained from the images.

9 HEALTH AND SAFETY

All work with hazardous and biohazardous agents will be conducted in accordance with The Hamner Institutes' currently operative Health, Safety and Environment guidelines and procedures. A review of safe handling procedures will be presented to personnel and documented. Ventilation, both local and general, will be utilized as necessary to minimize employee exposure to fiber test material. Minimum standard protective apparel and equipment for laboratory work will include disposable chemical resistant gloves, lab coats and safety glasses. A Health and Safety Research Protocol that provides details for the safe use of amphibole asbestos was submitted to the Sponsor on October 1, 2008. Additional animal care and inhalation safety procedures specific for protection of personnel working

with or close to fiber inhalation exposure systems were prepared and are currently being followed.

10 STUDY PERSONNEL

Principal Investigator	Earl Tewksbury, B.A.
Alternate Contact:	Brian Wong, Ph.D.
Alternate Contact:	Ed Bermudez, M.S., DABT

Additional personnel will be documented in the study file and presented in the final report.

11 PRESERVATION OF RECORDS AND SPECIMENS

All data documenting experimental details and study procedures and observations will be recorded and maintained as raw data. At the completion of the study, all reports, raw data, and retained samples will be maintained in the Testing Facility's Archives for a period of one year after submission of the signed final report. The Sponsor will be contacted in order to determine the final disposition of these materials. The Sponsor is responsible for all costs associated with the storage of these materials beyond one year from the issuance of the final report and for any costs associated with the shipment of these materials to the Sponsor or to any other facility designated by the Sponsor.

12 STATISTICAL EVALUATIONS

Appropriate statistical analysis (e.g., one-way analysis of variance and/or Dunnett's Test for normally distributed parametric data or Fisher's Exact Test for non-parametric data) will be conducted on collected data from component 1 of the study (e.g., body weights, BAL endpoints, cell proliferation, and histopathology). Comparisons will be made between control (air-only) and fiber treated groups.

13 REPORTING

One unbound hard copy and/or an electronic copy of a partially audited draft report will be submitted following termination of the study. After receipt and review of the Sponsor's comments, appropriate changes will be made and one hard copy and one electronic copy of a signed final report will be issued. The report will minimally include:

Summary
Introduction
Experimental Design
Materials and Methods
- Identification and appropriate characteristics of test materials
- Exposure concentrations with aerosol fiber analysis (bivariate size distribution)
- Description of rat strain, source, number, sex, body weight, etc.
- Statistics used
- Tissue fiber burden preparation
- Preparation for fiber counts
- Analytical methods for fiber counts (if EPA selects The Hamner Institutes for fiber counting option)
- Counting rules for fiber burden (if EPA selects The Hamner Institutes for fiber counting option)

Study Results
- Summary tables
- Statistical analyses
- Figures, where appropriate
- Inhalation exposure data
- Clinical signs, mortality data, and body weights
- Necropsy findings
- Inflammation endpoints (BAL)
- Histopathology
- Cell proliferation
- Fiber tissue burdens including bivariate size distribution (if EPA selects Hamner for fiber counting option)

Discussion
Conclusion
Appendices (as needed)
Senior personnel participating in the study

14 REGULATORY REFERENCES

14.1 Facilities Management/Animal Husbandry

Currently acceptable practices of good animal husbandry will be followed, e.g., *Guide for the Care and Use of Laboratory Animals*; National Academy Press, 1996. The Hamner Institutes for Health Sciences is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

14.2 Animal Welfare Act Compliance

This study will comply with all appropriate parts of the Animal Welfare Act regulations: 9 CFR Parts 1 and 2 Final Rules, Federal Register, Volume 54, No. 168, August 31, 1989, pp. 36112-36163 effective October 30, 1989 and 9CFR Part 3 Animal Welfare Standards; Final Rule, Federal Register, Volume 56, No. 32, February 15, 1991, pp. 6426-6505 effective March 18, 1991. The Sponsor should make particular note of the following:

1. The Sponsor's signature on this protocol documents for the study described, there are no generally accepted non-animal alternatives and the study does not unnecessarily duplicate previous experiments.

2. All procedures used in this study have been designed to avoid discomfort, distress and pain to the animals. All methods are described in this study protocol or in written laboratory standard operating procedures.

3. Any procedures outlined in this study protocol which are expected to cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedatives, analgesics or anesthetics unless the withholding of these agents is justified for scientific reasons, in writing, by the Sponsor and the Principal Investigator and approved by the IACUC; in which case the procedure will continue for the minimum time necessary. Documentation of the justification for withholding treatment for pain or distress and IACUC approval of the procedures will be made prior to study initiation on the IACUC Statement Review form.

