EPA FIBER PROJECT: SUBCHRONIC INHALATION EXPOSURE OF RATS TO AMPHIBOLE ASBESTOS

(1-DAY POST-EXPOSURE, 1-MONTH POST-EXPOSURE AND 3-MONTHS POST-EXPOSURE)

THE HAMNER INSTITUTES FOR
HEALTH SCIENCES PROTOCOL NO. 10026

EPL PROJECT NO.: 304-466
EPL PATHDATA NOS. 90126; 90186; 90187; 90188

"AMENDED" 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.

Street Address:
615 Davis Drive
Suite 500
Durham, NC 27713

Mailing Address:
P.O. Box 12766
RTP, NC 27709

(919) 998-9407

March 6, 2013

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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).

The specific aims of the study are to determine the biological potency of inhaled Libby amphibole (LA) fibers over the near-life span of the rat compared to the potency of inhaled amosite, a known fibrogenic amphibole asbestos fiber, and to develop fiber burden data to use in a dosimetry model of amphibole fiber deposition, clearance, and retention in the respiratory tract (head, trachea, lung lobes) and GI tract.

INTRODUCTION

This Hamner Institutes for Health Sciences Study 10026 was conducted in male F344 rats. The in-life portion of this study consists of a 90-day inhalation exposure (65 exposures), 6 hours/day, 5 days per week, followed by necropsy time points at 1-Day, 1-Month, 3-Months and 18-Months after the last exposure day.

Male F344 (CDF) rats were procured and housed a minimum of 10 days before commencing nose-only inhalation exposures. At the beginning of the inhalation exposures, the rats were about 10 weeks of age (about 200 grams). The rats were exposed in nose-only exposure tubes, to the LA2007 material, spanning a 10-fold concentration range, at low, medium, and high concentrations (1.0, 3.3, and 10.0 mg/m^3), or a UICC amosite sample as a positive control fiber at 3.3 mg/m^3, for 6 hr/day, 5 days/week, for 13 consecutive weeks (no exposure weekends). Rats exposed to filtered air serve as the negative control. See Table 1 for animal numbers and necropsy time points. Groups 1 – 5 were added by EPL for data collection purposes.

Rats were necropsied at 1-Day, 1-Month and 3-Months, after the end of the 13-week exposure (Table 1). The remainder will be necropsied at approximately 18-months. The earlier necropsy time points (1-Day, 1-Month, 3-Months) were chosen

based on prior studies, which suggest that early mediators are most predictive of final pathological responses. Also, the earlier time points are useful for the dosimetry model. The final necropsy time point is listed as approximately 18 months; it will actually be determined when the average cohort survival rate declines to 75% of the number from the beginning of the exposure (i.e. from 277 starting rats to approximately 208 surviving rats, as best as it can be scheduled logistically). From prior studies with the F344 rat, it is expected that the rats will be about 23 months old when this percent of rats are surviving, so they will have survived 2 months of life before exposure, 3 months during exposure, and 18 months after exposure (or 21 months from the start of the exposure). This is a relatively high survival rate for a chronic study, which is often carried out at least 24 months from the start of an exposure.

The critical endpoints to be examined in this study are outlined in Table 2. Rats reserved specifically for fiber burden analysis are shown in rows labeled "Fiber #". At each time point, 3 control rats and 6 amosite- or LA-exposed rats will be necropsied and organs removed for the evaluation of clearance/biopersistence (via tissue fiber burden analysis). At the 1-day, 1-month, and 3-month time points, 8 other animals were necropsied, and bronchoalveolar lavage (BAL) fluid and blood were taken, and lungs, parietal pleura, and other organs were taken for histopathology. The BAL and lung histopathology were carried out in the same animal by tying off the left lung lobe and lavaging the right lung lobes, then removing the left lung lobe for histopathology. At the final (~18 month) necropsy, BAL will be conducted on 8 of the surviving animals from the scheduled group of 50. The rest of the 50 rats assigned for final necropsy, including those with unscheduled early deaths, will be reserved for histopathology only, and also for blood samples at final scheduled necropsy. To facilitate exposure and necropsy scheduling animals will be assigned to necropsy groups (Table 3). Table 4 details the animal numbers assigned to each dose group, exposure level and end point.

The study design is shown in Table 1 below:

Table 1. Study Design

Group Nos.	Test Material	Concentration (mg/m^3)	No. of rats necropsied at each time point				Total in Each Exp. Group
			Post-Exposure Period				
			1-Day	1-Month	3-Months	18-Months*	
1	Air Control	0.0	11	11	11	53	86
2	Amosite	3.3	14	14	14	56	98
3	LA	1.0	14	14	14	56	98
4	LA	3.3	14	14	14	56	98
5	LA	10.0	14	14	14	56	98
	Total in Each Post-Exp Period		67	67	67	277	
	Total Animals in Study		**478**				

*Actual Post-Exposure Period = 75% survival (see above; projected at 23 months old; 18 months post-exposure).

The in-life portion of this study consists of a 90-day inhalation exposure (65 exposures), 6 hours/day, 5 days per week, followed by necropsy time points at 1-day, 1-month, 3-months and 18-months after the last exposure day.

The critical endpoints to be examined in this study are outlined in Table 2.

Table 2. Critical Endpoints

Group Nos.	Test Material	Concentration (mg/m^3)	Endpoint	No. of rats necropsied for each endpoint type			
				Post-Exposure Period			
				1-Day	1-Month	3-Months	18-Months*
1	Air Control	0.0	Fiber #**	3	3	3	3
			BAL***	8	8	8	8
			Histopath	8	8	8	50
2	Amosite	3.3	Fiber #**	6	6	6	6
			BAL***	8	8	8	8
			Histopath	8	8	8	50
3	LA	1.0	Fiber #**	6	6	6	6
			BAL***	8	8	8	8
			Histopath	8	8	8	50
4	LA	3.3	Fiber #**	6	6	6	6
			BAL***	8	8	8	8
			Histopath	8	8	8	50
5	LA	10.0	Fiber #**	6	6	6	6
			BAL***	8	8	8	8
			Histopath	8	8	8	50
		Totals	Fiber #	27	27	27	27
			BAL/Histopath	40	40	40	250

*Actual Post-Exposure Period = 75% survival (see above; projected at 23 months old; 18 months post-exposure).

**Only fiber analysis is conducted on animals listed in "Fiber #" rows.

***BAL (Bronchoalveolar Lavage) is conducted on same set of animals as histopath; but only on 8 from each group of 50 at the final necropsy.

To facilitate exposure and necropsy scheduling animals will be assigned to necropsy groups (Table 3).

Table 3. Necropsy Group Assignment

Necropsy Group #	Description	Number of Animals
1	1-Day Post-exposure Period, Histopath /BAL Animals from all dose groups	40
2	1-Day Post-exposure Period, Fiber Burden - Control, Amosite, and LA Low Dose Animals	15
3	1-Day Post-exposure Period, Fiber Burden - LA Mid and LA High Dose Animals	12
4	1-Month Post-exposure Period, Histopath /BAL Animals from all dose groups	40
5	1-Month Post-exposure Period, Fiber Burden - Control, Amosite, and LA Low Dose Animals	15
6	1-Month Post-exposure Period, Fiber Burden - LA Mid and LA High Dose Animals	12
7	3-Month Post-exposure Period, Histopath /BAL Animals from all dose groups	40
8	3-Month Post-exposure Period, Fiber Burden - Control, Amosite, and LA Low Dose Animals	15
9	3-Month Post-exposure Period, Fiber Burden - LA Mid and LA High Dose Animals	12
10	18-Months Post-exposure Period, Histopath /BAL (10 animals per dose group)	50
11	18-Months Post-exposure Period, Histopath /BAL (10 animals per dose group)	50
12	18-Months Post-exposure Period, Histopath /BAL (10 animals per dose group)	50
13	18-Months Post-exposure Period, Histopath /BAL (10 animals per dose group)	50
14	18-Months Post-exposure Period, Histopath /BAL (10 animals per dose group)	50
15	18-Months Post-exposure Period, Fiber Burden - Control, Amosite, and LA Low Dose Animals	15
16	18-Months Post-exposure Period, Fiber Burden - LA Mid and LA High Dose Animals	12

Table 4. Dose Group, Exposure Level, End Point, Sentinel Animals and Animal Identification Number

Dose Group	Exposure Level Conc. (mg/m^3)	End Point	Animal Identification Numbers 10026-xxx				
			Post-Exposure Period				Sentinel Animals
			1–Day	1–Month	3–Months	18–Months	
Air Control	0	Fiber Burden	101-103	104-106	107-109	110-112	1-4
		Histopath / BAL	113-120	121-128	129-136	137-186	
Amosite	3.3	Fiber Burden	201-206	207-212	213-218	219-224	5-8
		Histopath / BAL	225-232	233-240	241-248	249-298	
LA	1.0	Fiber Burden	301-306	307-312	313-318	319-324	9-12
		Histopath / BAL	325-332	333-340	341-348	349-398	
LA	3.3	Fiber Burden	401-406	407-412	413-418	419-424	13-16
		Histopath / BAL	425-432	433-440	441-448	449-498	
LA	10.0	Fiber Burden	501-506	507-512	513-518	519-524	17-20
		Histopath / BAL	525-532	533-540	541-548	549-598	

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) followed by additional injections, if necessary, but not exceeding the euthanasia dose of 200 mg/kg and exsanguinated by transection of the abdominal aorta.

For animals assigned to histopathology and bronchoalveolar lavage (BAL) evaluation, the left lung was ligated and the right lung lobes were lavaged with a total of 15 ml phosphate-buffered saline (PBS) five times with approximately 3 ml each time. BAL cells were isolated and the total number of cells enumerated. For animals assigned to the last time point (18-months post-exposure) 8 animals from each dose group will have the right lungs lavaged for BAL cells, the remaining, approximately 42 animals, will have left and right lung lobes collected for histopathology.