4. Animals experiencing more than momentary or slight pain or distress due to test material or emergency situations such as injury or illness will be treated by the Attending Veterinarian or Laboratory Animal Resources and Technical Support Facility (LARTSF) designee with approved analgesics or agents to relieve pain. If possible, the Principal Investigator will be consulted prior to treatment; however, the LARTSF staff is authorized to administer emergency treatment as necessary. Any subsequent treatment or euthanasia will be administered after consultation with the Principal Investigator. The Sponsor will be advised by the Principal Investigator of all emergency situations in as timely a manner as possible.

5. Methods of euthanasia used during this study are in conformance with the above referenced regulations.

14.3 Institutional Animal Care and Use Committee

Initiation and completion of this protocol is contingent on review and approval by The Hamner Institutes' IACUC Statement review committee.

14.4 Research Quality Standards

This study will be conducted under The Hamner's Research Quality Standards program (SOP QUA-022).

15 ALTERATION OF DESIGN

Alterations of this protocol may be made as the study progresses. No changes in the protocol will be made without the consent of the Sponsor. In the event that the Sponsor authorizes a protocol change verbally, such changes will be honored by the Testing Facility and will be followed by a written verification. All protocol modifications will be signed by the Principal Investigator and a Sponsor representative. Any modifications potentially affecting animal welfare will also be reviewed and signed by members of the Institutional Animal Care and Use Committee prior to the modification's implementation.

16 QUALITY ASSURANCE

This study may be subject to Quality Assurance (QA) quality assessments for a review of study records, procedures and project objectives. Quality Assurance personnel will work with the Principal Investigator to appropriately schedule these independent assessments.

17 REFERENCES

National Research Council (1996). Guide for the care and use of laboratory animals, Washington, D.C.: National Academic Press.

18 APPENDICES (ON FILE)

A copy of the IACUC statement for this study is on file at The Hamner Institutes for Health Sciences.

PROTOCOL AMENDMENT NO.: 1

Protocol Number: 10002

Protocol Title: EPA Fiber Project: Two-Week Range Finding Study – Inhalation Exposure of Rats to Amphibole Asbestos

Text/Attachment to be Amended:

8 POST MORTEM

8.1 Method of Euthanasia

Animals to be euthanized will be deeply anesthetized with sodium pentobarbital (intraperitoneal injection, approximately 50 mg/kg) and exsanguinated by transection of the abdominal aorta.

8.2 Necropsy Schedule

8.2.1 Moribund and Humane Euthanasia

Animals showing signs of severe debility, particularly if death appears imminent, will be euthanatized to prevent loss of tissues through autolysis. Necropsy should be performed immediately. If a necropsy cannot be performed on a euthanized animal or an animal found dead, the animal will be appropriately disposed due to tissue autolysis.

8.2.2 Terminal Necropsy

Necropsy of all surviving animals will be performed at the times shown in Tables 1 through 4.

8.3 Necropsy

8.3.1 Gross Necropsy

A complete macroscopic examination will be performed on all Component 1 histopathology animals and all unscheduled deaths (Components 1 and 2). All abnormal observations will be recorded. The necropsy will include examination of the external surface of the body and all orifices; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass.

Postmortem examinations of Component 1 histopathology animals at terminal necropsy shall be conducted by a pathologist. The pathologist will be provided under contract with Experimental Pathology Laboratories, Inc. (Durham, NC).

8.3.2 Component 1 Core Group Histopathology and Cell Proliferation (BrdU-Treated)

For animals assigned to histopathology and cell proliferation (Tables 1 and 5), seven BrdU-treated rats per group will have lungs and trachea removed for histopathology and cell proliferation evaluations. The trachea and lungs will be fixed *in situ* with 10% neutral buffered formalin (NBF) at approximately 30 cm pressure. The nasal cavities will be flushed with NBF. The head will be removed, skinned, trimmed of excess tissue, and stored in NBF for approximately 3 days. The heads will then be rinsed in running tap water, decalcified, and re-rinsed in water. Cross sectional blocks of the nasal cavity will be prepared (at least 4 levels), embedded in paraffin wax, sectioned (approximately 5 micrometers), deparaffinized, and stained with hematoxylin and eosin (H&E). The lungs (left pulmonary lobe, right cranial lobe, and additional parts, if indicated), trachea, duodenum, and relevant gross organ/tissue lesions will be fixed with NBF for 48 hours, rinsed, and stored in 70% ethanol, embedded in paraffin wax, sectioned (approximately 5 micrometers), deparaffinized, and stained with H&E (except

duodenum). Multiple sections of the tissues shall be taken. An additional set of slides from the lungs shall be stained with a collagen specific stain (e.g., Masson-Goldner's trichrome stain).