Once the right lung lobes were collected the ligature on the left lung lobe was removed. The trachea and left lung lobe were fixed *in situ* with 10% neutral buffered formalin (NBF) at approximately 30 cm of NBF 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 (6 levels) and embedded in paraffin wax. The left lung lobe, trachea, sternum, and relevant gross organ/tissue lesions 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, trachea, and sternum were stained with a collagen specific stain (Masson-Goldner's trichrome stain). Testes and epididymis were collected at necropsy, stored in fixative, and one section from the left testis and left epididymis evaluated histologically. Preparation of histological slides and microscopic examinations was performed by Experimental Pathology Laboratories, Inc. (EPL®) Durham, NC.

Lung, trachea, sternum, testis and epididymis were evaluated via light microscopy by an EPL pathologist and selected findings are presented in Tables 5-7.

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. Some findings not suitable for grading were recorded as present (P). Gross findings at necropsy were correlated with histological findings when possible.

Definition of Histological Terms:

Alveolar epithelial hyperplasia was diagnosed when there was proliferation of cuboidal type II alveolar cells without evidence of significant inflammation or macrophage infiltration as seen in more common spontaneous subpleural lesions.

Alveolus inflammation was characterized by an infiltration of macrophages, lymphocytes and occasional neutrophils in alveoli and alveolar ducts resulting in a concomitant increase in the thickness of the alveolar and alveolar duct walls. A constituent of this process were occasional granulomas which are aggregates of macrophages with rare giant cells. The location of this inflammation was mainly centriacinar (around terminal bronchioles, alveolar ducts and alveoli). This inflammation was associated with the presence of asbestos fibers in alveolar macrophages. In more severely affected areas of inflammation, cytotoxicity was evident and characterized by pyknotic nuclei and karyorrhectic debris.

Bronchiolization was characterized by the appearance of cells that resemble bronchiolar epithelial cells at locations distal to the terminal bronchioles and thus occurring in alveolar ducts and adjacent alveoli. The presence of cilia was a helpful diagnostic criteria.

Bronchiole epithelial hyperplasia was characterized by increase in the number of cells in the epithelium of the terminal bronchioles some of which show karyomegaly and anisokaryosis.

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. Fibrosis was evident along alveolar walls frequently increasingly evident at branchpoints into alveolar sacs. Occasionally in fibrotic areas foci of multilamellar mineralization were evident.

Foreign body was diagnosed when asbestos fibers were evident in sections. These were mostly contained in macrophages some of which were completely engulfed while others were partially engulfed; syncytial giant cells were occasionally evident. Asbestos fibers were seen within alveolar macrophages and also within cells in the interstitium.

Chronic inflammation focal was the term used to describe discrete lesions which were 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. Occasionally these foci had minimal proliferation of alveolar type II cells.

RESULTS

The results are summarized with a brief discussion to communicate this phase of the study. However, the overall conclusions may be subject to change when the results and discussion are integrated across all necropsy time points in the 18-Month post-exposure report.

A summary of selected histological findings in the lungs at 1-Day, 1-Month and 3-Months post-exposure is given in Tables 5, 6 and 7. Other histological findings are in Summary Tables, Tables of Individual Microscopic Findings and Macro/Micro Correlation Tables in Appendices A-C for 1 Day, 1 Month and 3 Months, respectively.

For Animal #238 (Amosite 1-Month), the right caudal lung was submitted for histological evaluation instead of the left lung.

Table 5. 1-Day Post-exposure Summary of Incidence and Severity [ ] of Selected Findings in the Lung (number of animals = 8 per group)

Exposure	Bronchiole Epithelial Hyperplasia	Alveolus Inflammation	Interstitial Fibrosis	Foreign Body*	Bronchiolization	Inflammation Chronic Focal
Control	0	0	0	0	0	2 [0.3]
Amosite 3.3 mg/m^3	0	8 [1.0]	8 [1.0]	8	8 [1.0]	1 [0.1]
Libby amphibole 1.0 mg/m^3	0	8 [1.0]	8 [1.0]	8	7 [0.9]	4 [0.5]
Libby amphibole 3.3 mg/m^3	0	8 [1.0]	8 [1.0]	8	8 [1.0]	0
Libby amphibole 10.0 mg/m^3	8 [1.0]	8 [2.0]	8 [1.0]	8	8 [1.0]	0

[ ] = Mean Severity Grade for the group is calculated by dividing the total of severity grades for a finding/lesion in a group by the number of that tissue examined in that group.
*Not graded

Table 6. 1-Month Post-exposure Summary of Incidence and Severity [ ] of Selected Findings in the Lung (number of animals = 8 per group)

Exposure	Bronchiole Epithelial Hyperplasia	Alveolus Inflammation	Interstitial Fibrosis	Foreign Body*	Bronchiolization	Inflammation Chronic Focal	Alveolar Epithelial hyperplasia
Control	0	0	0	0	0	5 [0.6]	0
Amosite 3.3 mg/m^3	1 [0.1]	8 [1.1]	8 [1.0]	8	7 [0.9]	6 [0.8]	0
Libby amphibole 1.0 mg/m^3	0	8 [1.0]	7 [0.9]	8	7 [0.9]	6 [0.8]	0
Libby amphibole 3.3 mg/m^3	0	8 [1.0]	8 [1.0]	8	7 [0.9]	4 (0.5)	0
Libby amphibole 10.0 mg/m^3	8 [1.0]	8 [2.0]	8 [1.0]	8	8 [1.4]	0	1 (0.1)

[ ] = Mean Severity Grade for the group is calculated by dividing the total of severity grades for a finding/lesion in a group by the number of that tissue examined in that group.
*Not graded

Table 7. 3-Months Post-exposure Summary of Incidence and Severity [ ] of Selected Findings in the Lung (number of animals = 8 per group)

Exposure	Bronchiole Epithelial Hyperplasia	Alveolus Inflammation	Interstitial Fibrosis	Foreign Body*	Bronchiolization	Inflammation Chronic Focal	Alveolar Epithelial hyperplasia
Control	0	0	0	0	0	2 [0.3]	0
Amosite 3.3 mg/m^3	0	8 [1.0]	8 [1.0]	8	8 [1.0]	2 [0.3]	0
Libby amphibole 1.0 mg/m^3	0	8 [1.0]	8 [1.0]	8	8 [1.0]	1 [0.1]	0
Libby amphibole 3.3 mg/m^3	0	8 [1.1]	8 [1.0]	8	8 [1.0]	2 [0.3]	0
Libby amphibole 10.0 mg/m^3	0	8 [1.4]	8 [1.0]	8	8 [1.0]	0	1 [0.3]

[ ] = Mean Severity Grade for the group is calculated by dividing the total of severity grades for a finding/lesion in a group by the number of that tissue examined in that group.
*Not graded

Air Control

A minimal degree of focal chronic inflammation was observed as a spontaneous lesion in the lungs of 2 to 5 control animals at each of the three time points.

Amosite 3.3 mg/m^3

Minimal alveolus inflammation, interstitial fibrosis, bronchiolization and foreign body (asbestos fiber) were evident in most animals from this exposure group at each time point. Minimal spontaneous focal chronic inflammation was seen in the lungs of 1 to 6 animals at each time point.

Libby amphibole 1.0 mg/m^3

Minimal alveolus inflammation, interstitial fibrosis, bronchiolization and foreign body (asbestos fiber) were evident in most animals from this treatment group. All findings were evident in all animals other than bronchiolization which was evident in 7/8 rats at 1-Day post-exposure and 7/8 rats at 1-Month post-exposure, and fibrosis which was present in 7/8 animals at 1-Month post-exposure. Minimal spontaneous focal chronic inflammation was seen in the lungs of 1 to 6 animals at each time point.

Libby amphibole 3.3 mg/m^3

Minimal alveolus inflammation, interstitial fibrosis, bronchiolization and foreign body (asbestos fiber) were evident in all animals from this exposure group at each timepoint other than at 1-Month post-exposure where only 7/8 rats had bronchiolization. Minimal spontaneous focal chronic inflammation was seen in the lungs of 0 to 4 animals.

Libby amphibole 10.0 mg/m^3

Mild alveolus inflammation, minimal bronchial epithelial hyperplasia, bronchiolization, interstitial fibrosis, and foreign body (asbestos fiber) were evident in all animals from this treatment group at all time points. This exposure level was associated with an increased severity of alveolar inflammation in comparison to the other groups and was the only exposure group to have minimal bronchiolar epithelial hyperplasia at the 1-Day and 1-Month time points. One of 8 animals had minimal/mild alveolar epithelial hyperplasia at the 1-Month and 3-Month time points. At three months the severity of the alveolus inflammation was less than at the earlier time points.

CONCLUSIONS

Similar responses were evident in the lungs of animals exposed to Amosite 3.3 mg/m^3, Libby amphibole 1mg/m^3 and Libby amphibole 3.3 mg/m^3 as all groups had minimal alveolus inflammation, interstitial fibrosis, bronchiolization and foreign body (asbestos fiber) evident in the lungs of exposed animals.

The most intense response was seen in the lungs of the animals exposed to 10.0 mg/m^3 Libby amphibole which was the only group to have bronchiole epithelial hyperplasia evident in the lungs of exposed animals at 1-Day and 1-Month post-exposure. The exposure level was also associated with an increase in severity of the alveolar inflammation at 1-Day and 1-Month and which decreased at the 3-Month time point.

Essentially, the responses at all time points were similar for each of the exposure groups. The foreign materials persisted and continued to be sequestered in macrophages and sometimes syncytial giant cells were evident.

GABRIELLE A. WILLSON, B.V.M.S., MRCVS
F.R.C. Path.
Pathologist

6 Mar 13
Date

GAW/dc

REASON FOR CHANGE

1. To correct incorrectly calculated total cell counts reported in Appendix B of the BAL Cytology Report and to update narrative statements subsequent to the statistical analysis of the revised total cell count data for the 3-months post-exposure samples.

2. To include corrected Appendix A “3-Months Post-Exposure Total Cell Counts by Cell Types” which was used for statistical analysis.

3. NOTE: Changes were made only to the “Attachment (Bronchoalveolar Lavage Cytology Report)”. There were no changes made to the pathology report.

QUALITY ASSURANCE FINAL CERTIFICATION

QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: EPA Fiber Project: Subchronic Inhalation Exposure of Rats to Amphibole Asbestos - 1 Day, 1 Month and 3 Month timepoints

Client Study: Protocol No. 10026

EPL Principal Investigator: Dr. Gabrielle Willson

EPL Project Number: 304-466

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	Jan. 19, 2011; Feb. 3, 2011; May 17, 2011; Aug. 5, 2011; Aug. 16, 2011; May 29, 2012; Oct. 11, 2012; Oct. 24, 2012	Jan. 19, 2011; Feb. 3, 2011; May 17, 2011; Aug. 5, 2011; Aug. 16, 2011; May 29, 2012; Oct. 11, 2012; Oct. 24, 2012
Necropsy Records Review	Feb. 10, 2011; May 3, 2011; May 19, 2011; June 16, 2011; Aug. 16, 2011	Feb. 10, 2011; May 3, 2011; May 19, 2011; June 16, 2011; Aug. 16, 2011
Project Setup	Jan. 21, 2011; May 25, 2011; May 26, 2011; June 20, 2011; June 24, 2011; Aug. 23, 2011; Sept. 15, 2011; Oct. 31, 2011	Jan. 21, 2011; May 25, 2011; May 26, 2011; June 20, 2011; June 24, 2011; Aug. 23, 2011; Sept. 15, 2011; Oct. 31, 2011
Data Review	Jan. 27, 2011; Jan. 28, 2011; May 3, 2011; May 17, 2011; June 2, 2011; June 9, 2011; June 27, 2011; July 21, 2011; Sept. 1, 2011; Sept. 19, 2012	Jan. 27, 2011; Jan. 28, 2011; May 3, 2011; May 17, 2011; June 2, 2011; June 9, 2011; June 27, 2011; July 21, 2011; Sept. 1, 2011; Sept. 19, 2012
Draft Pathology Report	Dec. 12, 14 & 15, 2011; Oct. 10, 2012	Dec. 15, 2011; Oct. 10, 2012
Final Pathology Report	Oct. 31, 2012	Oct. 31, 2012
Amended Final Report	March 6, 2013	March 6, 2013

Date of last quarterly facility inspection: October 2012

Jane J. Hollingsworth
EPL Quality Assurance Unit

March 6, 2013
Date

APPENDIX A

1-DAY POST-EXPOSURE

SUMMARY TABLE

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90126 GAW
TEST SYSTEM : RAT, 1-DAY, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL SACRIFICE GROUP (K0)

Sex	Males				
Dose Group	01	02	03	04	05
No. Animals per Dose Group	8	8	8	8	8
LUNG, LEFT No.Examined	8	8	8	8	8
- Bronchiole Epithelial Hyperplasia	-	-	-	-	8
- Alveolus Inflammation	-	8	8	8	8
- Interstitium Fibrosis	-	8	8	8	8
- Foreign Body	-	8	8	8	8
- Bronchiolization	-	8	7	8	8
- Inflammation Chronic Focal	2	1	4	-	-
TESTIS, LEFT No.Examined	8	8	8	8	8
- Tubular Atrophy	2	1	5	3	1
EPIDIDYMIS, LEFT No.Examined	8	8	8	8	8
- Aspermia	-	-	-	1	-

Group 01, AIR CONTROL, males: AMPHIBOLE ASBESTOS (0 mg/m3)
Group 02, AMOSITE, males: AMPHIBOLE ASBESTOS (3.3 mg/m3)
Group 03, LA/1.0, males: AMPHIBOLE ASBESTOS (1 mg/m3)
Group 04, LA/3.3, males: AMPHIBOLE ASBESTOS (3.3 mg/m3)
Group 05, LA/10.0, males: AMPHIBOLE ASBESTOS (10 mg/m3)

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90126 GAW
TEST SYSTEM : RAT, 1-DAY, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, AIR CONTROL

ANIMAL NUMBER :	113 MK0	114 MK0	115 MK0	116 MK0	117 MK0	118 MK0	119 MK0	120 MK0
LUNG, LEFT	-	+	+	-	-	-	-	-
- Inflammation Chronic Focal	.	1.	1.	.	.	.	.	.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	+	-	-	-	+	-	-	-
- Tubular Atrophy.	2.	.	.	.	2.	.	.	.
EPIDIDYMIS, LEFT	-	-	-	-	-	-	-	-
STERNUM	-	-	-	-	-	-	-	-

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90126 GAW
TEST SYSTEM : RAT, 1-DAY, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 02, AMOSITE

ANIMAL NUMBER :	225 MK0	226 MK0	227 MK0	228 MK0	229 MK0	230 MK0	231 MK0	232 MK0
LUNG, LEFT	+	+	+	+	+	+	+	+
- Alveolus Inflammation.	1.	1.	1.	1.	1.	1.	1.	1.
- Interstitium Fibrosis.	1.	1.	1.	1.	1.	1.	1.	1.
- Foreign Body.	P.	P.	P.	P.	P.	P.	P.	P.
- Bronchiolization	1.	1.	1.	1.	1.	1.	1.	1.
- Inflammation Chronic Focal	.	.	.	.	1.	.	.	.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	-	-	-	-	+	-	-	-
- Tubular Atrophy.	.	.	.	.	2.	.	.	.
EPIDIDYMIS, LEFT	-	-	-	-	-	-	-	-
STERNUM	-	-	-	-	-	-	-	-

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90126 GAW
TEST SYSTEM : RAT, 1-DAY, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 03, LA/1.0

ANIMAL NUMBER :	325 MK0	326 MK0	327 MK0	328 MK0	329 MK0	330 MK0	331 MK0	332 MK0
LUNG, LEFT	+	+	+	+	+	+	+	+
- Alveolus Inflammation.	1.	1.	1.	1.	1.	1.	1.	1.
- Interstitium Fibrosis.	1.	1.	1.	1.	1.	1.	1.	1.
- Foreign Body.	P.	P.	P.	P.	P.	P.	P.	P.
- Bronchiolization	1.	1.	1.	1.	1.	1.	1.	.
- Inflammation Chronic Focal	.	.	.	1.	.	1.	1.	1.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	+	+	+	+	+	-	-	-
- Tubular Atrophy.	2.	3.	2.	3.	2.	.	.	.
EPIDIDYMIS, LEFT	-	-	-	-	-	-	-	-
STERNUM	-	-	-	-	-	-	-	-

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90126 GAW
TEST SYSTEM : RAT, 1-DAY, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, LA/3.3

ANIMAL NUMBER :	425 MK0	426 MK0	427 MK0	428 MK0	429 MK0	430 MK0	431 MK0	432 MK0
LUNG, LEFT	+	+	+	+	+	+	+	+
- Alveolus Inflammation.	1.	1.	1.	1.	1.	1.	1.	1.
- Interstitium Fibrosis.	1.	1.	1.	1.	1.	1.	1.	1.
- Foreign Body.	P.	P.	P.	P.	P.	P.	P.	P.
- Bronchiolization	1.	1.	1.	1.	1.	1.	1.	1.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	-	-	+	-	+	-	+	-
- Tubular Atrophy.	.	.	4.	.	2.	.	4.	.
EPIDIDYMIS, LEFT	-	-	+	-	-	-	-	-
- Aspermia	.	.	P.	.	.	.	.	.
STERNUM	-	-	-	-	-	-	-	-

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90126 GAW
TEST SYSTEM : RAT, 1-DAY, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 05, LA/10.0

ANIMAL NUMBER :	525 MK0	526 MK0	527 MK0	528 MK0	529 MK0	530 MK0	531 MK0	532 MK0
LUNG, LEFT	+	+	+	+	+	+	+	+
- Bronchiole Epithelial Hyperplasia.	1.	1.	1.	1.	1.	1.	1.	1.
- Alveolus Inflammation.	2.	2.	2.	2.	2.	2.	2.	2.
- Interstitium Fibrosis.	1.	1.	1.	1.	1.	1.	1.	1.
- Foreign Body.	P.	P.	P.	P.	P.	P.	P.	P.
- Bronchiolization	1.	1.	1.	1.	1.	1.	1.	1.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	-	-	-	-	-	-	-	+
- Tubular Atrophy.	.	.	.	.	.	.	.	1.
EPIDIDYMIS, LEFT	-	-	-	-	-	-	-	-
STERNUM	-	-	-	-	-	-	-	-

APPENDIX B

1-MONTH POST-EXPOSURE

SUMMARY TABLE

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90186 GAW
TEST SYSTEM : RAT, 1 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL SACRIFICE GROUP (K0)

Sex	Males				
Dose Group	01	02	03	04	05
No. Animals per Dose Group	8	8	8	8	8
LUNG, LEFT No.Examined	8	8	8	8	8
- Bronchiole Epithelial Hyperplasia	-	1	-	-	8
- Alveolus Inflammation	-	8	8	8	8
- Interstitium Fibrosis	-	8	7	8	8
- Foreign Body	-	8	8	8	8
- Bronchiolization	-	7	7	7	8
- Alveolar Epithelial Hyperplasia	-	-	-	-	1
- Inflammation Chronic Focal	5	6	6	4	-
TESTIS, LEFT No.Examined	8	8	8	8	8
- Tubular Atrophy	1	2	4	4	2
MEDIAST. LYMPH NODE No.Examined	-	-	-	-	1
- Hyperplasia	-	-	-	-	1
EPIDIDYMIS, LEFT No.Examined	8	8	8	8	8
- Sperm Granuloma	1	-	-	1	-