The rib cage (pleural tissues) will also be removed and preserved in NBF for possible future analysis. Samples of the left ventricular tissue (cut longitudinally into 3 separate samples for storage) and thoracic aorta (trim off connective tissue and cut into 2 separate samples for storage) will be removed and frozen in liquid nitrogen and stored at -70°C (or colder) for potential future analysis.

Preparation of histological slides and microscopic examinations will be performed by Experimental Pathology Laboratories, Inc. (Durham, NC). Evaluations of the lung and tracheal tissues will include assessment of collagen deposition at the broncho-alveolar junction, pulmonary fibrosis, interstitial fibrosis, peribronchiolar lesions, and pleural changes. The severity of fibrotic response and the area of the lung showing fibrosis will be quantified.

Additional cross sections of the lungs, tracheal, and duodenal (for background reference on each slide) tissues will be immunostained with BrdU for cell proliferation evaluation. Cell proliferation will be quantitated as labeling index or unit length labeling index (ULLI). The anatomical sites selected for evaluation will be defined by the histopathology data collected above.

8.3.3 Component 1 Core Group Inflammation Evaluation (non-BrdU-treated)

For animals assigned for inflammation evaluation (Tables 1 and 5), seven (non-BrdU-treated) rats per group will have their lungs lavaged for the collection of cells and BAL fluid. The trachea will be cannulated and the lungs lavaged with a total of 25 ml phosphate-buffered saline (PBS). BAL cells will be isolated and the total number of cells enumerated. Slide preparations of BAL cells will be prepared and stained with Diff-Quik or similar stain, and the cell differential percentages determined, including the quantification of macrophages,

monocytes, lymphocytes, neutrophils, and eosinophils. Lavaged lungs will be saved in liquid nitrogen, as separate lobes in separate tubes.

Cell-free BAL fluid supernatant will be assessed for total protein, lactate dehydrogenase, β-glucuronidase, N-acetyl-β-d-glucosaminidase, and alkaline phosphatase. Excess supernatant will be saved in 3 separate tubes for possible future cytokine analysis.

Serum and plasma (citrate will be the anticoagulant used) samples will also be collected and stored at -80°C for potential future analysis.

8.3.4 Component 2 Clearance/Biopersistence Evaluation

For animals assigned to clearance/biopersistence evaluation (Tables 2, 3, 4, and 5), the following tissues will be collected for fiber burden analysis: URT, larynx, trachea, lungs (5 lobes), pleural cast, lung-associated lymph nodes, esophagus, stomach, small intestine (duodenum/jejunum/ileum), cecum, and colon/rectum. Further information about the necropsy and casting procedure is available in SOP EPT-125-00. Details of the digestions and preparation of GI tissue samples will be provided in a protocol amendment. Tissues will be stored frozen at -70°C (or colder) until analysis.

Frozen tissue samples will be freeze dried using a Freezone® freeze drying system (Labconco, Kansas City, MO). Samples will be weighed before and after 6 hours of freeze drying and then again following overnight freeze drying to ensure complete drying.

Freeze dried samples will be either digested or ashed. Tissue digestion procedures may include acid or basic solutions. Options for ashing include low temperature or high temperature plasma asher (LTA-504, Anatech, Springfield, VA). Samples will be weighed before and after 24 hours of ashing and then again following another 24 hours to ensure complete ashing. Samples that do not readily reach a stable weight will

continue to be ashed for no longer than 96 hours. Specific informationb on the procedures used will be documented in the data file.

8.3.5 Counting of Fibers (option to be considered by the Sponsor)

Ashed samples will be suspended in water and spotted on a filter. Filters will be sputter-coated (Hummer V, Anatech, Springfield, VA) and examined for fibers using a scanning electron microscope (SEM) (JOEL, Tokyo, Japan). Images of fibers will be captured by SEM and analyzed using Image Pro-Plus (Version 5.0 for Windows 2000/XP, Media Cybernetics Inc, MD). Measurements of fiber diameter, fiber length and the number of fibers will be obtained from the images.

Amended to Read:

8 POST MORTEM

8.1 Method of Euthanasia

Animals to be euthanized will be deeply anesthetized with sodium pentobarbital (intraperitoneal injection, approximately 50 mg/kg followed by additional injections, if necessary, but not to exceed the euthanasia dose of 200 mg/kg) and exsanguinated by transection of the abdominal aorta.

8.2 Necropsy Schedule

8.2.1 Moribund and Humane Euthanasia

Animals showing signs of severe debility, particularly if death appears imminent, will be euthanatized and appropriately disposed.

8.2.2 Terminal Necropsy

Necropsy of all surviving animals will be performed at the times shown in Tables 1 through 4.