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90186 GAW
TEST SYSTEM : RAT, 1 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, AIR CONTROL

ANIMAL NUMBER :	121 MK0	122 MK0	123 MK0	124 MK0	125 MK0	126 MK0	127 MK0	128 MK0
LUNG, LEFT	+	+	+	-	+	+	-	-
- Inflammation Chronic Focal	1.	1.	1.	.	1.	1.	.	.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	-	+	-	-	-	-	-	-
- Tubular Atrophy.	.	4.	.	.	.	.	.	.
EPIDIDYMIS, LEFT	-	+	-	-	-	-	-	-
- Sperm Granuloma.	.	P.	.	.	.	.	.	.
STERNUM	-	-	-	-	-	-	-	-

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90186 GAW
TEST SYSTEM : RAT, 1 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 02, AMOSITE

ANIMAL NUMBER :	233 MK0	234 MK0	235 MK0	236 MK0	237 MK0	238 MK0	239 MK0	240 MK0
LUNG, LEFT	+	+	+	+	+	+	+	+
- Bronchiole Epithelial Hyperplasia.	.	.	.	.	.	1.	.	.
- Alveolus Inflammation.	1.	1.	1.	1.	1.	1.	2.	1.
- Interstitium Fibrosis.	1.	1.	1.	1.	1.	1.	1.	1.
- Foreign Body.	P.	P.	P.	P.	P.	P.	P.	P.
- Bronchiolization	1.	.	1.	1.	1.	1.	1.	1.
- Inflammation Chronic Focal	1.	1.	.	1.	1.	1.	.	1.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	-	+	-	+	-	-	-	-
- Tubular Atrophy.	.	1.	.	1.	.	.	.	.
EPIDIDYMIS, LEFT	-	-	-	-	-	-	-	-
STERNUM	-	-	-	-	-	-	-	-

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90186 GAW
TEST SYSTEM : RAT, 1 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 03, LA/1.0

ANIMAL NUMBER :	333 MK0	334 MK0	335 MK0	336 MK0	337 MK0	338 MK0	339 MK0	340 MK0
LUNG, LEFT	+	+	+	+	+	+	+	+
- Alveolus Inflammation.	1.	1.	1.	1.	1.	1.	1.	1.
- Interstitium Fibrosis.	.	1.	1.	1.	1.	1.	1.	1.
- Foreign Body.	P.	P.	P.	P.	P.	P.	P.	P.
- Bronchiolization	1.	1.	1.	1.	1.	.	1.	1.
- Inflammation Chronic Focal	1.	1.	1.	1.	.	.	1.	1.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	-	+	+	-	-	-	+	+
- Tubular Atrophy.	.	1.	1.	.	.	.	1.	2.
EPIDIDYMIS, LEFT	-	-	-	-	-	-	-	-
STERNUM	-	-	-	-	-	-	-	-

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90186 GAW
TEST SYSTEM : RAT, 1 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, LA/3.3

ANIMAL NUMBER :	433 MK0	434 MK0	435 MK0	436 MK0	437 MK0	438 MK0	439 MK0	440 MK0
LUNG, LEFT	+	+	+	+	+	+	+	+
- Alveolus Inflammation.	1.	1.	1.	1.	1.	1.	1.	1.
- Interstitium Fibrosis.	1.	1.	1.	1.	1.	1.	1.	1.
- Foreign Body.	P.	P.	P.	P.	P.	P.	P.	P.
- Bronchiolization	1.	1.	1.	1.	1.	1.	1.	.
- Inflammation Chronic Focal	1.	1.	1.	.	.	1.	.	.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	+	-	-	-	+	+	-	+
- Tubular Atrophy.	1.	.	.	.	1.	4.	.	1.
EPIDIDYMIS, LEFT	-	-	-	-	-	+G	-	-
- Sperm Granuloma.	.	.	.	.	.	P.	.	.
STERNUM	-	-	-	-	-	-	-	-

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90186 GAW
TEST SYSTEM : RAT, 1 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 05, LA/10.0

ANIMAL NUMBER :	533 MK0	534 MK0	535 MK0	536 MK0	537 MK0	538 MK0	539 MK0	540 MK0
LUNG, LEFT	+	+	+	+	+	+	+	+
- Bronchiole Epithelial Hyperplasia.	1.	1.	1.	1.	1.	1.	1.	1.
- Alveolus Inflammation.	2.	2.	2.	2.	2.	2.	2.	2.
- Interstitium Fibrosis.	1.	1.	1.	1.	1.	1.	1.	1.
- Foreign Body.	P.	P.	P.	P.	P.	P.	P.	P.
- Bronchiolization	1.	2.	1.	1.	2.	2.	1.	1.
- Alveolar Epithelial Hyperplasia	1.	.	.	.	.	.	.	.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	-	-	+	-	+	-	-	-
- Tubular Atrophy.	.	.	1.	.	2.	.	.	.
MEDIAST. LYMPH NODE	'	'	'	'	'	'	+G	'
- Hyperplasia							2.	
EPIDIDYMIS, LEFT	-	-	-	-	-	-	-	-
STERNUM	-	-	-	-	-	-	-	-

MACRO/MICRO CORRELATION TABLE

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90186 GAW
TEST SYSTEM : RAT, 1 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

CORRELATION TABLE: NECROPSY - MICROSCOPY DOSE GROUP 04, MALE

NECROPSY OBSERVATION	CORRESPONDING MICROSCOPIC FINDING
	ANIMAL NO: 438
EPIDIDYMIS, LEFT	
- 01: 3mm diameter yellow nodule.	- Sperm Granuloma.

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90186 GAW
TEST SYSTEM : RAT, 1 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

CORRELATION TABLE: NECROPSY - MICROSCOPY DOSE GROUP 05, MALE

NECROPSY OBSERVATION	CORRESPONDING MICROSCOPIC FINDING
	ANIMAL NO: 539
MEDIASTINAL LYMPH NODE	
- 01: Enlarged, mild; discolored, pink.	- Hyperplasia, grade 2.

APPENDIX C

3-MONTHS POST-EXPOSURE

SUMMARY TABLE

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90187 GAW
TEST SYSTEM : RAT, 3 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL SACRIFICE GROUP (K0)

Sex	Males				
Dose Group	01	02	03	04	05
No. Animals per Dose Group	8	8	8	8	8
LUNG, LEFT No.Examined	8	8	8	8	8
- Alveolus Inflammation	-	8	8	8	8
- Interstitium Fibrosis	-	8	8	8	8
- Foreign Body	-	8	8	8	8
- Bronchiolization	-	8	8	8	8
- Alveolar Epithelial Hyperplasia	-	-	-	-	1
- Inflammation Chronic Focal	2	2	1	2	-
TESTIS, LEFT No.Examined	8	8	8	8	8
- Tubular Atrophy	2	1	3	3	2
TESTIS, RIGHT No.Examined	-	-	1	-	-
- Tubular Atrophy	-	-	1	-	-
EPIDIDYMIS, LEFT No.Examined	8	8	8	8	8
- Aspermia	-	-	1	-	-

Group 01, AIR CONTROL, males: AMPHIBOLE ASBESTOS (0 mg/m3)
Group 02, AMOSITE, males: AMPHIBOLE ASBESTOS (3.3 mg/m3)
Group 03, LA/1.0, males: AMPHIBOLE ASBESTOS (1 mg/m3)
Group 04, LA/3.3, males: AMPHIBOLE ASBESTOS (3.3 mg/m3)
Group 05, LA/10.0, males: AMPHIBOLE ASBESTOS (10 mg/m3)

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90187 GAW
TEST SYSTEM : RAT, 3 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, AIR CONTROL

ANIMAL NUMBER :	129 MK0	130 MK0	131 MK0	132 MK0	133 MK0	134 MK0	135 MK0	136 MK0
LUNG, LEFT	-	-	-	-	-	+	-	+
- Inflammation Chronic Focal	.	.	.	.	.	1.	.	1.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	-	-	-	-	-	-	+	+
- Tubular Atrophy.	.	.	.	.	.	.	1.	1.
TESTIS, RIGHT	.	.	.	.	.	.	.	.
EPIDIDYMIS, LEFT	-	-	-	-	-	-	-	-
STERNUM	-	-	-	-	-	-	-	-

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90187 GAW
TEST SYSTEM : RAT, 3 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 02, AMOSITE

ANIMAL NUMBER :	241 MK0	242 MK0	243 MK0	244 MK0	245 MK0	246 MK0	247 MK0	248 MK0
LUNG, LEFT	+	+	+	+	+	+	+	+
- Alveolus Inflammation.	1.	1.	1.	1.	1.	1.	1.	1.
- Interstitium Fibrosis.	1.	1.	1.	1.	1.	1.	1.	1.
- Foreign Body.	P.	P.	P.	P.	P.	P.	P.	P.
- Bronchiolization	1.	1.	1.	1.	1.	1.	1.	1.
- Inflammation Chronic Focal	.	1.	.	.	1.	.	.	.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	+	-	-	-	-	-	-	-
- Tubular Atrophy.	1.	.	.	.	.	.	.	.
TESTIS, RIGHT	.	.	.	.	.	.	.	.
EPIDIDYMIS, LEFT	-	-	-	-	-	-	-	-
STERNUM	-	-	-	-	-	-	-	-

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90187 GAW
TEST SYSTEM : RAT, 3 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 03, LA/1.0

ANIMAL NUMBER :	341 MK0	342 MK0	343 MK0	344 MK0	345 MK0	346 MK0	347 MK0	348 MK0
LUNG, LEFT	+	+	+	+	+	+	+	+
- Alveolus Inflammation.	1.	1.	1.	1.	1.	1.	1.	1.
- Interstitium Fibrosis.	1.	1.	1.	1.	1.	1.	1.	1.
- Foreign Body.	P.	P.	P.	P.	P.	P.	P.	P.
- Bronchiolization	1.	1.	1.	1.	1.	1.	1.	1.
- Inflammation Chronic Focal	.	.	.	.	.	.	1.	.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	-	+	+	+	-	-	-	-
- Tubular Atrophy.	.	3.	5.	2.	.	.	.	.
TESTIS, RIGHT	.	.	+G	.	.	.	.	.
- Tubular Atrophy.	.	.	5.	.	.	.	.	.
EPIDIDYMIS, LEFT	-	-	+	-	-	-	-	-
- Aspermia	.	.	P.	.	.	.	.	.
STERNUM	-	-	-	-	-	-	-	-

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90187 GAW
TEST SYSTEM : RAT, 3 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, LA/3.3

ANIMAL NUMBER :	441 MK0	442 MK0	443 MK0	444 MK0	445 MK0	446 MK0	447 MK0	448 MK0
LUNG, LEFT	+	+	+	+	+	+	+	+
- Alveolus Inflammation.	2.	1.	1.	1.	1.	1.	1.	1.
- Interstitium Fibrosis.	1.	1.	1.	1.	1.	1.	1.	1.
- Foreign Body.	P.	P.	P.	P.	P.	P.	P.	P.
- Bronchiolization	1.	1.	1.	1.	1.	1.	1.	1.
- Inflammation Chronic Focal	.	.	.	1.	.	.	.	1.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	+	-	-	-	+	-	+	-
- Tubular Atrophy.	1.	.	.	.	1.	.	2.	.
TESTIS, RIGHT	.	.	.	.	.	.	.	.
EPIDIDYMIS, LEFT	-	-	-	-	-	-	-	-
STERNUM	-	-	-	-	-	-	-	-

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90187 GAW
TEST SYSTEM : RAT, 3 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 05, LA/10.0

ANIMAL NUMBER :	541 MK0	542 MK0	543 MK0	544 MK0	545 MK0	546 MK0	547 MK0	548 MK0
LUNG, LEFT	+	+	+	+	+	+	+	+
- Alveolus Inflammation.	2.	1.	1.	1.	2.	1.	1.	2.
- Interstitium Fibrosis.	1.	1.	1.	1.	1.	1.	1.	1.
- Foreign Body.	P.	P.	P.	P.	P.	P.	P.	P.
- Bronchiolization	1.	1.	1.	1.	1.	1.	1.	1.
- Alveolar Epithelial Hyperplasia	2.	.	.	.	.	.	.	.
TRACHEA	-	-	-	-	-	-	-	-
TESTIS, LEFT	-	+	-	-	-	-	+	-
- Tubular Atrophy.	.	1.	.	.	.	.	1.	.
TESTIS, RIGHT	.	.	.	.	.	.	.	.
EPIDIDYMIS, LEFT	-	-	-	-	-	-	-	-
STERNUM	-	-	-	-	-	-	-	-

MACRO/MICRO CORRELATION TABLE

TEST ITEM : AMPHIBOLE ASBESTOS PATHOL. NO.: 90187 GAW
TEST SYSTEM : RAT, 3 MONTH, INHALATION DATE : 30-OCT-12
SPONSOR : THE HAMNER PathData®System V6.2d2

CORRELATION TABLE: NECROPSY - MICROSCOPY DOSE GROUP 03, MALE

NECROPSY OBSERVATION	CORRESPONDING MICROSCOPIC FINDING
	ANIMAL NO: 343
TESTIS (RIGHT)	
- 01: Discoloration, dark, mild.	- Tubular Atrophy, focal, grade 5.

EXPLANATION OF CODES AND SYMBOLS

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

ATTACHMENT

EPA FIBER PROJECT: SUBCHRONIC INHALATION EXPOSURE OF RATS TO AMPHIBOLE ASBESTOS:

BRONCHOALVEOLAR LAVAGE (BAL) CYTOLOGY

EPA FIBER PROJECT: SUBCHRONIC INHALATION EXPOSURE OF RATS TO AMPHIBOLE ASBESTOS: BRONCHOALVEOLAR LAVAGE CYTOLOGY

THE HAMNER INSTITUTES FOR HEALTH SCIENCES PROTOCOL NUMBER: 10026

EPL PROJECT NO.: 304-466

"AMENDED" FINAL BRONCHOALVEOLAR LAVAGE CYTOLOGY REPORT
(1 DAY, 1 MONTH AND 3 MONTHS POST-EXPOSURE)

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.

Street Address:
615 Davis Drive
Suite 500
Durham, NC 27713

Mailing Address:
P.O. Box 12766
RTP, NC 27709

(919)998-9407

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OF RATS TO AMPHIBOLE ASBESTOS:
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INTRODUCTION

This subchronic study was conducted to determine the biological potency of inhaled Libby amphibole (LA) fibers over the near-life span of the rat compared to the potency of inhaled amosite, a known fibrogenic amphibole asbestos fiber, and to develop fiber burden data to use in a dosimetry model of amphibole fiber deposition, clearance, and retention in the respiratory tract (head, trachea, lung lobes) and GI tract. 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. As noted in Table 1 below, at each scheduled necropsy conducted at 1 day, 1 month, and 3 months post-exposure, the same 8 animals designated for histopathology were also designated to have the right lung lavaged for BAL cytology.

Table 1. Experimental Design

Group Number[1]	Test Material	Concentration (mg/m^3)	No. of rats necropsied at each time point[2]				Total in Each Exp. Group
			Post-Exposure Period				
			1 day	1 month	3 months	18 months[3]	
1	Air Control	0.0	11	11	11	53	86
2	Amosite	3.3	14	14	14	56	98
3	LA	1.0	14	14	14	56	98
4	LA	3.3	14	14	14	56	98
5	LA	10.0	14	14	14	56	98
	Total in Each Post-Exp Period		67	67	67	277	
	Total Animals in Study		**478**				

[1]To facilitate the BAL cytology analysis, the exposure level (treatment) groups were numbered as follows: 1 – Air control, 2 – amosite 3.3 mg/m^3, 3 – LA 1.0, 4 – LA 3.3 mg/m^3, and 5 – LA 10 mg/m^3.

[2]At each scheduled necropsy conducted at 1 day, 1 month, and 3 months post-exposure, the same 8 animals designated for histopathology were also designated to have the right lung lavaged for BAL cytology.

[3]Actual Post-Exposure Period = 75% survival (see above; projected at 23 months old; 18 months post-exposure).

BRONCHOALVEOLAR LAVAGE CYTOLOGY PROCEDURES

Slide preparations of rat BAL cell samples that were stained with Siemens Diff-Quik Stain Set (Fisher Scientific) were provided to EPL for determination of the cell differential percentages. Except when samples were inadequate, three hundred (300) leukocytic 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 (40X 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. Due to inadequate samples the total cells counted were less than 300 for animal 436 (LA 3.3 mg/$m^{3,}$ 1 Month) and 545 (LA 10.0 mg/m^3, 3 Months). The differential cell count data for those two animals were recorded, but percentages were not calculated and the animals were excluded from the statistical analyses. Animal 120 (Air Control, 1 Day) had cell percentages calculated and included in the results tabulation, but was excluded from the statistical analysis due to an unexplained high neutrophil count (173 of 300 cells). For those animals for which 300 cells were counted, the total 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 cell counts recorded for individual photomicrographic images were included in the filed study raw data. Except as noted above, the total 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 for application to calculate the absolute (total) differential BAL cell counts used for the statistical analysis. The tabulated data for the differential counts of cells in BAL cytology preps were audited by the EPL Quality Assurance unit and the audited tabulated data were provided electronically to the Study Director for statistical analysis of the absolute (total) BAL differential cell counts that was completed by The Hamner Institutes for Health Sciences. The results of the statistical analysis were returned to EPL. Tables were provided by The Hamner Institutes for Health Sciences that summarize the statistically significant differences in cell counts by group for each necropsy interval and the total cell counts by type for each necropsy interval are incorporated as appendices in this report. Tabular and graphic information that further document the statistical analysis was filed as raw data.

RESULTS AND DISCUSSION

The results for the differential cell count of up to 300 cells in BAL cytology samples from each animal are presented in Appendix A. As previously described, percentages were not calculated and included for animal 436 (1 Month, LA 3.3 mg/m^3) and animal 545 (3 Months, LA 10.0 mg/m^3) that had less than 300 cells counted due to inadequate samples. These two animals and animal 120 (Air Control, 1 Day) that had an unexplained high neutrophil count, were also excluded from the statistical analysis. Statistically significant differences in the total number of cells for each type of cell included in the differential count at the one day, one month and 3 months post-exposure time points are presented in Appendix B. The total cell counts that were calculated using percentages in Appendix A are presented in Appendices C, D, and E for the one day, one month, and 3 month necropsy time points, respectively.

1 Day Post-exposure Samples

In comparison to the Air control group, the total numbers of lymphocytes and neutrophils of the LA 10.0 mg/m^3 were increased ($p<0.01$), total number of neutrophils of the LA 3.3 mg/m^3 was increased ($p<0.01$), and total number of neutrophils in the Amosite 3.3 mg/m^3 group was increased ($p<0.01$). The total numbers of neutrophils were also increased in the LA 10.0 mg/m^3 ($p<0.01$) and LA 1.0 mg/m^3 ($p<0.05$) when compared to the Amosite 3.3 mg/m^3 group.

1 Month Post-exposure Samples

At one month post-exposure the total numbers of neutrophils of the Amosite 3.3 mg/m^3 group and the LA 10.0 mg/m^3 groups were increased ($p<0.01$) when compared to the Air Control group. The total number of neutrophils was also increased ($p<0.01$) in the LA 10.0 mg/m^3 when compared to the Amosite 3.3 mg/m^3 group.