8.3 Necropsy

8.3.1 Gross Necropsy

A complete macroscopic examination will be performed on all scheduled Component 1 histopathology animals. All abnormal observations will be recorded. The necropsy will include examination of the external surface of the body and all orifices; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass. Postmortem examinations shall be conducted by a pathologist. The pathologist will be provided under contract with Experimental Pathology Laboratories, Inc. (Durham, NC).

8.3.2 Component 1 Core Group Histopathology and CellProliferation (BrdU-Treated)

For animals assigned to histopathology and cell proliferation (Tables 1 and 5), seven BrdU-treated rats per group will have lungs and trachea removed for histopathology and cell proliferation evaluations. The trachea and lungs will be fixed *in situ* with 10% neutral buffered formalin (NBF) at approximately 30 cm pressure. The nasal cavities will be flushed with NBF. The head will be removed, skinned, trimmed of excess tissue, and stored in NBF for approximately 3 days. The heads will then be rinsed in running tap water, decalcified, and re-rinsed in water. Cross sectional blocks of the nasal cavity will be prepared (at least 4 levels), embedded in paraffin wax, sectioned (approximately 5 micrometers), deparaffinized, and stained with hematoxylin and eosin (H&E). The lungs (2 sections - left lobe and right cranial lobe), trachea (2 sections – carina and anterior), and duodenum (1 section per left lung lobe block and 1 section per trachea block) will be fixed with NBF for 48 hours, rinsed, and stored in 70% ethanol, embedded in paraffin wax, sectioned (approximately 5 micrometers), deparaffinized, and stained with H&E. An additional set of slides from the lungs and trachea blocks shall be stained with a collagen specific stain (e.g., Masson's trichrome stain).

The pleural tissues (thorax to include rib cage, diaphragm, and thoracic spine) and relevant gross organ/tissue lesions will also be removed and preserved in NBF for possible future analysis. Samples of the left ventricular tissue (cut longitudinally into 3 separate samples for storage) and thoracic aorta (trim off connective tissue and cut into 2 separate samples for storage) will be removed and frozen in liquid nitrogen and stored at approximately -70°C (or colder) for potential future analysis.

Preparation of histological slides and microscopic examinations will be performed by Experimental Pathology Laboratories, Inc. (Durham, NC). Evaluations of the lung and tracheal tissues will include assessment of collagen deposition at the broncho-alveolar junction, pulmonary fibrosis, interstitial fibrosis, peribronchiolar lesions, and pleural changes. The severity of fibrotic response and the area of the lung showing fibrosis will be quantified (graded with severity scores).

Two additional sections of the left lung lobe and trachea (each containing the duodenum for background reference) will be immunostained with BrdU for cell proliferation evaluation. Cell proliferation will be quantitated as labeling index or unit length labeling index (ULLI). The anatomical sites selected for evaluation will be defined by the histopathology data collected above.

8.3.3 Component 1 Core Group Inflammation Evaluation (non-BrdU-treated)

For animals assigned for inflammation evaluation (Tables 1 and 5), seven (non-BrdU-treated) rats per group will have their lungs lavaged for the collection of cells and BAL fluid. The trachea will be cannulated and the lungs lavaged with a total of 25 ml phosphate-buffered saline (PBS). BAL cells will be isolated and the total number of cells enumerated. Slide preparations of BAL cells will be prepared and stained with Diff-Quik or similar stain,

and the cell differential percentages determined, including the quantification of macrophages, monocytes, lymphocytes, neutrophils, and eosinophils. Lavaged lungs will be saved in liquid nitrogen, as separate lobes in separate tubes.

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Freeze dried samples will be either digested or ashed. Tissue digestion procedures may include acid or basic solutions. Options

for ashing include low temperature or high temperature plasma asher (LTA-504, Anatech, Springfield, VA). Samples will be weighed before and after 24 hours of ashing and then again following another 24 hours to ensure complete ashing. Samples that do not readily reach a stable weight will continue to be ashed for no longer than 96 hours. Specific information on the procedures used will be documented in the data file.

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Reason for Amendment:

1) Due to concern for health and safety of personnel working with animals treated with fibers at necropsy, several procedural changes were made.

2) Clarification of necropsy, histology, and histopathology procedures were incorporated into the protocol following technical and professional discussions with the Sponsor (EPA) and with EPL.

3) All changes in the protocol were made to improve the evaluation of study specific endpoints related to the toxicological assessment and biopersistence of the test material.

Accompanying Amendments (e.g. IACUC, Biohazardous Agent, Radioactive Material)

None

Earl Lewis Study Director / Principal Investigator — 03/5/2010 Date

Dave E. Dodd Management for Funding — 3/5/10 Date

Stephen L. Gaunt Sponsor's Representative — 3/5/2010 Date