3 Months Post-exposure Samples

At 3 months post-exposure the total numbers of neutrophils in the LA 10.0 mg/m^3 group were increased ($p<0.01$) when compared to the Air control group and to the Amosite 3.3 mg/m^3 group. The total number of neutrophils of the LA 3.3 mg/m^3 group was increased ($p<0.05$) when compared to the Air control group.

Temporal Patterns of Total Cell Counts for Individual Cell Types

The results at the one day, one month and 3 months post-exposure intervals showed increases in the total neutrophils counts in the LA 10.0 mg/m^3 group when compared to the Air control and Amosite 3.3 mg/m^3. In comparison to the Air control group, total neutrophils of the LA 3.3 mg/m^3 were increased most markedly at one day post-exposure, were not different at one month, and were increased again at 3 months post-exposure. The LA 1.0 mg/m^3 group showed a difference in total neutrophils from the total neutrophils of the Amosite 3.3 mg/m^3 group only at one day post-exposure. The total counts of neutrophils of the Amosite 3.3. mg/m^3 group were increased at one day and one month post-exposure, but not at 3 months post-exposure.

A difference in total counts of lymphocytes was only noted for the LA 10.0 mg/m^3 when compared to the Air Control group at one day post-exposure.

CONCLUSIONS

The BAL cell differential cytology and total cell counts for individual cell types disclosed that Libby amphibole (LA) asbestos exposure of rats to 10.0 mg/m^3 for 90 days resulted in increased neutrophils in comparison to rats that were Air Control or exposed to Amosite 3.3 mg/m^3 at all post-exposure sampling intervals (one day, one month, 3 months). Neutrophils were increased in the LA 3.3 mg/m^3 group only at the one day and 3 month post-exposure necropsy samplings. The Amosite 3.3 mg/m^3 group only showed increased neutrophils in comparison to the Air control group at one day and one month post-exposure.

Henry G. Wall

HENRY G. WALL, D.V.M., Ph.D.
Diplomate, ACVP
Veterinary Pathologist

6 Mar 2013

DATE

HGW/dc

REFERENCES

Gao H, Hoesel LM, Guo R-F, Rancilio NJ, Sarma JV, Ward PA. 2006. Adenoviral-mediated overexpression of SOCS3 enhances IgG immune complex-induced acute lung injury. J Immunol 177:612-620.

Palmans E, Kips JC, Pauwels RA. 2000. Prolonged allergen exposure induces structural airway changes in sensitized rats. Am J Respir Crit Care Med 161:627-635.

REASON FOR CHANGE

1. To correct incorrectly calculated total cell counts reported in Appendix B of the BAL Cytology Report and to update narrative statements subsequent to the statistical analysis of the revised total cell count data for the 3-months post-exposure samples.

2. To include corrected Appendix A "3-Months Post-Exposure Total Cell Counts by Cell Types" which was used for statistical analysis.

3. NOTE: Changes were made only to the "Attachment (Bronchoalveolar Lavage Cytology Report)". There were no changes made to the pathology report.

Henry G. Wall

HENRY G. WALL, D.V.M., Ph.D.
Diplomate, ACVP
Veterinary Pathologist

6 Mar 2013
DATE

APPENDIX A

INDIVIDUAL ANIMAL BAL CELLS DIFFERENTIAL COUNTS AND PERCENTAGES – 1 DAY, 1 MONTH AND 3 MONTHS POST-EXPOSURE

Appendix A
Hamner Insitutes of Health Sciences Protocol Number 10026
EPL 304-466

Individual Animal BAL Cells Differential Counts and Percentages - 1 Day, 1 month, and 3 Months Post-exposure

Necropsy (elapsed time post-exposure)	Exposure Level Concentration (mg/m^3)	GP	ID	EOS	EOP	LYM	LYP	NEU	NEP	MAC	MAP	BAS	BAP	Total Cells Counted
1 day	Air Control	1	113	0	0.00	8	2.67	0	0.00	292	97.33	0	0.00	300
1 day	Air Control	1	114	0	0.00	1	0.33	0	0.00	299	99.67	0	0.00	300
1 day	Air Control	1	115	0	0.00	0	0.00	2	0.67	298	99.33	0	0.00	300
1 day	Air Control	1	116	0	0.00	4	1.33	3	1.00	293	97.67	0	0.00	300
1 day	Air Control	1	117	0	0.00	0	0.00	0	0.00	300	100.00	0	0.00	300
1 day	Air Control	1	118	0	0.00	0	0.00	1	0.33	299	99.67	0	0.00	300
1 day	Air Control	1	119	0	0.00	11	3.67	2	0.67	287	95.67	0	0.00	300
1 day	Air Control	1	120	0	0.00	17	5.67	173	57.67	110	36.67	0	0.00	300
1 day	Amosite 3.3	2	225	0	0.00	3	1.00	77	25.67	220	73.33	0	0.00	300
1 day	Amosite 3.3	2	226	0	0.00	15	5.00	48	16.00	237	79.00	0	0.00	300
1 day	Amosite 3.3	2	227	0	0.00	20	6.67	45	15.00	235	78.33	0	0.00	300
1 day	Amosite 3.3	2	228	0	0.00	11	3.67	50	16.67	239	79.67	0	0.00	300
1 day	Amosite 3.3	2	229	0	0.00	3	1.00	46	15.33	251	83.67	0	0.00	300
1 day	Amosite 3.3	2	230	0	0.00	7	2.33	58	19.33	235	78.33	0	0.00	300
1 day	Amosite 3.3	2	231	0	0.00	7	2.33	20	6.67	273	91.00	0	0.00	300
1 day	Amosite 3.3	2	232	0	0.00	11	3.67	47	15.67	242	80.67	0	0.00	300
1 day	LA 1.0	3	325	0	0.00	0	0.00	8	2.67	292	97.33	0	0.00	300
1 day	LA 1.0	3	326	0	0.00	37	12.33	27	9.00	236	78.67	0	0.00	300
1 day	LA 1.0	3	327	0	0.00	5	1.67	18	6.00	277	92.33	0	0.00	300
1 day	LA 1.0	3	328	0	0.00	4	1.33	8	2.67	288	96.00	0	0.00	300
1 day	LA 1.0	3	329	0	0.00	2	0.67	10	3.33	288	96.00	0	0.00	300
1 day	LA 1.0	3	330	0	0.00	11	3.67	15	5.00	274	91.33	0	0.00	300
1 day	LA 1.0	3	331	0	0.00	5	1.67	8	2.67	287	95.67	0	0.00	300
1 day	LA 1.0	3	332	0	0.00	8	2.67	32	10.67	260	86.67	0	0.00	300
1 day	LA 3.3	4	425	0	0.00	1	0.33	32	10.67	267	89.00	0	0.00	300
1 day	LA 3.3	4	426	0	0.00	4	1.33	52	17.33	244	81.33	0	0.00	300
1 day	LA 3.3	4	427	0	0.00	23	7.67	72	24.00	205	68.33	0	0.00	300
1 day	LA 3.3	4	428	0	0.00	25	8.33	90	30.00	185	61.67	0	0.00	300
1 day	LA 3.3	4	429	0	0.00	13	4.33	51	17.00	236	78.67	0	0.00	300
1 day	LA 3.3	4	430	0	0.00	18	6.00	58	19.33	224	74.67	0	0.00	300
1 day	LA 3.3	4	431	0	0.00	0	0.00	22	7.33	278	92.67	0	0.00	300
1 day	LA 3.3	4	432	0	0.00	16	5.33	60	20.00	224	74.67	0	0.00	300
1 day	LA 10.0	5	525	0	0.00	31	10.33	100	33.33	169	56.33	0	0.00	300

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Individual Animal BAL Cells Differential Counts and Percentages - 1 Day, 1 month, and 3 Months Post-exposure

Necropsy (elapsed time post-exposure)	Exposure Level Concentration (mg/m^3)	GP	ID	EOS	EOP	LYM	LYP	NEU	NEP	MAC	MAP	BAS	BAP	Total Cells Counted
1 day	LA 10.0	5	526	0	0.00	16	5.33	105	35.00	179	59.67	0	0.00	300
1 day	LA 10.0	5	527	0	0.00	19	6.33	133	44.33	148	49.33	0	0.00	300
1 day	LA 10.0	5	528	0	0.00	21	7.00	104	34.67	175	58.33	0	0.00	300
1 day	LA 10.0	5	529	0	0.00	6	2.00	101	33.67	193	64.33	0	0.00	300
1 day	LA 10.0	5	530	0	0.00	19	6.33	82	27.33	199	66.33	0	0.00	300
1 day	LA 10.0	5	531	0	0.00	26	8.67	105	35.00	169	56.33	0	0.00	300
1 day	LA 10.0	5	532	0	0.00	12	4.00	91	30.33	197	65.67	0	0.00	300
1 month	Air Control	1	121	0	0.00	5	1.67	0	0.00	295	98.33	0	0.00	300
1 month	Air Control	1	122	0	0.00	3	1.00	1	0.33	296	98.67	0	0.00	300
1 month	Air Control	1	123	0	0.00	9	3.00	6	2.00	285	95.00	0	0.00	300
1 month	Air Control	1	124	0	0.00	4	1.33	5	1.67	291	97.00	0	0.00	300
1 month	Air Control	1	125	0	0.00	6	2.00	0	0.00	294	98.00	0	0.00	300
1 month	Air Control	1	126	0	0.00	4	1.33	2	0.67	293	97.67	1	0.33	300
1 month	Air Control	1	127	0	0.00	0	0.00	0	0.00	300	100.00	0	0.00	300
1 month	Air Control	1	128	0	0.00	9	3.00	0	0.00	291	97.00	0	0.00	300
1 month	Amosite 3.3	2	233	0	0.00	2	0.67	34	11.33	264	88.00	0	0.00	300
1 month	Amosite 3.3	2	234	0	0.00	1	0.33	26	8.67	273	91.00	0	0.00	300
1 month	Amosite 3.3	2	235	0	0.00	0	0.00	32	10.67	268	89.33	0	0.00	300
1 month	Amosite 3.3	2	236	0	0.00	1	0.33	19	6.33	280	93.33	0	0.00	300
1 month	Amosite 3.3	2	237	0	0.00	0	0.00	16	5.33	284	94.67	0	0.00	300
1 month	Amosite 3.3	2	238	0	0.00	0	0.00	18	6.00	282	94.00	0	0.00	300
1 month	Amosite 3.3	2	239	0	0.00	0	0.00	60	20.00	240	80.00	0	0.00	300
1 month	Amosite 3.3	2	240	0	0.00	0	0.00	34	11.33	266	88.67	0	0.00	300
1 month	LA 1.0	3	333	0	0.00	0	0.00	24	8.00	276	92.00	0	0.00	300
1 month	LA 1.0	3	334	0	0.00	0	0.00	6	2.00	294	98.00	0	0.00	300
1 month	LA 1.0	3	335	0	0.00	0	0.00	4	1.33	296	98.67	0	0.00	300
1 month	LA 1.0	3	336	0	0.00	0	0.00	4	1.33	296	98.67	0	0.00	300
1 month	LA 1.0	3	337	0	0.00	0	0.00	5	1.67	295	98.33	0	0.00	300
1 month	LA 1.0	3	338	0	0.00	0	0.00	21	7.00	279	93.00	0	0.00	300
1 month	LA 1.0	3	339	0	0.00	0	0.00	0	0.00	300	100.00	0	0.00	300
1 month	LA 1.0	3	340	0	0.00	0	0.00	7	2.33	293	97.67	0	0.00	300
1 month	LA 3.3	4	433	0	0.00	2	0.67	42	14.00	256	85.33	0	0.00	300
1 month	LA 3.3	4	434	0	0.00	0	0.00	23	7.67	277	92.33	0	0.00	300

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Individual Animal BAL Cells Differential Counts and Percentages - 1 Day, 1 month, and 3 Months Post-exposure

Necropsy (elapsed time post-exposure)	Exposure Level Concentration (mg/m^3)	GP	ID	EOS	EOP	LYM	LYP	NEU	NEP	MAC	MAP	BAS	BAP	Total Cells Counted
1 month	LA 3.3	4	435	0	0.00	35	11.67	27	9.00	238	79.33	0	0.00	300
1 month	LA 3.3	4	436	0		3		16		56		0		75
1 month	LA 3.3	4	437	0	0.00	0	0.00	24	8.00	276	92.00	0	0.00	300
1 month	LA 3.3	4	438	0	0.00	21	7.00	36	12.00	243	81.00	0	0.00	300
1 month	LA 3.3	4	439	0	0.00	0	0.00	15	5.00	285	95.00	0	0.00	300
1 month	LA 3.3	4	440	0	0.00	3	1.00	12	4.00	285	95.00	0	0.00	300
1 month	LA 10.0	5	533	0	0.00	4	1.33	46	15.33	250	83.33	0	0.00	300
1 month	LA 10.0	5	534	0	0.00	1	0.33	71	23.67	228	76.00	0	0.00	300
1 month	LA 10.0	5	535	0	0.00	0	0.00	33	11.00	267	89.00	0	0.00	300
1 month	LA 10.0	5	536	0	0.00	0	0.00	41	13.67	259	86.33	0	0.00	300
1 month	LA 10.0	5	537	0	0.00	0	0.00	59	19.67	241	80.33	0	0.00	300
1 month	LA 10.0	5	538	0	0.00	0	0.00	85	28.33	215	71.67	0	0.00	300
1 month	LA 10.0	5	539	0	0.00	31	10.33	49	16.33	220	73.33	0	0.00	300
1 month	LA 10.0	5	540	0	0.00	0	0.00	46	15.33	254	84.67	0	0.00	300
3 months	Air Control	1	129	0	0.00	10	3.33	75	25.00	215	71.67	0	0.00	300
3 months	Air Control	1	130	0	0.00	13	4.33	0	0.00	287	95.67	0	0.00	300
3 months	Air Control	1	131	0	0.00	6	2.00	0	0.00	294	98.00	0	0.00	300
3 months	Air Control	1	132	0	0.00	21	7.00	2	0.67	277	92.33	0	0.00	300
3 months	Air Control	1	133	0	0.00	6	2.00	1	0.33	292	97.33	1	0.33	300
3 months	Air Control	1	134	0	0.00	10	3.33	3	1.00	287	95.67	0	0.00	300
3 months	Air Control	1	135	0	0.00	4	1.33	5	1.67	290	96.67	1	0.33	300
3 months	Air Control	1	136	0	0.00	1	0.33	2	0.67	297	99.00	0	0.00	300
3 months	Amosite 3.3	2	241	0	0.00	15	5.00	34	11.33	251	83.67	0	0.00	300
3 months	Amosite 3.3	2	242	1	0.33	36	12.00	47	15.67	215	71.67	1	0.33	300
3 months	Amosite 3.3	2	243	0	0.00	12	4.00	26	8.67	262	87.33	0	0.00	300
3 months	Amosite 3.3	2	244	0	0.00	6	2.00	30	10.00	264	88.00	0	0.00	300
3 months	Amosite 3.3	2	245	0	0.00	18	6.00	21	7.00	260	86.67	1	0.33	300
3 months	Amosite 3.3	2	246	0	0.00	9	3.00	29	9.67	262	87.33	0	0.00	300
3 months	Amosite 3.3	2	247	1	0.33	13	4.33	32	10.67	254	84.67	0	0.00	300
3 months	Amosite 3.3	2	248	0	0.00	14	4.67	33	11.00	252	84.00	1	0.33	300
3 months	LA 1.0	3	341	0	0.00	12	4.00	66	22.00	221	73.67	1	0.33	300
3 months	LA 1.0	3	342	0	0.00	9	3.00	7	2.33	284	94.67	0	0.00	300
3 months	LA 1.0	3	343	0	0.00	8	2.67	8	2.67	282	94.00	2	0.67	300

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Necropsy (elapsed time post-exposure)	Exposure Level Concentration (mg/m^3)	GP	ID	EOS	EOP	LYM	LYP	NEU	NEP	MAC	MAP	BAS	BAP	Total Cells Counted
3 months	LA 1.0	3	344	0	0.00	30	10.00	23	7.67	245	81.67	2	0.67	300
3 months	LA 1.0	3	345	0	0.00	6	2.00	3	1.00	289	96.33	2	0.67	300
3 months	LA 1.0	3	346	0	0.00	29	9.67	14	4.67	257	85.67	0	0.00	300
3 months	LA 1.0	3	347	0	0.00	11	3.67	6	2.00	280	93.33	3	1.00	300
3 months	LA 1.0	3	348	0	0.00	14	4.67	11	3.67	275	91.67	0	0.00	300
3 months	LA 3.3	4	441	0	0.00	12	4.00	47	15.67	240	80.00	1	0.33	300
3 months	LA 3.3	4	442	0	0.00	14	4.67	22	7.33	262	87.33	2	0.67	300
3 months	LA 3.3	4	443	0	0.00	14	4.67	32	10.67	249	83.00	5	1.67	300
3 months	LA 3.3	4	444	0	0.00	19	6.33	37	12.33	242	80.67	2	0.67	300
3 months	LA 3.3	4	445	0	0.00	41	13.67	59	19.67	200	66.67	0	0.00	300
3 months	LA 3.3	4	446	0	0.00	17	5.67	25	8.33	256	85.33	2	0.67	300
3 months	LA 3.3	4	447	0	0.00	20	6.67	66	22.00	214	71.33	0	0.00	300
3 months	LA 3.3	4	448	0	0.00	20	6.67	48	16.00	232	77.33	0	0.00	300
3 months	LA 10.0	5	541	0	0.00	17	5.67	54	18.00	228	76.00	1	0.33	300
3 months	LA 10.0	5	542	0	0.00	4	1.33	109	36.33	186	62.00	1	0.33	300
3 months	LA 10.0	5	543	0	0.00	10	3.33	81	27.00	203	67.67	6	2.00	300
3 months	LA 10.0	5	544	0	0.00	18	6.00	56	18.67	225	75.00	1	0.33	300
3 months	LA 10.0	5	545	0		26		45		76		0		147
3 months	LA 10.0	5	546	0	0.00	46	15.33	77	25.67	176	58.67	1	0.33	300
3 months	LA 10.0	5	547	0	0.00	27	9.00	67	22.33	205	68.33	1	0.33	300
3 months	LA 10.0	5	548	1	0.33	33	11.00	85	28.33	181	60.33	0	0.00	300

APPENDIX B

STATISTICAL SIGNIFICANCE OF CELL TYPES TABLES – 1 DAY, 1 MONTH, AND 3 MONTHS POST-EXPOSURE

Appendix B

The Hamner Protocol 10026 (EPL 304-466)

Statistical Significance of Cell Types Tables –
1 Day, 1 Month and 3 Months Post-exposure

Statistical Significance of Cell Types Table – One Day

Group A	Group B	Macrophage	Lymphocytes	Neutrophils
Air control	Amosite 3.3	–	–	++↑
Air control	LA 1.0	–	–	–
Air control	LA 3.3	–	–	++↑
Air control	LA 10.0	–	++↑	++↑
Amosite 3.3	LA 1.0	–	–	+↑
Amosite 3.3	LA 3.3	–	–	–
Amosite 3.3	LA 10.0	–	–	++↑

–	no significance		
+	significant at a p value < 0.05	↓	Group B decrease compared to Group A
++	significant at a p value <0.01	↑	Group B increase compared to Group A

Appendix B

The Hamner Protocol 10026 (EPL 304-466)

Statistical Significance of Cell Types Tables –
1 Day, 1 Month and 3 Months Post-exposure

Statistical Significance of Cell Types Table – One Month

Group A	Group B	Macrophage	Lymphocytes	Neutrophils
Air control	Amosite 3.3	–	–	++↑
Air control	LA 1.0	–	–	–
Air control	LA 3.3	–	–	–
Air control	LA 10.0	–	–	++↑
Amosite 3.3	LA 1.0	–	–	–
Amosite 3.3	LA 3.3	–	–	–
Amosite 3.3	LA 10.0	–	–	++↑

–	no significance		
+	significant at a p value < 0.05	↓	Group B decrease compared to Group A
++	significant at a p value <0.01	↑	Group B increase compared to Group A

Appendix B

The Hamner Protocol 10026 (EPL 304-466)

Statistical Significance of Cell Types Tables –
1 Day, 1 Month and 3 Months Post-exposure

Statistical Significance of Cell Types Table – Three Month

Group A	Group B	Macrophage	Lymphocytes	Neutrophils	Eosinophils	Basophils
Air control	Amosite 3.3	–	–	–	–	–
Air control	LA 1.0	–	–	–	–	–
Air control	LA 3.3	–	–	+↑	–	–
Air control	LA 10.0	–	–	++↑	–	–
Amosite 3.3	LA 1.0	–	–	–	–	–
Amosite 3.3	LA 3.3	–	–	–	–	–
Amosite 3.3	LA 10.0	–	–	++↑	–	–

–	no significance		
+	significant at a p value < 0.05		Group B decrease compared to Group A
++	significant at a p value <0.01	↑	Group B increase compared to Group A

Note: Eosinophils were observed in two animals in the Amosite 3.3 group and in one animal in the LA 10.0 group.

APPENDIX C

1 DAY POST-EXPOSURE TOTAL CELL COUNTS BY CELL TYPES

Appendix C
Protocol 10026 (EPL 304-466)
1 Day Post-exposure Total Cell Counts By Cell Types

Sample Number	rat ID	Group	Number of Macrophage	Number of Lymphocytes	Number of Neutrophils
1	113	Control	492,587.1	13513	0
2	114	Control	423,796.8	1403	0
3	115	Control	1,339,465.1	0	9035
4	116	Control	561,114.2	7641	5745
5	117	Control	788,700.0	0	0
6	118	Control	731,876.8	0	2423
7	119	Control	1,041,655.0	39959	7295
8	120	Control	162,081.4	25061	254901
9	225	Amosite 3.3	208,257.2	2840	72903
10	226	Amosite 3.3	959,534.0	60730	194336
11	227	Amosite 3.3	309,325.2	26340	59235
12	228	Amosite 3.3	625,967.2	28835	130976
13	229	Amosite 3.3	603,595.4	7214	110591
14	230	Amosite 3.3	537,578.8	15991	132662
15	231	Amosite 3.3	559,650.0	14330	41021
16	232	Amosite 3.3	385,844.6	17506	74950
17	325	LA 1.0	579,113.5	0	15887
18	326	LA 1.0	263,465.8	41293	30141
19	327	LA 1.0	709,279.1	12829	46092
20	328	LA 1.0	454,272.0	6294	12634
21	329	LA 1.0	837,024.0	5842	29034
22	330	LA 1.0	732,010.0	29415	40075
23	331	LA 1.0	840,365.3	14669	23365
24	332	LA 1.0	263,303.5	8111	32385
25	425	LA 3.3	413,939	1535	49626
26	426	LA 3.3	633,154	10354	134914
27	427	LA 3.3	501,064	56244	175992
28	428	LA 3.3	413,621	55869	201210
29	429	LA 3.3	774,978	42655	167467
30	430	LA 3.3	476,843	38316	123441
31	431	LA 3.3	573,813	0	45387
32	432	LA 3.3	564,879	40321	151300
33	525	LA 10.0	315,392	57838	186615
34	526	LA 10.0	377,055	33680	221165
35	527	LA 10.0	324,443	41632	291558
36	528	LA 10.0	717,459	86100	426441
37	529	LA 10.0	471,668	14664	246868
38	530	LA 10.0	558,233	50496	230009
39	531 *	LA 10.0	328,686	50589	204225
40	532 **	LA 10.0	676,007	41176	312217

* L1 dropped in Ice Bath - Spillage

** L1 has 3 washed L2 has 2 washes

APPENDIX D

1 MONTH POST-EXPOSURE TOTAL CELL COUNTS BY CELL TYPES

Appendix D
Protocol 10026 (EPL 304-426)
1 Month Post-exposure Total Cell Counts By Cell Types

Sample Number	rat ID	Group	Number of Macrophage	Number of Lymphocytes	Number of Neutrophils
1	121	Control	835313	14187	0
2	122	Control	841754	8531	2815
3	123	Control	1190160	37584	25056
4	124	Control	1027812	14093	17695
5	125	Control	1588090	32410	0
6	126	Control	976072	19184	1
7	127	Control	1174000	0	0
8	128	Control	1229475	38025	0
9	233	Amosite 3.3	1103432	8401	142067
10	234	Amosite 3.3	1021566	3705	97329
11	235	Amosite 3.3	848456	0	101344
12	236	Amosite 3.3	1024670	3623	69497
13	237	Amosite 3.3	872668	0	49132
14	238	Amosite 3.3	991230	0	63270
15	239	Amosite 3.3	936720	0	234180
16	240	Amosite 3.3	984326	0	125774
17	333	LA 1.0	1358748	0	118152
18	334	LA 1.0	925316	0	18884
19	335	LA 1.0	1116056	0	15044
20	336	LA 1.0	792715	0	10685
21	337	LA 1.0	916337	0	15563
22	338	LA 1.0	1145574	0	86226
23	339 *	LA 1.0	1532600	0	0
24	340	LA 1.0	1219996	0	29104
25	433	LA 3.3	718649	5643	117908
26	434	LA 3.3	770494	0	64006
27	435	LA 3.3	727773	107061	82566
28	436	LA 3.3	253464	13576	72360
29	437	LA 3.3	1075112	0	93488
30	438	LA 3.3	1304181	112707	193212
31	439	LA 3.3	1049085	0	55215
32	440	LA 3.3	1016975	10705	39287
33	533	LA 10.0	795802	12702	146402
34	534	LA 10.0	858876	3729	267495
35	535	LA 10.0	925600	0	114400
36	536	LA 10.0	1071269	0	169631
37	537	LA 10.0	847401	0	207499
38	538	LA 10.0	1003738	0	396762
39	539	LA 10.0	788664	111099	175629
40	540	LA 10.0	1673333	0	302967

APPENDIX E

3 MONTHS POST-EXPOSURE TOTAL CELL COUNTS BY CELL TYPES

Appendix E
Protocol 10026 (EPL 304-466)
3 Months Post-exposure Total Cell Counts by Cell Types

Sample Number	rat ID	Group	Number of Macrophage	Number of Lymphocytes	Number of Neutrophils	Number of Eosinophils	Number of Basophil
1	129	Control	629334	29241	219525	0	0
2	130	Control	938331	42469	0	0	0
3	131	Control	914438	18662	0	0	0
4	132	Control	728668	55244	5288	0	0
5	133	Control	959576	19718	3253	0	0
6	134	Control	567132	19740	5928	0	0
7	135	Control	909471	12513	15711	0	0
8	136	Control	611820	2039	4141	0	0
9	241	Amosite 3.3	728264	43520	98616	0	0
10	242	Amosite 3.3	438979	73500	95979	2021	0
11	243	Amosite 3.3	822823	37688	81689	0	0
12	244	Amosite 3.3	889416	20214	101070	0	0
13	245	Amosite 3.3	976598	67608	78876	0	0
14	246	Amosite 3.3	673489	23136	74575	0	2545
15	247	Amosite 3.3	1047622	53575	132020	4083	0
16	248	Amosite 3.3	683508	38000	89507	0	2685
17	341	LA 1.0	779871	42344	232892	0	0
18	342	LA 1.0	802139	25419	19742	0	0
19	343	LA 1.0	880310	25005	25005	0	3090
20	344	LA 1.0	644050	78860	60486	0	0
21	345	LA 1.0	702535	14586	7293	0	0
22	346	LA 1.0	759893	85773	41423	0	2927
23	347	LA 1.0	876462	34465	18782	0	0
24	348	LA 1.0	562670	28664	22526	0	0
25	441	LA 3.3	686880	34344	134543	0	2833
26	442	LA 3.3	787280	42100	66080	0	2975
27	443	LA 3.3	704504	39639	90567	0	0
28	444	LA 3.3	555171	43563	84855	0	4611
29	445	LA 3.3	663567	136058	195776	0	6669
30	446	LA 3.3	702607	46687	68589	0	5517
31	447	LA 3.3	428765	40093	132242	0	0
32	448	LA 3.3	688778	59410	142512	0	8907

Appendix E
Protocol 10026 (EPL 304-466)
3 Months Post-exposure Total Cell Counts by Cell Types

Sample Number	rat ID	Group	Number of Macrophage	Number of Lymphocytes	Number of Neutrophils	Number of Eosinophils	Number of Basophil
33	541	LA 10.0	611648	45632	144864	0	0
34	542	LA 10.0	415462	8912	243447	0	2211
35	543	LA 10.0	648549	31915	258768	0	6421
36	544	LA 10.0	600150	48012	149397	0	13363
37	545	LA 10.0	211252	72308	125100	0	5588
38	546	LA 10.0	591218	154480	258677	0	0
39	547	LA 10.0	702842	92574	229686	0	6892
40	548	LA 10.0	513046	93544	240918	2806	0